

## CD86: A Novel Prognostic Marker in Acute Lymphoblastic Leukemia Patients

Mona Ahmed Wahba, Heba Mostafa El-Maraghy, Asmaa Mohamed Abdel-Razik

Clinical Pathology Department, Faculty of Medicine, Ain Shams University

### ABSTRACT

**Background:** Acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in patients younger than 15 years, accounting for 76% of all leukemias in this age group. It accounts for only 20% of adult acute leukemias. The B7-family molecule CD86 is a type I transmembrane glycoprotein expressed on the surface of antigen presenting cells (APCs). Cell surface expression of CD86 provides an important co-stimulatory signal that profoundly influences immune responses. Optimal T-cell activation needs costimulatory signals via the interaction between costimulatory molecule CD28 on T lymphocytes and its ligands the B7-family molecules B7.2 (CD86) on APCs. Activation and differentiation of T lymphocytes plays an important role in mediating the pathogenesis of ALL.

**Objective:** This study aims to assess the expression of CD86 in acute lymphoblastic leukemia patients and correlate its expression with the clinical, hematological findings and response to therapy.

**Subjects and methods:** CD86 was measured in 35 newly diagnosed acute lymphoblastic leukemic patients and 20 age and sex matched healthy controls.

**Results:** A significant statistical difference between CD86 expression levels in patients versus controls was determined. There was a high statistically significant association between CD86 expression and poor outcome.

**Conclusion:** High CD86% and mean fluorescence intensity (MFI) expression appears to be a powerful prognostic indicator of unfavorable outcome in ALL. Analysis of CD86 percentage and MFI expression in addition to other standard prognostic markers at diagnosis may contribute to improve the management of ALL patients.

**Keywords:** Acute lymphoblastic leukemia, CD86.

### INTRODUCTION

Leukemia is a hematopoietic malignancy that results from the clonal proliferation of bone marrow cells with impaired differentiation, regulation, and cell death<sup>1</sup>.

Acute lymphocytic leukemia (ALL) is the most common hematological malignancy in childhood and accounts for about 20% of acute leukemia in adults<sup>2</sup>. Based on ontogenic classification, ALL is divided into T-lineage ALL and B-lineage ALL. T-cell acute lymphoblastic leukemia (T-ALL) is a rare, aggressive malignancy of thymocytes and corresponds to a heterogeneous group of leukemia arrested at various stages of lymphoid development<sup>3</sup>. CD86 is a member of B7 family, which consists of cell-surface proteins that regulate costimulatory or coinhibitory signals by binding to their ligands<sup>4</sup>.

Recognition of CD86 ligand by co-stimulatory CD28 and co-inhibitory CTLA-4 receptors plays an important role in influencing immune responses by proliferation and suppression of effector T cells respectively<sup>5</sup>.

CD86 is expressed on the surface of antigen presenting cells (APCs) as monocytes and dendritic cells (DCs), its expression was found to be associated with many hematological malignancies such as acute myeloid leukemia

(AML) and it was reported as a marker of poor prognosis in it<sup>6</sup>. Some of the most recent therapeutic developments for acute leukemia depend on the involvement of costimulatory pathways and molecules including CD86<sup>7</sup>.

### PATIENTS AND METHODS

#### Subjects:

#### Patients group:

The present study was carried out on thirty-five (35) newly diagnosed ALL patients, presented to the Hematology/Oncology Clinic, Ain Shams University Hospitals from June 2016 till September 2017. Patients were 22 children and 13 adults. They were 21 males and 14 females.

#### The study was approved by the Ethics Board of Ain Shams University.

The studied patients were subjected to complete history taking, clinical examination, complete blood picture (CBC), bone marrow (BM) aspiration and flow cytometry (FCM) immunophenotyping by Coulter (Epics-XL) FCM using the routine panel for acute leukemia. The CD86 expression was also detected by FCM.

#### Control group:

Twenty (20) healthy controls were enrolled into the study. 12 of them were males & 8 were females.

Patients received induction therapy & were followed up on day 28 to assess response to therapy by clinical evaluation as well as CBC.

Patients' complete remission (CR) was achieved if no lymphadenopathy or organomegaly detected, CBC returned to normal ranges, disappearance of blast cells from PB and the BM blast <5% (Scott et al., 2005)<sup>8</sup>.

**Methods:**

**Sample collection:**

Samples were collected under complete aseptic conditions using sterile vacutainers as follows:

- 1) Two ml of venous blood were aseptically collected from each patient, dispensed into a tube containing K-Ethylene Diamine Tetra-Acetic Acid (K-EDTA), to be used for CBC and preparation of Leishman-stained smears.
- 2) One ml fresh bone marrow sample was collected on EDTA for Leishman stained smears.

**Procedure:**

The BM aspirates or PB samples were processed within 24 hours of collection, being preserved at room temperature. 50 µL of diluted samples were delivered in two tubes labelled for the antibody used and the negative control. 10 µL of anti CD86 as well as of the negative control MoAb were added to the respective tubes. The tubes then were incubated in the refrigerator (2-8°C) for 10 minutes. 2 ml of PBS, as a wash buffer, were added to each tube and mixed. The tubes were centrifuged at 3000 rpm for 5 minutes and the supernatant was discarded. Cells were suspended in 500 µL PBS to be ready for analysis by the FCM.

Gating was done on the blast cell population based on forward (cell-size) and side scatter (granularity) properties, and CD86 expression was assessed.

**Statistical analysis**

All data were analyzed using the statistical package for social science (SPSS) operating system for IBM compatible PC.

Qualitative data were presented as numbers and percentages. Quantitative data were presented as mean ± standard deviation (SD), range and median. Student t and Chi square tests were used for intergroup comparison. Pearson's correlation (r) was used for correlating data while receiver operating characteristic (ROC) curve was used to assess the cutoff value of CD86 of the highest sensitivity and specificity. p-values less than 0.05 were considered statistically significant.

**RESULTS**

The present study was conducted on 35 newly diagnosed ALL patients. 20 age and sex matched healthy subjects, as a control group were included in the study.

**Comparison of CD86% expression and MFI among patients and control:**

On comparing the two groups, the present work detected a high statistical significant difference in CD86% expression as well as MFI among patient group than control group with (p-value=0.000 and p-value=0.003 respectively), as it was negative in all subjects among the control group, while it was positively expressed in all patients (Table 1).

**Table (1):** Comparison between control group and patients' group regarding CD86 expression percentage and MFI

Parameter	Control group	Patient group	Independent t-test		Significance
	Mean ± SD	Mean ± SD	t	p-value	
MFI	1.2 ± 0.3	1.6 ± 0.5	3.061	0.003	HS
CD86%	7.6 ± 3.6	61.3 ± 21	11.292	0.001	HS

(MFI= mean fluorescence intensity, HS=highly significant)

**Relationship between CD86 expression and all studied parameters:**

There was no significant correlation between CD86 expression and all studied parameters (Table 2 and 3).

**Table (2):** The relationship between CD86 expression, demographic & clinical data among the ALL patients

Parameter		CD 86 %	Independent t-test		Significance
		Mean ± SD	t	p-value	
Age	<18 years	59.4 ± 23.5	0.699	0.490	NS
	>18 years	64.5 ± 16.1			
Sex	Female	58.0 ± 18.3	0.735	0.467	NS
	Male	63.4 ± 22.8			
Hepatomegaly	Present	67.1 ± 18.3	1.843	0.074	NS
	Absent	54.4 ± 22.5			
Splenomegaly	Present	64.4 ± 19.7	1.065	0.295	NS
	Absent	56.7 ± 22.7			
Lymphadenopathy	Present	63.4 ± 23.0	0.870	0.391	NS
	Absent	56.7 ± 15.9			

(NS= non significant)

**Table (3):** Correlation between CD86 expression and laboratory data of the studied ALL patients

	CD 86 %		
	r	p-value	Significance
BM blasts%	-0.215	0.215	NS
TLC	0.169	0.333	NS
Hb	-0.005	0.976	NS
PLT	-0.150	0.389	NS

(BM=bone marrow, TLC=total leucocytic count, Hb= hemoglobin, PLT=platelets, NS=non significant).

**Association between CD86 expression and outcome:**

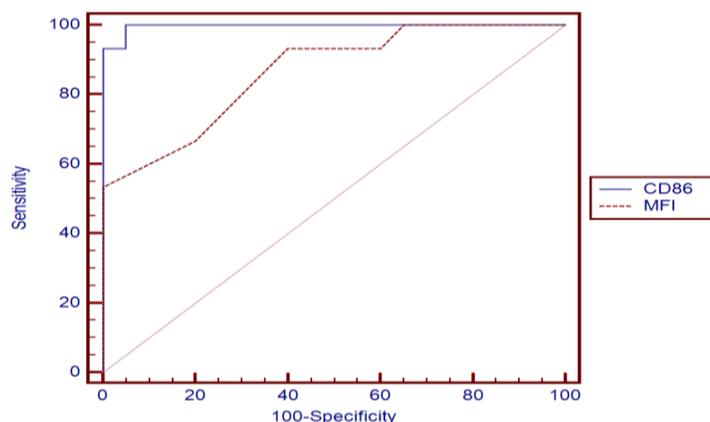
According to patients’ outcome, there was a high statistically significant association between CD86% and MFI expression and poor outcome (p=0.000 for both) (Table 4).

**Table (4):** The association between CD86% expression and mean fluorescence intensity (MFI) with patients’ outcome

Parameter		CD 86 %	Independent t-test		Sig.	MFI	Independent t-test		Sig.
		Mean ± SD	t	p-value		Mean ± SD	t	p-value	
Outcome	Unfavorable (Death or relapse)	81.2 ± 10.2	8.81	0.000	HS	1.9 ± 0.5	-4.673	0.001	HS
	Favorable (Complete remission)	46.2 ± 12.5				1.3 ± 0.3			

(HS=highly significant)

Using receiver operating characteristic (ROC) curve, the best cutoff point of CD86% expression to predict poor prognosis was >61% with sensitivity of 100% and specificity of 95%, while the best cutoff point for mean fluorescence intensity (MFI) was found > 1.4 with sensitivity of 93.3% and specificity of 60% (Figure 1).



**Figure (1):** Receiver operating characteristic curve (ROC) showing the best cutoff value for CD86% and MFI.

## DISCUSSION

Like all cancers, ALL probably develops as a result of a combination of an environmental trigger in the presence of genetic susceptibilities such as upregulation of oncogenes or loss of inherent tumor suppressor proteins<sup>9</sup>.

Recently, combination chemotherapy together with central nervous system prophylaxis has improved the treatment of ALL, and overall cure rates now approach to 80%, despite this improvement, about 20-25% of the patients still relapse<sup>10</sup>.

A number of clinical and biological factors at the time of presentation are relevant to the prognosis and affect the response to treatment. These prognostic factors include age, number of blasts, white blood cells (WBC) count, platelet count, cytogenetic abnormalities, extramedullary involvement (EMI) and immune phenotype<sup>11</sup>.

Immune responses of T cells against tumours first involve recognition by the T cell receptor (TCR) of tumour antigen-derived peptides in association with the MHC antigens. For the T cell to acquire the functions of an effector cell a second transmembrane signal event is required. This second signal is provided by costimulatory molecules, which are expressed on APCs as dendritic cells and certain macrophages and bind to the ligands of the costimulatory molecules that are expressed on T cells. Two of these costimulatory molecules are CD80 (B7.1) and CD86 (B7.2)<sup>12</sup>.

The expressions of CD80, CD86 on ALL and AML cells from newly diagnosed patients were variable, with high expression of CD86 on ALL cells<sup>13</sup>.

The patients of the study were 22 children and 13 adults, being more frequent in children. This was in accordance with **El-Sharkawy et al.**<sup>14</sup>, **Ahmed and Hassab**<sup>15</sup> and **Siegel et al.**<sup>16</sup> who proved that the frequency of ALL in children is more than in adults.

They were 21(60%) males and 14(40 %) females with male to female ratio 1.5:1. This was in accordance with previous studies by **Willman et al.**<sup>17</sup> and **Siegel et al.**<sup>16</sup> who confirmed a male predominance in ALL patients.

Among the patients, 54.3% had hepatomegaly and 57.1% had splenomegaly, this agrees with **Pui et al.**<sup>18</sup> and **El-Sharkawy et al.**<sup>14</sup> who found splenomegaly in 60% of patients but the percentage of hepatomegaly was higher than that detected in our patients being 83.9% in their patients.

The Egyptian study done by **Ahmed and Hassab**<sup>15</sup> detected lymphadenopathy in 73.3% of their patients. This is consistent with the present study detecting lymphadenopathy being 71.4% in our patients.

Regarding the blood picture, initial TLC was detected as  $> 50 \times 10^9/L$  in 34.3% of our patients. This finding is consistent with those reported by **El-Sharkawy et al.**<sup>14</sup> and **Kamazani et al.**<sup>19</sup> who detected TLC  $> 50 \times 10^9/L$  in 35.5% and 17% respectively of patients.

In this work, CD86 was positively expressed in all the studied patients, this finding agrees with **Mansour et al.**<sup>13</sup> who reported that CD86 was positively expressed in 65% of the studied ALL patients.

Our study revealed a high significant statistical difference between patients' group and control group regarding CD86% expression and MFI ( $p=0.000$  and  $p=0.003$  respectively). This was previously explained by **Mansour *et al.***<sup>13</sup>.

On analyzing the relationship of CD86 percentage and MFI expression with various studied standard prognostic factors, a non significant association was detected between them and all clinical and laboratory standard prognostic markers. This was in consistence with **Mansour *et al.***<sup>13</sup>. However, only a significant association with CD13 and CD7 ( $p=0.04$  for both) was found.

Follow up of all patients showed that there was a non significant association between patients' outcome and age, clinical or laboratory data. However, a high significant association was found between CD86% and MFI expression and patients' outcome.

## CONCLUSION

High CD86% and mean fluorescence intensity (MFI) expression appears to be a powerful prognostic indicator of unfavorable outcome in ALL. Analysis of CD86 percentage and MFI expression in addition to other standard prognostic markers at diagnosis may contribute to improve the management of ALL patients.

**Conflict of Interest Statement:** Nothing to declare.

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