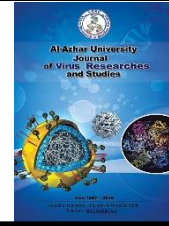




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The Role of CD133 and CD73 in Childhood B-Cell Precursor Acute Lymphoblastic Leukemia

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Abstract

Despite the fact that the prognosis for children with pediatric acute lymphoblastic leukemia (ALL) has progressed in the past few years, a large number of cases recur, frequently with illness that is refractory to treatment. We aimed to assess the expression of CD133 and CD73 on blasts of childhood Precursor B-lymphoblastic leukemia (B-ALL) as well as their relationship to clinical and pathological features and treatment response. A total of 41 patients and 40 apparently healthy normal participants were involved in this study. Patients were assigned into two groups: Group IA (n= 29 Newly diagnosed B-ALL), Group IB (n=12 Relapsed B-ALL). Flow cytometry was used to assess the expression of CD73 and CD133 on bone marrow B-ALL blasts as a percentage of positive cells expressing the markers. When compared to the control group, the proportion of CD73 and CD133 in Group IA and Group IB was significantly increased. Between Groups IA and IB, there was no significant variation in the proportion of CD73, however there was a substantial rise in the percentage of CD133 in Group IB compared to Group IA. We concluded that CD73 and CD133 could be used for detection of minimal residual disease (MRD) also CD133 expression is substantially linked with increased resistance to therapy, worse results, and a greater recurrence rate.

Keywords: CD73, CD133, Precursor B-Lymphoblastic Leukemia (B-ALL).

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common kind of childhood cancer, accounting for one-third of all childhood cancers. It has become a curable condition in more than 80% of patients with vigorous multimodality therapy; nonetheless, the treatment has high morbidity and mortality.

Cure rates have improved while therapeutic toxicity has been reduced because of the implementation of risk-adapted treatment procedures [1]. Acute lymphoblastic leukemia (ALL) appears to have a primitive cell origin and exhibits many immunophenotypic traits with healthy

progenitor cells, according to evidence. These leukemic stem cells may be resistive to existing therapeutic techniques, resulting in relapses. CD133 expression has been discovered in these cancer stem cells (CSC) [2]. CD133, also termed prominin-1, is a cell-surface trans-membrane glycoprotein found on the surface of stem cells, including normal and CSCs. Prominin-1 represents a marker of CSCs that has been demonstrated to be more precise in hematological malignancies than CD34 and may provide alternative to the usual CD34 monoclonal antibodies. In contrast to CD34 antigen, Prominin-1 antigen is lost very early during the differentiation process [3]. In acute leukemia, CD133 expression was linked to a more juvenile phenotype of the blast population and a poor prognosis. However, research on the expression of CD133 in ALL, specifically in pediatric ALL, is contradictory. A high level of CD133 expression has been discovered in a lot of investigations [4], while others have shown either a low level of expression [5] or no expression at all [6]. CD73, also known as ecto-5'-nucleotidase, is a glycosylphosphatidyl inositol (GPI)-anchored cell surface protein. CD73 expression is found in a variety of organs and cell types, including T and B cell subsets, endothelium and epithelial cells [7]. CD73 expression has been reported to be elevated in a variety of cancer cell lines and patient biopsies, such as breast cancer, gastric cancer, gallbladder cancer, colorectal cancer, and ovarian cancer, and has been related to poor prognosis in cancer patients [8]. In hematologic neoplasms, CD73 expression has been linked to leukemia subtype, differentiation, and development. High CD73 expression has been linked to a more aggressive clinical course in chronic lymphoblastic leukemia (CLL) [9]. We aimed to assess the expression of CD73 and CD133 on bone marrow B-ALL blasts, and the association of the two markers with the clinical and pathological characteristics and response to therapy.

2. Patients and Methods

In this case-control research, 41 patients with childhood B cell precursor lymphoblastic leukemia (B-ALL) were included and selected from El-Maady military hospital, during the period between March 2019 to March 2021. The subjects were divided into two categories: Group IA (n= 29 newly diagnostic B-ALL cases), Group IB (n=12 relapsed B-ALL cases). Forty apparently healthy individuals were enrolled as controls (patients undergoing BM aspiration as part of a routine examination for non-malignant hematological disorders).

2.1 Inclusion Criteria

Patients were fulfilling the 2016 World Health Organization (WHO) criteria of pediatric precursor B lymphoblastic leukemia (B-ALL).

2.2 Exclusion Criteria

Children with any other malignancies were excluded from this study.

2.3 Ethical Consideration

The current study was carried out in accordance with the World Medical Association's Helsinki declaration on human experimentation. It has got an approval by Al-Azhar University Review Board and written informed consents were obtained from the patient's parents.

2.4 Procedures and laboratory investigations

All patients evaluated underwent a thorough history taking, clinical examination and the following laboratory testing:

I. A complete blood count (CBC) test was conducted using the Sysmex KX 21N automated hematology analyzer (Kobe,

Japan), and chemical analyses (kidney and liver functions) were performed by Cobas c311 clinical chemistry autoanalyzer (Germany).

II. Morphological examination of peripheral blood smear and bone marrow aspiration.

III. The ALL diagnosis was established using multicolor flowcytometric analysis using basic panel of acute leukemia including the following:

CD34, CD13, CD33, CD14, CD117, CD10, CD19, CD22, CD2, CD3, CD5, CD7, CD4, CD8, MPO, TDT and HLA-DR. Samples were considered positive for a marker if $\geq 20\%$ of the gated blast cells expressing that marker, except for MPO and CD34 positivity was considered $\geq 10\%$.

IV. Molecular examination for the presence of t (9:22), t (1:19), t (12:21) and t (4:11) was performed by karyotyping and fluorescence in situ hybridization (FISH).

V. Detection of the expression of CD73 and CD133 on bone marrow B-ALL blasts at the time of diagnosis using flow cytometry FACs caliber and the Cell Quest Pro system software (BD Biosciences, San Jose, USA). Bone marrow samples were diluted with phosphate buffered saline (PBS) to a concentration of $10 \times 10^9/L$, and then 50 μ of each dilution was mixed with the following monoclonal antibodies: phycoerythrin (PE)-conjugated anti-human CD73 (Lot NO. 200018) and allophycocyanin (APC)-conjugated anti-human CD133 (Lot NO. 200012). Immunotech, Beckman Coulter, Marseille, France provided all monoclonal antibodies. After 20 minutes at room temperature in the dark, these samples were rinsed with PBS, centrifuged, washed again, and suspended in sheath fluid. Titration tests were done to determine the optimal antibody concentration. Prior to obtaining the samples, compensation parameters were established using color calibrate beads (BD, Biosciences, San Jose, USA, and lot no. 8192516). After adjusting the sample count for acquisition (50000 events),

unstained samples were obtained to identify sample auto-fluorescence.

Gating of blasts:

By employing linear amplification, we were able to identify blasts based on their FSC/SSC characteristics: Figure (1). Then blasts were evaluated for the expression of CD73 and CD133 using dot plot characteristics: Figure (2).

2.5 Statistical methods

The SPSS V. 26 was used to code and enter data (IBM Corp., Armonk, NY, USA). Moreover, the mean, median, standard deviation, minimum, and maximum values were used to represent quantitative data, while frequency (count) and relative frequency (%) were used to summarize categorical data. Non-parametric comparisons of quantitative variables were made using the Kruskal-Wallis and Mann-Whitney tests. To compare categorical data, the Chi square (χ^2) test was utilized. When the anticipated frequency is less than 5, the exact test was utilized instead. The correlations between quantitative variables were determined using the Spearman correlation coefficient. P-values less than 0.05 were considered statistically significant.

3. Results

There were 41 patients enrolled, 31 males and 10 females, ranging in age from 1 to 12 years, with a mean age of 6.76 ± 3.35 years. Patients were categorized into Group IA (n=29, newly diagnosed B-ALL cases) and Group IB (n=12, relapsed B-ALL cases). Table (1) summarizes the clinical characteristics of the cases examined. Control samples were forty individuals, 24 males and 16 females, ranging in age from two to twelve years, with an average age of 2 to 12 years, a mean age of 8 ± 2.7 years.

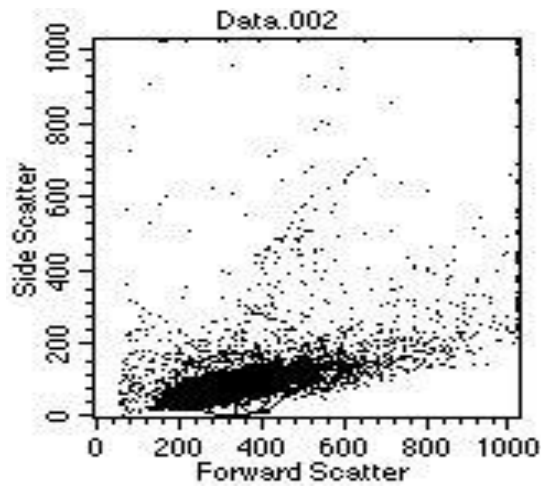
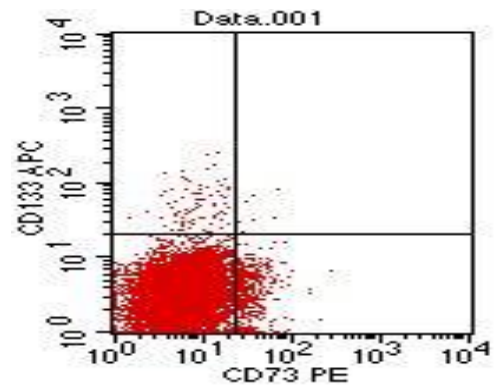
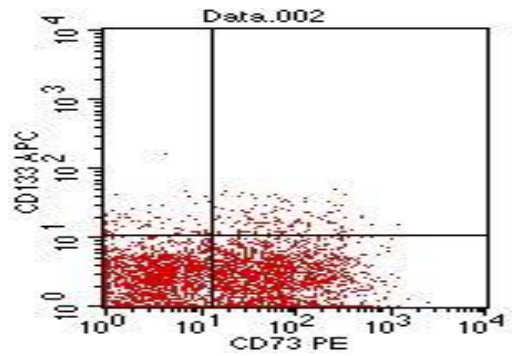


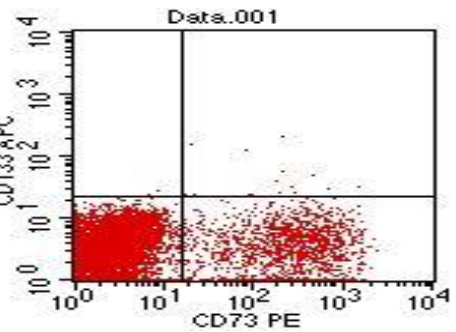
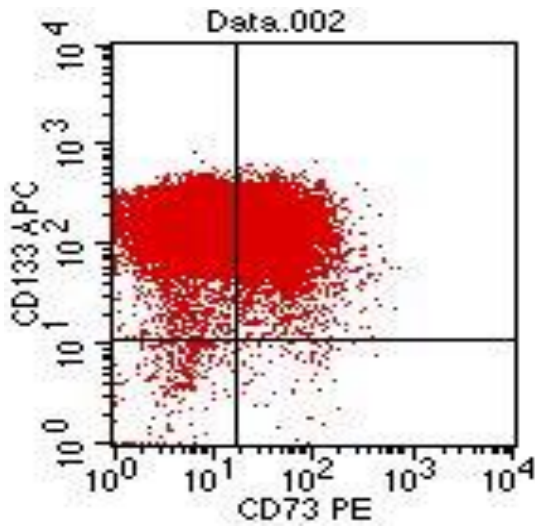
Figure (1): A typical dot-plot illustrating the gating of B-ALL blasts using FSC/SSC characteristics.



(A)



(B)



(B)

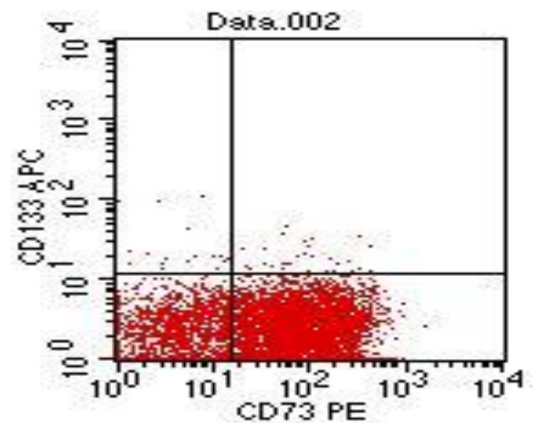
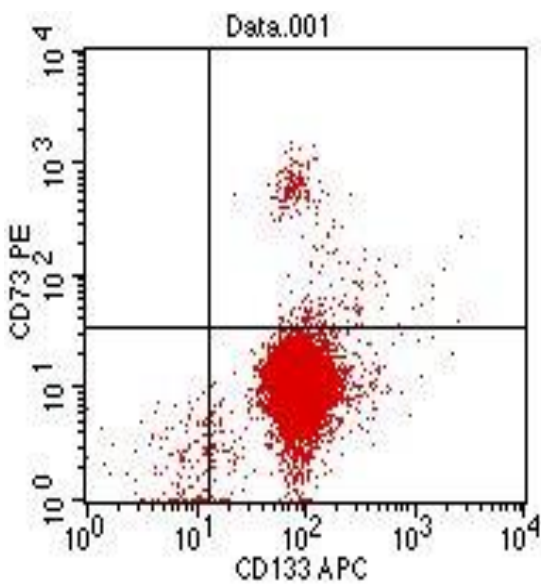


Figure (2): Representative dot-plots demonstrating CD73 and CD133 expression on relapsed cases of B-ALL.

Figure (3): Representative dot-plots showing expression of CD73 and CD133 on: (A) control, (B) newly diagnosed cases of B-ALL.

The expression of CD73 and CD133 in the investigated groups is summarized in Table (2). The proportion of CD73 shows highly significant increase in Groups IA and IB than in the control group ($P < 0.001$), but there was no significant difference between Groups IA and IB. The proportion of CD133 positive cells increased significantly in Groups IA and IB when compared to the control group ($P = 0.001$ and < 0.001 , respectively), and significantly in Group IB when compared to Group IA ($P = 0.018$). There was a substantial negative correlation between the percentage of CD73 and the count of RBCs ($r = -0.356$, $P = 0.023$) and a significant positive correlation between serum albumin and the percentage of CD73 ($r = 0.325$, $P = 0.023$). There was a significant negative correlation between

the percentage of CD133 and serum bilirubin, as well as the percentage of TDT expressed on blasts ($r = -0.322$, $P = 0.04$ and $r = -0.352$, $P = 0.024$, respectively), a highly significant negative correlation with the percentage of CD38 ($r = -0.466$, $P = 0.002$), and a significant positive correlation with the percentages of CD19 and CD117 expressed on blasts ($r = 0.379$, $P = 0.015$ and $r = 0.345$, $P = 0.027$, respectively). In newly diagnosed B-ALL cases there was 8/29 (27.3%) MRD positive cases, while in relapsed cases 11/12 (91.7%) were MRD positive. Correlation of the percentage of CD73 and CD133 with the percentage of blasts at day 14 and day 42 by morphological examination of bone marrow samples and flow cytometry detection of (MRD) is shown in Table (3).

Table (1): Clinical characteristics of B-ALL cases.

		Group I A (n=29, Newly diagnosed B-ALL)		Group I B (n=12, Relapsed B-ALL cases)	
		Count	%	Count	%
Gender	M	19	65.5	12	100
	F	10	34.5	0	0
Fever	P	15	51.7%	6	50.0%
Lymphadenopathy	P	15	51.7%	3	25.0%
Hepatomegaly	P	13	44.8%	3	25.0%
Splenomegaly	P	13	44.8%	4	33.3%
Bone ache	P	13	44.8%	3	25.0%
CNS infiltration	P	1	3.4%	1	8.3%

P: present, F: female, M: male.

Table (2): The percentage of CD73 and CD133 in the studied groups.

	Group I A (n=29, Newly diagnosed B-ALL cases)			Group I B (n=12, Relapsed B-ALL cases)			Group II (n=40)			P1 value	P2 value	P3 value
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mean ± SD	Median	Range			
CD 73 %	28.30 ± 31.10	14.60	0.30 – 95.00	17.11 ± 11.88	16.50	0.27 – 34.00	2.69 ± 1.68	2.80	0.20 – 5.60	< 0.001	< 0.001	0.832
CD 133%	25.97 ± 35.15	3.00	0.02 – 98.00	44.08 ± 33.52	30.50	13.00 – 100.00	0.98 ± 0.83	0.60	0.10 – 3.70	0.001	< 0.001	0.018

P1: Group IA vs Group II.

P2: Group IB vs Group II.

P3: Group IA vs Group IB.

Table (3): Correlation of the percentage of CD73 and CD133 with the percentage of blasts at day 14 and day 42 by morphological examination of bone marrow samples and flow cytometry detection of MRD.

	Group I (B-ALL cases) (n=41)			
	CD 73 %		CD 133%	
	Correlation Coefficient	P value	Correlation Coefficient	P value
Percentage of blast cells by morphology at day 14	-0.092	0.568	0.380	0.014
Percentage of MRD by flow cytometry at day 14	0.036	0.824	0.579	< 0.001
Percentage of blast cells by morphology at day 42	-0.112	0.485	0.423	0.006
Percentage of MRD by flow cytometry at day 42	-0.010	0.950	0.574	< 0.001

There was no significant correlation between CD73 percentage and blasts by morphology on days 14 and 42, or between the percentage of MRD on day 14 and day 42, whereas there was a significant positive correlation between CD133 percentage and blasts by morphology on day 14. Additionally, there was a highly significant positive connection between the percentage of blasts as determined by morphology at day 42 and the percentage of MRD as determined by flow cytometry at days 14 and 42.

4. Discussion

Acute lymphoblastic leukemia (ALL) is the most prevalent type of childhood cancer. It is characterized by uncontrolled clonal proliferation of lymphoid blasts with impaired differentiation potential into mature cells. [10]. Therefore, we aimed to investigate the expression of CD73 and CD133 on B-ALL blasts to see if there is any correlation of these markers with the prognosis, relapse and response to treatment. On the one hand, the proportion of CD73 in Group IA and Group IB was considerably greater than in the control group ($P < 0.001$). As a result, CD73 expression on precursor B-ALL blasts may

be used to identify MRD. Between Groups IA and IB, there was no statistically significant difference in the percentage of CD73 positive individuals ($P = 0.832$). On days 14 and 42, there was no significant correlation between the percentage of CD73 and the percentage of blasts determined by morphology, or the percentage of MRD determined by flow cytometry. This is in concordance with **Wieten et al., 2011**, they concluded that CD73 doesn't influence the prognosis of children with B-ALL blasts [11]. This agrees with **Wang et al., 2015**, they studied the CD73 expression on hematogones, blasts and different maturation stages of B-cells and they concluded that it can be used as a valuable marker for detection of MRD in B-ALL. [12] **Tembhare et al., 2018** showed high frequency of abnormal expression of CD73 on B-ALL blasts so they reported that the addition of that marker to the MRD panel improve the applicability of MRD monitoring. [13]. Also, **Sedek et al., 2018**, concluded that CD73 would be used as a reliable stable (to induction therapy) marker for MRD detection in B-ALL cases by flow cytometry. [14]. On the other hand, there was a highly significant increase in CD133 percent in Group IA and Group IB compared to the control group ($P = 0.001$ and

<0.001, respectively). Thus, the expression of CD133 on B-ALL blasts could be used for detection of MRD in precursor B-ALL. Additionally, the percentage of CD133 was much higher in Group IA than in Group IB (P 0.018). There was a significant positive correlation between the percentage of CD133 and the percentage of blasts by morphology at day 14, and a highly significant positive correlation between the percentage of blasts by morphology at day 42 or the percentage of MRD by flow cytometry at day 14 and day 42. Therefore, this marker could be used for prediction of the prognosis, response to treatment and relapse. These findings were consistent with **Elgendi et al., 2010** they explained that CD133 is an independent predictor in acute leukemia in children and associated with higher resistance to chemotherapy [15]. Also, **Tolba et al., 2013**, stated that CD133 is an independent predictor in acute leukemia, particularly ALL, and is related to treatment resistance, relapse and death. [16]. This agrees with **Ji et al., 2017**, they emphasized on the aberrant expression of CD133 in childhood B-ALL and the applicability of the marker to evaluate the disease progression. [17]. **Hagag et al., 2019** stated that CD133 is a useful prognostic marker for ALL patients [18]. **Riegg et al., 2021** showed that CD133 can be used as a target for treatment in B-ALL [19].

5. Conclusion

We concluded that CD73 and CD133 could be used for detection of MRD and could be used as targets for therapy of B-ALL. CD133 expression is substantially linked with increased resistance to therapy, worse results, and a greater recurrence rate.

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