



Expression of CD123 in B-Acute Lymphoblastic Leukemia as a Predictor of BCR/ABL Rearrangement and Disease Relapse

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:

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Original Research Article

Received: 25/09/2023

Accepted: 28/11/2023

Published: 01/12/2023

ABSTRACT

Introduction: CD123 is the alpha chain of the interleukin 3 receptor (IL-3R) and is normally expressed on hematopoietic progenitor cells, monocytes, B lymphocytes and endothelial cells. Leukemic stem cells can be detected using CD123, and its usefulness for measuring residual disease and potential involvement in disease relapse is being evaluated. It also regulates the growth, proliferation, survival, and differentiation of hematopoietic cells, along with immunity and inflammatory response.

Materials and Methods: Bone marrow or peripheral blood from 50 B-Acute Lymphoblastic Leukemia (B-ALL) patients were enrolled in the study. CD123 expression was studied by flow cytometry technique and correlated with clinical and hematological parameters as well as BCR-ABL status, MRD status and disease status.

Results: CD123 expression was found positive in 38% of patients. No significant correlation of CD123 expression with clinical and hematological parameters was observed. A significantly higher

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incidence of CD123 expression was noted in patients with BCR-ABL fusion (70%), relapse patients (67%) and MRD patients (67%).

Conclusion: CD123 can be used to predict BCR-ABL status in B-ALL patients and it has potential role to recognize high risk of relapse and helps to scrutinize high risk B-ALL patients who benefited with aggressive chemotherapy. Further, higher expression of CD123 in MRD patients can be used to evaluate minimal residual disease in follow-up B-ALL patients.

Keywords: B-Acute lymphoblastic leukemia; flow cytometry; CD123.

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disease marked by the clonal growth of leukemic cells in the bone marrow (BM), lymph nodes, thymus, and spleen. This is a diverse disease classified into multiple subtypes [1]. Acute lymphoblastic leukemia (ALL), which accounts for 75% of all leukemias in children under the age of 15, affects persons of all ages but is the most prevalent disease in youngsters. Among adults, it is more common in people older than 45. B acute lymphoblastic leukemia/lymphoma (B-ALL) is a hematologic malignancy derived from B-cell progenitors [2].

CD123 is the alpha chain of the interleukin 3 receptor (IL-3R) and is normally expressed on hematopoietic progenitor cells, monocytes, B lymphocytes and endothelial cells. Several hematologic neoplasms, including B-ALL, express CD123, but normal hematopoietic stem cells either express it less or don't express it at all [3]. Importantly, it has been reported that leukemic stem cells and more differentiated leukemic blast cells both express CD123 positively [4, 5]. Leukemic stem cells can be detected using CD123, and its usefulness for measuring residual disease and potential involvement in disease relapse is being evaluated [6]. It also regulates the growth, proliferation, survival, and differentiation of hematopoietic cells, along with immunity and inflammatory response [7,8]. This study aimed to assess the pattern of CD123 expression in B-ALL patients.

2. MATERIALS AND METHODS

2.1 Patient Characteristics

In this prospective study, 50 B-cell Acute Lymphoblastic Leukemia (B-ALL) patient

samples were collected at The Gujarat Cancer & Research Institute (G.C.R.I.) from of January 2023 to April 2023. First differential counts at diagnosis and clinicopathological data such as age, gender, karyotype and bone marrow were recorded from available hospital records files maintained at the Institutional Medical Record Department. Patients provided general consent to use their sample for the study. This study was approved by the Institutional Scientific Review Board and Ethics Committee.

2.2 Sample Collection

Bone marrow or peripheral blood samples of 50 patients (25 newly diagnosed + 25 follow-up patients) were collected in Ethylenediamine Tetra Acetic Acid (EDTA) vacuette.

2.3 Sample Preparation

100 µl BM/PB samples were stained with 5 µl CD45 (V500c) and 10 µl CD123 (FITC) monoclonal antibodies, then RBCs were lysed with 2 ml RBC lysing solution (1:10 Dilution), further, washed with 2 ml Phosphate Buffer Saline (PBS) and then resuspended with 500 µl PBS. The stained samples were analyzed in Flow Cytometer within 72 hours.

2.4 Sample Acquisition

Sample acquisition is done in BD FACSCanto II Flow Cytometer using FACSDiva software and total 1,00,000 cells or events were acquired for each sample.

2.5 Data Analysis

CD123 positive cells were calculated on dim CD45 blast population. The percentage of each positive population is noted from the "population hierarchy" table. Histogram is plotted and gated

to find the CD123 MFI (median fluorescence intensity) value of leukemic blasts.

2.6 Statistical Analysis

Statistical analysis was carried out using SPSS statistical software version 27. Receiver operating characteristic (ROC) curve was generated to know the sensitivity and specificity of the marker. Pearson's chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between two parameters. p values ≤ 0.05 were considered to be statistically significant.

3. RESULTS

Out of 50 B-ALL patients, CD123 expression was found in 19(38%) of all B Acute Lymphoblastic Leukemia patients whereas 31(62%) of patients did not express CD123 (Figs 1 & 2). The expression was further correlated with clinical and hematological parameters using the median as cut off. In correlation with clinical parameters, a trend of higher incidence of CD123 expression was found in male, absence

of hepatomegaly and lymphadenopathy as compared to their counterparts. In correlation with hematological parameters, a trend of higher incidence of CD123 expression was found in B-ALL patients with high blast cells, high lymphocytes and high platelets as compared to their counterparts. No significant correlation of CD123 expression with other clinical and hematological parameters was observed (Table.1).

3.1 CD123 Expression in Relation to BCR-ABL Status

Correlation of CD123 expression was evaluated with BCR-ABL status as shown in Table 2. BCR-ABL fusion was detected in 10 out of 45 B-ALL patients. A higher incidence of CD123 expression (7/10, 70%) was seen in BCR-ABL positive group ($p = 0.01$) as compared to low expression of CD123 expression (3/10, 30%). (Table 2). ROC curve analysis (AUC 0.723) suggests patients with CD123 MFI more than 130 have a higher chance of having BCR-ABL fusion genes (Fig. 3).

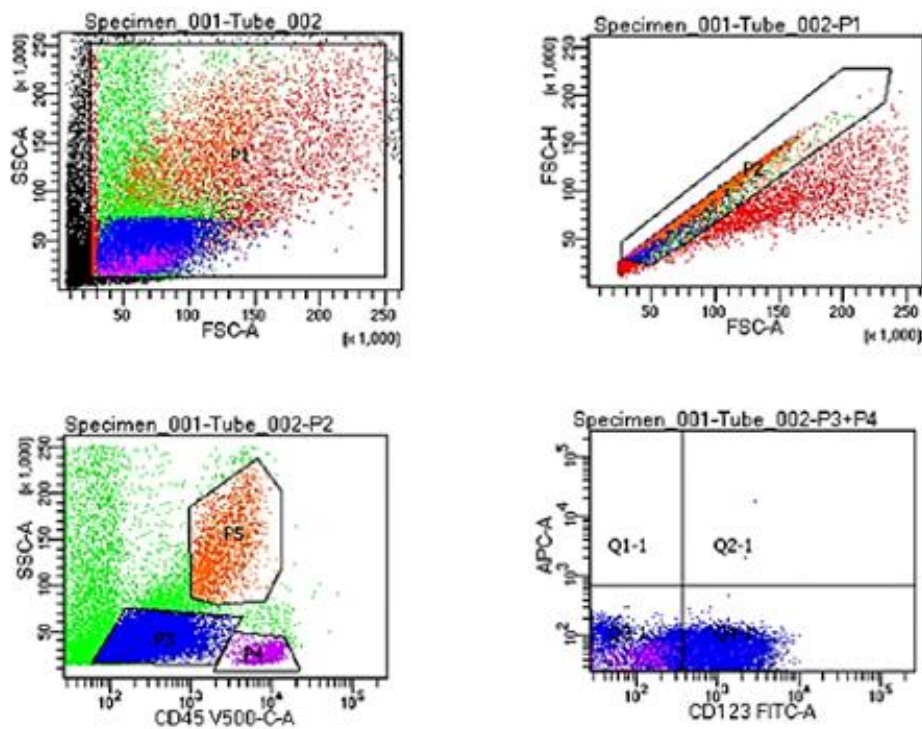


Fig. 1. Expression of CD123 in B-ALL

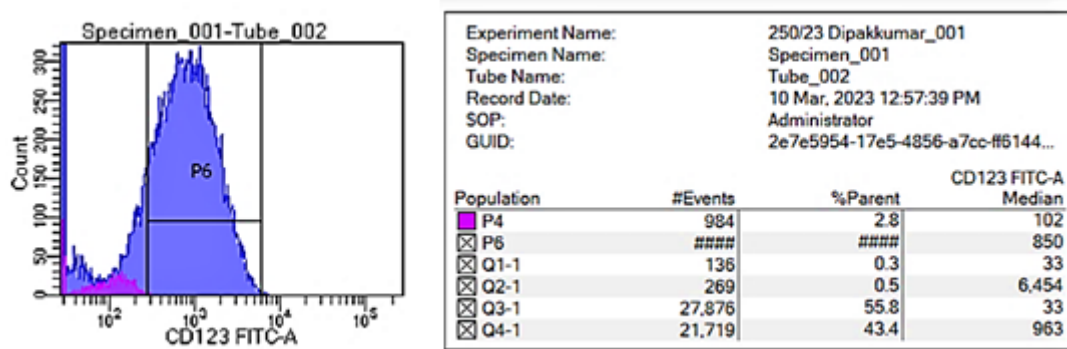


Fig. 2. Median fluorescence intensity of CD123 in B-lymphoblasts

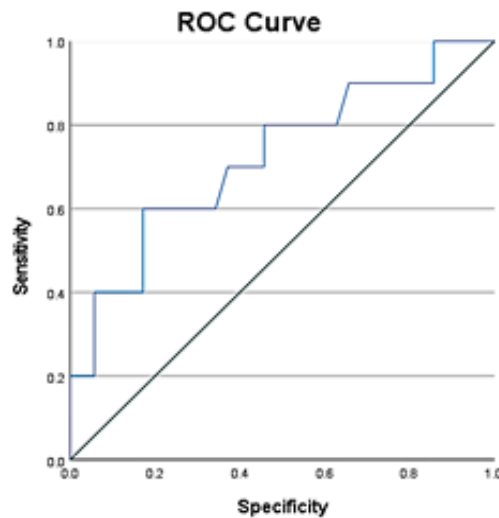


Fig. 3. ROC Curve showing CD123 expression with BCR-ABL

Table 1. correlation of CD123 expression with clinical and hematological parameters

Parameters	Total Patients N (%)	CD123 Negative N (%)	CD123 Positive N (%)	χ^2	R	p
Age	50(100)	31(62)	19(38)			
Adult	25(50)	15(60)	10(40)	0.08	0.04	0.77
Pediatric	25(50)	16(64)	9(36)			
Gender	50(100)	31(62)	19(38)			
Male	32(64)	18(56)	14(44)	1.24	0.16	0.26
Female	18(36)	13(72)	5(28)			
Splenomegaly	40(100)	24(60)	16(40)			
Positive	21(52)	13(62)	8(38)	0.07	-0.04	0.79
Negative	19(48)	11(58)	8(42)			
Hepatomegaly	40(100)	24(60)	16(40)			
Positive	23(58)	16(70)	7(30)	2.06	-0.23	0.15
Negative	17(42)	8(47)	9(53)			

Parameters	Total Patients N (%)	CD123 Negative N (%)	CD123 Positive N (%)	χ^2	R	p
Lymphadenopathy	21(100)	10(48)	11(52)	0.40	-0.14	0.52
Positive	5(24)	3(60)	2(40)			
Negative	16(76)	7(44)	9(56)			
Hemoglobin (gm/dl)	50(100)	31(62)	19(38)	0.19	-0.06	0.66
>8.9	23(46)	15(65)	8(35)			
≤8.9	27(54)	16(59)	11(41)			
RBC (cells/μl)	50(100)	31(62)	19(38)	0.26	0.07	0.61
>3.27×10 ⁶	24(48)	14(58)	10(42)			
≤3.27×10 ⁶	26(52)	17(65)	9(35)			
WBC (cells/μl)	50(100)	31(62)	19(38)	0.08	-0.04	0.77
>7.295×10 ³	25(50)	16(64)	9(36)			
≤7.295×10 ³	25(50)	15(60)	10(40)			
Platelets (cells/μl)	50(100)	31(62)	19(38)	1.20	0.15	0.27
>34×10 ³	24(48)	13(54)	11(46)			
≤34×10 ³	26(52)	18(69)	8(31)			
Lymphocytes (%)	50(100)	31(62)	19(38)	3.31	-0.26	0.07
>30	24(48)	18(75)	6(25)			
≤30	26(52)	13(50)	13(50)			
Polymorphs (%)	49(100)	30(61)	19(39)	0.002	0.007	0.96
>25	23(47)	14(61)	9(39)			
≤25	26(53)	16(61)	10(39)			
Blast cells (%)	31(100)	22(71)	9(29)	1.70	0.23	0.19
>52	15(48)	9(60)	6(40)			
≤52	16(52)	13(81)	3(19)			

(χ^2 = Chi-square; R = Pearson's correlation coefficient; p = p-value)

Table 2. correlation of CD123 expression with BCR-ABL status

	CD123 Expression (N%)		χ^2	R	p
	Positive N (%)	Negative N (%)			
BCR-ABL Status N (%)					
Positive	7(70)	3(30)	5.68	0.35	0.01
Negative	10(29)	25(71)			
Total	17(38)	28(62)			
MRD Status N (%)					
Positive	6(67)	3(33)	7.21	0.60	0.007
Negative	1(9)	10(91)			
Total	7(35)	13(65)			
Disease Status N (%)					
Relapse	4(67)	2(33)	3.75	0.35	0.05
Remission	6(25)	18(75)			
Total	10(33)	20(67)			

(χ^2 = Chi-square; R = Pearson's correlation coefficient; p = p-value)

3.2 CD123 Expression in Relation to MRD Status

Correlation of CD123 expression was evaluated with MRD status as shown in Table 2. MRD

Status of 20 patients was evaluated of which in 9 B-ALL patients shoed residual disease cells. In correlation with MRD status, higher incidence of CD123 positive expression was found in MRD positive group (67%, 6/9) (p = 0.007) as

compared to MRD negative group (9%, 1/11) ($p = 0.007$) (Table 2) (Fig. 4).

with CD123 MFI more than 108 have higher chance of having disease relapse (Fig.5)

3.3 CD123 Expression in Relation to Disease Status

Disease status of 30 patients was available out of which 20% (6/30) showed disease relapse and 80% (24/30) showed disease remission. CD123 expression was found positive in 33% (10/30) with 4 relapse patients and 6 remission patients. ($p = 0.05$). CD123 expression was found negative in 67% (20) with 2 relapse patients and 18 remission patients ($p = 0.05$) (Table 2). ROC curve analysis (AUC 0.820) suggests patients

4. DISCUSSION

A clonal hematologic malignancy - acute lymphoblastic leukemia (ALL) develops from T- or B-lymphoid progenitor cells. In the present study, CD123 expression was found in 38% (19/50) of the studied B-ALL patients. This incidence is lower than that reported by Angelova E et al [3] (164/183, 89.6%); Djokic M et al [9] (98/119, 82%), Hassanein NM et al [10] (45/50, 89%); Bras AE et al. [11], who detected CD123 expression in 85% of B-cell precursor (BCP)

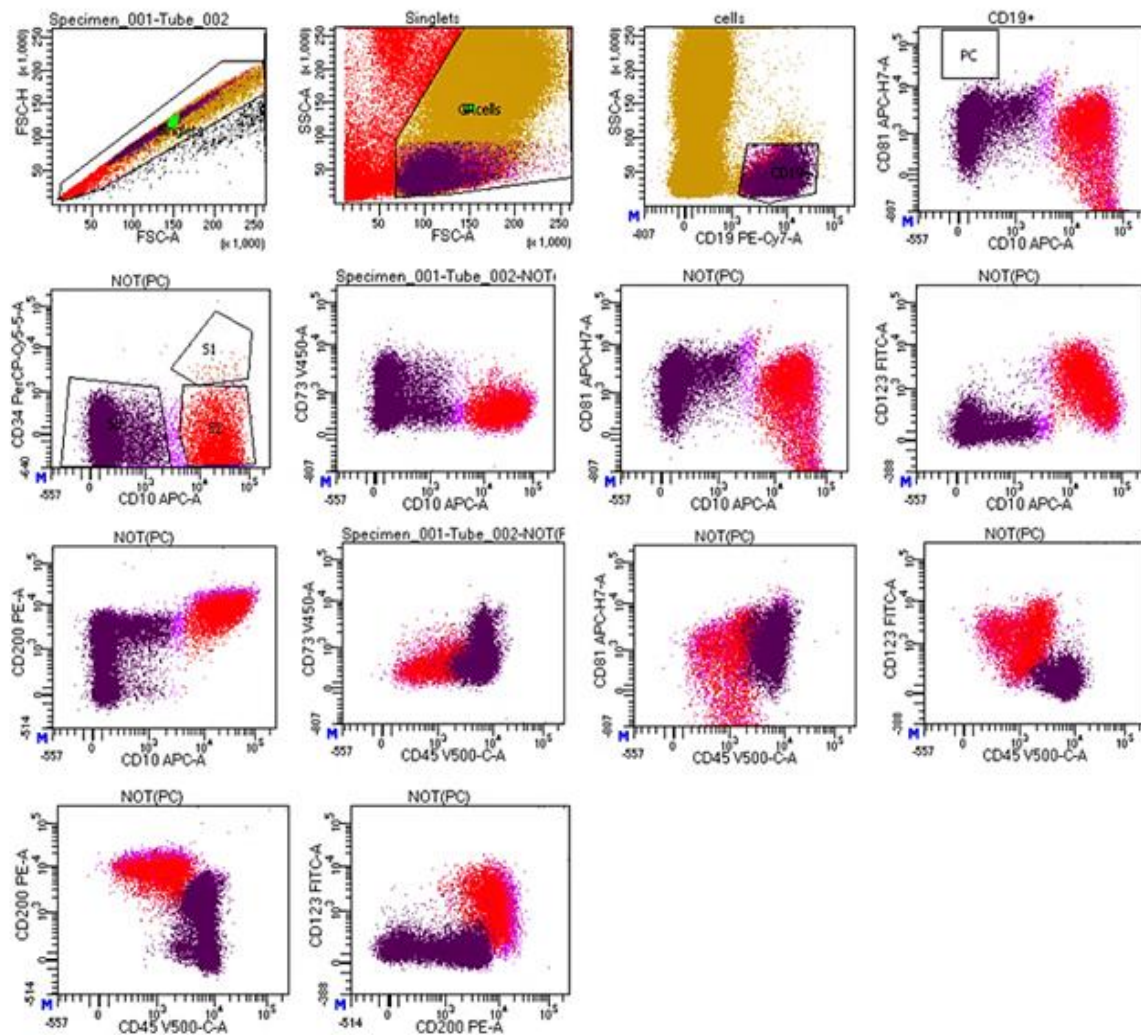


Fig. 4. Aberrant CD123 expression in B-ALL useful in MRD detection along with other markers

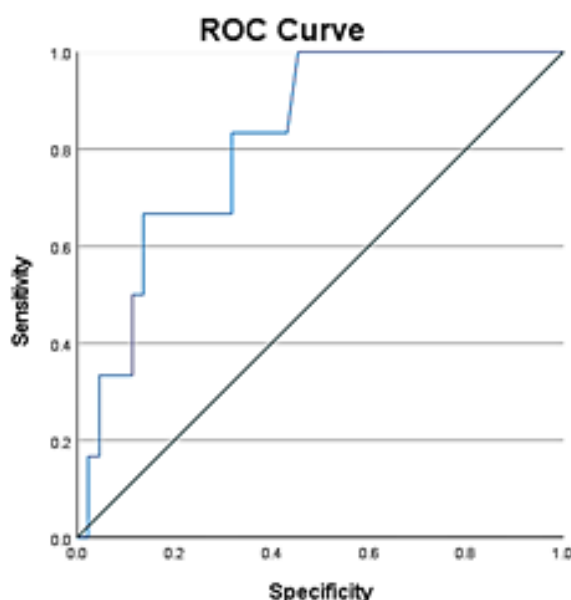


Fig. 5. ROC curve showing CD123 expression with disease status

ALL cases (224/ 262). In the present study, out of 50 B-ALL patients, CD123 expression was found in 44% male (14/32) and 28% female (5/18) patients. The study by Li Z et al. [12] has showed that out of 328 pediatric B-ALL patients, CD123 expression was found in 55% (105/190) male and 51% female (70/138) patients. In the current study, an expression of CD123 was not significantly correlated with hematological parameters such as hemoglobin, RBC, WBC, platelets, lymphocytes, polymorphs, and blast cells. Similarly, in the study by Aref S et al. [13], no significant correlation of CD123 expression was observed with hemoglobin, WBC, platelets, and blast cells count.

3-5% of children and 20-30% of adults have Philadelphia-positive (Ph+) B-ALL, and the frequency rises to roughly 50% in people over the age of 50. In the present study, the correlation of CD123 expression was evaluated with BCR-ABL fusion status. There was a strong association between CD123 positive expression and the BCR-ABL positive group ($p = 0.017$). BCR-ABL fusion was detected in 22% (10/45) of B-ALL cases, with 6% in children and 16% in adults. This finding is lower than that reported by Gadhia P et al. [14] (33.3%), Aref S et al. [13] (30%) and higher than that reported by Owaidah TM et al. [15] (17.5% (18/103).

In the present study, follow-up samples of 20 B-ALL patients were evaluated, and 9 patients showed minimal residual disease (MRD). Out of these 9 patients, 6 (67%) showed CD123 expression; this incidence is nearly similar to the study by Li Z et al. [12] (61.3%). Das N et al [6] reported that the presence of CD123 expression at baseline was substantially more frequently related to MRD-positive status ($p < 0.001$ for 10% or 20% and $p = 0.005\%$ for 5% of blasts expressing CD123).

In the present study, the disease status of 30 B-ALL patients was evaluated and out of these, 6 patients showed relapse, and 24 patients showed remission. Out of 6 relapse patients, CD123 expression was seen in 4 (66.6%) relapse patients and out of 24 remission patients, CD123 expression was seen in 6 (25%) remission patients. In contrast to this finding, Li Z et al [12] reported that expression of CD123 was seen in 39.3% of relapse patients and 53.3% of remission patients. This difference may be due to the fact that they were enrolled only pediatric B-ALL patients in their study. Thus, this study demonstrated that high expression of CD123 was correlated with BCR-ABL positivity, MRD positivity, and disease relapse.

5. CONCLUSION AND SUMMARY

CD123 can be used to predict BCR-ABL status in B-ALL patients, and it has the potential to recognize high-risk of relapse and help to scrutinize high-risk B-ALL patients who benefited from aggressive chemotherapy. Further, higher expression of CD123 in MRD patients can be used to evaluate minimal residual disease in follow-up B-ALL patients. However, inclusion of more number of patients is required for further conclusion.

CONSENT

Written informed consent was obtained from the patients and explained to them in their own language.

ETHICAL APPROVAL

The study was approved by the Institutional Scientific Review Board and Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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