EXPRESSION OF CD123 IN B-ACUTE LYMPHOBLASTIC LEUKEMIA AS A PREDICTOR OF BCR/ABL REARRANGEMENT AND DISEASE RELAPSE

ABSTRACT

Introduction: CD123 is the alpha chain of the interleukin 3 receptor (IL-3R) and 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% patients. No significant correlation of CD123 expression with clinical and hematological parameters was observed. A significant higher 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 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. MATERIAL 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.) in the duration 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 the 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) + 10 µl CD123 (FITC) monoclonal antibodies, lysed with 2 ml RBC lysing solution (1:10 Dilution), washed with 2 ml Phosphate buffer saline (PBS) and then resuspended with 500 µl PBS. Then, Subjected to Flow Cytometer within 72 hours.

2.4 SAMPLE ACQUISITION

Sample acquisition is done in FACSCanto Ⅱ (Flow Cytometer) instrument using FACSCDiva software. During acquisition, global worksheet is kept open and total 1,00,000 cells or events are acquired.

2.5 DATA ANALYSIS

For analysis, global worksheet is changed to normal worksheet**.** CD123 positive cells are selected from Dim CD45 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 positive in 19(38%) patients and negative in 31 (62%) patients (Figure. 1 & 2). The expression was further correlated with clinical and hematological parameters. 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 higher blast cells count, lymphocytes and platelets as compared to their counterparts. No significant correlation of CD123 expression with other clinical and hematological parameters was observed (Table.1).

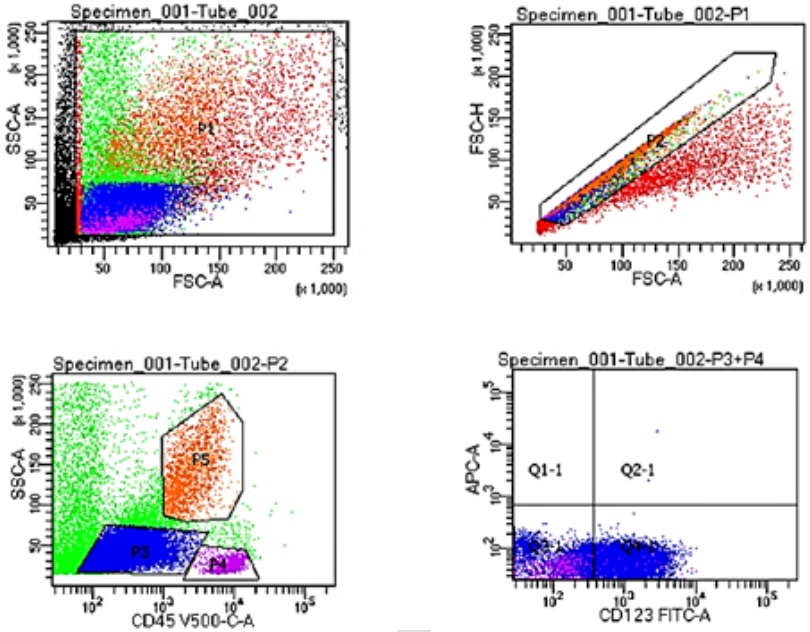
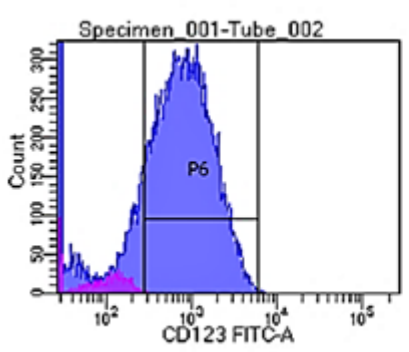


Figure 1. Expression of CD123 in B-ALL



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Tube Name:	Tube_002
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SOP:	Administrator
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Population	#Events	%Parent	CD123 FITC-A Median
P4	984	2.8	102
P6	####	####	850
Q1-1	136	0.3	33
Q2-1	269	0.5	6,454
Q3-1	27,876	55.8	33
Q4-1	21,719	43.4	963

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

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

Parameters	Total Patients N (%)	CD123 Negative N (%)	CD123 Positive N (%)	□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)			
Lymphadenopathy	**21(100)**	**10(48)**	**11(52)**			
Positive	5(24)	3(60)	2(40)	0.40	-0.14	0.52
Negative	16(76)	7(44)	9(56)			
Hemoglobin (gm/dl)	**50(100)**	**31(62)**	**19(38)**			
>8.9	23(46)	15(65)	8(35)	0.19	-0.06	0.66
≤8.9	27(54)	16(59)	11(41)			
RBC (cells/µl)	**50(100)**	**31(62)**	**19(38)**			
$>3.27^{x}10^{6}$	24(48)	14(58)	10(42)	0.26	0.07	0.61
$\leq 3.27^{x}10^{6}$	26(52)	17(65)	9(35)			
WBC (cells/µl)	**50(100)**	**31(62)**	**19(38)**			
$>7.295^{x}10^{3}$	25(50)	16(64)	9(36)	0.08	-0.04	0.77
$\leq 7.295^{x}10^{3}$	25(50)	15(60)	10(40)			
Platelets (cells/µl)	**50(100)**	**31(62)**	**19(38)**			
>34x103	24(48)	13(54)	11(46)	1.20	0.15	0.27
≤34x103	26(52)	18(69)	8(31)			
Lymphocytes (%)	**50(100)**	**31(62)**	**19(38)**			
>30	24(48)	18(75)	6(25)	3.31	-0.26	0.07
≤30	26(52)	13(50)	13(50)			
Polymorphs (%)	**49(100)**	**30(61)**	**19(39)**			
>25	23(47)	14(61)	9(39)	0.002	0.007	0.96
≤25	26(53)	16(61)	10(39)			
Blast cells (%)	**31(100)**	**22(71)**	**9(29)**			
>52	15(48)	9(60)	6(40)	1.70	0.23	0.19
≤52	16(52)	13(81)	3(19)			

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

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

	CD123 Expression (N%)		□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)			

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

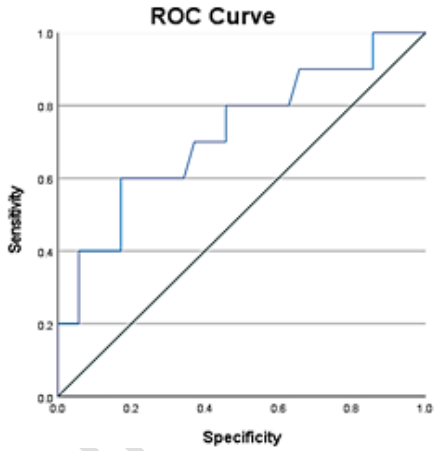

Figure 3. ROC Curve showing CD123 expression with BCR-ABL

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 as positive in 10 out of 45 B-ALL patients. A higher incidence of CD123 positive expression (7/10, 70%) and low expression of CD123 negative expression (3/10, 30%) was seen in BCR-ABL positive group (*p = 0.01*). A lower incidence of CD123 positive expression (29%, 10/35) and a higher incidence of CD123 negative expression (25/35, 71%) was seen in BCR-ABL negative group (*p* = 0.01) (Table.2). ROC curve

analysis (AUC 0.723) suggests patients with CD123 MFI more than 130 have higher chance of having BCR-ABL fusion (Figure.3).

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 from that it was detected as positive in 9 out of 20 B-ALL patients and a higher incidence of CD123 positive expression was seen in MRD positive group (67%, 6/9) (p = 0.007) (Figure 4). A lower incidence of CD123 positive expression was seen in MRD negative group (9%, 1/11) (p = 0.007) (Table.2).

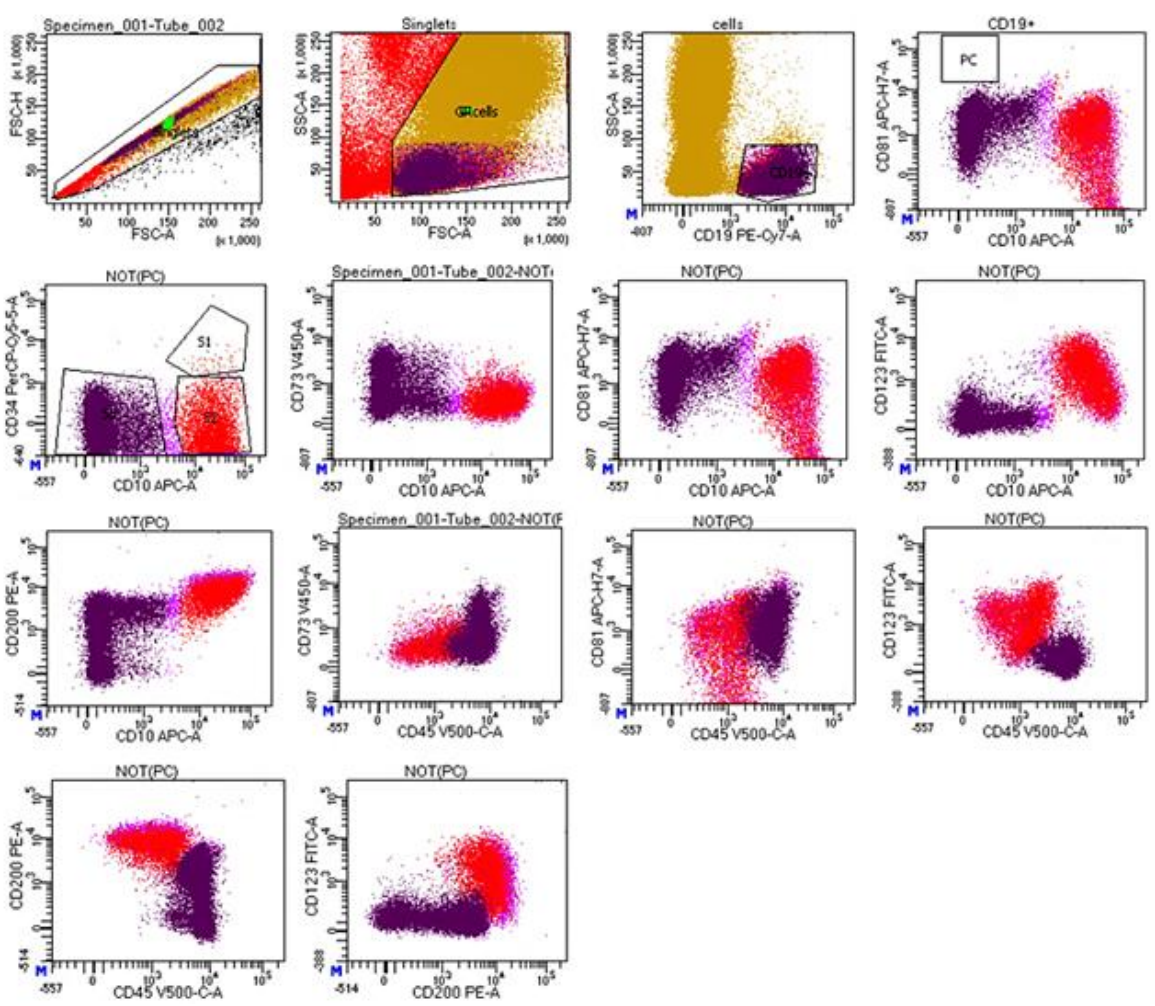

Figure 4. Aberrant CD123 expression in B-ALL useful in MRD detection along with other markers

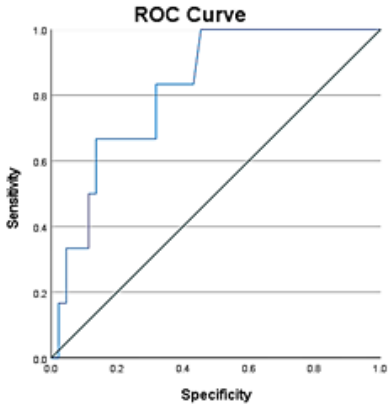

Figure 5. ROC curve showing CD123 expression with disease status

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

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%) and Bras AE et al [11] who detected CD123 expression in 85% of B-cell precursor (BCP) 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. In the study by Li Z et al [12] has shown 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, expression of CD123 was not significantly correlated with the 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, correlation of CD123 expression was evaluated with BCR-ABL fusion status. There was strong association between CD123 positive expression and 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 to 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%) patients 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 with MRD-positive status ($p< 0.001$ for 10% or 20% and $p = 0.005$% for 5% of blasts expressing CD123).

In the present study, disease status of 30 B-ALL patients were 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% relapse patients and 53.3% remission patients. This difference may be due to 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

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.

CONSENT

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

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