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RESEARCH ARTICLE

Diagnostic value of CD117 in differential diagnosis of acute leukemias

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Abstract C-kit receptor (CD117) and its ligand, stem cell factor, play a key role in normal hematopoiesis. It has been demonstrated that its expression extremely increases in leukemias with myeloid commitment. We analyzed findings on CD117 expression together with other myeloid related markers in 203 de novo acute leukemias, referred to Iranian immunophenotyping centers: Iranian Blood Transfusion Organization (IBTO) and Baghiatallah Hospital (BH). All cases were characterized based on the French American British cooperative group (FAB) and European Group for Immunological Classification of Leukemias (EGIL). The cases comprised of 111 acute myeloblastic leukemia (AML), 86 acute lymphoblastic leukemia (ALL), and 6 acute undifferentiated leukemia (AUL). CD117 was positive in 75 % of AML and 50 % of AUL, whereas none of the ALL cases was positive for this marker. Although CD117 was positive in 100 % of M5a cases, no M5b positive was found (p=0.036). The calculated specificity for myeloid involvement was 100 % for CD117 and CD33, and 98 % for CD13 and CD15 (p<0.001). The calculated sensitivity for myeloid involvement was 83, 76, 64, and 41 % for CD13, CD117, CD33, and CD15, respectively (p<0.001). We concluded that CD117 expression is a specific and rather sensitive marker for differential diagnosis between AML and ALL, and except for M5 subtypes, it fails to determine FAB subtypes; lack of expression in M5 can identify M5b. Therefore, it should be included in the routine primary panel for diagnosis of acute leukemias.

Keywords Acute Leukemia · Immunophenotyping · CD117 · AML · ALL

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Introduction

The proto-oncogene c-kit encodes a 145 KD transmembrane tyrosine kinase receptor that its ligand is stem cell factor (SCF) [1, 2]. This receptor together with its ligand plays a key role in hematopoiesis [3, 4]. Expression of c-kit can be demonstrated by immunophenotyping with monoclonal antibodies clustered under CD117 [1, 5]. This receptor is expressed in a minority (<5 %) of bone marrow progenitor cells, and demonstrate myeloid lineage commitment [6]. It has been demonstrated that its expression extremely increases in a variety of human leukemia cell lines, especially those committed to the myeloid, erythroid, and megakaryocytic lineages [6–8]. In addition, its expression is reported in a minority of early human thymocytes, in murine prothymocytes, and natural killer cells

[9]. In hematological malignancies, CD117 expression is almost restricted to acute myeloblastic leukemia and somewhat to multiple myeloma, Hodgkin disease, and anaplastic large cell lymphoma [10–12]. There have been a number of reports suggesting that CD117 expression is the most reliable marker for identifying myeloblasts, but its specificity for myeloid leukemias is contradicted. Hoehn D. et al. suggested that FLT3 mutation is rare in T-ALL, and its presence supports T/myeloid lineage. They concluded that CD117 alone has high prognostic value for acute lymphoblastic leukemia (ALL). The immunophenotypic profile of TdT, CD7, CD13, CD34, and CD117 (bright) is helpful for predicting FLT3 mutation, with a sensitivity of 100 % and specificity of 94 % [13]. Few data exist on diagnostic value of CD117 and other myeloid-related markers (CD13, CD33, and CD15) in differential diagnosis of acute myeloblastic leukemia (AML) from ALL, and there are no other studies that investigate the role of this marker in Iranian population. Therefore, in this paper, we study the diagnostic efficacy of CD117 in Iranian acute leukemia patients to primary screening of leukemias.

Materials and methods

Patients

Bone marrow aspiration and peripheral blood samples of 203 consecutive patients with de novo acute leukemia referred to Iranian immunophenotyping centers (Iranian Blood Transfusion Organization (IBTO) and Baghiatallah Hospital (BH)) were analyzed and included in this study. All cases were characterized and diagnosed by: (1) morphological and cytochemical features of leukemic blasts in peripheral blood and bone marrow films according to the French American British (FAB) criteria, and (2) immunological features using a comprehensive panel of MoAbs against myeloid- and lymphoid-associated antigens, according to the European Group for the Immunological Classification of Leukemias (EGIL) recommendations. All samples had over 20 % of blasts. Written informed consent was obtained and the project was approved by the Research Ethics Committee of the IBTO (Tehran-Iran).

Of 203 patients, 115 subjects (57 %) were adults (age between 16 and 90 years) and 88 patients (43 %) were children (age between 2 and 16 years). They were classified as follows: (1) AML (n=111), in which blasts were positive with one or more myeloid markers. Patients categorized into seven groups according to the FAB functional classes named as M0 (n=6), M1 (n=28), M2 (n=23), M3 (n=24 (M3=20 and M3v=4)), M4 (n=21 (M4=17 and M4E=4)), M5 (n=8 (M5a=6 and M5b=2)), and M6 (n=1). (2) ALL (n=86), 13 T cell lineage and 83 B cell lineage. Based on the level of differentiation of the gated blasts, the B cell lineage ALLs were further sub classified into three subtypes: early pre-B (Tdt+, CD10+, CD20-, sIg-, cIg-), pre-B (Tdt+, CD10+, CD20+, sIg-, cIg+), and B cell (Tdt-, CD10-, CD20+, sIg+, cIg-). (3) The remaining six cases were considered acute undifferentiated leukemia (AUL), as they could not be classified by cytochemistry and/or the expression of lymphoid and myeloid markers. Blast cells of these six cases were only positive for CD45 (100 %), CD38 (83 %), HLA-DR (50 %), CD7 (50 %), CD34 (33 %), and Tdt (33 %). No cases were diagnosed as acute biphenotypic leukemia (BAL) or mix-lineage leukemia.

Sample collection

Aliquot of 2 ml of bone marrow aspirates are used for immunophenotyping assay. For prevention of sample clotting, 0.02 mg/ml EDTA was added to specimens.

Immunological tests

Immunophenotypic analyses were performed using Coulter Epics Profile II and Epics XL. Coulter Q-prep/Immunoprep System was used for blast preparation for immunophenotyping. All of the MoAbs were bought from DAKO, Denmark. Bone marrow aspirates and peripheral blood specimens were analyzed using the following: (a) fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (MoAbs): CD10, CD15, CD34, CD33, CD38, CD16, CD13, CD20, CD7, CD23, CD22, and Tdt and (b) RPE-conjugated MoAbs: CD45, CD14, CD5, CD20, CD2, CD19, HLA-DR, CD3, CD4, and CD8. The expression of C-kit receptor antigen (CD117) was evaluated using RPE-conjugated MoAb clone 104 D2, DAKO, Denmark. The reaction was considered positive when antigen expression was present in 20 % or more of the gated blast cells.

Statistical analysis

Data was analyzed by SPSS 16 (SPSS Inc., Chicago) and results were presented as mean±SD and the p value less than 0.05 was considered as significant. Receiver operating characteristic (ROC) curves were constructed to establish a sensitivity-specificity relationship. Cutoff values that provided the best combination of sensitivity and specificity were determined by ROC curve analysis. Coefficient of variances of the test, sensitivity (true-positive/true-positive+false-negative), specificity (true-negative/true-negative+false-positive), positive predictive value (PPV, true-positive/true-positive+false-positive), negative predictive value (NPV, true-negative/true-negative+false-negative), positive likelihood ratio (LR+, sensitivity/1-specificity), and negative likelihood ratio (LR-, 1-sensitivity/specificity) were calculated.

Table 1 Patients' characteristics

	Adult pts. n=92	Children n=111	Total n=203
Age			
Median	43 Y.	8.4 Y.	27.4 Y.
Range	16–90 Y.	2–16 Y.	2–90 Y.
Sex			
Male	60 %	51 %	57 %
Female	40 %	49 %	43 %
Diagnosis			
AML	74 %	33 %	55 %
ALL	21 %	66 %	42 %
AUL	5 %	1 %	3 %

Pts. patients, *Y*. years

Results

Specimens of 92 adult patients and 111 children with acute leukemia were analyzed for CD117 expression. Patients' characteristics are shown in Table 1. The expression of CD117 antigen greater than 20 % occurred in the majority (76 %) of the AML (84/111) patients. Frequency of CD117 expression within FAB subtypes is shown in Table 2. CD117 antigen was only restricted to more undifferentiated granulocytic leukemias based on the FAB classification: 83 % of M0, 86 % of M1, and 78 % of M2 were CD117 positive, whereas 62 % of M3 were positive for this antigen. CD117 positive AMLs were significantly less frequent among M3, M4, and M5 subtypes: 62 % (15/24) of M3, 71 % (15/24) of M4, and 75 % (6/8) of M5 resulted positive. Of the eight cases with pure monocytic leukemia, all six M5a AML expressed CD117, whereas none of the two M5b AML did. While 76 % of AML cases and half of the six AUL were c-kit positive, none of the ALL samples expressed this antigen. Results on the reactivity with CD117 in the acute leukemias are shown in Table 3. We also tested the expression of other myeloid related markers (CD13, CD33, and CD15) in all of the 203 patients.

Table 2 Frequency of CD117 positive cases within FAB subtypes

FAB subtype	$CD117^+$	Total	%
M0	5	6	83
M1	24	28	86
M2	18	23	78
M3	15	24	62
M3	12	20	60
M3v	3	4	75
M4	15	21	71
M4	12	17	70
M4E	3	4	75
M5	6	6	75
M5a	6	8	100
M5b	0	2	0
M6	1	1	100
Total	84	111	76

Table 3 CD117 expression in acute leukemias (number and proportion of positive cases)

Acute leukemia	No. of positive cases/total	Positive cases (%)
AML	84/111	76
AUL	3/6	50
ALL	0/86	0
Total	87/203	43

Using cutoff point of 20 % for CD117 expression, the corresponding sensitivity was 75.7 % and specificity was 100 %. The positive and negative predictive values for this marker were 100 and 76.1 %, respectively. Table 4 shows the positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio, and negative likelihood ratio of CD13, CD33, CD15, and CD117 in blasts of BM samples of the studied subjects. The highest (100 %) specificity and highest (89.8 %) accuracy could be obtained by detection of CD117, CD13, and CD33, respectively. In addition, the highest (69.083 %) LR+ and the highest (100 %) PPV could also be obtained by detection of CD13, CD33, and CD117. The highest (82.9 %) sensitivity could be obtained by measurement of CD13 in sample of the patients. Furthermore, the highest (81.7 %) NPV and the lowest (0.0) LR− could be obtained by detection of CD13, CD33, and CD 117. Figures 1 and 2 show the flow cytometry analysis with double markers expression (CD13+, CD117+) for AML and (CD13−, CD117−) for ALL patients.

Furthermore, according to age of subjects, samples were divided into two age range (children≤16 years and adult> 16 years). Data analysis also confirmed that CD117 expression significantly (p<0.05) differs in samples from adult

Table 4 Diagnostic Value of CD13, CD33, CD15, and CD117 in determination of myeloid lineage involvement (AML)

	CD13	CD33	CD15	CD117
Sensitivity	82.9 %	64 %	41.4 %	75.7 %
Specificity	98.8 %	100 %	98.8 %	100 %
Accuracy	89.8 %	79.7 %	66.5 %	86.3 %
PPV	98.8 %	100 %	97.9 %	100 %
NPV	81.7 %	68.3 %	56.7 %	76.1 %
LR+	69.083	–	34.5	–
LR-	0.173	0	0.593	0
P value	<0.001 %	<0.001 %	<0.001 %	<0.001 %
Kappa	0.798 %	0.608 %	0.372 %	0.231 %

PPV positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR-* negative likelihood ratio

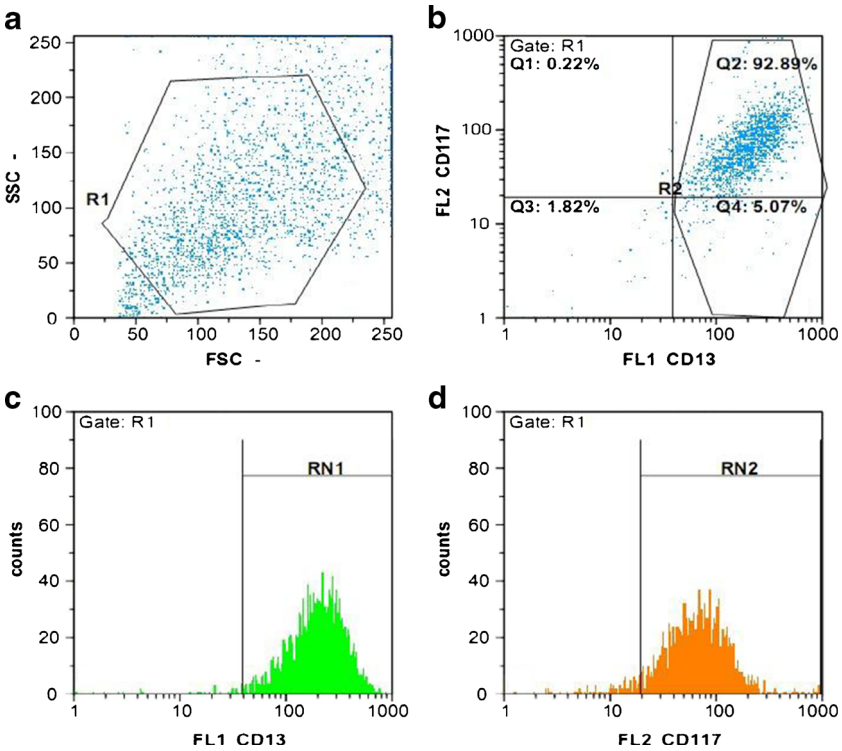

Fig. 1 Flow cytometry analysis of myeloid blasts with double markers expression (CD13+, CD117+) for AML patients. **a** Forward scatter (*FSC*)/side scatter (*SSC*) plot of CD117, CD13/++, with the *R1* gate indicated. The percentage of events in gate *R1* is listed. Events are represented as *blue points* (**a** and **b**). **c** and **d** show the flow cytometry histograms for double markers analysis of CD117 and CD13/++. **c** CD13 (FL1) histogram. **d** CD117 (FL2) histogram

subclasses of age range classification, and adult sample populations tends to yield larger observations of positive results for CD117 than the child sample population (Table 5).

Discussion

Flow cytometric analysis of acute leukemias provides the most rapid and reliable method for distinguishing different

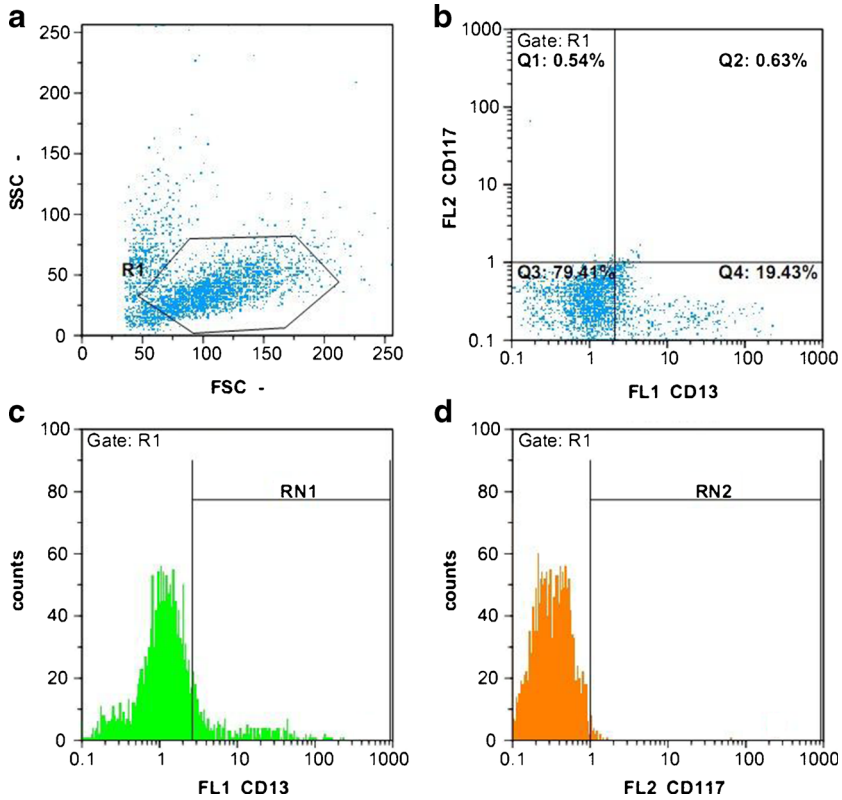

Fig. 2 Flow cytometry analysis of lymphoid blasts with double markers expression (CD13−, CD117−) for ALL patients. **a** Forward scatter (*FSC*)/side scatter (*SSC*) plot of CD117, CD13/—, with the *R1* gate indicated. The percentage of events in gate *R1* is listed. Events are represented as *blue points* (**a** and **b**). **c** and **d** show the flow cytometry histograms for double markers analysis of CD117 and CD13/—. **c** CD13 (FL1) histogram. **d** CD117 (FL2) histogram

Table 5 Expression of myeloid related markers (CD13, CD33, CD15, and CD117) in AML and ALL according to age classification

	AML				ALL			
	Children		Adults		Children		Adults	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
CD13 (% of patients)	21.7	4.72	60.38	13.21	0.07	70.89	0.0	27.85
CD33 (% of patients)	19.81	6.6	44.34	29.25	0.0	72.15	0.0	27.85
CD15 (% of patients)	10.38	16.04	28.3	45.28	0.07	70.89	0.0	27.85
CD117 (% of patients)	17.9	8.5	56.6	17	0.0	72	0.0	28

Children ≤16 year and Adults >16 year

lineage involvement by the neoplastic process [12, 14]. However, differences in reagents, gating, and staining techniques, and thresholds for positivity may account for the discrepancies between groups working in this field. A normalization and standardization procedure such as EGIL recommendations and related consensus represent a step forward in the accurate classification of these disorders [15, 16]. Demonstration of CD117 expression in acute leukemia is thought to be a reliable marker in identifying myeloid differentiation [1, 5, 6]. Different studies show that the frequency of CD117 expression in AML varies between 23 up to 87 % [17]. Accordingly, we analyzed 203 cases with de novo acute leukemia referred to our immunophenotyping centers for expression of four myeloid related markers (CD13, CD33, CD15, and CD117) and compared the sensitivity, specificity, PPV, and NPV of them to detect myeloid involvement. The overall frequency of 76 % CD117-positive AML in our study is consistent with the results of other investigators who analyzed at least over 50 patients and chose a comparable cutoff level for positivity in flow cytometric analysis (20 %) [2, 3, 9, 11] CD117 was found on >20 % of blast cells in 76 % of the AML cases. This finding is in contrast to some previous reports and could be due to different antibody specificities or to differences in the sensitivity of the detection methods employed [1, 4, 5, 17, 18]. Furthermore, the expression of Cd117 was detected in all FAB subtypes, except for M5b, while in other investigations no clear correlation was found between CD117 and FAB subtypes [6, 19, 20]. Scilicet, CD117 expression is not restricted to any particular, undifferentiated phenotype as previously proposed [18, 19]. The frequent expression of CD117 in AML and lack in ALL emphasizes its diagnostic value in recognizing myeloid differentiation in acute leukemia as demonstrated by the present study [1, 5, 7, 17, 20]. Nevertheless, in at least one third of patients with AML, determination of CD117 expression fail to prove myeloid differentiation [12]. In these instances, a broader diagnostic approach will still be necessary.

Our findings clearly establish the high specificity of c-kit for leukemic cells committed to the myeloid lineage and thus, support its value as a diagnostic reagent in the characterization of acute leukemias. Although the sensitivity of c-kit for detection of AML seems to be slightly lower than CD13, particularly in cases with a monocytic component, its specificity is higher because this marker and CD33 are expressed in a significantly larger proportion of ALL cases. The high incidence of CD117-positive cases within the M2 category (81 %) is difficult to interpret. Although CD117 antigen is irrespective of FAB classification and may be retained in more advanced maturation levels of the granulocytic lineage in acute monocytic leukemia (M5), it is strictly restricted to earlier stages (M5a). This interesting finding is in agreement with those of Josep FN et al. [21].

There are very few published clinical studies about the value of myeloid-related markers in differential diagnosis of acute leukemias. In our present study, using a cutoff point of 20 % for expression of CD markers on myeloid or lymphoid blasts, the sensitivity and specificity of CD117 were 75.7 and 100 %, respectively. Hoehn D et al. [13], by measurement of TdT, CD7, CD13, CD34, and CD117 in bone marrow aspirates, could predict FLT3 mutation in ALL patients. The related sensitivity and specificity for these markers were 100 and 94 %, respectively [13]. We showed that measurement of CD117 or CD33 combined with CD13 is a very useful marker for differentiation of acute leukemias because of its high specificity. The specificity for CD117 in BM aspirates is the highest values that have been reported for this test to date.

In summary, flow cytometric analysis of CD117 is a useful and reliable marker in the differential diagnosis of acute leukemias. Because it is a myeloid lineage related and strongly specific marker, it should be included in the primary panel of markers and routinely tested in all new diagnosed cases. In addition, we could not find any association between CD117 expression and morphological FAB subtypes, except for M5. Its expression in acute monocytic leukemia was restricted to immature morphology (M5a). It can be concluded that determination of CD117, CD13, and CD33 in BM aspiration might be an accurate diagnostic tool for screening and monitoring acute leukemias. However, further studies with a larger sample size are recommended.

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Conflicts of interest None

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