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Cluster of Differentiation 45: An Adjunct to Flowcytometric Diagnosis of Leukemias

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Abstract

Background: This Cross sectional comparative study was performed to compare the mean fluorescence intensity of cluster of differentiation 45 between healthy individuals and patients with acute lymphoblastic leukemia.

Patients and methods: Thirty three healthy individuals (mean age 11 years) and 41 patients with B cell acute lymphoblastic leukemia (mean age 7 years) were enrolled in the study in department of Immunology, Armed Forces Institute of Pathology, Rawalpindi, from Jan 2009 to April 2011, after ethics committee approval and obtaining informed consent. For immunophenotyping analysis, blood was stained with monoclonal antibodies using lyse wash procedure. For each subject, panel for staining consisted of cluster of differentiation 3, cluster of differentiation 5, cluster of differentiation 7, cluster of differentiation 10, cluster of differentiation 13, cluster of differentiation 14, cluster of differentiation 19, cluster of differentiation 20, cluster of differentiation 33, cluster of differentiation 34, cluster of differentiation 45, Human Leukocyte Antigen DR and intracytoplasmic myeloperoxidase and terminal deoxynucleotidase. Cells were then analyzed on Becton Dickinson FACSCalibur flow cytometer using Cell Quest Pro software. For each subject, mean fluorescence intensity was calculated in software along with geometric mean and standard deviation.

Results: Mean of geometric means of cluster of differentiation 45 expression on blasts of patient population was considerably low (145) as compared to lymphocytes of healthy population (764), being statistically significant ($p<0.0001$).

Conclusion: cluster of differentiation 45 is useful in differentiating mature lymphocytes from blasts in ALL cases.

Keywords: Flow cytometry, acute lymphoblastic leukemia, cluster of differentiation 45, mean fluorescence intensity.

Introduction

Flow cytometry, over the past decade, has evolved as an indispensable tool in the diagnosis of hematological malignancies.[1,2] Historically, the diagnosis in these cases relied predominantly on morphologic and cytochemical features, but now, it is supplemented with immunophenotypic, cytogenetic, and molecular data to define clinically relevant diagnostic categories. Multiparameter flow cytometry gives detailed antigen expression profiles in acute leukemias, which in conjunction with morphology, often suggests a definitive diagnosis or a narrow differential. Improvements in flow-cytometry instrumentation, availability of wide range of antibodies and fluorochromes, improved gating strategies and routine use of multiparameter techniques have dramatically improved the diagnostic utility of flowcytometry.[3,4] Besides being a powerful diagnostic tool for leukemias and lymphomas, more recently, it has aided in development and monitoring of antibody based treatment strategies.[5]

Cluster of differentiation 45 (CD45) is a protein tyrosine phosphatase that is present in all leukocytes with brightest expression on lymphocytes.[6] This molecule finds widespread uses in flow cytometry as it is used to differentiate mature lymphoid neoplasms (bright expression) from acute lymphoblastic leukemia (ALL) and plasma cell neoplasms (PCN) (weaker intensity).[3] In addition, it is of prognostic significance as its absence is

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associated with longer event free survival in childhood B-cell ALL.[7] Also CD45/side scatter (SSC) gating strategy permits efficient discrimination between blasts and normal cells facilitating analysis of blasts present in low proportions.[8] Thus, measurement of CD45 MFI can adjunct the flow cytometric diagnosis of leukemias if difference in intensity of its expression is established in different forms of leukemia and healthy individuals. In the present study we aimed to determine if CD45 increased diagnostic accuracy of flow cytometry by comparing its MFI in healthy individuals with patients having acute lymphoblastic leukemias.

Patients and methods

This cross sectional comparative study was carried out at Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi, from Jan 2009 to Jan 2011, after approval by ethical and research committee of the institute. The patient population consisted of 41 patients (19 males, 22 females) of B cell acute lymphoblastic leukemia (B-ALL) with the mean age of 7 years (range 1.5-16 years). Characteristics of study subjects are summarized in table 1. Thirty three healthy individuals (27 males, 6 females) with mean age 11 years (range 2-23 years) were enrolled in the study after informed consent as the control group. Individuals giving history of any illness within the past 3 months were not included in the control group.

For immunophenotyping analysis, 3 ml of peripheral blood sample (as advised by referring hematologists) was taken in ethylenediaminetetraacetate (EDTA) tubes and stored at room temperature until staining. This was then stained with monoclonal antibodies (Becton Dickinson, USA) using lyse wash procedure, according to manufacturer's recommendations. For each subject, panel for staining consisted of cluster of differentiation 3, cluster of differentiation 5, cluster of differentiation 7, cluster of differentiation 10, cluster of differentiation 13, cluster of differentiation 14, cluster of differentiation 19, cluster of differentiation 20, cluster of differentiation 33, cluster of differentiation 34, cluster of differentiation 45, Human Leukocyte Antigen DR and intracytoplasmic myeloperoxidase and terminal deoxynucleotidase. Following staining, cells were then analyzed on Becton Dickinson FACSCalibur flowcytometer using Cell Quest Pro software. Ten thousand cells were analyzed in each tube and dot plot diagram was made with CD45 FITC on logarithmic scale along X axis and side scatter (SSC) on linear scale along Y axis. For each subject, mean fluorescence intensity (MFI) was calculated in software along with geometric mean and standard deviation. The data was entered in SPSS version 13.0 and compared for statistical difference of patients versus healthy controls, using t test in openepi software. A p value of < 0.5 was considered significant at 95% confidence interval.

Results

A total of 33 healthy individuals and 41 patients with ALL were studied. For each subject Cell Quest Pro software calculated the geometric mean of CD45 MFI which was entered in SPSS software and mean of all geometric means, for each group was computed (Table 1). It was then entered in openepi software along with sample size and standard deviation to apply t test and calculate the p value. The results are summarized in table 1. Mean total leukocyte count and mean lymphocyte/blasts percentage of healthy population fell well within normal range (mean 7640 cells/ul and 33.3%)

Table 1: Characteristics and statistical analysis of healthy individuals and patients of B-ALL.

Group	Male: Female	Mean age (years)	Mean total leukocyte count (TLC) cells/ul	Mean lymphocyte/ blasts percentage	Mean of geometric means ± SD	Range (min-max)	p value
Healthy (n=33)	4.5:1	11	7640 ± 3130	33.3 ± 11.8 (lymphocytes)	764 ± 274	1098 (131-1229)	-
B-ALL (n=41)	0.86:1	7	41208 ± 7622	54.1 ± 12.6 (blasts)	145 ± 207	881 (5-886)	<0.0001

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as compared to blasts of patients (mean 41208 cells/ul and 54.1%). Mean of geometric means of CD45 MFI was 764 in healthy individuals vs. 145 in patients and the difference was statistically significant ($p<0.0001$). Figure 1 shows CD45 vs. SSC dot plot for an ALL patient, clearly showing one population of normal lymphocytes with bright CD45 expression and a second population of blasts with weaker CD45 expression. Figure 2 shows the distribution of geometric means of two groups along their respective means.

Discussion

In the past few years, flow cytometry has emerged as the most useful tool in diagnosis of hematological malignancies.[9] However, there is a great deal of variability in intra and interlaboratory reproducibility of results mainly owing to wide range of reagents and methods being used for sample preparation and data acquisition and analysis. A major factor for this difference is the cross lineage antigen expression, both in ALL and AML.[10] Besides, there is considerable variability among different laboratories in selection of monoclonal antibodies and consensus leukemia panel has been difficult to reach despite efforts.[3]

Cluster of differentiation 45 is a cell surface glycoprotein with a cytoplasmic tyrosine phosphatase domain that catalyzes the removal of phosphates from tyrosine residues and is believed to function in both the activation and suppression of lymphocytes as well as in T cell maturation.[11] CD45 carries the advantage that it is a pan leukocyte marker and is invariably expressed in all forms of leukemias. However, when measured by flow cytometry, its intensity of expression varies according to the stage of maturation in different forms of leukemias and healthy individuals. Here lies the utility of CD45 which can adjunct the diagnosis especially in cases which have limited expression of lineage specific antigens.[12] Hadi et al. have previously shown that CD45 expression is considerably brighter in healthy individuals than typical and atypical chronic lymphocytic leukemia (CLL) cases ($p< 0.001$).[13] Carulli et al. have also showed dim expression of CD45 in CLL versus non-Hodgkin's lymphoma (NHL).[14] Similarly Nakamura et al. have determined brighter CD45 expression in T-ALL than in B-ALL ($p<0.05$).[15] DiGiuseppe has reviewed varied intensity of CD45 in different forms of B-ALL and T-ALL.[16] Reviewing different researches, it is common among all that CD45 expression is brighter on mature lymphocytes than on blasts that helps differentiate blasts from mature lymphocytes in CD45-SSC dot plot in flow cytometric analysis. In our analysis the difference of geometric means between healthy individuals and ALL patients was quite large (geometric means: 764 & 145, $p <0.0001$) and statistically significant, proving that at least in ALL patients, CD45 expression is considerably weaker on blasts as compared to mature lymphocytes. Therefore in flow cytometric immunophenotyping, reduced/ normal expression of CD45 in combination with

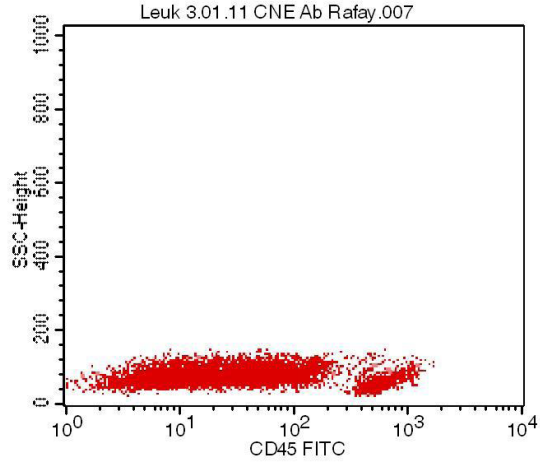


Figure 1: CD45 vs. SSC in an ALL patient.

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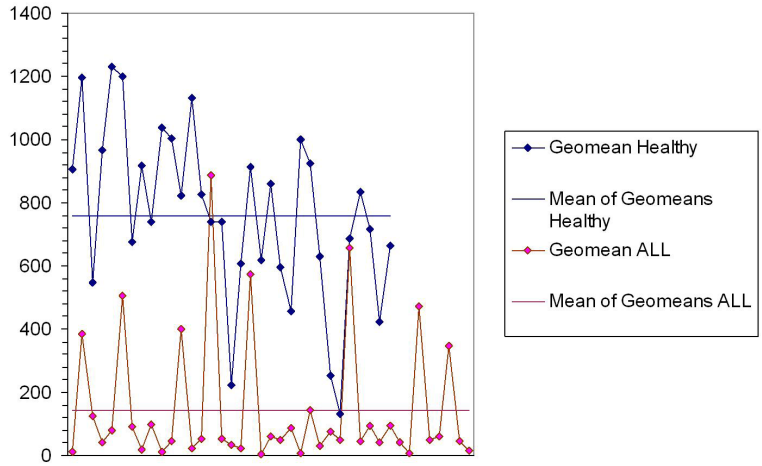


Figure 2: Geometric means distribution of healthy controls and ALL patients along their respective means.

lineage specific markers can provide valuable information about the diagnosis.

Conclusion

CD45 is useful in differentiating mature lymphocytes from blasts in ALL cases, along CD45-SSC dot plot in flow cytometric immunophenotyping. In future, this marker can further be studied among different forms of acute leukemias, chronic leukemias and in lymphomas.

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