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Original Research Article

A Study of Aberrant Phenotypes in Acute Leukemia by Flowcytometry

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ABSTRACT

Introduction: Immunophenotyping is an essential method for diagnosis and classification of acute leukemia and its extensive use could identify blast cell subpopulations with aberrant phenotypes rarely seen in normal haemopoiesis. Aberrant phenotypes in acute leukemia have a variable frequency and are helpful for detection of minimal residual disease and for determination of prognosis. Our study aimed to analyze the frequency of aberrant expression in acute leukemias.

Methods: We prospectively investigated the phenotype of blast cells from 50 acute leukemia patients using a large panel of monoclonal antibodies by multiparametric flowcytometry.

Results: 50 cases of acute leukemia were analyzed using multiparametric flowcytometry. Out of which 33 cases were Acute Myeloid Leukemia (AML), 15 cases were Acute Lymphocytic Leukemia (ALL) and 2 cases were Mixed Phenotypic Acute Leukemia (MPAL). 29/50 cases (58.0%) had conventional phenotypes while 21/50 cases (42.0%) showed aberrant expression. 18/33 cases (54.54%) of AML and 3/15 (20.0%) of ALL cases demonstrated aberrant phenotype in our study. Among the AML cases, CD7, 8/33 cases (24.24%) was the most commonly expressed aberrant lymphoid marker. Paired aberrancy was seen in 3/21 cases (14.28%). Among the ALL cases, CD13, 2/3 cases (66.66%) was most commonly expressed aberrant myeloid marker.

Conclusion: Our study demonstrates that aberrant phenotypes were frequently expressed in acute leukemias and seen more commonly in AML than ALL. The existence of aberrant leukemic associated phenotypes can become a valuable tool for the detection of minimal residual disease however its prognostic importance need to be further evaluated.

Key words: Aberrant phenotype, Acute leukemia, Flowcytometry.

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INTRODUCTION

Acute leukemias are a heterogeneous group of malignancies with varying clinical, morphologic, immunologic and molecular features and display characteristic patterns of surface antigenic expression. Immunophenotyping of leukemia cell results in broad classification of Acute leukemia into Acute Myeloid leukemia (AML), Acute Lymphocytic leukemia (ALL) and Mixed Phenotypic Acute leukemia (MPAL) based on expression of different subsets of surface molecules, now defined as cluster of differentiation (CD) antigens, by the precursor or blast cell. The expression of CD markers on leukocytes can be determined by flowcytometry.[1]

The flowcytometric immunophenotyping rely on the concept that neoplastic cells show a great similarity to normal hematopoietic precursors but they frequently display unusual expression of CD markers called aberrant expression of markers that allow their specific identification and differentiation from normal cells. The aberrant phenotypes shows either co-expression of a particular lymphoid or myeloid associated antigens showing lineage infidelity; asynchronous antigen expression, in which early antigens are co expressed with more mature ones or antigen over expression and existence of abnormal light scatter patterns.[2]

Occurrence of aberrant phenotype has been reported in acute leukemias with varying frequency. Aberrant expression has relevance to clinical prognosis and can help in identification of minimal residual disease on follow up. This is a first such comprehensive study done in Upper Assam, India. The aim of our study was to evaluate the occurrence of aberrant phenotypes in newly diagnosed cases of acute leukemia.

MATERIALS AND METHODS

The study was carried out in the DBT Health Care Flowcytometry Laboratory, Department of Pathology, Assam Medical College, Assam, India for a period of one year from May, 2013 to June, 2014. A total number of 50 cases of acute leukemia were included in the study, which were subjected to routine haematological investigations and cytochemistry followed by multiparametric flowcytometry. Diagnosis of acute leukemia was made on routinely stained bone marrow aspiration and blood smears. Immunophenotyping was carried out on bone marrow or peripheral blood.

Complete blood count was done by using Sysmex XS-800i and peripheral blood film stained by Giemsa stain to find the presence of blast cells. A total of 500 cells of WBC were counted and blasts cells over 20% are regarded as acute leukemias. Then whole blood or aspirate samples were prepared by cell Stain-Lyse-Wash

method for immunoflourescence staining with different antibodies which were conjugated with fluorochromes (i.e APC H7, PE cy7, FITC, PE, APC and PerCPcy5.5). Cell washing was done with phosphate buffer saline (PBS) (NaH2PO4.2H2O, Na2HPO4 and NaCl). When whole blood is added to the monoclonal antibody reagent, the fluorochrome labelled antibodies in the reagent bind specifically to leucocyte surface antigens. The stained samples were then treated with FACS Lysing solution (NH4CL) which lyses erythrocytes under gentle hypotonic conditions while preserving the leucocytes.

The permeablizing solution containing 15% formaldehyde and 50% diethylene glycol and proprietary permeablizing agent used for intracellular staining of antigens such as MPO, CD79a, CD3 Cytoplasmic and Tdt. Data acquisition and analysis were performed on a FACS Canto 2 flow cytometer (Becton Dickinson, San José, USA) using BD FACS Diva software. Identification of blast cells was performed using side scatter (SSC) versus CD 45 intensity and SSC versus forward scatter (FSC) parameter dot plots. The percentage of gated abnormal population expressing a particular CD marker was analyzed whether expression was positive or negative (> 20% for surface antigen and >10% for cytoplasmic antigen). AML cases were studied for aberrant expression of any B or T cell markers and ALL cases were studied for aberrant expression of myeloid markers.

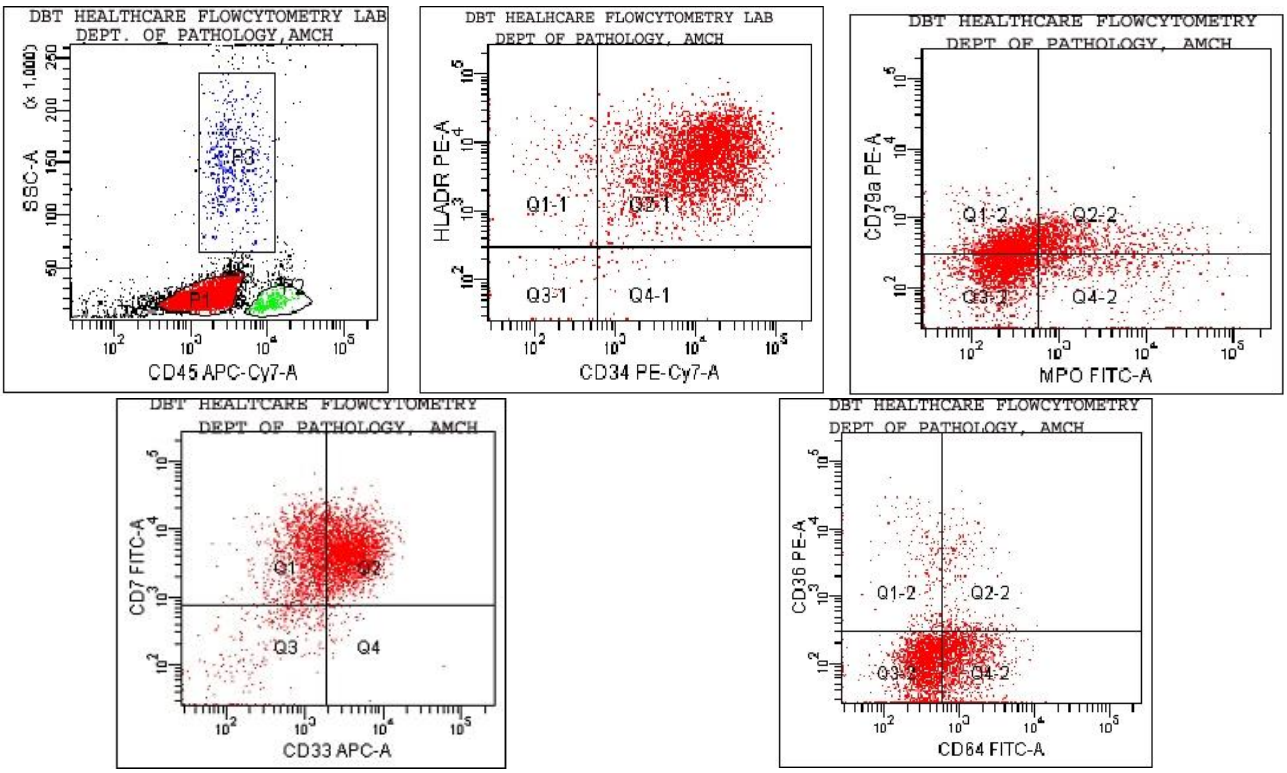


Figure 1. Immunophenotype of AML with aberrancy of CD7 and CD79a

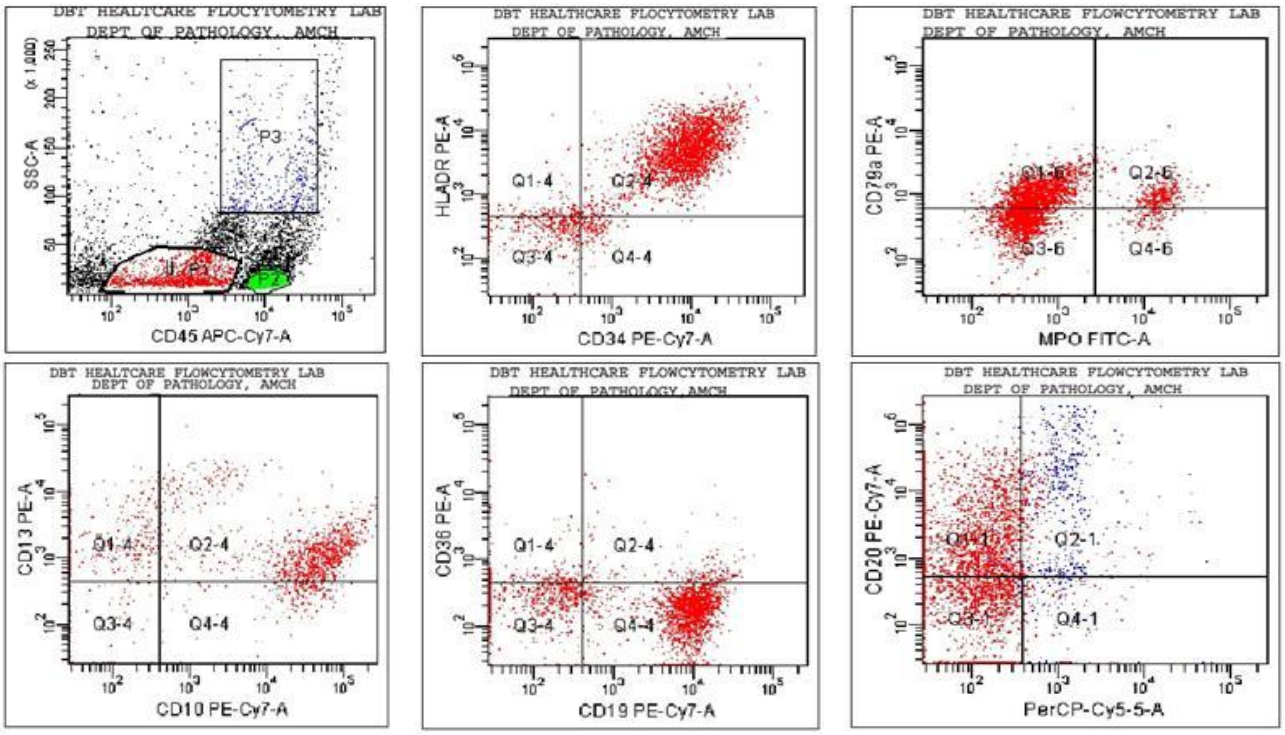


Figure 2. Immunophenotype of B-ALL with Aberrancy of CD13

RESULTS

In the present study 50 patients of acute leukemia were analyzed to find out the frequency of aberrant antigens in leukemia (33 AML cases + 15 ALL cases + 2 MPAL cases). ALL cases were further subdivided into B-ALL and T-ALL. Lymphoid and myeloid cell subsets in various stages of maturation have distinct immunophenotype as given in this table (Table 1).

Out of these 50 cases 29 (58.0%) had conventional immunophenotypes as they showed expression of lineage specific markers while 21 (42.0%) cases showed aberrant expression of CD antigens (Table 2).

In 18 patients, lymphoid lineage associated antigens were present on acute myeloid leukemia cases while 3 cases showed myeloid associated antigens expression on acute lymphoid leukemia cases. These cases were considered as aberrant immunophenotypes (Table 3).

Table 1: Lineage Specific Markers

Myeloid markers	B cell markers	T cell markers
CD 13	CD 19	CD 5
CD 33	CD 20	CD 2
Anti MPO	CD 79a	CD 3
CD 11c , CD 64, CD 36	CD 10	CD 7
CD 14, CD 117	TdT	TdT
CD41, CD42		

Table 2: Patterns of CD markers expression in Acute Leukemia

	No. of Cases	Conventional cases / %	Aberrant Cases / %
AML	33	15 (45.45)	18 (54.55)
B ALL	14	11 (78.75)	3 (21.42)
T ALL	1	1 (100.0)	0

Table 3: Aberrant Expression of CD markers in Acute Leukemia

CD Markers	AML (18/33)	ALL (3/15)
	No of cases / %	No of cases / %
CD 7	8(24.24)	
CD 79a	5 (15.15)	
CD 19	4(12.12)	
CD 2	4 (12.12)	
CD 20	3 (9.09)	
CD 13	-	2 (13.33)
CD 64	-	1 (6.66)

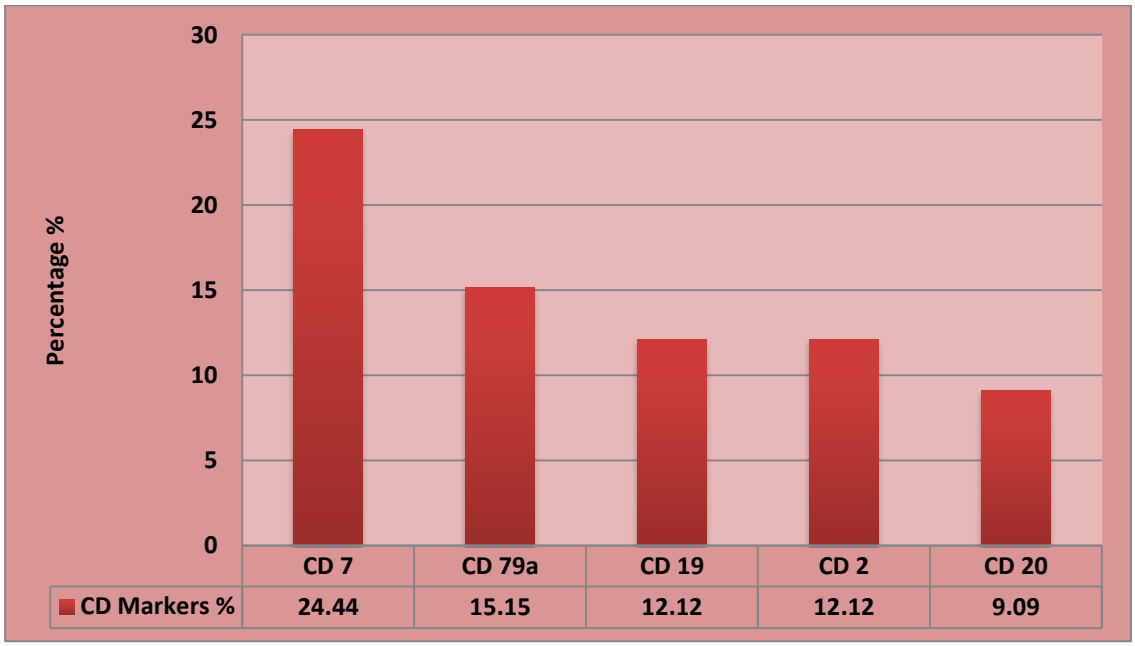
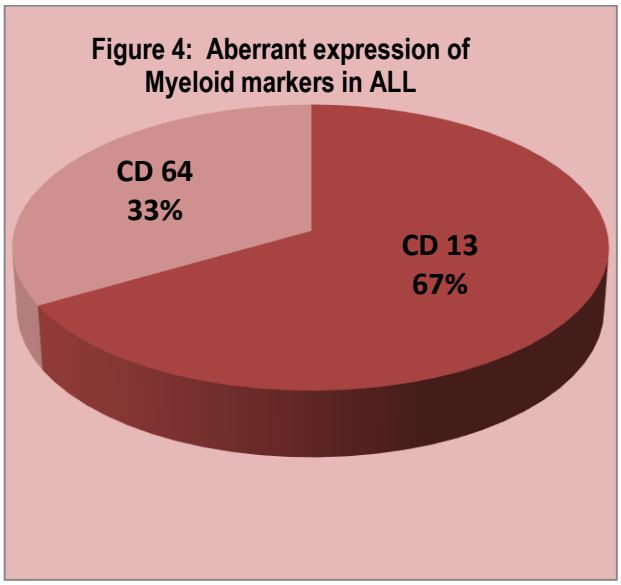


Figure 3: Aberrant expression of Lymphoid markers in AML

In 33 patients with AML, we found CD7 expression was the most common aberrant phenotype in 8 cases (24.24%) followed by CD79a in 5 cases (15.15%). CD 19 and CD 2 were expressed in 4 cases (12.12%) each. Least expressed was CD 20 in 3 cases (9.09%). Paired aberrant expressions were seen as CD7/CD79a, CD2/CD79a and CD19/CD79a 1 case each. Triple aberrant expression of CD 2, CD 7, CD 79a was seen in 1 case. CD79a was most commonly shared with other aberrant phenotypes. (Fig 3).

In 15 patients with ALL, we found 14 cases of B ALL and 1 case of T ALL. Out of the B ALL cases, 3 cases (20.0%) showed aberrant expression. No aberrant expression was found in the T ALL case. CD 13 was the most common aberrant phenotype, 2 cases (13.33%) followed by CD 64 in 1 case (6.66%). (Fig 4)



DISCUSSION AND CONCLUSION

Acute leukemias are broadly defined as having myeloid or lymphoid differentiation according to the expression of surface and/or cytoplasmic antigens associated with their normal myeloid or B/T-lymphoid counterparts. Detection of aberrant phenotypes is of clinical importance not only for accurate diagnosis of acute leukemia but potentially also for AML and ALL sub-classification. In this study 50 cases of acute leukemia (33 AML cases + 15 ALL cases + 2 MPAL case) were analyzed using multiparametric flowcytometry. 29/50 cases (58.0%) had conventional phenotypes while 21/50 cases (42.0%) showed aberrant expression of CD markers. Aberrant antigen expression in acute leukemia is a frequent occurrence as reported by Naghmana Mazhar et al.[3]

18/33 cases (54.54%) of AML demonstrated aberrant phenotype in our study compared to 48% as reported by Khalidi et al.[4] and 30% as reported by Zhu et al.[5]

Among the aberrant AML cases, CD7 was the most commonly expressed lymphoid marker seen in 8/33 cases (24.24%) in agreement to the results of Bahia et al.[6] WHO also reported that CD7 was the most frequent lymphoid associated antigen in AML. The same results were reported by Shen et al.[7] where CD7 was most common aberrant expression (12.8%). Further aberrant expression of CD7 antigen in AML is associated with poor prognosis as shown by Saxena et al.[8] and Cruse JM et al.[9].

In 15 patients with ALL, only the B ALL cases showed aberrant expression, total 3 cases (20.0%). CD 13 expression was the most common aberrant phenotype in 2 cases (13.33%) followed by CD 64 in 1 case (6.66%) which is similar to other studies.[3,8]

Studies by Suggs JL et al.[10] have reported CD 13, CD 33 and MPO aberrant expression in T ALL. However in our study, no aberrant expression was found in T ALL probably due to smaller sample size.

To conclude, flowcytometry helps in the diagnosis of aberrant phenotypes in acute leukemia. Aberrant expression adds important information for prognosis and at the same time, could be of help when looking for minimal residual disease during morphologic remission. Relevance of these markers in prognosis and treatment needs to be studied further.

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ETHICAL APPROVAL: Ethical approval was given by the institutional ethical committee for conducting the present study.

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Conflict of Interest: None Declared.

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