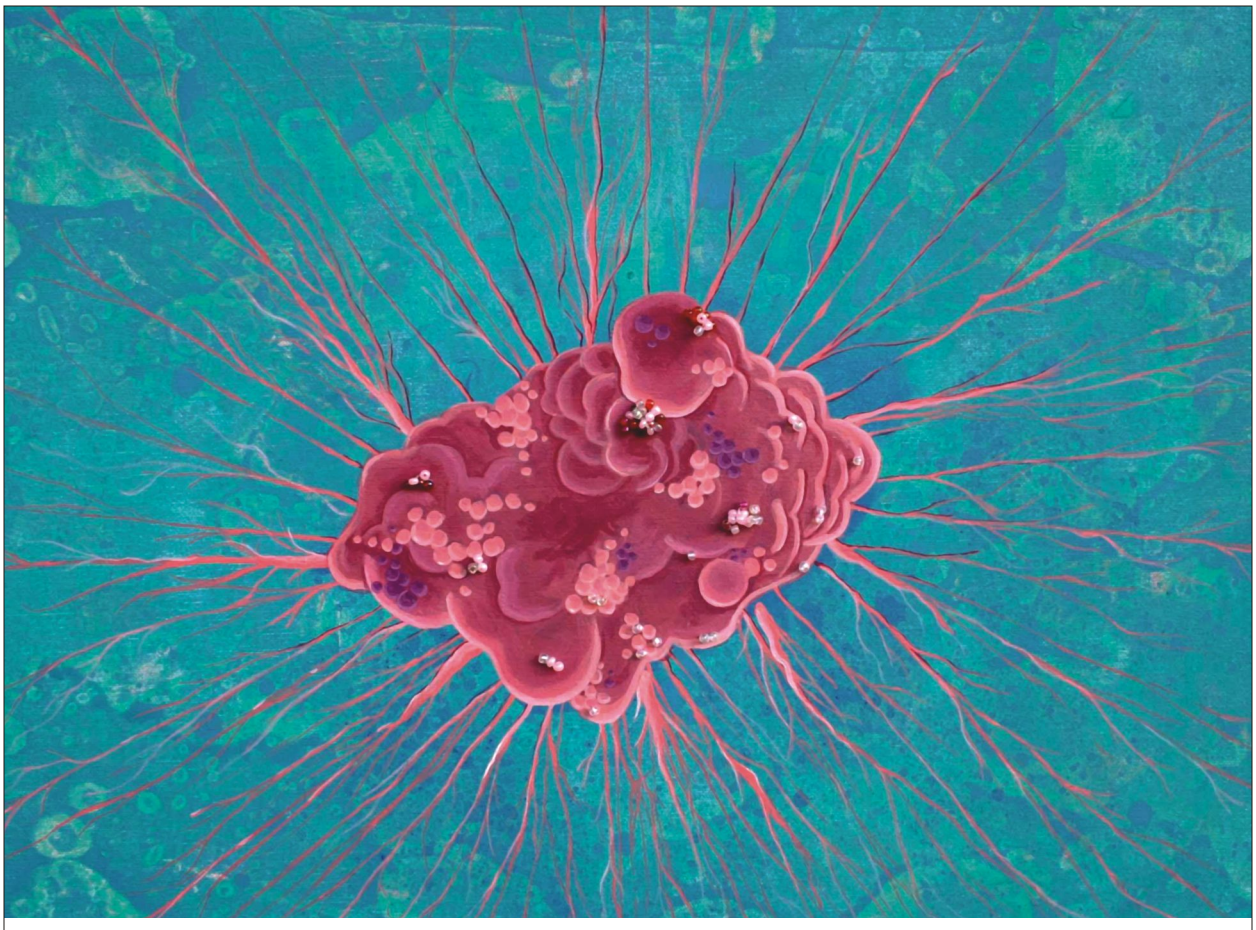

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Research Article
Expression of Aberrant Antigens in Adult Acute Leukemia: A Study in Bangladesh

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Abstract

Background and Objective: Aberrant antigen expression can adversely influence the clinical response, remission rate and overall survival in patients with acute leukemia. The aim of this study was to observe the frequency of expression of aberrant antigens in Acute Leukemia (AL) of adult patients. **Materials and Methods:** A cross sectional prospective study of acute leukemia was done over one year period (March, 2015-February, 2016). Multi parametric Flow Cytometric Immunophenotyping (FCI) was performed on peripheral blood and/or bone marrow aspirates collected from provisionally diagnosed acute leukemia patients of age ≥18 years. The FCI was performed with a complete panel of fluorochrome monoclonal antibodies. The co-expression of CD markers on myeloid and lymphoid population was analyzed. **Results:** A total of 64 AL cases were diagnosed by FCI, of which 31 cases had Acute Myeloid Leukemia (AML), 21 had B-Acute Lymphatic Leukemia (ALL) and 10 T-ALL. The remaining 2 cases (3.1%) were diagnosed as Mixed Phenotype Acute Leukemia (MPAL). Overall 40.3% (25/62) of the patients showed expression of aberrant CD markers. Among the 31 AML cases, aberrant expression of CD7 was in 7 (22.5%) cases followed by CD19 (12.9%) 4/31 and TdT (3.2%) 1/31 cases. Among 21 B-ALL cases aberrantly expressed antigens were CD13 (23.8%) 5/21 and CD33 (19.05%) 4/21. Of the 10 T-ALL cases, CD33 was expressed in 2(20%) cases and cyCD79a, CD117 and CD13 in (10%) 1/10 case each. Both cyCD79a and CD13 was expressed in one case. **Conclusion:** Quest for aberrant antigen expression should be given adequate emphasis as this may be of prognostic value.

Key words: Acute leukemia, flow cytometry, aberrant antigen, CD markers, mixed phenotype acute leukemia

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Introduction of immunophenotyping for typing of Acute Leukemia (AL) has improved the diagnostic accuracy as well as ensured better prognosis of acute leukemia patients. Immunophenotyping has significantly contributed to the reproducibility of the typing results of AL, besides immunophenotyping can be considered particularly useful for identifying poorly differentiated subtypes of acute leukemia, Acute Myeloid Leukemia (AML) with lymphoid marker expression and Acute Lymphatic Leukemia (ALL) with myeloid marker expression. Immunological studies of leukemic blasts have become critical also for identifying biphenotypic and bilineal acute leukemias[1]. At present, while the prognostic value of individual antigen expressions is still controversial, it is important in the immunologic detection of minimal residual disease, especially in AML, as it seems to be important in the monitoring of acute leukemia patients in remission[2].

Classification of hematopoietic neoplasms was done in 2008 on the basis of morphologic, immunophenotypic, cytogenetic and molecular features for the diagnosis and sub-classification of acute leukemias[3]. Multiparameter high-resolution flow cytometry has been developed to precisely identify lineage characteristics of leukemia based on co-expression and correlation of lineage-associated antigens. Cytogenetic abnormalities and prognosis is correlated with some immunophenotypes[4].

Among the acute leukemia cases, 46% of ALL cases and 48% of AML cases were reported[5] to have aberrant expression of a single antigen associated with another cell lineage, most commonly CD2[5] and CD7[6] in AML and CD33 in ALL[7,8].

From a prognostic point of view, aberrant antigen expression can adversely influence the clinical response, remission rate and overall survival in patients with acute leukemia[3,9-11]. This study was done to assess the frequency of aberrant antigen expression in acute leukemia in adult patients of Bangladesh which may add some prognostic information to physicians for better management of the patients.

MATERIALS AND METHODS

This cross-sectional prospective study was conducted from March, 2015-February, 2016. Peripheral blood and bone marrow aspirates were collected from Department of Hematology, Sir Salimullah Medical College Hospital (SSMCH) and of other tertiary institutes of Dhaka. Morphologic features of all the samples were reviewed by qualified hematologist of Department of Clinical Pathology, BSMMU. Rest of the laboratory works were done in the Department of Microbiology and Immunology, BSMMU. The protocol was approved by the Institutional Review Board (IRB) of BSMMU[12].

Study population: Seventy adult patients aged 18 years or above, attending the Department of Hematology of BSMMU and other institutes who were newly diagnosed as acute leukemia by cytomorphology were included in the study. Informed written consent was taken from all patients. Consecutive sampling procedure was followed for this purpose. Patients who were suffering from chronic myeloid leukemia with blastic crisis, myelodysplastic syndrome and other myeloproliferative disorders or have received chemotherapy for acute leukemia were excluded from the study[12].

Sample collection: Preferred sample was bone marrow aspirate and in case of unavailability peripheral blood was collected. Bone marrow aspirate (2 mL) was collected from 29 patients. Collection was performed by experienced personnel of the Hematology Department of the respective institutes from posterior superior iliac spine after ensuring strict asepsis and necessary precautions. Peripheral blood (2 mL) from 41 patients was collected mostly from antecubital vein with aseptic precautions. All the samples were collected in EDTA tubes[12].

Morphologic assessment: All specimens were obtained and prepared for morphologic examination using standard techniques. Smears were air dried and stained by Leishman stain followed by light microscopy[12].

Methods for immunophenotyping: Sample collected in EDTA tube was immediately transported to the lab for immunophenotyping. Measured amount of sample was taken in previously marked tubes to ensure approximate cell concentration of 10^6 mL^{-1}. Pre-titrated volume of specific antibodies or antibody cocktails were added to specific tubes followed by incubation in dark for 20 min. Lysing solution BD FACSLyse™ (1X) was added and incubated further for 10-12 min. Then temperature regulated centrifugation was done at 200-300 g for 5 min at 25°C and supernatant discarded. Washing and centrifugation process was repeated once. Cells were finally re-suspended in 0.5 mL sheath fluid or PBS with 2% paraformaldehyde. This was done for surface markers, but for staining of intracellular markers, 0.5 mL of permeabilizing solution Perm2™ (1X) was added to the tubes after centrifugation and incubated for 10 min in the dark. Then cells were washed by sheath fluid and centrifuged at 300 g

for 5 min and supernatant discarded. Addition of pre-titrated volume of antibodies or antibody cocktail against intracellular antigens was done followed by incubation in dark for 10-15 min. Then the steps are same as for extracellular markers as washing, centrifugation and final preparation in sheath fluid or PBS.

Following marker combinations of fluorochrome tagged Monoclonal Antibodies (MoAb) were added to different tubes for detection of various cellular markers by flow cytometry:

- For T-cell: Cytoplasmic (cy) CD3, CD5, CD7
- For B-cell: CD19, CD10, cyCD79a
- For myeloid cells: CD13, CD33, CD117, CD14, CD15, CD64 and Cytoplasmic Myeloperoxidase (cyMPO), CD235a, CD41a
- Pan leukocyte marker: CD45
- Precursor marker: CD34, TdT, HLA-DR

Four color flow cytometry immune-phenotyping was performed according to the instructions provided in BD FACSVerse™ System User's guide, © 2011, Becton, Dickinson and Company by collecting 10,000 ungated list mode events. The blast gating strategy included using dot plots of CD45 expression versus side scattering (SSC) and also a second gating strategy using forward scattering (FSC). Back gating was also done when required. Analyses of different parameters of the gated cells were done by standard method. Any antigenic marker was considered positive if 20% or more of the blast cells reacted with a particular antibody[12].

RESULTS

In this study, among 70 morphologically diagnosed acute leukemia patients, 64 patients were confirmed by flow cytometry. Samples from these 64 patients were further analyzed to see the antigen expression pattern. Among them 31 cases had AML, 21 had B-ALL and 10 T-ALL. The remaining 2 cases (3.1%) were diagnosed as mixed phenotype acute leukemia and they were excluded from this calculation as they are regarded as distinct entity. Overall 40.3% (25/62) of the patients showed expression of aberrant CD markers in flow cytometric analysis. The expression rate of lineage infidelity was found in 38.7% (12/31) of the AML patients which was 42.9% (9/21) for B-ALL and 40% (4/10) for T-ALL (Table 1).

Among the 31 AML cases, CD7 was expressed in 7(22.5%) cases while CD19 and TdT expression were found in 4 (12.9%) and 01 (3.2%) cases, respectively. Among 21 B-ALL cases, 5 (23.8%) cases were positive for CD13 and 4(19.04%) cases for CD33. Of the 10 T-ALL cases, expression of CD33 was found in 2(20%) cases and cyCD79a, CD117 and CD13 was expressed in 1 (10%) case each. Among these one cases expressed both cyCD79a and CD13 (Table 2).

Flow cytometric dot plots depicted in Fig. 1 illustrates abnormal cell clusters (low SSC with dim to moderate CD45 expression) were gated (P1) showing bright staining for CD33, CD13, cytMPO, HLA-DR and CD19. The overall features are consistent with acute myeloid leukemia with aberrant expression of lymphoid marker CD19.

Table 1: Frequency of aberrant antigen expression in acute leukemia cases (n = 62)

	Aberrant antigen expression					
Acute leukemia	Absent		Present		Total	
cases	Number	Percentage	Number	Percentage	Number	Percentage
AML	19	61.3	12	38.7	31	50.0
B-ALL	12	57.1	09	42.9	21	32.3
T-ALL	06	60.0	04	40.0	10	16.1
Total	37	59.7	25	40.3	62	100

AML: Acute myeloid leukemia, ALL: Acute lymphatic leukemia

Table 2: Aberrant antigens in acute leukemia cases (n = 62*)

	AML (n = 31)		B-ALL (n = 21)		T-ALL (n = 10)	
Antigenic markers	Number	Percentage	Number	Percentage	Number	Percentage
TdT	01	3.2	-	-	-	-
CD7	7	22.5	-	-	-	-
CD19	4	12.9	-	-	-	-
cyCD79a	-	-	-	-	1	10.0
CD13	-	-	5	23.80	1	10.0
CD33	-	-	4	19.05	2	20.0
CD117	-	-	-	-	1	10.0

*2 mixed-phenotype acute leukemia (MPAL) cases have been excluded as they have been placed in a distinct group

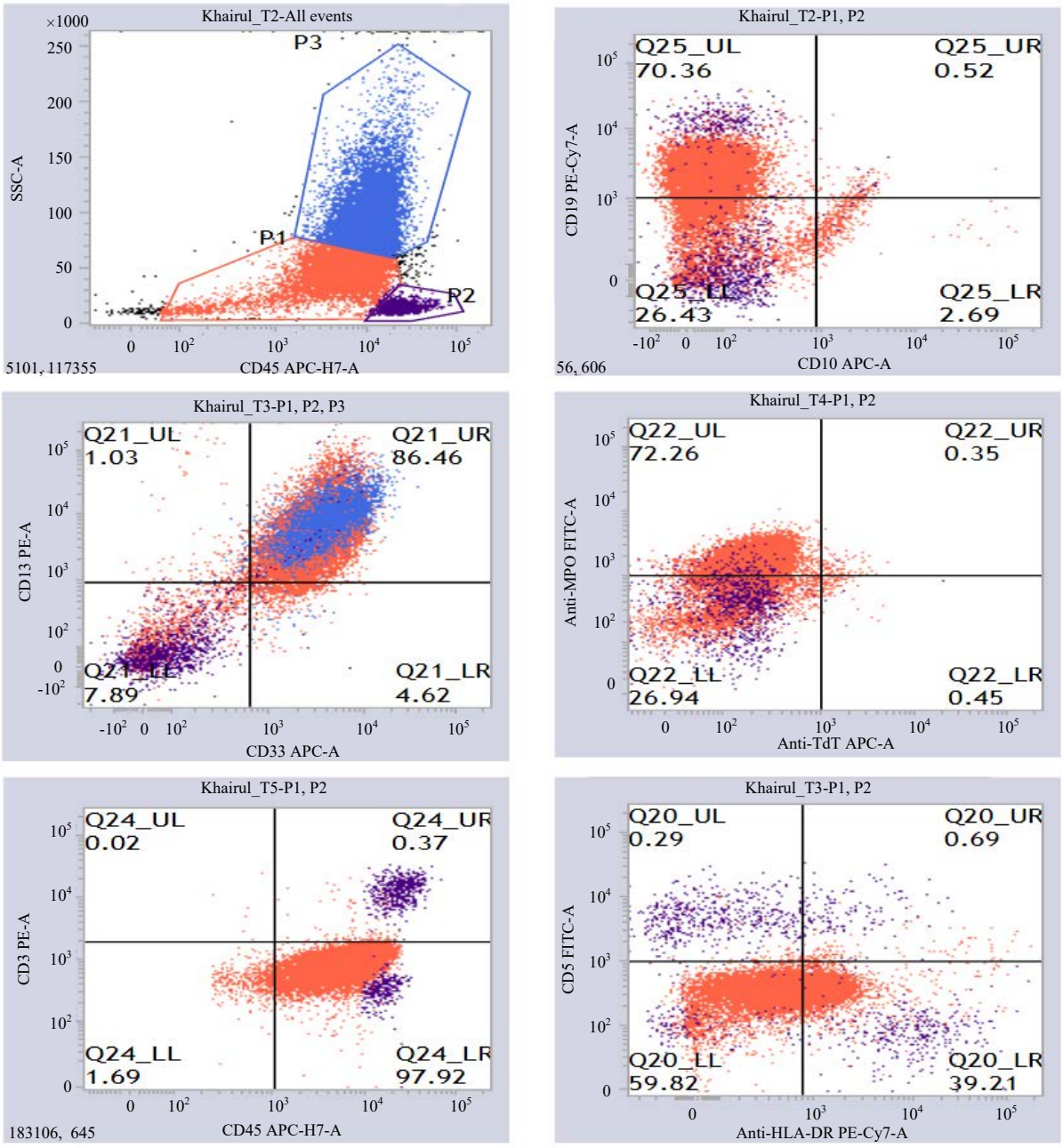

Fig. 1(a-f): Flow cytometric immunophenotyping of a case of acute myeloid leukemia with aberrant expression of CD19, (a) Low SSC with dim to moderate CD45 (P1) expression, (b) Blast cells expressing CD19, (c) Positivity of blast cells for both CD13 and CD33, (d) Blast cell positivity for MPO but negativity for TdT, (e) CD3 negativity of blast cells and (f) HLA-DR positivity of blast cells

*This dot plot diagram is of a patient of this study analyzed in our department

DISCUSSION

Proper categorization is of utmost importance for management of acute leukemia. Inclusion of multi parameter flow cytometry for characterization of AL has revolutionized the leukemia diagnostics. In the current study 64 acute leukemia patients were analyzed by flow cytometry to see their antigen expression pattern.

In the current study out of the 31 AML cases 12 (38.7%) showed aberrant expression of lymphoid markers. Mazher *et al.*[13] showed that aberrant expression of lymphoid markers in AML is 43% in Pakistani population. Feki *et al.*[14] showed this rate to be 44.4%. So, the study result is more or less consistent with the current findings.

Aberrant expression of CD7 was the mostly expressed aberrant marker in AML cases with a percentage of 22.5% which is similar to other study findings[13,15,16]. Overall prognosis is poor in AML patients with CD7 expression[17].

Expression of CD19 was found in 12.9% of AML cases which is within the range of previous studies (4, 1.8 and 11%)[13,18,19]. A little variation is notable in the results of different studies but current study findings do not contradict those results. CD19 expression in AML cases warrants the search for specific cytogenetic defect t (8;21)[20,21].

Aberrant expression of TdT was found in 3.2% (1/31) AML case in the current study which is slightly lower than other studies (22 and 6%)[13,18]. In the current study most AML patients were young adult which may be a possible explanation of the finding.

Aberrant expression of myeloid antigens (My+ B-ALL) was found in 42.85% (9/21) cases of which CD13 and CD33 were aberrantly expressed in B-ALL. The significance of expression of myeloid antigen in B-ALL is controversial regarding patient prognosis[22,23].

CD13 was aberrantly expressed in 5/21 (23.8%) B-ALL cases which is within the range (36.5 and 20%, respectively)[13,24].

Expression of CD33 in B-ALL was seen in 4/21 (19.05%) cases that is also within range of other study findings (29 and 15%, respectively)[13,24].

Aberrant antigen expression was found in 40% (4/10) cases of T-ALL in the current study. Findings from other researchers also support this data[23,25]. This promiscuous expression was noted for CD13, CD33, CD117 and cyCD79a.

CD33 was expressed in 2/10 (20%) cases of T-ALL which is almost similar to the findings of Mazher *et al.*[13] and Vitale *et al.*[23] (28 and 25%, respectively).

Aberrant expression of CD13 was seen in 10% of T-ALL cases that is a bit lower than other studies[12,22]. It may be explained by the very small number of T-ALL cases in the current study.

cyCD79a was found to be expressed in 1/10 (10%) case of T-ALL and findings from Lai *et al.*[26] is consistent with this result with an expression rate of 13.8%.

Aberrancy of CD117 (C-KIT) in T-ALL is sometimes associated with immaturity, but its prognostic significance is still not established. In the current study 1/10 (10%) T-ALL case showed expression of CD117 which is nearly consistent with other studies (4 and 9%, respectively)[27,28].

Mixed Phenotype Acute Leukemia (MPAL) is a distinct form of acute leukemia where single leukemic blast express antigenic markers of more than one lineage (biphenotypic) or presence of blasts of more than one lineage at a time (bilineal)[6,29]. MPAL represented 2-5% of acute leukemia in adult[30]. The prognosis for MPAL is poor comparing to other acute leukemia, with an overall survival of 18 months[31,32].

In present study out of 2/64 (3.12%) cases showed the features of MPAL which is consistent with the previous studies. Both were bilineal by flow cytometric analysis. One case was B+My and another was B+T MPAL. In both cases B lineage was determined by strong expression of CD19 and dim or moderate expression of CD79a and CD10. In case of the B+My MPAL MPO confirmed the myeloid component. cyCD3 confirmed the T-lineage in case of B+T MPAL. Flow cytometric assessment is unique in these cases as MPAL diagnosis by morphology is very critical in most of the cases.

Presence of aberrant phenotype was noted in a significant number of adult acute leukemia patients in Bangladesh which pave the pathway for future research to correlate it with patients' prognosis.

CONCLUSION

Multiparameter flow cytometry has become an essential tool for characterizing acute leukemia in recent years. A poor prognosis with current drug regimens can be predicted from expression of aberrant markers.

SIGNIFICANCE STATEMENT

This study revealed that substantial numbers of adult patients with acute leukemia in Bangladesh present with aberrant phenotypes and this finding will help future researchers to find more correlation between aberrant markers and cytogenetic abnormalities, therapeutic response and overall prognosis of the patients.

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