Original Article

Clinical and hematological correlates of aberrant immunophenotypic profiles in adult and pediatric acute myeloid leukemia at presentation

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ABSTRACT

Background: Aberrant phenotypes in acute leukemia have been reported with varying frequencies in independent studies and their association with prognostic factors is still a matter of debate.

Aim: This study aims to identify the frequency of aberrant immunophenotypes in *de novo* acute myeloid leukemia (AML) and to evaluate their association with initial clinical and hematological features.

Materials and Methods: A total of 181 patients of *de novo* AML were included during the time (July 2010–June 2012). The immunophenotype of all cases of AML was studied by using flow cytometry.

Results: Aberrant lymphoid antigen expression was seen in 43.1% cases. Most frequent aberrant lymphoid antigen was CD7, seen in 26.5% cases. All French-American-British (FAB) subtypes except AML-M3 expressed aberrant lymphoid antigens. The expression was most common in AML-M4 in the current study. CD34 expression in AMLs was significantly associated with the expression of aberrant lymphoid antigens. Lymphoid antigen expression in adult AML was significantly associated with higher white blood cell (WBC) count (>50,000/mm^3) and higher number of peripheral blasts (>70%).

Conclusion: In summary, CD7 is the most common aberrant lymphoid antigen expressed in AML. FAB subtype AML-M3 is usually not associated with aberrant lymphoid antigen expression. AML cases with CD34 positivity are more likely to express aberrant lymphoid markers. The current study also supports that aberrant lymphoid antigen expression in adult AML is associated with adverse presenting hematological features (WBC count >50,000/mm^3, peripheral blasts >70%). Pediatric Ly + AML cases are not associated with adverse presenting clinical and biological features.

KEY WORDS: Aberrant, acute leukemia, acute myeloid leukemia, immunophenotyping

INTRODUCTION

Aberrant phenotypes in acute leukemia are characterized by variation in the patterns of antigen expression on neoplastic cells as compared to the process of normal hematopoietic maturation. In acute myeloid leukemia (AML), these aberrancies include cross-lineage expression (expression of lymphoid antigens in AML) or/and asynchronous antigen expression where early antigens are co-expressed with mature ones.[1,2] Immunophenotyping is an indispensable tool for proper lineage assignment and identification of any aberrant phenotypes. It has been reported that aberrant phenotypes in AML occur with varying frequency (30%–88%) and there is still a controversy about its prognostic implication.[3-7] This wide variation in incidence may be because of differences in flow cytometry (FCM) instruments, reagents, criteria for aberrancy, and variation in the binding patterns of monoclonal antibody clones. Although immunophenotyping is a valuable tool in acute leukemia diagnosis; many hospitals in India do not have the availability of flow cytometer. Consequently, those cases which do not reach referral tertiary care centers miss a proper lineage characterization and identification of any aberrant phenotypes. Identification of these aberrant

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phenotypes may be instrumental in making diagnosis, disease monitoring, and making specific treatment decisions.[8] This particular study was planned for a span of 2 years to identify various immunophenotypic aberrancies in a large series of AML cases which were uniformly characterized using FCM. Initial clinical and hematological features were studied in relation to the expression of aberrant markers.

MATERIALS AND METHODS

Procedure

A prospective study was conducted in the department of hematology at a tertiary hospital for 2 years. All newly diagnosed cases of AML during this period were included in the study. All the patients underwent bone marrow examination for light microscopic evaluation and immunophenotyping on FCM. Written informed consents were obtained from all the patients. The study was approved by the Ethical Committee of the Institution.

Bone marrow examination

Bone marrow examination was done from the posterior superior iliac spine using Jamshidi bone marrow aspiration needle (No. 16 for adults and No. 18 for pediatric patients). Approximately 1 ml of bone marrow aspirate was taken separately for making smears and in ethylenediaminetetraacetic acid (EDTA) vials for FCM. Bone marrow biopsy was taken in all cases with Jamshidi trephine biopsy needle (No. 11 for adults and No. 13 for pediatric cases). It was fixed in 10% buffered formalin. Smears were examined using May–Grunwald Giemsa and special stains using myeloperoxidase (MPO) and Periodic acid–Schiff. All cases were evaluated morphologically based on the FAB criteria.[9]

Immuno-phenotyping

Approximately 1 ml of bone marrow aspirate was collected in EDTA vials. Samples were analyzed within 24 h of collection. Immunophenotypic analysis was performed in all cases, and it was based on a pretitrated four-color combination cocktail comprising of fluorescein isothiocyanate (FITC), phycoerythrin (PE), and allophycocyanin (APC) conjugated monoclonal antibodies (Moab), i.e., CD19-FITC, CD10-PE, CD20-APC, CD22-PE, CD1a-PE, CD2-FITC, CD3-PE, CD4-PE, CD8-FITC, CD5-PE, CD7-FITC, CD13-PE, CD33-APC, CD117-APC, CD14-FITC, CD11b-PE, CD34-PE, human leukocyte antigen-DR-FITC on the surface of leukemic cells and intracytoplasmic Igμ chain-PE/FITC, cytoplasmic CD3-FITC/APC, CD79a-PE, MPO-FITC antigens and nuclear TdT-APC, along with CD45PerCP for gating of blasts. All antibodies were procured from BD Biosciences, California, USA. Briefly, 50 μL of blood sample was taken in each test tube and processed with 2 ml of red blood cell lysing solution. The sample was washed twice with phosphate buffered saline (PBS) and re-suspended in PBS and counts were adjusted to approximately 1×10^6 cells per tube. Pretitrated cocktails of Moab were added and incubated in dark for 20–30 min at room temperature. The cells were then again washed with PBS to remove any unbound Moab, and the cell button was resuspended in 500 μL of PBS. The nuclear and cytoplasmic antigens were processed using permeabilization with "BD FACS Permeabilizing solution 2" for 10 min. Flow cytometric analysis was performed on flow cytometer (BD FACS caliber/BD FACS Canto II) and analyzed with cell quest/FACS Diva, respectively. Results were obtained by gating the blast cells with side scatter analysis versus CD45PerCP gating. FCM data were analyzed based on dot plots. Negative controls were simultaneously run in every case. For surface and intracytoplasmic antigens, marker positivity was considered when more than 20% of blast cells were positive with the exception of anti-MPO, which was reported as positive at a cutoff of 10%. Besides isotype control for assessing nonspecific binding in MPO staining, lymphocyte population in each sample was assessed as the internal negative control for MPO.

RESULTS

Patient characteristics

A total of 181 newly diagnosed cases of AML were included in the study. The patient age range was wide (range, 1–81 years; median 30 years) with male/female ratio (M: F) of 1.42:1. Among all AML cases, 135 cases were adults (range, 17–81 years; median 40 years) with M: F ratio of 1.1:1. Forty-six cases were in the pediatric age group with M: F ratio of 1.23:1.

Expression of aberrant phenotype

Overall, 78 (43.1%) AML cases showed aberrant lymphoid antigens. Aberrancy for T-lineage associated markers (CD7/CD4/CD8) was most common, comprising 73% (57/78) of total aberrancies. Aberrancy for B-lineage associated markers (CD19/CD10) constituted 17.9% (14/78), followed by both B and T-cell aberrancy of 8.9% (7/78). Among all antigens, the most common aberrant lymphoid antigen was CD7 (26.5% of cases), followed by CD19 (11% of cases). The expression of CD7 was more common in childhood AML (28.2%) as compared to adult AML cases (25.9%) though the difference was not statistically significant. CD19 expression was more common in adult AML cases (12.6%) than childhood AML cases (6.5%). The detailed expression of aberrant B and T-lymphoid antigens is described in Table 1.

Table 1: Lymphoid antigen expression in 181 (46 pediatric and 135 adult) cases of acute myeloid leukemia

	Total, *n* (%)	Pediatric, *n* (%)	Adult, *n* (%)	*P*
Aberrant B-lymphoid antigens				
CD19	20 (11)	3 (6.5)	17 (12.6)	NS
CD10	5 (2.8)	2 (4.3)	3 (2.2)	NS
Aberrant T-lymphoid antigens				
CD7	48 (26.5)	13 (28.2)	35 (25.9)	NS
CD4	16 (8.8)	3 (6.5)	13 (9.6)	NS
CD8	1 (0.6)	0 (0)	1 (2.1)	NS

NS=Nonsignificant

Correlation of aberrant phenotype with French–American–British subtypes

All FAB subtypes except AML-M3 showed expression of aberrant phenotypes. Overall, the most common FAB subtype with aberrant lymphoid phenotype was AML-M4, present in 76% (16/21) cases. The aberrant phenotypic expression was next seen in AML-M0 and AML-M1 subtypes, constituting 62.5% each (10/16 and 5/8 cases, respectively). The association between aberrant lymphoid antigen expression and FAB subtype is shown in Tables 2 and 3. CD7 expression was seen in all FAB subtypes except AML-M3 and AML-M6. CD7 expression was prominently seen in AML-M0 and AML-M7 (70%, 7/10 cases). CD19 expression was most frequently seen in AML-M5 (50%, 8/16 cases) followed by AML-M2 (25%, 5/20 cases).

Table 2: Frequency of lymphoid marker expression in different French-American-British subtypes

Aberrant lymphoid antigens and FAB subtypes		
FAB subtype	Total cases	Lymphoid marker positivity (%)
M0	16	10 (62.5)
M1	8	5 (62.5)
M2	48	20 (41.6)
M3	13	0 (0)
M4	21	16 (76)
M5	46	16 (34.7)
M6	4	1 (25)
M7	25	10 (40)
Total	181	78

Table 3: Distribution of various lymphoid antigens among different FAB subtypes

Aberrant lymphoid phenotypes	M0	M1	M2	M3	M4	M5	M6	M7	Total
CD7	7	3	10	-	10	7	-	7	44
CD4	2	2	5	-	-	-	1	2	12
CD8	-	-	-	-	1	-	-	-	1
CD19	-	0	4	-	-	4	-	1	9
CD10	1	-	-	-	3	1	-	-	5
CD7 and CD19	-	-	-	-	1	2	-	-	3
CD4 and CD19	-	-	1	-	-	2	-	-	3
CD4, CD7, and CD19	-	-	-	-	1	-	-	-	1
Total	10	5	20	-	16	16	1	10	78

Relation of aberrant phenotype with adverse prognostic factors

Based on the positivity of aberrant lymphoid antigens (Ly+), AML cases were stratified into two groups: Ly + AML and Ly − AML. The expression of aberrant lymphoid antigens in both adult and pediatric AML cases was compared with known prognostic factors. The clinical and biological characteristics of Ly + AML and Ly − AML are summarized in Table 4. No difference was found between Ly + AML and Ly − AML group with regard to age, gender, median hemoglobin, median white blood cell (WBC) count, median platelet count, and clinical features at presentation such as lymphadenopathy, hepatomegaly, and splenomegaly. On analyzing the adult and pediatric groups separately, CD34 expression was significantly more in adult Ly + AML group than adult Ly − AML group ($P = 0.001$). Higher WBC count (>50,000/mm^3) was seen more in adult Ly + AML group than adult Ly − AML group ($P = 0.050$). Higher peripheral blasts% (>70%) was seen in adult Ly + AML than adult Ly − AML ($P = 0.039$). No difference was seen between pediatric Ly + AML cases and Ly − AML cases with respect to CD34 expression, WBC count (>50,000/mm^3), and peripheral blasts (>70%).

DISCUSSION

In the present study of 181 newly diagnosed cases of AML, aberrant expression of lymphoid antigens was seen in 43.1% of AML cases. The degree of lymphoid antigen expression in AML has varied between 30%–88% in various reports.[3-7] This wide variation in the incidence of aberrant phenotypes can be attributed to factors like differences in the criteria used for aberrancy, the cutoff value for confirming the positivity of a particular antigen, number of antigens studied, sample size and differences in the instruments, reagents, and monoclonal antibody clones used for FCM. In the current study, a cutoff

Table 4: Lymphoid antigen expression in adult and pediatric acute myeloid leukemia cases in relation to clinical and biological features

	Adult AML (*n*=135)			Pediatric AML (*n*=46)		
	Ly+ AML (v=61)	Ly− AML (*n*=95)	*P*	Ly+ AML (*n*=17)	Ly− AML (*n*=29)	*P*
Age*	36.5 (18-70)	40.0 (17-81)	NS	7.0 (1-15)	6.0 (1-14)	NS
Gender						
Female	21	43	NS	8	9	NS
male	19	52		9	20	
WBC count*	19.25 (1-386)	13.6 (1-404)	NS	31.1 (3-205)	7.9 (0-150)	NS
Hemoglobin level*	7.35 (3.3-11.3)	7.11 (3.1-12.2)	NS	7.5 (3.9-11.6)	7.3 (3.1-11.2)	NS
Platelet count*	27.5 (7-662)	27.0 (4-367)	NS	32.0 (7-288)	34.0 (2-178)	NS
Lymphadenopathy	7	24	NS	10	12	NS
Hepatomegaly	19	49	NS	14	21	NS
Splenomegaly	20	45	NS	11	16	NS
CD34	36	49	0.001	10	17	NS
WBC (>50,000/mm^3)	12	13	0.050	6	12	NS
Platelet (<30,000/mm^3)	22	50	NS	8	12	NS
Peripheral blasts (>70%)	17	24	0.039	8	10	NS

*Nonnormally distributed data: Median (range). AML=Acute myeloid leukemia, WBC=White blood cell count

value of ≥20% of blast cells positivity was used to characterize the presence of a particular antigen, and a uniform FCM method was used to characterize all acute leukemia cases. The highest frequency of 88% of aberrant phenotypes in AML is reported by Bahia *et al*.[6] This particular study also included the asynchronous expression of antigens as the criteria for aberrancy. However, excluding the asynchronous expression of antigens, aberrant lymphoid antigens were seen in 34% of AML cases.

CD7 was the most frequently expressed lymphoid antigen, seen in 26.5% (48/181) cases in our study. The aberrant expression of CD7 was more frequently seen in pediatric AMLs (28.2%) in comparison to adult-AMLs (25.9%). Second-most common aberrantly expressed lymphoid antigen was CD19 in 11% cases, followed by CD4 in 8.8% cases, CD10 in 2.8% cases, and CD8 in 0.6% cases. Majority of studies have identified CD7 as the most frequent lymphoid antigen, as seen in 20.5% cases by Zheng *et al*., 25.7% cases by Bahia *et al*., 37% by Legrand *et al*.[6,10,11] There are few studies where other lymphoid markers were found to be expressed more frequently than CD7. Bhushan *et al*. found CD19 as the most common lymphoid antigen (32%).[3] CD7 was seen in 15% of cases in this study. Reading *et al*. found CD4 as the most common lymphoid antigen (61%), followed by CD7 (24%).[2] Khalidi *et al*. found CD20 as the most common lymphoid antigen (17%).[5] It is believed that CD7 is expressed early in hematopoietic ontogeny and is usually co-expressed with early antigens.[12] Supporting this view, we found CD34 expression in 75% of CD7-positive AML cases. Few studies have reported an inferior outcome in AML cases showing CD7 expression.[12-15] Co-expression (CD34 + CD7 + AML) is reported to be associated with multiple drug resistant proteins and a worse prognosis.[8]

Aberrant lymphoid phenotypes in AML were seen in all FAB subtypes except AML-M3. The expression was most common in AML-M4 in 76% of cases, followed by AML-M0 and AML-M1 in 62.5% cases each and AML-M2 in 41.6% cases. Similar to our results, the aberrant lymphoid marker was not a common finding in AML-M3 in many other studies.[6,7,15-17] However, there is still no consensus on the most common FAB subtype associated with lymphoid antigen expression. Our study finds AML-M4 to be the most common FAB subtype. Bhushan *et al*. reported AML-M5 to be the most common FAB subtype associated with lymphoid antigen expression and AML-M2 was the most common FAB subtype with Bahia *et al*.[3,6] In the present study, CD7 was seen in all FAB subtypes except AML-M3 and AML-M6. Similarly, Bahia *et al*. found CD7 in all FAB subtypes except AML-M3 and AML-M6.[6] Thalhammer-Scherrer *et al*. did not find CD7 positive cases with AML-M3, AML-M6, and AML-M7 morphology.[18] Bahia *et al*. and Zheng *et al*. found thatCD19 expression was highest in AML-M2.[6,10] We could not find an association between CD19 expression and AML-M2. In our study, CD19 expression was most frequently noted in AML-M5.

A number of clinical and biological features are known to predict the prognosis in AML. Association between aberrant lymphoid antigens and prognostic factors is still debatable. Some studies have reported AML with aberrant phenotypes to be associated with poor prognosis,[1,19,20] while other studies reported favorable prognosis[21] and few other studies report no prognostic value.[10] In our study, we compared Ly + AML and Ly − AML patients with adverse prognostic factors. No significant association was seen between the aberrant phenotypes and adverse prognostic factors in children. In adults, the expression of CD34 was significantly more in Ly + AML patients than Ly − AML cases ($P = 0.001$). However, contrary to our observations, Kawai S *et al*. found CD34 to be more associated with pediatric Ly + AML cases.[22] Bhushan *et al*. did not find any association between CD34 expression and aberrant phenotypes.[3] The expression of CD34 on leukemic blasts might be attributed to a developmental arrest at an immature stage or an aberrant continuous spectrum of expression.[8] CD34 expression in AML is regarded as a poor prognostic factor.[23] Leukocytosis (>50,000/mm^3) is regarded as a negative prognostic factor in AML. In our study, Ly + AML adult patients were more associated with leukocytosis (WBC count >50,000/mm^3; $P = 0.050$) than Ly − AML patients. Peripheral blasts (>70%) were significantly more in Ly + AML patients ($P = 0.039$). The current study definitely highlights an association between aberrant lymphoid antigens expression in adult AML cases and adverse presenting hematological factors. Nevertheless, this study had its limitations, the biggest being that it could not take into account the follow-up data of the patients to document correlation between immune-phenotype of leukemic cells and treatment response since that might have more categorically established overall prognostic significance of expression of lymphoid markers in AML. In fact, the aim of the current study was mainly to generate data from Indian population regarding the expression of aberrant markers in AML cases.

CONCLUSION

In summary, we conclude that in our series, 43.1% of AML cases showed aberrant lymphoid antigens. CD7 was the most common aberrant lymphoid antigen expressed in AML. FAB subtype AML-M3 is not associated with the expression of aberrant lymphoid antigens. Childhood Ly + AML cases are not associated with adverse presenting clinical and hematological factors. Adult AML cases with higher WBC count (>50,000/mm^3), higher blast count% (>70%), and expression of CD34 antigen are more likely to express aberrant lymphoid antigens. The identification of these aberrant phenotypes may help in making a proper diagnosis, monitoring minimal residual disease, and making alternate treatment decisions. Longer period studies evaluating the treatment responses in these patients are needed to definitely comment on the prognostic significance of expression of aberrant markers in AML cases.

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Conflicts of interest
There are no conflicts of interest.

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