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Original Article

Lymphoid associated antigen expression in new cases of Acute Myeloid Leukemia

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ABSTRACT

Background: Occurrence of aberrant phenotype has been reported in acute leukemias with varying frequency though its prognostic importance remains controversial. In acute myeloid leukemias, aberrant phenotype, as high as 88 %, has been reported. To evaluate the occurrence of aberrant lymphoid phenotypes and to correlate their presence with various French American British classification, 100 cases of fresh acute myeloid leukemias were analyzed for lymphoid markers CD 4,7,8,10 and 19.

Materials and Methods: Whole blood or bone marrow aspirate collected in EDTA were processed by standard method and subjected to immunophenotyping for B Cells marker CD 19 and 10 and T cell marker CD 4, 7 and 8.

Results: Aberrant lymphoid markers were seen in 35(35%) cases. All FAB subtypes except M7 showed aberrancy for the markers studied. However it was the most common in M0 (100%), followed by M2 (51.9%). T cell aberrancy was the most common, comprising 62.8% (22/35) of total aberrancy. CD 7 was the most common aberrantly expressed marker, seen in 20% AML, followed by CD 4(14%) and CD 19 (8%).

Conclusion: Occurrence of lymphoid phenotypes is frequent in pediatric as well adult AML. Though T cell markers are more common, only B cell as well as both B and T cell markers may be co expressed.

INTRODUCTION

Occurrence of aberrant phenotype has been reported in acute leukemias with varying frequency though its prognostic importance remains controversial.[1] The aberrant phenotypes are classified into different types: co-expression of lymphoid-associated antigens or 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.[2,3] In acute myeloid leukemias (AML), aberrant phenotype as high as 88 % has been reported.[2]

To evaluate the occurrence of aberrant lymphoid phenotypes and to correlate their presence with various French American British classification (FAB subtypes), 100 cases of newly diagnosed AML were analyzed for lymphoid markers CD 4, 7,8,10 and 19. Mixed lineage acute leukemias, chronic

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Table 1: Aberrant lymphoid marker expression in different AMLs

FAB TYPE	TOTAL NUMBER	LYMPHOID MARKER POSITIVITY (%)
M0	9	9(100)
M1	15	4(26.7)
M2	27	14(51.9)
M3	9	2(22.2)
M4	20	3(15)
M5	14	2(14.3)
M6	5	1(20)
M7	1	0(0)
TOTAL	100	35

myeloid leukemias (CML) in myeloid blast crisis, AML on therapy or relapsed cases were excluded. Those AMLs in which above markers were not applied were also excluded. Diagnosis of acute leukemia was made on routinely stained bone marrow aspiration, trephine biopsies and blood smears. Immunophenotyping was carried out on bone marrow or peripheral blood smears.

MATERIALS AND METHODS

Whole blood or bone marrow aspirate collected in EDTA were subjected to RBC lysis in 5 volumes of ammonium chloride lysis solution for 10 minutes. The samples were then washed and resuspended with phosphate buffered saline. The cells were stained with four colour antibody cocktail. The flourochromes used were FITC, PE, APC and PerCP. AMLs were studied for B Cells marker CD 19 and 10 and T cell marker CD 4, 7 and 8. Data acquisition and analysis were performed on a FACScanto flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) using FacsDiva software. Identification of blast cells was performed using forward scatter (FSC) versus side scatter (SSC) parameters and/or CD45 intensity versus SSC dot plots. The percentage of gated myeloid cells expressing a particular CD marker was used to determine whether expression was positive or negative. Expression of a CD marker by less than 20% of the gated population was considered negative.

RESULTS

Total 100 AML from 81 adults and 19 pediatric patients were subjected to immunophenotyping with above markers during the study period out of which aberrant lymphoid markers were seen in 35(35%) cases.

These included 28 adults and 7 children. Age ranged from 7 to 75 years. 25 cases expressed one, 8 cases expressed two, 1 case expressed three and 1 expressed all four markers studied. The FAB classification of these 100 AMLs and frequency of lymphoid marker expression are shown in table 1. All FAB subtypes except M7 showed aberrancy for the markers studied. However it was most common in M0 (100%), followed by M2 (51.9%).

Table 2: Distribution of aberrant T cell and B cell markers in AML

T Ly +	M0	M1	M2	M3	M4	M5	M6	M7	TOTAL
7	2	2	2	-	3	2	-	-	11
4	2	1	3	2	-	-	-	-	8
4,7	-	-	3	-	-	-	-	-	3
B Ly+									
19	2	-	2	-	-	-	-	-	4
10	1	-	1	-	-	-	-	-	2
Both T & B Ly+									
10,7	1	1	1	-	-	-	-	-	3
19,4	1	-	-	-	-	-	-	-	1
19,7	-	-	-	-	-	-	1	-	1
4,7,19	-	-	1	-	-	-	-	-	1
4,7,8,19	-	-	1	-	-	-	-	-	1
	9	4	14	2	3	2	1	0	35

Table 3: Individual lymphoid markers expression in different AMLs

FAB subtypes (Total AML=100)	Lymphoid markers expressed				
	CD 4	CD 7	CD 8	CD 10	CD 19
M0	3	3	-	2	3
M1	1	3	-	1	-
M2	8	8	1	2	4
M3	2	-	-	-	-
M4	-	3	-	-	-
M5	-	2	-	-	-
M6	-	1	-	-	1
M7	-	-	-	-	-
TOTAL	14	20	1	5	8

Expression of various lymphoid markers, on different FAB subtypes, is shown in table 2 and table 3. T cell aberrancy was most common, comprising 62.8% (22/35) of total aberrancy. B cell aberrancy was 17.1% (6/35) of total aberrancy where as both T and B cell aberrancy was seen in 20% (7/35). CD 7 was the most common aberrantly expressed marker, being seen in 20% AML. These were followed by CD 4(14%) and CD 19 (8%).

Apart from M7, CD 7 was not seen in M3 and CD 4 was not seen in M6. CD 4 positivity in M4 and M5 were not considered aberrant. The FAB subtype which showed more than two aberrant markers was M2. AML M3 only showed aberrant CD 4 expression.

DISCUSSION

Expression of lymphoid antigens in AML have been found to be variable depending upon the markers studied, sample size and criteria of aberrancy used. Bahia et al, who included asynchronous expression of antigens also as aberrancy in AML, reported frequency as high as 88%.[2] But in their study also lymphoid antigen expression was seen in 34.2% AML which is close to our data of 35 %. Bhusan B et al. found lymphoid-associated antigens in almost half of the samples with AML (49%).[1]

In study of Reading CL et al. coexpression of T lymphoid and B lymphoid with myeloid occurred in 38% and 13% of AML samples respectively.[4] We found co expression of T lymphoid and B lymphoid with myeloid in 29% and 13% of all AML samples respectively.

CD 7 is the most common aberrant marker found in AML in most studies. In a study by Zheng J et al. CD7 was the most common lymphoid marker (20.5%) in AML patients, followed by CD2 (12.5%) and CD19 (10.0%). Other lymphoid markers such as CD5, CD8, CD10 and CD20 were detected in lower than 5% of all cases.[5] In study of Bahia DM et al. also the most frequent lymphoid antigen was CD7 (25.7%) followed by CD2 (11.4%) and CD19 (8.6%).[2] CD7 was positive in 37%, CD19 in 16%, and CD10 in 10% and CD8 in 0% in study of Legrend O et al.[6] CD7 expression was seen in 32.6% AML in another study.[7] However some studies also find other lymphoid markers to be more common than CD7. Reading CL et al. found CD 4 to be most common(61%) followed by CD7(24%) and CD 19 (11%).[4] Bhusan B et al. found expression of CD19 to be more common than CD7.[1] In one study CD20 was the most commonly expressed lymphoid antigen (17%).[8] Like most other studies we also found CD 7 to be expressed most commonly and CD 8 and 10 positivity in less than 5% AMLs. In this study CD 2 was not applied in all AMLs so it was not evaluated.

The clinical relevance of lymphoid antigen expression in AML has been highly controversial. Some studies have reported Ly+AML to be associated with the poor prognosis .[9-11] But some reported it to be associated with favorable prognosis[12] whereas other suggest it to be of no prognostic value.[5]

Initial reports in pediatric AML suggested that cases expressing CD2 and CD7 antigens were biologically different to other forms of AML, and had a poor response to standard induction chemotherapy protocols.[13,14] Kita K et al. found that CD7 positive AML patients were younger males who had a higher incidence of hepatomegaly and CNS involvement than CD7 negative AML patients. They responded poorly to standard chemotherapy for AML and had an unfavorable outcome.[15] In contrast, Ball and colleagues described a high remission rate and improved remission duration and survival in adult AML patients expressing CD2 and/or CD19 antigens, suggesting an improved outcome for these patient.[11] No significant correlations of CD10 with prognosis have been reported in some studies.[16,17]

Lymphoid expression in AML shows some correlation with FAB subtypes of AML .In study of El-Sissy EH et al CD7 was mostly confined to FAB M1 and M2.[18] Bahia DM et al. found CD7 in all FAB subtypes except M3 and M6.[2] Kita K et al. also found CD7 in all FAB subtypes except M3 though it was more common in M1 and M2.[15] In a study from Taiwan aberrant CD7 expression was observed in all AML-non M3 subtypes, most frequently in AML-M7 (4/6, 67%) In their study CD19 expression was only observed in AML-M2 (5/36, 14%).[19] Zheng J et al. and Bahia DM et al also found that CD19 was expressed at highest rate in AML M2.[2,5]

In our study CD7 was seen in all FAB types except M3 and M7. However highest frequency was seen in M0. In our study CD 19 was not limited to M2 but was seen in M0 and M6 as well and in fact showed highest expression in M0 (15.6%).

Khalidi HS et al. found increased frequency of CD2 expression in AML-M3, increased frequency of CD20 in AML-M5 and increased frequency of CD5 expression in AML-M5a.[8] One study found CD2 almost exclusively co-expressed in FAB subtypes M3 and M4Eo.[20] These markers were not available for evaluation in all cases in this study and thus were excluded however among the 15 cases in which CD2 was applied only 3 (2FAB M2 and 1 FAB M3) showed positivity in this study (data not shown).

Aberrant lymphoid marker is not a common finding in AML M3. In study of Chen et al, in AML non-M3 aberrant antigen expression was identified in 56/96 (58%) cases, in contrast to 2/15 (13%) AML-M3 cases (P = 0.001).[19] All the cases of M3 subtypes of children and adults were negative for lymphoid markers in another study. They also reported higher frequency of Ly+AML phenotype in children compared to adults (59% and 45 %respectively) and also higher CD19 expression in pediatric AML than adults (52%vs 32%) though these differences were not statistically significant.[1] We also found lymphoid antigen less frequently in M3 (n=2, 22.2 %) as compared to non M3 AMLs (n=33, 36.2%) Among 100 AML 19 were pediatric cases in this study. Co-expression of lymphoid phenotype were comparable in adults and pediatric AML (n=28, 34.6%) adults Vs n=7, 36.8%) pediatric) and so was expression of CD 19 (7.4% adult Vs 10.5% pediatric).

CONCLUSION

Occurrence of lymphoid phenotypes is frequent in pediatric as well adult AML. Though T cell markers are

more common, only B cell as well as both B and T cell markers may be co expressed. Relevance of these markers in prognosis and treatment needs to be studied.

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