FREQUENCY OF ABERRANT EXPRESSION OF CD MARKERS IN CASES OF ACUTE LEUKEMIA

MUHAMMAD M. KHURRAM*
SAGHIR A. JAFRI*
ABDUL MANNAN**
AFTAB NADEEM***
ASIF JAMAL*

SUMMARY: In the present study 100 patients of acute leukemia were studied to find out the frequency of aberrant antigens in AML, B-ALL and T-ALL of which 73% cases were of lymphoid lineage and 27% cases were of myeloid lineage. 74% cases showed expression of lineage specific markers and were considered as conventional immunophenotypes while 26% cases showed expression of CD antigens which were not of that lineage upon which they were expressing. Some myeloid lineage associated antigens were present on acute lymphoblastic leukemia cases and lymphoid associated antigens showed their expression on acute myeloid leukemia cases. These cases were considered as aberrant immunophenotypes. The cases of acute lymphoblastic leukemia were further subcategorized as B-cell and T-cell acute lymphoblastic leukemia. The data from this study suggested that either the commonly described myeloid, B-cell and T-cell differentiation pathways are incorrect or blasts from cases of acute leukemia do not represent their normal counterparts. To explain these mixed immunophenotypes it is suggested that leukemic cells may have aberrant markers because of their abnormal genetic programme resulting in lineage infidelity. In this scenario the precursor cells may retain features of one lineage that they should have lost during commitment to another cell line. As a result of the leukemic process cells with aberrant immunophenotypes are immortalized in a precommitment phase of differentiation resulting in lineage promiscuity. This study strengthens the theories of lineage infidelity and lineage promiscuity by taking a critical and comparative approach of frequencies of aberrant antigens in acute leukemia in population of Pakistan.

Key words: Aberrant, CD antigen, Leukemia, AML, B-ALL, T-ALL.

INTRODUCTION

Acute leukemia is a cloned expansion of tumor cells in bone marrow, blood or other tissues. The acute leukemia is classified as acute myeloid leukemia (AML) or acute lymphoid leukemia (ALL) based on the lineage of the blast cells under the control of cytokines i-e colony stimulating factors. Precursor cells from different lineages or blast cells in acute leukemia express different subsets of surface molecules many of which are now defined as cluster of differentiation (CD) antigens. The expression of CD antigens on leukocytes is determined by flow cytometry (4).

It has been demonstrated that several immunophe-

* From Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.
** From Sultan Qaboos University Hospital, Department of Pathology, Musqat, Sultante Oman.
*** From Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore, Pakistan.

Table 1: Proportional (%) frequency of aberrant cases and conventional cases.

Title	No. of cases	Aberrant cases	%	Conventional cases	%
AML	27	15	55.55	12	44.44
B-ALL	55	6	10.90	49	89.09
T-ALL	18	5	27.77	13	72.22
Grand total	100	26	26	74	74

notypes of blast cells from cases of acute leukemia do not exhibit the features of normal cellular differentiation but show the expression of aberrant markers (1,9). For example blast cells from some cases of B-lineage acute lymphoblastic leukemia may lack certain B-cell antigen or possess T-cell and /or myeloid antigens. Likewise blasts from cases of T-cell acute lymphoid leukemia may lack certain T-cell antigens or possess B-cell and/or myeloid determinant. This phenomenon is called as ''aberrant expression" of CD markers (3).

The majority of studies on determination of frequency of aberrant CD markers in acute leukemia by flow cytometry are from the western countries with only very few reports from Taiwan (7). The frequency of aberrant lymphoid antigens was expressed in 24% of acute myeloid leukemia in northern Taiwan (2) a similar frequency to those from the West, the incidence of aberrant lymphoid antigen expression in acute myeloid leukemia was extremely variable ranging from 13% to 60% (10).

Determination of frequency of aberrant CD antigen expression in acute leukemia has been studied in the west with very few reports from Taiwan. In Pakistan such a study has not been traced. This study is a unique study in Pakistan which can be helpful for the correct and prompt diagnosis of acute leukemia, identification of acute undifferentiated leukemia and minimal residual disease. The major objective of the present study was to determine the frequency of aberrant expression of CD markers in case of acute leukemia.

MATERIALS AND METHODS

Sample collection

A total of 100 patients (Aged 1 to 50 years) were studied. 3ml peripheral blood/3ml bone marrow aspirate samples were taken in vials containing ethylenediamine tetra-acetate (EDTA).

Sample processing

Complete blood count was done by using Sysmex XE-2100 and peripheral blood film stained by Giemsa stain to find the presence of blast cells. (Lewis et al 2001). After the evaluation of blast cells the whole blood or aspirate samples were prepared by cell lysing and fixing method for immunoflourescence staining with different antibodies named as CD markers which were conjugated with fluorochromes (i.e., FITC, PE and PerCP) and cell washing was done with phosphate buffer saline (PBS) ($NaH_2PO_4{\cdot}_2H_2O$, Na_2HPO_4 and NaCl). When whole blood is added to the monoclonal antibody reagent, the fluorochrome labeled 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. Sodium azide (NaN_3) was in immunofluorescent assays using biotinylated primary labeling reagents. Cell fix was used for the fixation of peripheral blood cell suspension after immunofluorescence staining with monoclonal antibodies and prior to flow cytometric analysis is a buffered solution contains less than 10% formaldehyde and 1% sodium azide. The prepared samples were acquired in flow cytometry instrument using Cell Quest software (8).

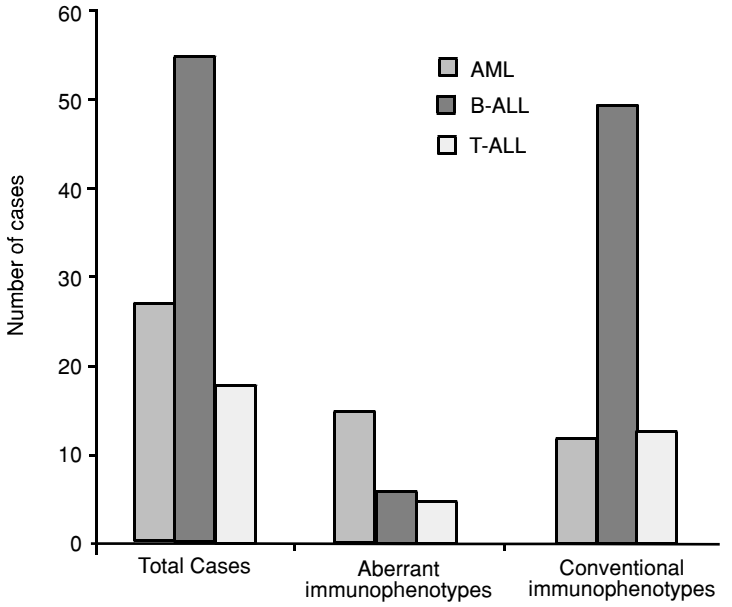
Data analysis

Data was analyzed using Cell Quest software. The fluorescence intensities of the blast population were compared with those of the internally negative cell population for different CD marker expressions. If the blasts showed fluorescence between 1 and 2 of side scatter histogram (SSC) they were interpreted as positive expression of CD markers applied (8).

RESULTS

During this study 100 consecutive diagnosed patients of acute leukemia were studied and frequency

Figure 1: Histogram showing the distribution of total, aberrant and conventional immunophenotypes.



of aberrant expression of CD antigens was noted. Out of 27 cases of AML 15 cases, out of 55 cases of B-ALL 6 cases and out of 18 cases of T-ALL 5 cases showed aberrant expression of CD antigens so adding 15, 6 and 5 respectively total proportion of aberrant cases among total study population becomes 26(26%). The proportional frequency of aberrant cases in AML cases is 55.55%, in B-ALL cases is 10.90% and in T-ALL it is 27.77% so orderly more aberrancy seen in AML then in T-ALL and then in B-ALL cases shown in (Table 1) and (Figure 1) also shows the distribution of study population as total cases, aberrant cases and conventional cases.

Three single antigens (CD7, CD19 and CD4) and three antigens (CD7 + CD19) and (CD2+CD7) in pairs were aberrantly expressed in 15 cases of AML. The complete demographic expression of aberrant antigens in AML cases is shown in (Table 2). CD7 showed aberrancy in 10(37.03%) out of 27 cases of AML which were mostly males and age range was 11 to 20 years of age group. CD19 showed aberrancy in 2(7.40%) out of 27 cases of AML which were mostly males and age range was 1 to 20 years of age groups. CD4 showed aberrancy in 1(3.70%) out of 27 cases of AML which was male and age range was1 to 10 years of age group. Two antigens CD7 and CD19 showed aberrancy in 1(3.70%) out of 27 cases of AML which was male and age range was 1 to 10 years of age group. Two antigens CD7 and CD2 showed aberrancy in 1(3.70%) out of 27 cases of AML which was male and age range was 11 to 20 years of age group.

In 5(9.09%) cases of B-ALL single aberrant antigen expression seen and in 1(1.81%) case of B-ALL paired aberrant antigen expression was seen. CD13 showed aberrancy in 5(9.09%) cases out of 55 cases of B-ALL. The aberrant cases were mostly males and 4 patients were in age range from 1 to 20 years, while one case was in age range of 41-50 years of age group. Two antigens (CD11b and CD7) showed aberrancy in 1(1.81%) case out of 55 cases of B-ALL. The aberrant case was male and age range was 11 to 20 years of age group.

In T-ALL 4 antigens (CD13, CD117, CD11c and CD14) showed single aberrant expression while 2 antigens (CD13 and CD117) showed paired aberrant expression. CD13 showed aberrancy in 1(5.55%) case out of 18 cases of T-ALL which was male and age range was 21 to 30 years of age group. CD117 showed aber-

Table 2 : Frequency of positive CD marker expression.

CD Markers	AML Cases (27/100)		B-Cell All Cases (55/100)		T-Cell All Cases (18/100)	
	Number	Percent (%)	Number	Percent (%)	Number	Percent (%)
CD 45	26	(96.29)	43	(78.18)	18	(100)
CD 34	20	(74.07)	29	(52.72)	8	(44.44)
CD 13	12	(44.44)	5	(9.09)	2	(11.11)
CD 33	13	(48.14)	0	(0)	0	(0)
MPO	26	(96.29)	0	(0)	0	(0)
HLADR	23	(85.18)	54	(98.18)	3	(16.66)
CD 117	23	(85.18)	0	(0)	2	(11.11)
CD 11b	5	(18.51)	1	(1.81)	0	(0)
CD 11c	14	(51.85)	0	(0)	1	(5.55)
CD 10	0	(0)	55	(100)	0	(0)
CD 19	3	(11.11)	54	(98.18)	0	(0)
CD 20	0	(0)	24	(43.63)	0	(0)
Tdt	1	(3.70)	50	(90.90)	8	(44.44)
CD 79a	0	(0)	54	(98.18)	0	(0)
CD 2	1	(3.70)	0	(0)	12	(66.66)
CD 3	0	(0)	0	(0)	8	(44.44)
CD 5	0	(0)	0	(0)	11	(61.11)
CD 7	12	(44.44)	1	(1.81)	18	(100)
Cy CD 3	0	(0)	0	(0)	12	(66.66)
CD 4	1	(3.70)	NA		NA	(0)
CD 14	Exceptional		NA		1	(5.55)

rancy in 1(5.55%) case out of 18 cases of T-ALL which was male and age range was 11 to 20 years of age group. CD11c showed aberrancy in 1(5.55%) case out of 18 cases of T-ALL which was male and age range was 1 to 10 years of age group. CD14 showed aberrancy in 1(5.55%) case out of 18 cases of T-ALL which was male and age range was 21 to 30 years of age group. Two antigens (CD13 and CD117) showed paired aberrancy in 1(5.55%) out of 18 cases of T-ALL which was male and age range was 21 to 30 years of age group.

DISCUSSION

Immunologic marker studies of the acute leukemia through flow cytometry have greatly improved the precision of diagnosis of acute leukemia by providing specific information regarding the lineage and stage of maturation of the malignant cells. In this study the immunophenotypes of blasts from 100 patients of acute leukemia to define the frequency of aberrant immunophenotypes were noted from different parts of Pakistan.

More than 70% of these cases exhibited conventional B-cell, T-cell and myeloid immunophenotypes. The proportional frequency of aberrancy in AML cases is 55.55%, in B-ALL cases is 10.90% and in T-ALL it is 27.77% as shown in (Table 1). Orderly more aberrant expression of CD antigens was seen in AML, then in T-ALL and then in B-ALL cases. Aberrant myeloid antigen (MyAg) expression was presented in 10-40% of patients of acute lymphoblastic leukemia (ALL) in other studies collectively while in this study it was presented as 11 % (Figure 1).

Frohling *et al.* (6) and Camos and Calomer (4) proposed that only 1 of 21 cases of T-ALL showed CD33 expression and none expressed CD13 while in present study in B-ALL CD13 expression was seen in 5% cases and only 1 of 18 cases of T-ALL showed CD13 expression and none expressed CD33. Two antigens (CD11b and CD7) showed paired aberrancy in 1 (1.81%) case out of 55 cases of B-ALL and this paired aberrancy was not seen in other studies. Interestingly in other studies 4 (25%) of 21 cases of T-ALL also showed expression of CD10 but none expressed CD19 and CD 20.

Weir and Borowitz (2001) and Auewarakul et al. (2) stated that the B-cell associated antigen CD19 was seen in 7(4%) of 175 cases of AML. The T-cell associated antigen CD7, CD2 and CD4 were seen in 66(27%) out of 246, 30(19%) out of 154 cases and in 22(14%) out of 154 cases of AML respectively. While in this study CD7 and CD19 showed aberrancy in 10(37.03%)cases 2(7.40%)cases out of 27 cases of AML respectively and CD7 and CD19 showed paired aberrancy in 1(3.70%) out of 27 cases of AML.

The authors (1,5,9) investigated that thirty to fifty percent (30-50%) of patients with B-ALL showed co-expression of a single myeloid-associated antigens, such as CD13 and CD33, whereas 2 or more myeloid antigens were expressed in approximately 8% of cases. Cytoplasmic MPO has also been described in B-ALL but present study has no complains of aberrancy of MPO in B-ALL. Typically such cases involved only one or occasionally 2 or more brightly expressed B or lymphoid lineage antigens (e.g., CD10, CD19, CD22 or c CD79a). Problems with lineage assignment of a T-ALL can arise because the T-cell associated antigens CD7,CD2,CD4 and CD5 can all also be aberrantly expressed by AML blasts. In addition expression of myeloid antigens such as CD13 and CD33 are quite frequently observed in T-ALL, about 10% to 20% of patients express a single myeloid antigen with a small percentage (6%) expressing 2 or more myeloid antigens. As patients with ALL frequently express myeloid associated antigens, patients with AML also quite frequently express lymphoid associated antigens. The T-cell related antigens CD7, CD5 and CD2 (in decreasing order of frequency of expression) are expressed in about 20% to 40% of cases, whereas the B-lineage antigen CD19 and CD20 occur in about 10% to 25% of AML cases. While in this study CD7 showed aberrancy in 10(37.03%) out of 27 cases of AML and CD7 and CD2 showed paired aberrancy in 1(3.70%) out of 27 cases of AML (Table 2) and CD19 showed aberrancy in 2(7.40%) out of 27 cases of AML but no aberrant claim is given to CD5 and CD20 in present study. In this study we also described some other important aberrant combinations of CD antigens like CD11b and CD7 showed paired aberrancy in 1(1.81%) case out of 55 cases of B-ALL CD13 and CD117 showed paired aberrancy in 1(5.55%) out of 18 cases of T-ALL, CD117 and CD14 showed aberrancy in 1(5.55%) case and 1(5.55%) case out of 18 cases of T-ALL respectively has seen which were not described by other studies.

In this study it is agreed that genotype is reflected in the immunophenotype of leukemic blasts, there is no absolute concordance between the immunophenotype and genotype categories, but such type of frequencies of aberrant expression of CD antigens in acute leukemia including this study in population of Pakistan emerged many questions of molecular and medical nature which are still not known. So studies should be done on pre-screened subsets of patients with focus on genotypic correlation with a specific pattern of antigen expression.

REFERENCES

1. Armstrong SA, Look AT: Molecular genetics of acute lymphoblastic leukemia. J Clin Oncol, 23(26): 6306-15, 2005.

2. Auewarakul CU, Promsuwicha O, Yaowaluk UP, Kovit P, Surapol I: Immunophenotypic profile of adult acute myeloid leukemia (AML): analysis of 267 cases in Thailand. Asian Pacific journal of allergy and immunology / launched by the Allergy and Immunology Society of Thailand, 21(3):153-60, 2003.

3. Borowitz MJ, Falletta JM: Leukemias and lymphomas of thymic differentiation. Clin Lab Med, 8:119-134, 1988.

4. Camos M, Colomer D: Molecular biology in acute leukemia. Clin Transl Oncol, 8(8); 550-559, 2006.

5. Digiuseppe JA: Acute lymphoblastic leukemia: diagnosis and detection of minimal residual disease following therapy. Clin Lab Med, 27(3): 533-549, 2007.

6. Frohling S, Scholl G, Gillil DG, Levine RL: Genetics of myeloid malignancies; pathogenetic and clinical implications. J Clin Oncol, 23(26): 6285-95, 2005.

7. Hsiao CH, Tang JL, Yao M, Tsay W, Wang CH, Chen YC, Shen MC, Tien HF: High incidence of CD 56 expression and relapse rate in acute myeloid leukemia patients with t (8; 21) in Taiwan. J Formos Med Assoc, 101(6): 393-398, 2002.

8. Lewis SM, Bain BJ, Bates I: In: Reference ranges and normal values. Dacie and Lewis practical haematology. 9th ed. London: Churchill Livingstone, pp 9-18, 2001.

9. Valk PJ, Verhaak RG, Beijen MA: Prognostic ally useful gene expression profiles in acute myeloid leukemias. Engl J Med, 350(16):1617-1628, 2004.

10. Weir EG, Borowitz MJ: Flow cytometry in the diagnosis of acute leukemia. Semin Hematol, 38(2):124-138, 2001.

Correspondence:
Muhammad Moin Khurram
Institute of Molecular Biology
and Biotechnology,
The University of Lahore,
Lahore, PAKISTAN.
e-mail: moinmalik56@hotmail.com