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Role of Immunophenotyping in Myeloperoxidase Negative Acute Leukemias

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ABSTRACT

INTRODUCTION

Inspite of the advances in cytochemistry , accurate diagnosis before the onset of specific therapy has been a difficult domain in the field of leukemias. The present study is designed to establish the role of immunophenotyping by flowcytometry in diagnosis of acute leukemias.

MATERIAL & METHODS-

-50 cases of acute leukemias were studied from Dec2009 to Aug2011 in SCB Medical College .All cases of APML and clearly myeloid cases with prominent auer rods and strong MPO positivity were excluded from the study. Morphology, cytochemistry and immunophenotypic profiles(using multiparameter BD FACS Caliber)of the cases were evaluated and compared.

OBSERVATION

Out of 50 cases, 24(48%)cases were of pre-B ALL, 15(30%) cases of pre-T-ALL and 11(22%)cases were of AML. In addition ,immunophenotyping corrected the lineage from ALL to AML in 3(6%) cases. Out of 24 cases of pre-B ALL,11(45%) showed aberrant myeloid antigen expression and 2 cases were CD10 negative.8 cases of pre -T-ALL showed aberrant myeloid antigen expression.

CONCLUSION

The study highlights the pivotal role played by immunophenotyping of acute leukemias in specific lineage determination before the onset of therapy.

INTRODUCTION

Acute leukemias ,a heterogenous group of malignancies with varying morphological, immunological and molecular characteristics. While the diagnosis and classification of these malignancies were originally based on morphologic features supplemented by cytochemical studies, the diagnosis now requires a complex battery of specialised tools that include immunophenotyping and genetics.[1]

Acute leukemias are divided into myeloid (AML) and lymphoid leukemias (ALL) which substantially differs in response to therapy and prognosis, hence accurate differentiation between the two is mandatory for therapeutic decisions. 2 Subclassification of each group is also of increasing importance, as therapy continues to evolve for specific genetic subgroups of leukemias . Improvements in instrumentation and availability of wide range of antibodies and fluorochromes has improvised phenotyping of cells, leading to easier identification of abnormal population.[3] Along with lineage determination ,it also provides information about potential targets for drug therapy nd guides in selecting molecular tools like FISH and PCR.[4]

The present study was designed to undertake immunophenotyping of cases of acute leukemias attending the department of Pathology and Clinical hematology of S.C.B. Medical College and Hospital, Cuttack. The study is aimed at highlighting the pivotal role of immunophenotyping by flowcytometry in establishing the diagnosis of acute leukemias and to evaluate the expression of commonly used immunomarkers ,their patterns and their relationship to initial clinical and biological features in acute leukemia.

MATERIAL AND METHODS

50 cases were evaluated which included detailed history taking, clinical examination and routine laboratory investigations of the cases. Peripheral smear and bone marrow examination was done to establish the diagnosis. The samples were then subjected for immunophenotypic analysis for lineage assignment and subtyping.

All MPO negative acute leukemias were immunophenotyped whereas cases which were proved to be clearly myeloid based on morphology and cytochemistry were excluded. However, cases of acute myeloid leukemias not showing presence of auer rods and with ambiguous MPO positivity were subjected to immunophenotyping.

IMMUNOPHENOTYPING-

Bone marrow and peripheral blood samples were immunophenotyped using multiparameter flowcytometry. Primary panel of antibodies comprising of CD45, CD34, HLADR, CD10, CD19, CD7, CD5, CD13, CD33 and CD117 ,cCD79a ,cCD3 and cMPO were used. 5 Listmode data were acquired on a FACS Calibur flowcytometer(BD Biosciences). Data were analysed using cell quest pro multiparameter analysis software. Acquired data were gated on CD45 dim vs side scatter to isolate the blast population. An antigen was considered positively expressed when at least 20% of the gated cases expressed the antigen.

RESULTS

Out of 50 cases studied, 41cases were assigned ALL and 9 cases were assigned AML on the basis of morphology and cytochemistry .Out of 09 cases of AML diagnosed on basis of cytomorphology , 6 were subclassified as AML-M1 ,2 cases as AML-M5 and 1 case as AML-MO. They were selected for immunophenotyping to confirm the myeloid lineage as none of these cases showed presence of auer rods and definite MPO positivity. Cases of ALL were subclassified into pre-B and pre-T ALL using the primary antibody panel. The cases of AML were included only to confirm

the myeloid lineage but no subclassification was done due to the limited panel of antibodies. Immunophenotyping revealed lymphoid lineage in 38 cases but demonstrated myeloid lineage in 3 out of total 41 cases of ALL diagnosed morphologically. Similarily, immunophenotyping confirmed lineage in 8 out of 9 cases of AML included in the study. So,overall lineage correction was done in 4 out of 50 cases, comprising of 8% of total cases studied.

The 3 cases with lymphoid morphology / cytochemistry but myeloid phenotype 1 case with myeloid morphology but lymphoid phenotype are summarised in table- (1,2)

Characteristics of cases showing lineage correction (ALL to AML) after flowcytometric analysis.(TABLE1)

morphology	cytochemical staining	Antigens expressed	Antigen not expressed
ALL-L2	MPO NEGATIVE, PAS INCONCLUSIVE	CD34 ,HLADR, CD13,CD33 CD117 ,cMPO	CD10, CD19, CD5, CD7
ALL-L2	MPO < 3% PAS NEGATIVE	CD34, HLADR, CD13, CD 117 dim CD7 ,cMPO	CD10, CD19, CD5
ALL-L1	MPO NEGATIVE PAS SPARSE POSITIVITY	CD 13,CD33, CD34,HLADR, CD7 ,cMPO	CD19, CD10, CD5

Characteristics of cases showing lineage correction (AML to ALL) after flowcytometric analysis.(TABLE 2)

morphology	Cytochemical staining	Antigens expressed	Antigen not expressed
AML-MO	MPO positivity-1% , PAS negative	CD34, CD5, CD7, cCD3	CD13, 33,117, CD19, CD10 HLADR

24 cases were of pre-B ALL and 15 cases were of pre-T-ALL. Only 20% of T-ALL showed block positivity with PAS stain, while 87% of B-ALL showed PAS positivity. CD19 was positive in 100% cases and CD10 was positive in 91.6% cases of B-ALL (table 3). CD7 was positive in 100% cases and CD5 was found to be positive in 93.3% cases of T-ALL .Only 26.6% of T-ALL showed positivity for HLADR .(table 4)

Reactivity of surface Antigens in B-ALL(n=24)(TABLE 3)

Markers	**Children (%) (n=5)**	**Adults(%) (n=19)**	**Males(%) (n=18)**	**Females (%) (n=6)**	**Cases (+ve)**	**Sensitivity (%)**
CD19	5(100)	19 (100)	18(100)	6(100)	24	100
CD10	5(100)	17 (89.5)	17(94.4)	5(83.3)	22	91.6
HLADR	3(60)	16 (84.2)	15(83.3)	4(66.7)	19	79.2
CD13	2(40)	6 (31.6)	6(33.3)	2(33.3)	08	33.3
CD33	0(0)	4 (21.1)	4(22.2)	0(0)	04	16.6
CD34	5(100)	18 (94.7)	18(100)	5(83.3)	23	95.8
CD5	1(20)	2 (10.5)	2(11)	1(16.7)	03	12.5
CD7	0(0)	1 (5)	1 (5)	0(0)	01	4

Reactivity of surface Antigens in T-ALL(n=15)(TABLE 4)

Marker	Children (%) (n=3)	Adults(%) (n=12)	Males(%) (n=11)	Females (%) (n=4)	Cases+ve	Sensitivity
CD5	3(100)	11(91.6)	11(100)	3(75)	14	93.3
CD7	3(100)	12(100)	11(100)	4(100)	15	100
HLADR	0(0)	4(33.33)	3(27.3)	1(25)	04	26.6
CD13	0(0)	3(25)	3(27.2)	0(0)	03	20
CD33	0(0)	2(16.7)	2(18)	0(0)	02	13.3
CD34	2(66.7)	11(91.6)	9(81.8)	4(100)	13	86.6
CD10	0 (0)	2 (16.7)	2(18)	0(0)	02	13.3
CD19	0(0)	1(8.3)	0 (0)	1(25)	01	6.7

The frequency of aberrant phenotypes in ALL was studied. 45.8% cases of B-ALL and 33.3% cases of T-ALL expressed aberrant myeloid markers, with overall positivity of aberrant markers in ALL being 41%. CD13 was the most common myeloid marker expressed aberrantly in ALL, comprising of 61.5% of aberrant markers in B-ALL and 60% of aberrant markers in T-ALL. CD33 was expressed in 30.8% of B-ALL and 40% of T-ALL. Only one case (7.7%) of B-ALL showed positivity for CD117 while none of the T-ALL cases showed positivity for CD117.(table-5)

-PERCENTAGE OF DIFFERENT ABERRANT ANTIGENS EXPRESSED IN ALL(TABLE 5)

IPT	CD 117	CD13	CD33	TOTAL
B-ALL	1(7.7%)	8(61.5%)	4(30.8%)	13
T-ALL	0(0)	3(60%)	2(40%)	05
TOTAL	1(5.5%)	11(61%)	6(33.5%)	18

Out of 11 cases of AML diagnosed immunophenotypically, 4 cases(36.4%) did not show any aberrant markers ,6 cases (54.5%) showed aberrant T-lymphoid marker and 1 case(9.1%) showed aberrant B- lymphoid marker. The clinical and haematological parameters of these categories were compared which did not reveal any significant difference in the three groups. CD13 was positive in 100% cases . CD117 and CD33 was positive in 9(81%) cases. Aberrant expression of T-lymphoid markers, CD7 and CD5 was seen in 5(45.5%) cases and 4(36.4%) cases respectively.

DISCUSSION

Lineage assignment is critical for optimal therapy for acute leukemia ,as treatment regimen for AML and ALL differ markedly.

In our study, 41cases were assigned ALL and 9 cases were assigned AML on the basis of cytomorphology . ALL-L2 (52.6%) subtype was the most common subtype encountered by us. But in contrast to the present study, Choudhary et al6 reported ALL-L1(73.3%) to be more common than ALL-L2. In the present study, the maximum number of cases of ALL belonged to 21-30 yrs age group .. The male to female ratio was 2.6:1. According to the study by SEER , 2011 , the median age at diagnosis for acute lymphocytic leukemia was 13 years of age..The sex-ratio was of 1.3.[7]

Samples used for immunophenotyping was bone marrow in all but 8 cases of ALL where the peripheral blood showed a TLC higher than 25,000/dl. Peripheral blood could be used in these cases as we acquired 25,000 cells during flowcytometric analysis.

Lineage correction

Among the 41 cases of ALL based on cytomorphology, immunophenotyping demonstrated lymphoid lineage in all but 3 (7%) cases. All the 3 cases were positive for CD34,CD13,CD33 ,HLADRand cMPO. The bone marrow slides were again reviewed .Two cases showed occasional MPO positivity in atleast 3 % cases and were assigned AML-M1. The third case was MPO negative ,PAS negative and assigned AML-M0. Among the 9 cases of AML diagnosed on the basis of morphology, immunophenotyping demonstrated myeloid lineage in all but 1(1.1%) case. It showed positivity for CD5, CD7 and CD34, cCD3 with aberrant expression of CD33. Overall , lineage correction was done in 4 out of 50 cases, comprising of 8 % of the study group.

Misbah Qadir et al in 2006 ,in their retrospective analysis of cases of acute leukemia showed lineage correction by using flowcytometry in 2% of cases.8 The higher percentage of lineage correction in our study can be attributed to the difference in efficacy of staining methods and subjective variations in assessment of morphology.

Out of 39 cases of ALL confirmed by immunophenotyping, 24(61.5%) cases were pre B -ALL and 15 (38.5%) cases were pre T -ALL. The study by **Fatima Bachir et al** showed a higher incidence of B-ALL (77.4%) than T-ALL (22.8%).[9] **S .Sazawal et al** in their study , showed a declining trend in T-ALL in India, comprising of only 22%of all ALL cases detected.10 Our study how-

ever, shows a comparatively higher incidence of T-ALL.

CD19 was positive in 100% cases.CD10 was positive in 89.5% cases of B-ALL in adults and in 100% cases of B-ALL in children. CD7 was positive in 100% cases of T-ALL in our study. CD5 was found to be positive in 91.6% of adult T-ALL and 100% of childhood T-ALL. Only 33.3%of adult T-ALL showed positivity for HLADR . The sensitivity of these markers is in accordance to the studies by Tiensiwakul et al(1999)11,Yusuf RJ et al (2001)12 , and Rajalakshmy KR et al (2001)13. CD19 was the most commonly expressed of all B-lineage associated antigens detected with a positivity rate of 100%.In T-ALL, the positive expression of CD5 and CD7 was about 90% in all previous studies.

Aberrant antigen expression in ALL

The frequency of aberrant phenotypes in ALL and their prognostic significance remains controversial. In our study, overall positivity of aberrant marker in ALL was 41%. CD13 was the most common myeloid marker expressed aberrantly in ALL, comprising of 61.5% of aberrant markers in B-ALL and 60% of aberrant markers in T-ALL. CD33 was expressed in 30.8% of B-ALL and 40% of T-ALL. Only one case (7.7%) of B-ALL showed positivity for CD117 while none of the T-ALL cases showed positivity for CD117.

This correlated well with the study by Wenxiu SHU et al (2005),which showed aberrant myeloid expression in 39.5% of ALL cases.14 CD13 was the commonest aberrant antigen expressed in the above study. Similar results were noted by Sobol et al (1987) 15and Vitale et al(2007).16

Out of the 11 cases of AML, aberrant expression of T-lymphoid markers, CD7 and CD5 was seen in 5(45.5%) cases and 4(36.4%) cases respectively. CD10 was positive in 1 case. These finding differed with the study by **Bharat Bhusan et al** 17who found Ly+ AML phenotypes in 45% cases ,with CD7 positivity in only 17% cases and CD5 positivity in 16% cases. This difference could be attributed to the small size of our study group and also due to the exclusion of major FAB groups of AML from the study.

CONCLUSION

Immunophenotype pattern determined by flowcytometry is not only essential for subtyping of acute leukemias but also caries prognostic significance. Aberrant expression of markers is also indicator of few chromosomal rearrangement . So, experience in interpretation in flowcytometry plays a very important role in making a correct diagnosis.

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