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Immunophenotyping in Acute Leukemia: A Clinical Study

Ashish Gupta[1], Abhijit Pal[1], Silas Supragya Nelson[2]

[1]Assistant Professor, Department of Medicine, NSCB Medical College, Jabalpur, Madhya Pradesh, India, [2]Associate Professor, Department of Medicine, NSCB Medical College, Jabalpur, Madhya Pradesh, India

Abstract

Background: Acute leukemia area a group of neoplastic disorders characterized by proliferation and accumulation of immature hematopoeitic cells in bone marrow, blood, and other tissues. The present study was conducted to have a detailed understanding of immunophenotyping profile, the morphologic and immunophenotypic discrepancy and importance of immunophenotyping in diagnosis of acute leukemia.

Objectives: To study immunophenotyping profile in acute leukemia (acute myeloid leukemia [AML], acute lymphoid leukemia, and mixed lineage leukemia) and to study its importance in diagnosis.

Materials and Methods: This study was performed in Medical College, Jabalpur. 160 patients diagnosed morphologically with AML, acute lymphoblastic leukemia and mixed lineage leukemia seen were included in the study.

Results: Only in 73% cases of acute leukemia did find similarity in morphological appearance and immunophenotyping. In remaining 27% cases morphological findings did not correlate with immunophenotyping expression. Diagnosis in these 27% patients changed after immunophenotyping.

Conclusions: It is imperative and absolutely essential to ascertain the lineage of leukemia by immunophenotyping before starting on treatment as more than 25% of patients would not respond or later relapse if treatment is initiated on morphological diagnosis.

Key words: Cytogenetics, Leukemia, Lineage leukemia, Immunophenotyping

INTRODUCTION

Acute leukemia area group of neoplastic disorders characterized by proliferation and accumulation of immature hematopoeitic cells in bone marrow and blood and other tissues.

Velpeau in 1827 reported the first accurate description of case of leukemia. In 1845, Bennet published a report of series of patients who died with enlarged spleen and changes in color and consistency of blood. He coined the term "Leukocythemia." Virchow introduced the term "leukemia" which he derived from Greek meaning white blood. Neumann in 1869 described that white blood cells (WBC) were made in bone marrow. Moser in 1869 described aspiration of bone marrow as the means to diagnose acute leukemia. Negalia in 1900 described myeloblast and divided leukemia into acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML). The development of histochemical stains, cytogenetics, immunologic, molecular, and biochemical markers have helped to define the lineage of acute leukemia.[1]

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Acute leukemia is diagnosed on doing morphologic evaluation of bone marrow based on suspicion created by altered hematological profile. The malignant cells are called blasts. The normal percentage of blasts in marrow is <5%. For diagnosis of acute leukemia the percentage of blasts in marrow is >20%. Morphologically that is how blasts appear under microscope, leukemia are classified into two types AML and ALL. AML is subclassified into M0-M7 and ALL has three subtypes L1-L3. After

Corresponding Author: Dr. Silas Supragya Nelson, Silver Oak Compound, Napier Town, Jabalpur, Madhya Pradesh, India. Phone: +91-9826111066. E-mail: silasnelson@gmail.com

diagnosing acute leukemia morphologically the cells are subjected to cytochemistry.[2]

Every blood cell expresses certain cytoplasmic and surface proteins which are called cluster differentiation (CD) antigens. Every level of differentiation has a unique set of expression of CD antigens. Immunophenotyping is the identification and quantification of cellular antigens through flourochrome labeled monoclonal antibodies. The immunophenotypic identification and classification of cells began in late 1970 with revolution in immunology by the discovery of T- and B-cells.[3] The technology then expanded to the analysis of other cells as myeloid cells in various stages of maturation. As a result of the progress in the understanding of molecular biology and cytogenetics of acute leukemia, WHO again classified leukemia from morphologic classification to immunological and cytogenetic classification in 1985. Morphological classification of acute leukemia may not always be correct and hence clinically immunophenotype analysis since then has become critical part of initial diagnosis and classification of acute leukemia.[4] In addition, immunophenotyping provides prognostic information not available by other techniques provides a sensitive means to monitor the progress of patients after chemotherapy and aids in detection of minimal residual disease.

The present study was conducted to have a detailed understanding of immunophenotyping profile, the morphologic and immunophenotypic discrepancy and importance of immunophenotyping in diagnosis of acute leukemia.

Aims and Objectives

To study the immunophenotypic profile in acute leukemia (AML, ALL and mixed lineage leukemia) and to study its importance in diagnosis.

MATERIALS AND METHODS

The 160 patients diagnosed morphologically with AML, acute lymphoblastic leukemia and mixed lineage leukemia seen were included in study.

Eligibility Criteria

1. Patients of all age groups were eligible
2. Only previously untreated patients were included
3. The requirement for the diagnosis
    - Presence of >20% blasts in bone marrow aspirate with appropriate clinical syndrome
    - The morphological diagnosis to be supplemented by immunological diagnosis
    - If possible, the diagnosis to be supplemented by cytogenetic studies.
4. No cases of chronic leukemia were eligible.

Investigations to be Completed on Patient Entry

1. Clinical information
2. Nutritional status assessed based on Indian Academy of Pediatric classification (weight for age)
3. Routine laboratory investigations
4. Hematology: Hemoglobin, total leukocyte count, differential leucocyte count, peripheral smear red blood cell indices, platelet counts
5. Biochemistry: Renal function test, liver function tests, serum electrolytes, serum uric acid and serum lactic acid dehydrogenase
6. X-ray chest
7. Bone marrow aspirate and sample evaluation for cytochemistry with periodic acid-Schiff (PAS) and Sudan black B stains, immunophenotyping and cytogenetics
8. Other laboratory and imaging as par clinical indications
9. Diagnosis was based on immunological classification rather morphologic classification. Immunophenotyping was primarily performed by three color flowcytometric analysis of bone marrow aspirate or peripheral blood. After mononuclear cell enrichment by centrifugation over histopaque 1077, the peripheral blood and bone marrow specimens were studied for surface antigen expression using panel of monoclonal antibodies. Blasts were gated for the analysis with the use of CD45 antigen expression and right angle light scatter as described by Borowitz. The antigens detected and antibodies used were CD45, human leukocyte antigens (HLA) DR, CD34, CD13, CD33, CD117, CD54, CD56, CD41, CD61, CD2, CD3, CD4, CD5, CD7, CD8, PAN T, CD10, CD19, CD22, CD79, and PAN B. For all the above markers, blasts were considered positive if 20% or more expressed an antigen. For myeloperoxidase (MPO), it was considered to be positive when more than 3% of blasts reacted positively with anti-MPO.
10. Treatment of patient was according to immunologic diagnosis. ALL patients were kept on NCI MCP 841 or BFM 90 ALL protocol. AML patients were treated with standard 7 + 3 protocol (cytosine arabinoside 100-200 mg/m^2 × 7 days + daunorubicin 45-60 mg/m^2 × 3 days) or 5 + 2 protocol (cytosine arabinoside 100-200 mg/m^2 × 5 days + daunorubicin 45-60 mg/m^2 × 2 days) depending on age and performance status of patients
11. ALL patients were evaluated on day 8 for assessing the cytoreduction and response to chemotherapy. The peripheral blood smear on day 8 was assessed for the

presence or absence of blasts. If blasts were seen their percentage and absolute count was recorded

12. AML patients were evaluated 21-28 days after induction chemotherapy for demonstration of morphological remission. This was followed by 2-3 courses of intermediate dose (2 g/m^2 q12 hourly × 3 days) cytosine arabinoside as post induction consolidation therapy after achieving morphological remission
13. If AML patient failed to achieve morphologic remission after 1st course of 7 + 3 therapy, he was reinduced by 2nd course of induction 7 + 3 chemotherapy regimen in same dose
14. All patients were periodically assessed for any complication, mortality and response of treatment.

Support Measures and Management of Tumor Lysis

Neutropenic patients with absolute neutrophil count <500 with fever >100°F were hospitalized and immediate intravenous (IV) antibiotics to be started. Patients managed according to standard febrile neutropenia guidelines.

Patients with platelet counts <15,000/uL or overtly bleeding received platelet rich plasma transfusions. Irradiated blood products were administered. To the possible extent single donor platelets were transfused.

All patients were hydrated and given allopurinol (10 mg/kg for 7 days, then 5 mg/kg) during initial induction therapy. IV fluids at 3 L/m^2 preferably half normal salione without added potassium (unless hypokalemia) were given until WBC count was below 10,000/cmm, lymphadenopathy and organomegaly were reduced in size by 75%.

Patients of ALL with WBC of >1 Lakh/cmm were treated with prednisolone 10 mg/m^2 twice a day until the WBC count falls below 50,000/cmm prior to commencing induction protocol. If no response to prednisolone occurs within 24 h, induction protocol was started under careful surveillance of electrolytes and urine output.

Observations

There were 160 cases of acute leukemia in the study.

The distribution of cases was according to imunophenotyping profile into AML (83 cases: 51%), ALL (66 cases: 42%) and mixed lineage leukemia (11 cases: 7%).

AML

There were 83 cases (51%) of AML in the study.

Bone marrow profile of AML patients

Marrow was hypercellular in all cases.

In cytochemistry PAS stain was positive in 18 (22%), inconclusive in 5 (6%) and negative in 51 (60%) cases of AML. Sudan black was positive in 55 (66%), inconclusive in 5 (6%) and negative in 20 (24%) patients. Bone marrow blasts ranged between 14% and 95% and average percentage of blasts in marrow was 38%. Auer rods were seen in 15 (18%) of cases and absent in 68 (81%) of cases.

Bone marrow profile in various subtypes of AML

Marrow was hypercellular in all cases.

PAS was positive in 2 (25%) of AML M0, 5 (27%) cases of AML M1, 13 (40%) of AML M2, 3 (42%) of AML M3, 1 (12%) of AML M4, 2 (25%) of AML M5, and in no case of AML M6. PAS was inconclusive in 1 (4.4%) of AML M1, 2 (6%) cases of AML M2, and 1 (13%) cases of AML M5. PAS was negative in 5 (75%) of AML M0, 22 (68%) of AML M1, 17 (53%) of AML M2, 3 (57%) of AML M3, 8 (88%) of AML M4, 5 (62%) of AML M5, and 1 (100%) of AML M6.

Sudan black was positive in 6 (100%) of AM M0, 13 (63%) cases of AML M1, 22 (66%) of AML M2, 5 (86%) of AML M3, 6 (66%) of AML M4, 4 (50%) of AML M5, and in 1 (100%) cases of AML M6. Sudan black was inconclusive in 1 (9%) of AML M1, 1 (3%) of AML M2, 1 (11%) of AML M4, and 1 (13%) cases of AML M5. Sudan black was negative in 2 (27%) of AML M1, 10 (30%) of AML M2, 1 (13%) of AML M3, 2 (23%) of AML M4, and 3 (37%) of AML M5.

Bone marrow blasts ranged from 14% to 95%. Average number of blasts was 66.7% in AML M0, 71.2% in AML M1, 67.6% in AML M2, 82% in AML M3, 74% in AML M4, 73.1% in AML M5, and 65% in AML M6.

Auer rods were seen in 100% cases of AML M0, 3 (13%) cases of AML M1, 5 (20%) cases of AML M2, 5 (91%) cases of AML M3, 3 (33%) cases of AML M4, and were not seen in 17 (86%) of AML M1, 28 (80%) of AML M2, 1 (9%) of AML M3, 6 (66%) of AML M4, 8 (100%) of AML M5, and 1 (100%) of AML M6.

Immunophenotyic profile of AML patients

The expression of HLA-DR was highest in AML MO - 3 (50%). It was positive in 6 (33%) of M1 MI, 4 of ML M2, 4 (44%) of AML M4, and 3 (33%) of AML M5 and was absent in AML M3 and AML M6.

The expression of CD34 was highest in AML M1 - 6 (33%). It was positive in 2 (7%) of AML M2 and 1 (12%) of AML

M5 and was absent in AML M0, AML M3, AML M4, and AML M6.

The expression of CD 13 was highest in AML M6 - 1 (100%). It was positive in 1 (11%) cases of AML M0, 9 (43%) of AML M1, 3 (10%) of AML M2, 3 (42%) of AML M3, and 7 (77%) of AML M4, and 3 (37%) of cases of AML M5.

The expression of CD 33 was highest in AML M4, AML M5 and AML M6, - 100%. It was positive in 75% cases of AML M0 and AML M1, 28 (86%) of AML M2 and 3 (57%) of AML M3.

The expression of CD117 was highest in AML M6 - 1 (100%). It was positive in 3 (50%) cases of AML M0, 8 (40%) of AML M1, 10 (31%) of AML M2 2 (28%) of AML M3, and 7 (75%) of AML M4, and 3 (37%) of cases of AML M5.

MPO was expressed in all cases of AML M0, AML M1, AML M2, AML M4 and AML M6. It was positive in 5 (85%) cases of AML M3 and 6 (75%) cases of AML M5.

Lymphoid antigens CD22 and CD79 were seen in 1 (1%) of AML patients (AML M2).

In the present study positive lymphoid markers were CD22 and CD79 positive in 1 patient of AML M2.

The high lymphoid antigen expression was not observed in our study as we have included cases expressing both myeloid and more than one lymphoid antigen in multiple lineage leukemia.

Acute Lymphoblastic Leukemia

Bone marrow profile of ALL patients

Marrow was hypercelular in all cases.

In cytochemistry PAS stain was positive in 42 (62%), inconclusive in 2 (3%), and negative in 19 (28%) cases of ALL. Sudan black was positive in 3 (4.4%), inconclusive in 2 (3%) and negative in 62 (91%) patients. Bone marrow blasts ranged between 43% and 99% and average number of blasts in marrow was 85.2%.

Morphological diagnosis in ALL patients

Morphologically 60% (89%) marrow were suggestive of ALL, 3 (4%) suggestive of AML and 4 (6%) were suggestive of acute undifferentiated leukemia.

Immunophenotypic profile of ALL patients

B lymphoid antigens

CD10: 38 (67%) and CD22: 34 (60%) were the most common B lymphoid antigens expressed. HLA-DR was expressed in 22 (34%), CD34 in 21 (32%), CD79 in 17 (30%), CD19 in 24 (42%) and PAN B was expressed in 31% of all B-cell ALL cases.

T lymphoid antigens

CD3 7 (78%) was the most commonly expressed T-cell antigen. CD2 was expressed in 3 (33%), CD5 in 5 (56%), CD7 in 5 (56%) and PAN T was seen in 4 (44%) of all T-cell ALL cases.

There were a total of 57 (83%) cases of B-cell ALL and 9 (17%) cases of T-cell ALL.

Mixed Lineage Leukemia

Bone marrow profile of mixed lineage leukemia (MLL) patients

Marrow was hypercelular in all cases.

In cytochemistry PAS stain was positive in 3 (27%), inconclusive in 0 (0%) and negative in 8 (72%) cases of ALL. Sudan black was positive in 3 (27%), inconclusive in 1 (9%) and negative in 7 (63%) patients. Bone marrow blasts ranged between 65% and 90% and average number of blasts in marrow was 83.1% Auer rods were absent in all 100% of cases.

Immunophenotypic profile of MLL patients

Myeloid antigens

HLA-DR was present in 9% (1) cases, CD34 in 9% (1) cases, CD33 in 63% (7) cases CD117 in 18% (2) cases and MPO positive in 81% (9) cases.

B lymphoid antigens

PAN B (54%) was the most common B lymphoid antigens expressed. VCD 10 in 9%, CD22 in 9% and CD79 in 9% were other B lymphoid antigens expressed.

T lymphoid antigens

PAN T 45% was the most common expressed T-cell antigen. CD3 in 18% CD5 in 9% CD7 in % and CD8 in 9% cases were the other T lymphoid antigens expressed.

When biphenotyping score was calculated, off the total 11 cases, 4 (36%) patients had biphenotypic leukemia and 7 (64%) patents had bilineage leukemia.

DISCUSSION

There were 160 cases of acute leukemia in the study.

The distribution of cases was according to imunophenotyping profile into AML (83 cases: 51%), ALL (66 cases: 42%) and mixed lineage leukemia (11 cases: 7%).

AML

Distribution of AML patients according to FAB subtype is shown in Table 1.

According to one of the study conducted,[1] MPO activity is present in the primary (azurophilic granules) of both myeloid and monocytic precursors. In cytochemistry PAS stain was positive in 18 (22%), inconclusive in 5 (6%) and negative in 51 (60%) cases of AML. Sudan black was positive in 55 (66%), inconclusive in 5 (6%) and negative in 20 (24%) patients. Bone marrow blasts ranged between 14% and 95% and average percentage of blasts in marrow was 38%. Auer rods were seen in 16 (19%) of cases and absent in 67 (81%) of cases (Table 2).

Morphologically 61 (73%) marrow were suggestive of AML, 15 (18%) suggestive of ALL, 3 (3%) were reported as chronic myeloid leukaemia blastic phase, 1 (1%) was suggestive as acute undifferentiated leukemia, biphenotypic leukemia, myelodysplastic syndrome, and bilineage leukemia (Table 3).

Hoffbrand *et al.*,[5] San Miguel *et al.*,[6] Griffin *et al.*[7] in their studies have shown that the morphological diagnosis is not very reliable in conclusively diagnosing acute leukemia. Immunophenotyping is mandatory for establishing the confirmed diagnosis of acute leukemias and their subtypes. In up to 25% cases the immunophenotyping for cell antigen marker change the lineage of leukemia similar to that seen in our study.

Marrow was hypercellular in all cases (Table 4).

Positive staining with Sudan black B stains intracellular lipids which are found in secondary granules of both myeloid and monocyte precursors.

Griffin *et al.*[7] observed MPO positive in >95% cases of AML and PAS positive in 70% cases and negative in 8% cases.

Ghosh *et al.*[8] have found MPO positive in 97% AML.

Estey *et al.*[9] in their study of 180 acute leukemia cases found MPO to be very specific marker for myeloid antigen, the overall positivity of anti MPO in AML was 92%. Anti MPO

Table 1: Distribution of AML according to FAB subtypes

FAB subtype	Number of patients (%)
AML M0	5 (6)
AML M1	20 (24)
AML M2	33 (39)
AML M3	6 (7.2)
AML M4	9 ((10.8)
AML M5	8 (9.6)
AML M6	1 (1)
AML M7	0

AML: Acute myeloid leukemia , FAB: French american british classification

Table 2: Bone marrow profile of AML patients

Cytochemistry (%)						Blasts (%)		Auer rods	
PAS			Sudan			Mean	Range	Present	Absent
Positive	Incon	Negative	Positive	Incon*	Negative				
18 (22)	5 (6)	51 (61)	55 (66)	5 (6)	20 (24)	32	14-95	16 (19)	67 (81)

PAS: Periodic acid-Schiff, AML: Acute myeloid leukemia

Table 3: Morphological diagnosis of AML patients

Total number	Morphological diagnosis (%)						
	AML	ALL	AUL	CML (BP)	Biphenotypic	MDS	Bilineage
83	61 (73)	15 (18)	1 (1)	2 (3)	1 (1)	1 (1)	1 (1)

AML: Acute myeloid leukemia, ALL: Acute lymphocytic leukemia, CML: Chronic myeloid leukemia, AUL: Acute undifferentiated leukemia, MDS: Myelodysplastic syndrome

Table 4: Bone marrow profile of AML patients according to FAB subtype

FAB subtype	Number of cases	Cellularity	Cytochemistry (%)						Blasts (%)		Auer rods (%)	
		Hyper (%)	PAS			Sudan			Mean	Range	Present	Absent
			Positive	Incon*	Negative	Positive	Incon*	Negative				
M0	4	4 (100)	1 (25)	-	3 (75)	4 (100)	-	-	66.7	45-91	4 (100)	-
M1	22	22 (100)	6 (27)	10 (4.4)	15 (68)	14 (63)	2 (9)	6 (27)	71.2	22-95	3 (13)	19 (86)
M2	33	33 (100)	13 (40)	2 (6)	17 (53)	22 (66)	1 (3)	10 (30)	67.6	14-95	7 (20)	26 (80)
M3	7	7 (100)	3 (42)	-	4 (57)	6 (86)	-	1 (13)	82	67-90	6 (91)	1 (9)
M4	9	9 (100)	1 (12)	-	8 (88)	6 (66)	1 (11)	2 (23)	74	41-90	3 (33)	6 (66)
M5	8	8 (100)	2 (25)	1 (13)	5 (62)	4 (50)	1 (13)	3 (37)	73.1	56-93	-	8 (100)
M6	1	1 (100)	-	-	100%	100%	-	-	65	65	-	100
M7	-	-	-	-	-	-	-	-	-	-	-	-

PAS: Periodic acid-Schiff, AML: Acute myeloid leukemia, FAB: French american british classification, Incon: Inconsequential

was negative in all but two ALL which later was classified as biphenotypic leukemia.

Table 5: Immunophenotype profile of AML patients

FAB subtype	HLA-DR (%)	CD34 (%)	CD13 (%)	CD33 (%)	CD117 (%)	MPO (%)
MO	3 (50)	0 (0)	1 (11)	5 (75)	3 (50)	6 (100)
M1	7 (33)	6 (30)	9 (43)	15 (57)	8 (40)	20 (100)
M2	10 (13)	2 (7)	3 (10)	28 (86)	10 (31)	33 (100)
M3	0 (0)	0 (0)	3 (42)	3 (57)	2 (28)	5 (85)
M4	4 (44)	0 (0)	7 (77)	9 (100)	7 (75)	9 (100)
M5	3 (33)	2 (12)	3 (37)	8 (100)	3 (37)	6 (75)
M6	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)

HLA: Human leukocyte antigens, AML: Acute myeloid leukemia, MPO: Myeloperoxidase

Table 6: Lymphoid antigen expression in AML patients

FAB subtype	CD10	CD19	CD22	CD79	CD2	CD3	CD4	CD5	CD7	CD8
M0	-	-	-		-	-	-	-	-	-
M1	-	-	-		-	-	-	-	-	-
M2	-	-	1 (3%)	1 (3%)	-	-	-	-	-	-
M3	-	-	-	-	-	-	-	-	-	-
M4	-	-	-	-	-	-	-	-	-	-
M5	-	-	-	-	-	-	-	-	-	-
M6	-	-	-	-	-	-	-	-	-	-

AML: Acute myeloid leukemia

Table 7: Comparison between the immunophenotyping observations of presentation of lymphoid markers in AML all figures represent % of patients having positive antigen

Subtype	CD10	CD19	CD2	CD3	CD5	CD7
M0	0/0*	0/0*	0/0*	0/0*	0/0*	29/0*
M1	0/0*	7/0*	7/0*	7/0*	0/0*	20/0*
M2	5/0*	21/0*	5/0*	0/0*	5/0*	14/0*
M3	0/0*	14/0**	29/0*	0/0*	0/0*	0/0*
M4	0/0*	0/0*	9/0*	20/0*	0/0*	0/0*
M5	0/0*	0/0*	17/0*	17/0*	17/0*	17/0*
M6	0/0*	0/0*	0/0*	0/0*	0/0*	0/0*

AML: Acute myeloid leukemia

Blasts with L1 or L2 morphology showing block PAS positivity will be classified as lymphoblasts; therefore, a combined MPO RAS staining has relevance in developed countries.

Considering the significance of cytogenetics and immunopnenotyping in the diagnosis, treatment decision and prognosis of acute leukemia, consensus panel of WHO proposed a new revised classification for acute leukemia.[10]

Immunophenotypic observations in our study (Table 5) were similar to those observed by Khalidi *et al.*[11] and Ghosh *et al.*[8] and in their study. Similarly, the expression of lymphoid antigens (Table 6) in AML was similar to the observations of by Ghosh *et al.*[8]

Acute Lymphoblastic Leukemia

Comparison between the immunophenotyping observations of presentation of lymphoid markers in AML all figures represent % of patients having positive antigen. (Table 7).

In cytochemistry PAS stain was positive in 41 (62%), inconclusive in 2 (3%) and negative in 18 (28%) cases of ALL. Sudan black was positive in 3 (4.4%), inconclusive in 2 (3%) and negative in 62 (91%) patients (Table 8). Morphologically 60% (89%) marrow were suggestive of ALL, 3 (4%) suggestive of AML and 4 (6%) were suggestive of acute undifferentiated leukemia.

Data from India varies from center to center. In general there is a relatively high incidence of T-cell ALL. This was particularly high in the series by Kamat *et al.*[12] (43%). Similar data from Naeem and Hayee from Lahore reported 36% T-cell ALL.[13]

B lymphoid antigens

CD10: 38 (67%) and CD22: 34 (60%) were the most common B lymphoid antigens expressed. HLA-DR

Table 8: Bone marrow profile of ALL patients

Cytochemistry (%)						Blasts (%)		Auer rods (%)	
PAS			Sudan			Mean	Range	Present	Absent
Positive	Incon*	Negative	Positive	Incon*	Negative				
41 (62)	2 (3)	18 (28)	3 (4)	2 (3)	61 (93)	85.2	43-99	0 (0)	66 (100)

PAS: Periodic acid-Schiff, AML: Acute myeloid leukemia

Table 9: Immunophenotypic profile of ALL patients

Stem cell (%)		B-cell (%)					T-cell (%)				
HLA DR	CD34	CD79	CD22	CD10	CD19	PAN B	CD3	CD2	CD5	CD7	PAN T
22 (34)	21 (32)	17 (30)	34 (60)	38 (67)	24 (42)	20 (31)	7 (78)	3 (33)	5 (33)	5 (56)	4 (44)

ALL: Acute lymphocytic leukemia, HLA: Human leukocyte antigens

was expressed in 22 (34%), CD34 in 21 (32%), CD79 in 17 (30%), CD19 in 24 (42%) and PAN B was expressed in 31% of all B-cell ALL cases.

T lymphoid antigens

CD3 in 7 (78%) was the most common expressed T-cell antigen. CD2 was expressed in 3 (33%), CD5 in 5 (56%), CD7 in 5 (56%), and PAN T was seen in 4 (44%) of all T-cell ALL cases (Table 9).

There were a total of 57 (83%) cases of B-cell ALL and 9 (17%) cases of T-cell ALL.

Pui *et al.*[14] have reported incidence of Sudan black to be positive in 2.7% cases of ALL Therefore, Sudan black B used alone to differentiate lymphoid from myeloid leukemia may be misleading. Arber has found MPO positive in 23% cases of ALL.

Data from the western series indicate a high incidence of pre B (80-87%) immunophenotype.

In the present study the immunophenotypic diagnosis of pre B ALL was seen in 83% and T-cell ALL was seen in 16% cases (Table 10) which was similar to observations of Shanta *et al.*[15] and Magrath *et al.*[16] but less than the observations of Schrappe *et al.*,[17] and Tsuchida *et al.*[18] This difference in the incidence of distribution of various subtypes of ALL may have some regional influences which needs further evaluation.

Mixed Lineage Leukemia

Marrow was hypercelular in all cases.

In cytochemistry PAS stain was positive in 3 (27%), inconclusive in 0 (0%) and negative in 8 (72%) cases of ALL. Sudan black was positive in 3 (27%), inconclusive in 1 (9%) and negative in 7 (63%) patients. According to one of the study conducted[19] in their series observed PAS positivity in 50% of cases and remaining 50% were PAS negative in. Sudan B was positive in 25% and negative in 75% of cases. The findings were comparable to the present study.

Myeloid antigens

HLA-DR was present in 9% (1) cases, CD34 in 9% (1) cases, CD33 in 63% (7) cases CD117 in 18% (2) cases and MPO positive in 81%(9) cases.

B lymphoid antigens

PAN B (54%) was the most common B lymphoid antigens expressed. VCD 10 in 9%, CD22 in 9% and CD79 in 9% were other B lymphoid antigens expressed.

T lymphoid antigens

PAN T 45% was the most common expressed T-cell antigen. CD3 in 18% CD5 in 9% CD7 in % and CD8 in 9% cases were the other T lymphoid antigens expressed (Table 11).

When biphenotyping score was calculated, off the total 11 cases, 4 (36%) patients had biphenotypic leukemia and 7 (64%) patents had bilineage leukemia (Table 12).

Kantarjian *et al.*[20] observed the following immunophenotype in their study.

T-cell markers: CD2 - 42%; CD3 - 0%; CD4 - 24%, CD5 - 14%; CD7 - 57%; CD8 - 0. B-cell markers: CD10 - 14%; CD19 - 28%; CD20 - 28%; SLG - 0%.

Myeloid markers: CD11b - 0%; CD13 - 57%; CD14 - 0%; CD154 - 28%; CD33 - 57% MPO – 100%.

Non lineage restricted markers: HLA-DR - 85%; CD34 - 57%.

The observations of Kantarjian *et al.* are comparable to the observations seen in the present study.

CONCLUSION

Only in 73% cases of acute leukemia did find similarity in morphological appearance and immunophenotyping. In remaining 27% cases morphological findings did not correlate with immunophenotyping expression.

Table 10: Distribution of ALL cases according to lineage

B-cell ALL (%)	T-cell ALL (%)
57 (83)	9 (16)

ALL: Acute lymphocytic leukemia

Table 11: Immunophenotypic profile of mixed lineage leukemia patients

Myeloid (%)						Lymphoid (%)										
HLA DR	CD34	CD13	CD33	CD117	MPO	CD10	CD19	CD22	CD79	CD3	CD4	CD5	CD7	CD8	PAN B	PAN T
1 (9)	1 (9)	-	7 (63)	2 (18)	9 (81)	1 (9)	-	1 (9)	1 (9)	2 (18)	-	1 (9)	1 (9)	1 (9)	6 (54)	5 (45)

HLA: Human leukocyte antigens, MPO: Myeloperoxidase

Table 12: Immunophenotypic classification of mixed lineage leukemia patients

Number of patients	Biphenotypic leukemia (%)	Bilieneage leukemia (%)
11	4 (36)	7 (64)

Diagnosis in these 27% patients changed after immunophenotyping.

It is therefore imperative and absolutely essential to ascertain the lineage of leukemia by immunophenotyping before starting on treatment as more than 25% of patients would not respond or later relapse if treatment is initiated on morphological diagnosis.

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