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Original Research Article

Flowcytometric Evaluation and Morphological and Cytochemical correlation of 150 cases of Acute Leukemia

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Abstract

Background: Immunophenotyping by flowcytometry is well conceived & fundamental tool to diagnose & subtype hematological malignancy, especially acute leukemia. By detecting various antigens presenting in various parts of cell, it is possible to know cell lineage & immaturity of the cell or group of cells. Apart from diagnostic importance, this specialized tool is also useful in prediction of prognosis & detection of minimal residual disease. Now, immunophenotyping can diagnose and type also those acute leukemias where morphology and cytochemistry fail.
Aim: Study of Immunophenotypic patterns and their correlation with morphology and cytochemistry in North Indian Population.
Materials and methods: Short clinical details and complete blood count of 150 patients were noted in the department of hematology of tertiary health centre. Sample of each patient was processed as per protocol and run on *FACS CALIBUR OF BD BIOSCIENCES, USA*. Dot plot data of each patient was analyzed and result was released.
Results: AML, B-ALL and T-ALL comprised 38%, 49%, and 13% of all cases. Almost all blasts were expressing dimCD45 with no significant differences between the subtypes. CD34 have different expressions in AML subtypes, usually negative in APML. Aberrant expression of CD7 and CD19 were expressed in 5% and 3.4%of all cases of AML respectively. In 40% cases, morphology and Cytochemical studies clinched the diagnosis. 60% cases essentially needed Flowcytometric evaluation for diagnosis and subtyping of acute leukemias.
Conclusion: Flowcytometric analysis of the patterns and intensity of antigen expression in blasts improved the diagnosis of AML and ALL in our centre. All cases do not require Immunophenotyping for diagnosis. Simultaneous use of conventional morphology, cytochemistry and flowcytometry reduce diagnostic cost of acute leukemia. Immunophenotyping results of our acute leukemia patients were comparable to international published studies.
Keywords: Acute leukemia, Immunophenotyping, antigens, antibodies, flowcytometry, morphology, cytochemistry.

1. Introduction

Acute leukemia is a hematological disorder defined by presence of 20% or more blasts in peripheral blood, bone marrow or other tissue [1-3]. It is divided into Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL) and Biphenotypic Acute Leukemia (BAL) on the basis of morphological, cytochemical & *antigenic characteristics*. Further sub classification of AML & ALL has been done on the basis of morphology, cytochemistry, cytogenetics, immunophenotyping and molecular studies [1,2]. *Immunophenotyping* has become very useful and reliable tool to diagnose & subtype acute leukemia precisely into different subtypes [3,4[. Morphology, cytochemistry, cytogenetic, immunophenotyping and molecular markers are complementary to each other in diagnosing, subclassifying and prognosticating acute leukemia but Immunophenotypic studies have many added advantages over other methodologies [5,6]. Other diagnostic methods are useful in the diagnosis of only few subtypes of acute leukemias but flow-cytometric studies are helpful in diagnosis of all types of acute leukemias. It can acquire & analyze large number of

cells & large number of samples in very short period of time [6,7]. Flowcytometric studies are capable to detect antigens presenting on cell membrane, in cytoplasm or nucleus, thereby simultaneously detecting & differentiating various types of cells in given population of cells. *Multiparametric flowcytometer is capable to detect different antigens on same cell, both surface and cytoplasmic* [7, 8].

French-American-British (FAB) & recent WHO 2008 classifications require help of immunophenotyping in diagnosing and differentiating the acute leukemia. Especially, the diagnosis of FAB types: AML-M0, AML-M5, AML-M6 & AML-M7 and typing of ALL and CML-BC into T-lymphoid and B-lymphoid need Flowcytometric studies. Lymphoid blasts are further divided into T-lymphoid & B-lymphoid on the basis of presence of respective markers [9-11]. Now, diagnosis of acute Biphenotypic & bilinear leukemia has become possible due to clinical application flowcytometer. It is also useful in detecting minimal residual disease (MRD) [8].

Malignant cells can be recognized by their aberrant antigen expression and/or by their numerical excess. The antigen expression in leukemic cells may be similar to their normal progenitor cells along with presence of one or more markers of immaturity i.e. CD34, HLA-DR or TdT [9-11]. In few cases of AML, one may find scattering of blasts in lymphoid zone in dot plot presentation with expression of CD13 & CD33 but absence of antigens of immaturity. Like this , numerical excess of CD7 alone may diagnose a case of T-ALL & at the same time , simultaneous presence of two antigens on same cell or group of cells; normally not presenting, may diagnose a case of acute leukemia i.e. peripheral blood or bone marrow cells showing co-expression of CD4/CD8 is diagnostic of T-ALL [12,13,14]. Similarly presence of CD10 may denote blasts in morphologically suggestive acute leukemias because this antigen is normally expressed on immature B-lymphoid cell in ontogenesis [16]. When cells of interest are expressing T-Lymphoid, B-lymphoid or myeloid markers in significant concentration, *detection of lineage specific markers i.e. MPO, cyCD22, cyCD79a or CyCD3 clinch the diagnosis* [15, 16, 17]. Final typing of acute leukemia depends on the presence of lineage specific marker.

In AML-MO, blasts are negative for MPO cytochemistry, immunophenotyping of blasts is essential to differentiate between lymphoblast & myeloblasts [15].

Purpose of retrospective review of these data is to show diagnostic importance of immunophenotyping in acute leukemia & also to correlate Flowcytometric findings with morphological, cytochemical and epidemiological characteristics of patients in north Indian population.

2. Materials & Methods

In this retrospective study, short clinical & hematological profiles along with detailed Immunophenotypic features of 150 acute leukemia cases of department of hematology of a tertiary health centre of North India were noted and analyzed. Short clinical findings were noted. Blood samples were collected in EDTA vials and complete blood count of peripheral EDTA-Whole Blood or bone marrow-EDTA was done on hematology analyzer, especially to know Total Leukocyte Count to calculate volume of sample for flowcytometry and also peripheral blood smears were made to do morphological and cytochemical studies .*One million cells were taken in each & every case* & thus volume of sample accordingly adjusted. In all cases, morphological study and cytochemical myeloperoxidase (MPO) staining was done in place of Anti-MPO staining for azurophilic granules for detection of myeloblasts in undifferentiated leukemias. Irrespective of MPO cytochemistry result, every sample was prepared & stained as per standard protocol for sample analysis for clinical flowcytometry by using *antibodies of BD Biosciences (USA)* and *run on 3-color Flowcytomer of BD FACS Calibur (USA)***.** One standard panel of antibodies was used in first run & whenever needed, additional panel of antibodies was used on same sample. Standard panel for acute leukemia was: *Forward/Side scatter (tube without antibodies) , Mouse IgG1 FITC/Mouse IgG1 PE, FITC or PE coated AntiCD10/CD19, AntiCD2/CD13, AntiCD7/CD33, AntiCD22/CD34 per tube in pairing* & whenever needed additional antibodies **i.e**.*Anti-CD3 (surface or cytoplasmic), Anti-CD117, CyAnti-CD22, Anti-TDT*, & other few antibodies were used. Staining for cytoplasmic CD22 or CD3 was done whenever diagnostic confusion occurred due to the presence of both T-lymphoid & B-lymphoid markers in almost equal concentration.

3. Result

Immunophenotypic analysis of 150 cases, having suspicion of acute leukemia was included in this study. Flowcytometric findings were correlated with clinical, hematological and cytochemical findings. Interpretation of these data showed that acute leukemias usually present with high total leukocyte count, thrombocytopenia and anemia (Fig.1). They uncommonly present with normal or decreased TLC with aleukemic or subleukemic features in peripheral blood (case no.5, 9, 16 & 25 of table 1 and 40, 68 & 57 of table 2). The age ranged from 3 years to 56 years with mean age of 30 years. The weakness and bleeding were common clinical features respectively due to varying degree of anemia and thrombocytopenia. The lymphadenopathy was the most common presentation in the ALL. *The blasts were > 20% in peripheral blood in 99% cases*. Most common acute leukemia was B-ALL (49%), age ranged from 3 years to 56 years ; average age was 22.16% & T-ALL constituted only 13% of total acute leukemia cases, age ranged from 6 years to 76 years, average age was 20.2 years (Fig. 2). Precursor lymphoblastic leukemia had male predominance (male/Female ratio was 5:1). In B-ALL, CD19 and common

ALL antigen (CD10+) were most common antigen expression (Fig. 3). In seven B-ALL cases, co-expression of myeloid markers was seen, one expressed only CD13 (Table 2, Case No.14) & four expressed only CD33 (Table 2, case no. 18,23,30 & 43) & two expressed both CD13 & CD33 (Table 2, Case No.14,17), thus most common co-expression was of CD33.T-ALL usually presented with very high TLC (Table 3 Case No.4,9,13,14,16,17 &19) & mediastinal mass **(**Table 3 Case No. 5 ,7) & in younger age, average age was 20 years. T-ALL had Immunophenotypic expression of CD2, CD7, CD5, and CyCD3 (specific marker for T lymphoid lineage), Tdt (Fig. 6, 7) .Common expression of CD4/CD8 was found (table 3, case no.6, 9, and 11). AML was second most common type in this study & comprised 38% of total acute leukemia ,age ranged from 2 years to 71 years, surprisingly both cases were of AML-M7 **(**Case No.6, 8**).** They most commonly expressed CD13 and CD33 (Fig. 8, 9).

Two cases of AML-M3 (Table 1, Case No. 24, 28) was diagnosed morphologically showed many auer rods or faggot cells (Fig. 10) & also Immunophenotypic study was done & both showed only CD13 & CD33 & negative for the marker of immaturity i.e. CD34 & also B & T lymphoid markers .Further, both Acute promyelocytic leukemia (APML) cases were confirmed with molecular studies by showing presence of t (15; 17). One case of AML-M6 was detected morphologically and also confirmed with immunophenotyping and cytochemistry. In AML-M6a, all blasts showed presence of myeloid markers & morphologically, erythroblasts constituted 80% of the total cellularity in bone marrow. (Table 1, Case No. 29). AML-M4 & AML-M5 expressed positivity for CD14 along with other myeloid markers (Table 1, Case No. 2, 7, 26, 27). One case of AML expressed only CD13 & CD33 and not markers of immaturity & also negative for B-lymphoid & T-lymphoid markers but scattering of the cells was in lymphoid region. Other case had expression of CD33 & CD117 only & diagnosed AML with more confidence due to expression of CD117 and MPO was positive (CD117 is more specific marker than CD13 & CD33 (Fig. 9, 11). In this study, two cases of AML-M7; both were diagnosed on the basis of presence of CD41 along with myeloid markers & CD34. Most common co-expression in AML was of CD19 & CD7 but none expressed both in same case.

Figure 1: Hematological parameters with age distribution of acute leukemia mean age, Hb (gm/dl), TLC (x10^{3}cumm), and PLT ((x10^{3}cumm)

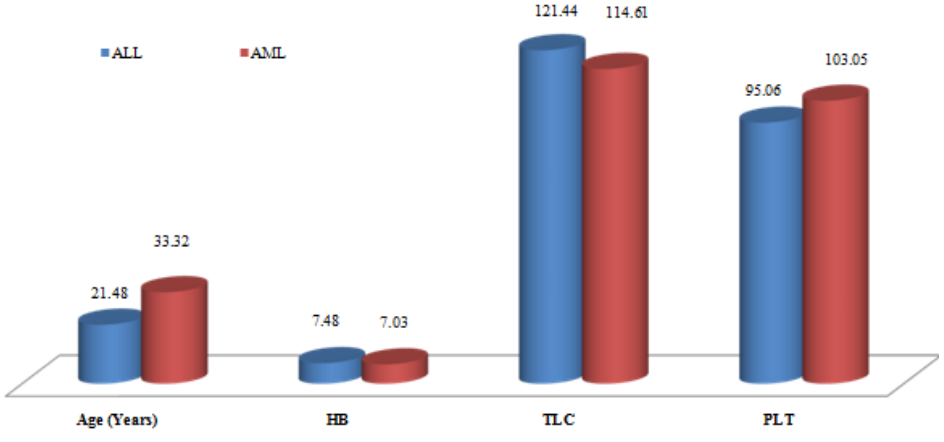
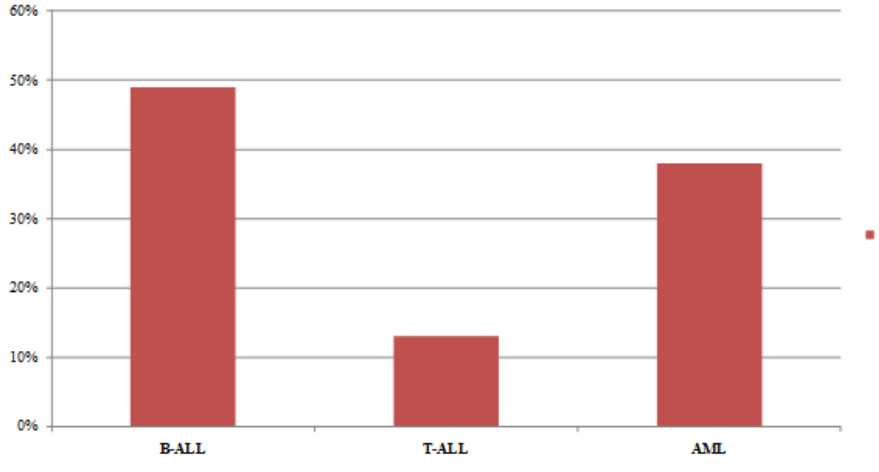


Figure 2: Percentage distribution of Acute Leukemia



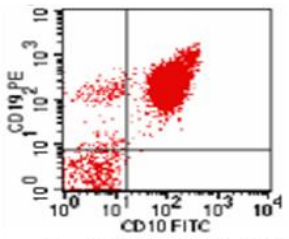


Fig. 3 CD10+/CD19+ B-ALL

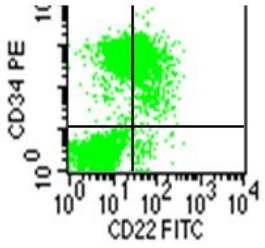


Fig. 4 CD34+/CD22% B-ALL

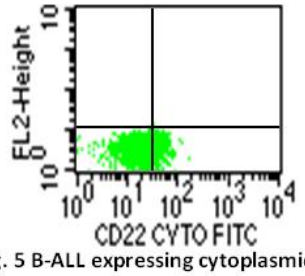


Fig. 5 B-ALL expressing cytoplasmic CD22

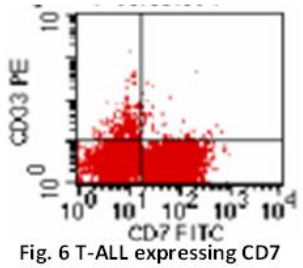


Fig. 6 T-ALL expressing CD7

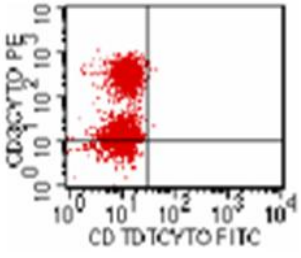


Fig. 7 Cytoplasmic CD3+ T-ALL

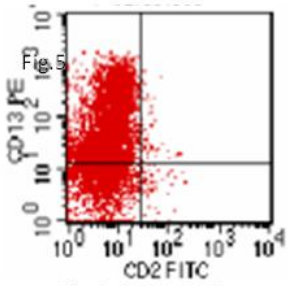


Fig. 8 CD13+ AML

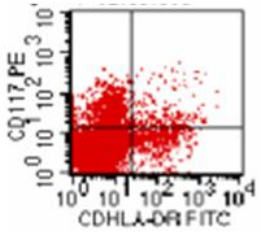


Fig. 9 HLA-DR+/CD117+ AML

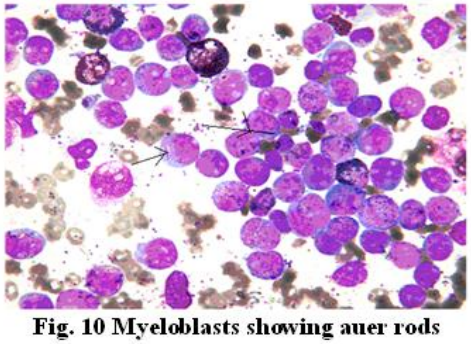


Fig. 10 Myeloblasts showing auer rods

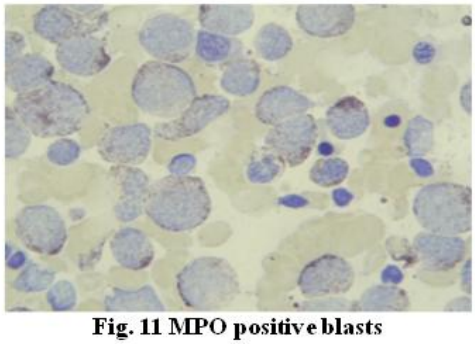


Fig. 11 MPO positive blasts

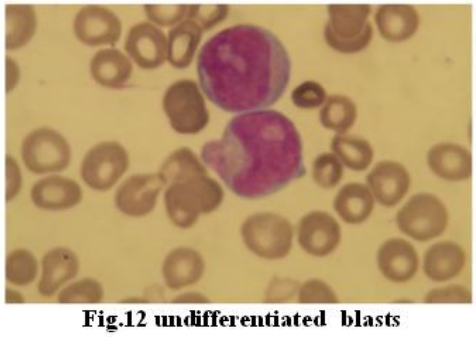


Fig.12 undifferentiated blasts

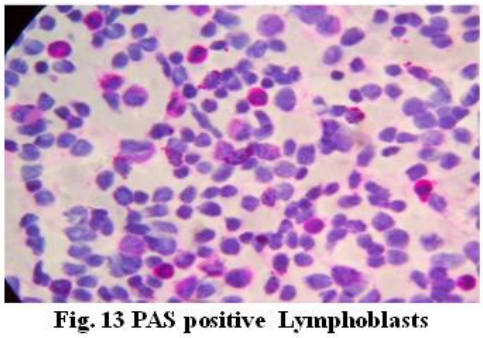


Fig. 13 PAS positive Lymphoblasts

Table 1: Hematological parameters and antigenic expression in AML

Case No	Age	Sex	Hb	TLC	PLT	Clinical diagnosis	Immunophenotypic expression of blasts	Diagnosis
1.	8yrs	F	12.3	50.79	53	?AML	CD13 & CD33, CD34	AML
2	20yrs	M	6.3	222.36	42	?Acute leukemia	CD13+, CD33+ & CD34+,CD14+	AML, morphology AML-M4
3	8yrs	M	13.6	19.6	13	?Acute leukemia	CD13+,CD33+,CD34+, CD117+	AML
4	13yrs	F	6.2	92.1	91	Acute leukemia	CD13+, CD33+, CD117+, CD34+	AML
5	23yrs	M	8	134	24	AML	CD13+, CD33+ CD117+	AML
6	71yrs	M	5.7	2.6	28	pancytopenia	CD33+, CD41+/CD34+	AML-M7
7	61yrs	M	9.3	134.0	27	CML-BC	CD13+, CD33+, CD34+, CD117+, CD14+	AML-M4
8	2yrs	F	5.6	269	28	?ALL	CD19+,CD13+,CD33+,34+, CD41+/CD117+	AML-M7
9	19yrs	M	5.9	11.1	108	?ALL	CD2+, CD13+, CD33+, 34+	AML
10	51yrs	M	7.2	30.53	162	Anémia under évaluation	CD13, CD33, CD34, &CD7	AML
11	58yrs	M	5.8	12.8	9	Acute leukemia	CD34+, CD13+, CD33+	AML
12	32 yrs	F	6.4	389	68	?AML	CD13+,CD33+,CD34+,CD117+	AML
13	40 yrs	M	7.7	335	25.8	Acute Leukemia	CD13+,CD33+CD34+CD117+	AML
14	53 yrs	M	4.7	18.9	77	?MDS	CD13+,CD33+,CD34+	AML
15	26 yrs	M	7.6	344.6	259	?ALL	CD13+,CD33+,CD34+,CD117+	AML
16	15 yrs	M	3.1	10.7	77	Acute leukemia	CD13+,CD33+,CD34+,CD117+,CD19+	AML with co-expression of CD19
17	38 yrs	F	3.1	331.7	55	Acute leukemia	CD13+,CD33+,CD34+	AML
18	37 yrs	F	6.3	18.38	155	?AML	CD13+,CD33+,CD34+,CD19+	AML with co-expression of CD19
19	71 yrs	M	7.1	33.95	9	?ALL	CD13+,CD3+,CD34+	AML
20	43 yrs	F	6.2	76.1	15	Relapse AML	CD34+,CD13+,CD33+,CD7+	AML
21	45 yrs	F	8.1	33.52	22	?ALL	CD13+,CD33+, CD34+	AML
22	5 yrs	M	6.5	445.5	65	?AML	CD13+,CD19+,CD33+,CD117+	AML with co-expression of CD 19
23	57 yrs	F	6.6	30.3	81	?AML	CD13+,CD33+,CD117+,CD34+	AML
24	18 yrs	M	11.4	84.6	67	?AML	CD13+, CD33+, t(15;17) found	AML-M3
25	30yrs	F	8.42	7.2	119	Acute leukemia	CD13+,CD33+, CD34+, CD117+	AML
26	3yrs	M	5.3	37.2	62	AML	CD13+CD33+,CD45+/CD14+	AML-5b
27	2yrs	M	6.7	78.3	47	?ALL	CD13+, CD33+, CD34+, CD14+	AML-M5b
28	46yrs	F	6.1	12	08	?AML	CD13+, CD33+,CD117+, Blasts –multiple auer rods	APML
29	36yrs	F	5.8	424.5	490	?AML	CD13+, CD33+, CD34+, CD117+	AML-M6
30	42yrs	M	6.7	34.7	244	?acute leukemia	CD13+, CD33+, CD34+	AML
31	20yrs	F	4.4	4.4	50	Acute leukemia	CD13+, CD33+, CD34+	AML
32	25yrs	M	6.5	4.4	7	Acute leukemia	CD13+, CD33+, CD34+, CD7+	AML with co-expression of CD7
33	22yrs	M	3.4	4.4	65	Acute leukemia	CD13+, CD33+, CD34+, 10+	AML with co-expression of CD10
34	54yrs	M	6.0	22	14	?CML	CD13+, CD33+, CD117%	AML
35	3yrs	F	6.4	22	10	?acute leukemia	CD13+, CD33+,CD117%	AML
36	39yrs	M	5.4	390.9	477	AML	CD13+,CD33+, HLA-DR+	AML
37	77yrs	M	9.3	468	40	AML	CD13+, CD33+,CD34+	AML
38	10yrs	M	5.54	94.96	197	Acute leukemia, BMT failed	CD13+, CD33+,CD117+	AML
39	66yrs	F	9.4	27.57	120	?AML	CD45+,CD34,CD13+, CD33+	AML
40	25yrs	F	4.0	104.5	199	?AML	CD13+, CD33+, CD117+	AML
41	71yrs	F	10.5	5.18	16	?AML	CD13+, CD33+,CD34+	AML
42	35yrs	M	7.4	89.89	271	AML	CD13+, CD33+, CD34+	AML
43	45yrs	M	6.5	60.6	90	?AML	CD45+,CD34,CD13+, CD33+	AML
44	35yrs	M	10.5	45.6	445	?AML	CD45+,CD34,CD13+, CD33+	AML
45	20 yrs	F	8.0	190.7	98	AML	CD45+,CD34,CD13+, CD33+	AML
46	8yrs	M	9.3	200	97	Acute leukemia	CD13+, CD33+, HLA-DR+, CD117+	AML
47	10yrs	F	8.4	29	97	Acute leukemia	CD34,CD13+, CD33+,CD117+	AML
48	12yrs	M	5.3	155	97	Acute leukemia	CD34, CD13+, CD33+, CD117+	AML
49	21yrs	F	8.0	119.0	145	AML	CD13+,CD33+ CD45+,CD34,	AML
50	19yrs	M	7.2	162.7	227	?ALL	CD13+, CD33+,CD34+	AML
51	75yrs	M	7.8	65	55	?Leukemia	CD13+, CD33+,CD34+	AML
52	78yrs	M	9.8	80	65	?Leukemia	CD13+, CD33+,CD34+	AML
53	56YRS	F	6.9	19.2	121	Anemia? cause	CD13+, CD33+,CD34+	AML
54	15yrs	F	2.8	30.1	90	?ac leukemia	CD13+, CD33+,CD34+	AML
55	40yrs	F	6.5	80	15	?AML	CD13+, CD33+,CD34+	AML
56	22yrs	M	4	221	133	Acute leukemia	CD13, CD33+,CD34+	AML
57	33yrs	F	8.3	102.8	113	?AML	CD13+, CD33+,CD34+	AML

Table 2: Hematological parameters and antigenic expression in B-ALL

Case No	Age	Sex	Hb	TLC	PLT	Clinical diagnosis	Immunophenotypic expression of blasts	Diagnosis
1.	5yrs	F	5.7	133.7	175	Acute leukemia	CD10+/CD19+,CD22+/CD34+ CD20+	B-ALL
2	12yrs	F	8	28.55	38.0	ALL	CD10+/CD19+,CD20+, CD34+	B-ALL
3	52yrs	M	6	50	20	?Acute leukemia	CD10+, CD19+, CD22+,Tdt^{w+}	B-ALL
4	3yrs	F	5.9	28.9	88	ALL	CD10+/CD19+, CD22+/CD34+	B-ALL
5	36yrs	M	10.2	91.8	63	AML	CD10+/CD19+,CD13+, CD33+,CD117-,CYCD22^{W+}	B-ALL with co-expression CD13 & CD33
6	6yrs	M	5.9	11.7	155	Pancytopenia	CD10+/CD19+,CD22+	B-ALL
7	11yrs	M	8.9	14.24	165	?Histiocytosis	CD10+/CD19+ CD45+,CD34,	B-ALL
8	17yrs	F	12.1	152	6	NHL/ALL	CD10+/CD19+	B-ALL
9	23yrs	M	6.1	204	166	Acute leukemia	CD10+/CD19+,CD22+/CD34+	B-ALL,CALLA+
10	54yrs	M	7.8	110.87	52	?AML	CD10+/CD19+,CD13+CD33+CD34+	B-ALL
11	5yrs	M	7.2	31.6	25	Acute leukemia	CD10+/CD19+,CD22+	B-ALL,
12	5yrs	M	7.2	31.6	25	Acute leukemia	CD10+/CD19+,CD22+	B-ALL,
13	9yrs	M	6.8	3.84	31	?Acute leukemia	CD10+/CD19+,CD22+/CD34+	B-ALL
14	10yrs	M	7.4	39.64	19	?Acute leukemia	CD10+/CD19+, CD13+, CD22+/CD34+	B-ALL with co-expression of CD13
15	38yrs	M	9.8	50.6	48	Acute leukemia	CD45+,CD34,CD10+/CD19+	B-ALL
16	42yrs	M	3.7	33.88	143	Acute leukemia	CD10+/CD19+,CD13+,CD33+,C22+/CD34+	B-ALL
17	16yrs	M	11yrs	10.65	41	?acute leukemia	CD10+/CD19+, CD13+, CD33+, CD22+	B-ALL with co-expression of CD13, CD33
18	4 yrs	M	9.4	10.9	37	?ALL	CD10+/CD19+/CD33+	B-ALL with co-expression of CD33
19	17 yrs	F	7.0	56.97	111	?ALL	CD10+/CD19+,CD22+/CD34+	B-ALL
20	56 yrs	F	3.0	18.54	5	Acute leukemia	CD10+/CD19+,CD22+/CD34+	B-ALL
21	16 yrs	M	8.1	621.67	33	?ALL	CD19+,CD34+,CD10-,CD34,CD45	B-ALL, CALLA-
22	11 yrs	M	12.2	304.2	307	?ALL	CD45+,CD34,CD10+/CD19+,	B-ALL
23	15yrs	M	3.6	299.2	158	Acute leukemia	Cd10+/CD19+,CD33+,CD34+	B-ALL with co-expression CD33
24	55yrs	M	3.9	121.1	33	?ALL	CD10+/CD19+,CD22+/CD34+ CD20+, CD45+	B-ALL
25	15yrs	F	4.2	193	48	?ALL	CD10+/CD19+, CD22+/CD34+	B-ALL
26	14yrs	M	6.2	62.5	94	ALL	CD45+,CD34,CD19+, CD22+	B-ALL
27	12yrs	F	7.5	60	15	? ALL	CD10+/CD19+,CD22+/CD34+, CD20+/CD117+	B-ALL
28	33yrs	F	6.1	118.2	14	Acute leukemia	CD10+/CD19+, CD22+,CD34,CD45	B-ALL
29	44yrs	M	5.5	44.9	89	?AML	CD10+/CD19+, CD22+/CD34+	B-ALL
30	57yrs	M	3.5	14.34	155	?acute leukemia	CD19+, CD34+, 13% CD33+	B-ALL with co-expression CD33
31	17yrs	M	7.3	19.91	95	Acute leukemia	CD19+, CD22+/CD34+, CD33+	CALLA-, B-ALL
32	22yrs	M	6.7	13	162	?ALL	CD10+/CD19+,CD22+, CD20+,	CALLA+, B-ALL
33	16yrs	M	7.9	133.9	41	ALL relapse	CD10+/CD19+, CD22+, CD20+	B-ALL
34	31yrs	M	2.9	42.8	13	?CML-BC	CD10+/CD19+,CD22+/CD34+	B-ALL
35	38yrs	M	3.95	139	5	Acute leukemia	CD10+/CD19+, CD22+,CD34+	B-ALL, CALLA+
36	16yrs	M	8.0	90	40	?ALL	CD10+/CD19+,CD33+,CD34+	B-ALL
37	34yrs	M	11.23	3.64	14	Acute leukemia	CD45+,CD34,CD19+, CD34+,	B-ALL
38	21yrs	M	7.28	242	7	?Acute leukemia	CD20+/CD19+, CD13+,CD45	B-ALL
39	33yrs	M	9.1	19.1	98	ALL/Burkitts Lymphoma	CD20+/CD19+, CD34+,CD45	B-ALL
40	43yrs	M	13.3	41.8	20	Acute leukemia	CD19+, CD34+,CD45	B-ALL
41	31yrs	F	12.4	171	43	?acute leukemia	20+/CD19+, CD22+ CD34+	B-ALL
42	52yrs	M		55	29	?Acute leukemia	CD13+, CD33+, CD2+, CD7+,cyCD22+	B-ALL
43	51yrs	M	27.1	6.8	9	?acute leukemia	CD10+/ CD19+, CD22+CD33+	B-ALL with co-expression of CD33
44	45yrs	F	7.9	167.5	22	Acute leukemia	CD10+/CD19+, CD22+	B-ALL
45	45yrs	M	4.6	5.7	26	ALL	CD10+/CD19+ CD34+	B-ALL
46	7yrs	M	3.9	7.3	36	ALL	10+/CD19+,CD22+ CD34+	B-ALL
47	5yrs	M	7.0	33.68	22	?ALL	CD10+/CD19+, CD22+/CD34+	B-ALL
48	4yrs	M	9.1	62.1	96	?ALL	CD19+,CD22+,CD34+,CD13+, CD33+	B-ALL
49	23yrs	M	3.95	56.97	131	AML-M7	CD10+/CD19+,CD13+, CD34+CD33+,CD117+,CD22+	B-ALL
50	5yrs	M	11	16	140	FUC ALL	CD10+/CD19+ CD34+	B-ALL
51	26yrs	M	4.1	299.2	34	?ALL	CD10+/CD19+, CD34+, CD33+	B-ALL
52	20yrs	F	4	48.5	16	?ALL	CD10+/CD19+ CD34+	B-ALL
53	5yrs	F	6.7	3.2	68	?acute leukemia	CD10+/CD19+ CD34+	B-ALL
54	40yrs	M	10.7	60	362	ALL on chemo	CD10+/CD19+, CD34+, CD22+	B-ALL
55	18yrs	M	6.4	694.9	194	ALL	CD10+/CD19+ CD34+	B-ALL
56	21yrs	M	7.3	73.9	280	ALL relapse	CD10+/CD19+ CD34+	Relapsed B-ALL
57	19yrs	M	4.3	7.2	205	ALL	CD10+/CD19+ CD34+	B-ALL
58	6yrs	M	5.3	46.78	175	?acute leukemia	CD10+/CD19+ CD34+	B-ALL, CALLA+
59	6yrs	M	7.0	7.62	84	?acute leukemia	CD10+/CD19+, CD34+	B-ALL, CALLA+
60	5yrs	M	7.0	53.7	291	Acute leukemia	CD10+/CD19+ CD34+	B-ALL,CALLA+
61	21yrs	M	6.8	81.1	245	ALL	CD10+/CD19+ CD34+	B-ALL, CALLA+
62	56yrs	M	5.5	652.1	378	?CLPD	CD10+/CD19+,CD13+, CD33+	B-ALL
63	15yrs	M	6.3	96	7	Lymphoma	CD10+/CD19+ CD34+	B-ALL
64	17yrs	M	8.9	15.16	224	?Acute leukemia	CD10+/CD19+ CD34+	B-ALL
65	26yrs	M	6.2	19	198	?leukemia	CD10+/CD19+ CD34+	B-ALL
66	13yrs	M	8.2	93.2	149	Leukemia relapse	CD10+/CD19+ CD34+	Relapsed B-ALL

Table 2 Continue.....

Case No	Age	Sex	Hb	TLC	PLT	Clinical diagnosis	Immunophenotypic expression of blasts	Diagnosis
67	14yrs	M	7.6	46.8	184	Acute leukemia	CD10+/CD19+ CD34+	Relapsed B-ALL
68	18yrs	M	6.1	10.1	52	?ALL	CD10+/CD19+ CD34+	B-ALL
69	8yrs	M	8	140	10	Acute leukemia	CD10+/CD19+ CD34+	B-ALL
70	3yrs	M	2.0	30.52	16	?ALL	CD10+/CD19+ CD34+	B-ALL
71	6yrs	F	9.1	103.1	79	ALL completed therapy? relapsed	CD10+/CD19+ CD34+	B-ALL
72	3yrs	M	5.3	30.35	21	?Acute leukemia	CD10+/CD19+ CD34+	B-ALL
73	2yrs	M	8.6	77.6	70	?Acute leukemia	CD10+/CD19+ CD34+	B-ALL

Table 3: Hematological parameters and antigenic pattern in T-ALL

Case No	Age	Sex	Hb	TLC	PLT	Clinical diagnosis	Immunophenotypic expression of blasts	Diagnosis
1	21 yrs	F	12.7	70	89	? AM,? NHL	CD2+,CD7+,CYCD3+	T-ALL
2	40 yrs	M	9.7	121	179	?ALL	CD3+/CD5+,CD2+,CD7+,CD34+	T-ALL
3	8 yrs	M	-	FNA of Lymph node	-	?Acute leukemia	CD2+,CD7+,CD37+CD5-CD4-/CD8-	T-ALL
4	14yrs	M	11.7	102.3	170	ALL	CD2+, CD7+, CD5+/CD3+, cyCD3+, TdT^{w+}	T-ALL
5	21yrs	M	-	FNA of Mediastinal mass	-	? NHL	CD2+, CD7+, CD3, CD13+, CD33+,	T-ALL
6	76yrs	F	7.4	532	55	?Leukemia	CD7+, CD2+, CD5+, CD3+,CD4+, CD8-, CD34-, Tdt+	T-ALL
7	8yrs	F	-	FNA of Mediastinal mass	-	Lymphoma	CD2+, CD7+, CD4+, CD5+, CD8+CD34+	T-lymphoblastic lymphoma/ T-ALL
8	26yrs	M	5.8	3.95	490	?AML	CD2, CD7, CD5, CD4, CD8,CD13, CD33, CD34	T-ALL
9	18 yrs	M	7.5	674.79	163	?Lymphoma/leukemia	CD2+, cytCD3+, CD5+, CD7+, CD4/CD8+, CD34+	T-ALL
10	8yrs	F	-	Cervical lymph node	-	?Lymphoma	CD7+, CD5+/CD34+, Tdt+	T-ALL
11	18yrs	M	8.1	106.11	41	?Acute leukemia	CD2+, CD7+, CD5+/CD3+, CD4+/CD8+	T-ALL
12	8yrs	F	10	10.6	44	Acute leukemia	CD2+, CD7+, CYCD3+	T-ALL
13	16yrs	M	6.0	290	25	Acute leukemia	CD2+,CD7+,CYCD3+	T-ALL
14	14yrs	M	9.7	196	154	Anterior .Mediastinal mass	CD7+, CD3+, CD5+,CD4+/CD8+,Tdt+,CyCD3+	T-ALL
15	14yrs	M	9.7	196	154	Anterior .Mediastinal mass	CD7+, CD3+, CD5+,CD4+/CD8+,Tdt+,CyCD3+	T-ALL
16	6yrs	M	6.9	357.17	51	?Acute Leukemia	CD2+, CD7+, cyCD3+, CD13+, CD33+	T-ALL, with co-expression myeloid markers
17	14 yrs	F	10.0	356.35	77	Acute leukemia	CD45+,CD34+,CD2+, CD7+	T-ALL
18	13yrs	M	9.1	114.72	121	?ALL	CD45+,CD34+,CD2+, CD7+, CYCD3+	T-ALL
19	11yrs	M	4.7	848.7	90	?ALL	CD45+,CD34CD2+, CD7+	T-ALL
20	10yrs	F	7.9	100.17	45	?Acute Leukemia	CD2+, CD7+, cyCD3+, CD13+,	T-ALL, with co-expression CD13

M=Male, F=Female, CD=Cluster of differentiation, AML=Acute myeloid leukemia, ALL=Acute lymphoblastic leukemia, Yrs=years, HB: Hemoglobin, PLT: platelet, TLC: Total Leukocyte count; Hemoglobin: gm/dl, TLC: $X10^3$/cumm, Platelet: $X10^3$/cumm

4. Discussion

In modern era of medicine, when exact diagnosis is needed to manage the patient & also to explain prognosis, immunophenotyping is very useful for acute leukemia [4]. It has diagnostic accuracy of almost 99 %. It can type acute leukemia into AML & ALL and ALL is further subclassified into B-ALL and T-ALL. AML is the second most common type of acute leukemia diagnosed in both adults and children**,** commoner in adults that are also confirmed in this study (Fig. 2). Further subtyping of AML into seven FAB subtypes is possible with the help of flowcytometer and cytochemical MPO (Fig. 3-11, 13) [13]. There are four important methods of diagnosis in acute leukemia i.e. morphology, cytochemistry, cytogenetic & immunophenotyping [5]. Each one has got diagnostic & prognostic importance but obviously immunophenotyping is the best amongst these [10, 16, 22]. It is not possible to differentiate FAB AML-MO, ALL & AML-M7 by morphology & cytochemistry because apart from morphological similarities, undifferentiated myeloblasts and megakaryoblasts are negative for MPO & SBB. ALL is the most common type of acute leukemia diagnosed in both adults and children, commoner in children. PAS staining is very useful in delineating lymphoblasts but further subtyping in T-lymphoid and B-lymphoid is possible by immunophenotyping only [31,37].

Now, we can do immunophenotyping in these cases & we have specific markers for particular lineage i.e. CD13 CD33, CD117 & MPO for myeloblasts , CD10, CD19, CyCD22 for B-ALL, CD2, CD7 & CyCD3 for T-ALL ,CD41 & CD61 for AML-M7.Small megakaryoblasts morphologically mimic lymphoblasts, so Immunophenotypic studies are needed to differentiate [1,14,15]. In the diagnosis of AML-M4 & AML-M5, Flowcytometric studies have got definitive role, positivity of the blasts for CD14 can type AML-M4 & AML-M5 (monocyte, promonocyte and monoblast express CD14 antigen), nonspecific esterase

(NSE) staining for monocytes & monoblasts did not give satisfactory result. In some cases of AML-M6, erythroblasts could mimic lymphoblasts, thus immunophenotyping for antigen glycophorin A & hemoglobin A is needed [23,24,26]. Whenever in particular case , antigens of more than one lineage is present in almost equal number & concentration on same population of cells, we can diagnose the case by using specific markers for particular lineage & scoring system is also used to make final diagnosis i.e.cyCD22 or CD79A, cyCD3 & anti-MPO are specific for B-lymphoblasts, T-lymphoblasts, & myeloblasts respectively [8,11]. Cytogenetic studies for Philadelphia chromosome and other chromosomal abnormalities, and molecular marker t(BCR:ABL) are useful for prognosis determination in cases of ALL and t(15;17) is diagnostic in APML, Cytogenetic and molecular studies are useful for classification of acute leukemia as per WHO 2008 classification. Histopathology and immunohistochemistry of lymph nodes and bone marrow biopsy could be useful in diagnosis of selected cases of acute leukemia.

5. Conclusion

Acute Leukemia is major health problem in North India with prevalence of all subtypes. Although, accurate diagnosis is not possible without immunophenotyping but this diagnostic modality is very costly. Prior to establishing antibody panel, morphological and cytochemical studies of peripheral blood and/or bone marrow will definitely help in decreasing the number of antibodies, thus cost of the test. In every suspected acute leukemia case, MPO -Cytochemistry can be used in place of monoclonal MPO antibody. PAS is also useful.

In the diagnosis & prognostication, Flowcytometric studies have got immense importance. *Acute lymphoblastic leukemia* (ALL) is the most common type of acute leukemia and commoner in younger children and has better prognosis than AML. Differentiation into B-lymphoblasts & T-lymphoblast has prognostic significance, because T-ALL has worse prognosis than B-ALL. Here again role of immunophenotyping is well documented. In B-ALL, positive expression of CD10 and CD19 is enough for diagnosis in morphologically suggestive cases. Positivity of CD34 along with CD19 is needed when CD10 is negative provided negative markers for T-lymphoid and myeloid lineages are present. In CML-BC, MPO cytochemistry has no role to play because myeloblasts are either negative or show dim positivity. CML-BC myeloid is commoner than CML-BC lymphoid. CD34 expression is more intense & commoner in more immature blasts, both in ALL & AML. In AML-MO, blasts show positivity for CD34 but negative for cytochemical MPO.

As far as diagnosis and sub classification of acute leukemia are concerned, immunophenotyping is very reliable diagnostic method. It is essential for diagnosis of undifferentiated acute leukemia: minimally differentiated AML, subtyping of ALL into B-ALL and T-ALL, BAL and also useful for detection of MRD. In developing country like India, especially in north India where financial constraint is major factor, judicious use of antibodies with cytochemistry and morphology, would definitely help in cutting down the cost of flowcytometric tests and thus would encourage more and more tertiary health centre of developing regions in establishing flowcytometric laboratory. The review data of this study is comparable with international published data of acute leukemia. Drawbacks of this study are that cytogenetic and molecular correlations are not done.

Disclosures

The authors have no conflict of interest.

Informed consent was taken from each patient or parents in case of minors and unconscious patient for this original article.

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