

## SPECTRUM OF ACUTE LEUKEMIAS AND ABERRANT MARKERS EXPRESSION BASED ON FLOWCYTOMETRY IN A TERTIARY CARE CENTRE

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### ABSTRACT

**Objective:** To determine frequency of different types of leukemias and aberrant CD markers expression on these types.

**Study Design:** Descriptive study.

**Place and Duration of Study:** Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi from Jul 2015 to Dec 2015.

**Material and Methods:** All peripheral blood and bone marrow samples to confirm the suspicion of acute leukemia with flow cytometric immunophenotyping were included in the study. Cells were stained with lineage specific monoclonal antibodies against cell specific CD markers through lyse wash procedure. Cell acquisition and analysis was done on Cell Quest software in multi parameter flow cytometer. Data was entered in SPSS v 20.0 to determine the frequencies of different types of leukemias and aberrant CD markers expression.

**Results:** Over 6 months, 102 males and 49 females were tested with mean age  $26 \pm 21$  years. Commonest leukemia was AML M2. Among 69 pediatric cases with mean age  $7.4 \pm 5.8$  years, precursor B ALL was commonest. Among 82 adults with mean age  $41.5 \pm 15.7$  years, AML M2 was commonest leukemia. Total 32 cases (18 children and 12 adults) expressed cross lineage aberrant markers, CD13, CD33 and CD7.

**Conclusion:** Aberrant CD markers expression must be kept in mind during lineage assignment of acute leukemias while performing flow cytometric immunophenotyping.

**Keywords:** Aberrant CD markers, Acute leukemia, Flow cytometry, Immunophenotyping.

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### INTRODUCTION

Acute leukemias have traditionally been classified using French-American-British (FAB) classification for acute leukemias. This classification was last revised in 2008 by World health Organization (WHO) and now incorporates not only morphology, but also flow cytometry (FCM), cytochemistry, immunohistochemistry (IHC) and cytogenetics in classifying acute leukemias<sup>1</sup> Flow cytometry has exuberantly helped making more accurate diagnosis through identification of cluster of differentiation (CD) markers on leukemic cells surface<sup>2</sup>. Availability of wide range of multicolor monoclonal antibodies, improved characterization of CD markers on cell surface and cytoplasm according to cell

maturation stages, improved gating strategies and availability of multilaser instruments have all contributed to improved diagnosis, treatment and monitoring of the disease<sup>3-4</sup>.

Flowcytometry contributes in this particular field in many ways. It helps confirming the morphological diagnosis and also defines the maturation stage of the leukemia. It is also capable of identifying aberrant antigens expression, which otherwise cause diagnostic confusion<sup>5</sup>. Many leukemic blasts in addition to lineage specific markers, also express markers of other lineages, so called "aberrant" expression of markers. Presence of absence of these aberrant markers may also be associated with poor or favorable prognosis<sup>6</sup>. This aberrant expression may occur in any lineage, B lymphocytes, T lymphocytes or myeloid blasts and more than markers from one lineage may be expressed on a separate lineage blast<sup>7</sup>.

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In our tertiary care center, we are providing flowcytometric diagnostic services for a wide range of diseases prominent of which are leukemias, lymphomas and primary and secondary immunodeficiency disorders. Patients or samples are referred to our department from all over the country, this includes both from armed forces and civilian personnel. In this study we presented spectrum of leukemias diagnosed as myeloid, T lymphoid or B lymphoid, and their stages thereof. This will help understanding predominant disorders in our region. Also we provide detailed analysis of aberrant expression of CD antigens across different lineages. Knowing what CD markers are aberrantly expressed and onto which lineages is certain to

within 6 hours. All the monoclonal antibodies for staining of cells were procured from Becton-Dickinson (BD) Biosciences, San Jose, CA, USA. Primary panel constituted antibodies against CD3, CD5, CD7 for T lineage cells blasts, CD19, CD20 for B lineage blasts and CD13, CD33, CD117 for myeloid lineage blasts. Additionally it included antibodies against CD45, CD10, CD34 and HLA-DR. Isotype control used was mouse anti IgG1FITC/IgG2PE. Wherever indicated the primary panel was extended up to (secondary panel) antibodies against cytoplasmic (cyt) CD3, CD4, CD8, myeloperoxidase (MPO), terminal deoxynucleotidyl transferase (TdT), CD79, CD41, CD61 and glycophorin. All these monoclonal antibodies were labeled with either of fluorescein isothiocyanate (FITC), phycoerythrin (PE) or

**Table-I: Total number of leukemia cases and their distribution.**

S No.	Leukemia	Pediatric	Adults	Total
1	AML M0	1	1	2
2	AML M1	2	0	2
3	AML M2	12	28	40
4	AML M3	7	13	20
5	AML M4	4	12	16
6	Precursor B ALL	20	13	33
7	Pre B ALL	14	3	17
8	Mature B ALL	0	2	2
9	T ALL	3	6	9
10	MPAL	6	4	10
	Total	69	82	151

improve diagnostic accuracy.

## **MATERIAL AND METHODS**

This descriptive study was carried out, from Jul 2015 to Dec 2015 in Immunology Department of Armed Forces Institute of Pathology, after approval by institutional ethical committee. Sampling was done with non-probability consecutive sampling. Total 151 (102 males and 49 females) peripheral blood or bone marrow samples referred to us for immunophenotyping of acute leukemias were included in the study, regardless of age and gender.

Samples for immunophenotyping were received in EDTA bottles and were processed

peridone chlorophyll protein (PerCP). Staining of cells was done with standard lyse wash procedure according to manufacturer's instructions. The stained samples were analyzed on BD FAC Scalibur flowcytometer using Cell Quest software provided by the manufacturer. At least 10,000 cells were selected for analysis through forward scatter side scatter (FSc/SSc) gating technique. The expression/ absence of different CD markers was determined by quadrant application using isotype control. Cell populations were defined as positive for a particular CD marker if more than 20% of the leukemic blasts events were above isotype control threshold, otherwise they were defined negative.

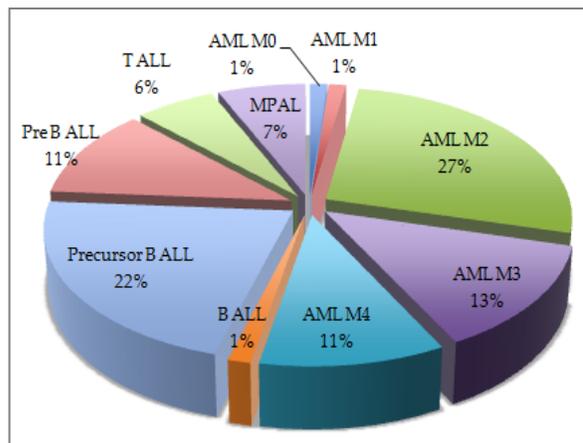
The percentage of abnormal cells with or without expression of analyzed CD markers was entered in Statistical package for Social Sciences (SPSS) version 20.0. These were then analyzed for frequencies and percentages of acute myeloid leukemia (AML) subtypes (ranging from M0 to M7), acute lymphoblastic leukemia (ALL) subtypes including both B and T lymphocytes ALL, and multi phenotypic acute leukemias (MPAL) and aberrant CD markers expression. Frequency and percentage of each individual leukemia and subtypes were calculated. These were also individually calculated for pediatric and adult age groups. Expression of aberrant markers CD13, CD33 and CD7 was calculated in each leukemia and age group.

**RESULTS**

Over a course of 6 months of study period, we received a total of 151 samples (either peripheral blood or bone marrow) for immunophenotyping of acute leukemias. This included 102 males (67.5%) and 49 females

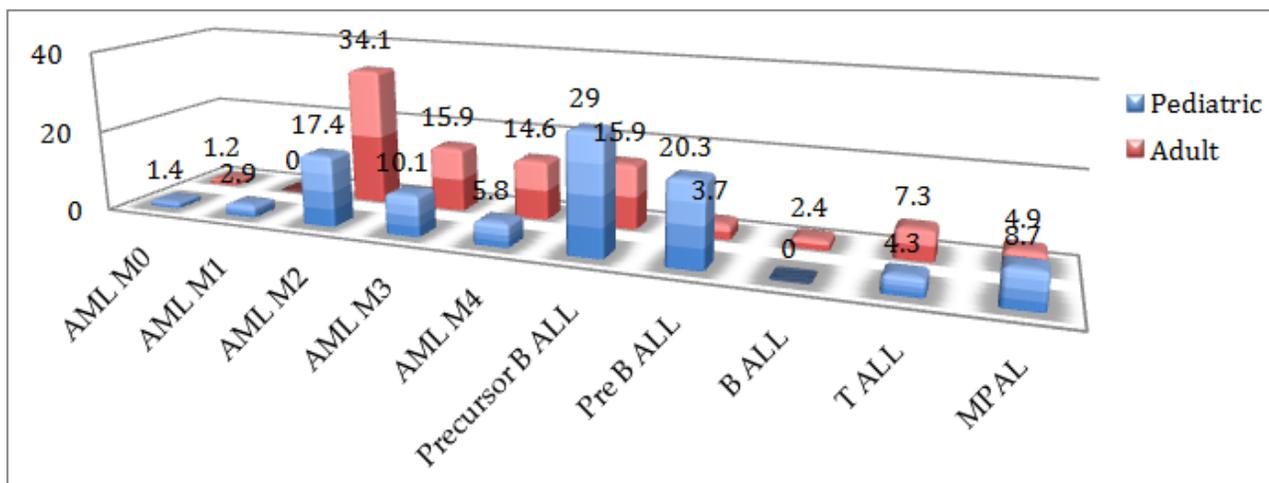
analysis, subjects were divided into pediatric (age at or below 18 years) and adult (age above 18 years) groups containing 69 and 82 subjects respectively<sup>8</sup>.

Among the pediatric 69 cases, mean age was



**Figure-1: Percentage distribution of leukemias among all patients (n=151).**

7.4 ± 5.8 years with mean blast percentage 69.5 ± 23.7. Among adult 82 cases, mean age was 41.5 ±



**Figure-2: Frequency distribution of leukemias among pediatric (n=69) and adult (n=82) age groups, given in percentage of total.**

(32.5%) with mean age 26 ± 21 years. table-I shows total number of leukemia cases identified and their distribution according to leukemia subtypes. Fig-1 shows percentage distribution of leukemias among all subjects indicating that AML M2 was the most commonly found followed by Precursor B ALL For further

15.7 years with mean blast percentage 73.5 ± 18.5. Fig-2 gives frequency distribution of different types of leukemias according to pediatric and adult age groups, given in percentages. Precursor B ALL is the predominant leukemia in children (20 cases of 69 total, 29%) with AML M2 being its analogue in adults (28 cases of 82 total, 34%).

No case of mature B ALL in children and AML M1 was detected in adults. Another interesting finding was occurrence of 6 cases of multiphenotypic acute leukemia (MPAL) in children (8.6%) and 4 cases in adults (4.9%), constituting a total of 10 cases (6.6%).

Table-II gives aberrant markers found and their distribution. Myeloid markers CD13 and CD33 were aberrantly (either together or separately) expressed in 21 cases of precursor B ALL and 1 case of T ALL (only CD33). CD7 was only aberrantly expressed lymphoid marker in 1, 2 and 7 cases of AML M0, M1 and M2 respectively. In total 18 children and 12 adults expressed aberrant markers constituting 32 cases

did not give distribution in separate age groups in their 100 studied patients<sup>10</sup> Koju et al have also determined ALL to be more common but when analyzed separately, AML (with maturation, M2) is more common in adults and ALL in children, in conjunction with our findings<sup>11</sup>. The difference might be due to the fact that our study population consisted of more adults than children (82 vs 69). Consensus is that AML M2 and precursor B ALL are the predominant leukemias in adults and children respectively.

We also determined that 21.2% cases express at least one aberrant marker, CD13, CD33 or CD7. Khurram et al<sup>10</sup> found 26% cases aberrantly expressed either of CD7, CD19, CD4, CD2, CD13,

**Table-II: Expression of aberrant markers and their distribution.**

S No.	Leukemia (number of cases with aberrant expression)	Aberrant marker	Pediatric group (n=69)	Adult group (n=82)	Total (n=151)
1	Precursor B ALL (21)	CD13+CD33	4	7	11
		CD13	6	1	7
		CD33	3	0	3
2	T ALL (1)	CD33	0	1	1
3	AML M0 (1)	CD7	0	1	1
4	AML M1 (2)	CD7	2	0	2
5	AML M2 (7)	CD7	3	4	7
	Total (32)		18 (26.1%)	14 (17.1%)	32 (21.2%)

(21.2%) of total 151.

## DISCUSSION

Flowcytometry has become an indispensable tool in diagnosing acute leukemias, in conjunction with morphology, cytochemistry and cytogenetic analysis. It helps determining not only type of blasts/abnormal cells but also their maturational stage. Determination of different types of CD markers has made tremendous contribution towards better classification of leukemias both for diagnosis and prognosis. Deviations from normal CD markers expression has become possible using flowcytometry.<sup>9</sup> We have determined that AML M2 is the dominant leukemia, however, when taken separately, it is the third commonest leukemia in children, preceded by precursor and pre B ALL. Khurram et al studied B ALL as the commonest but they

CD117, CD11c, CD14. Koju determined 37.6% cases with aberrant expression although they did not mention exactly which CD markers were involved<sup>11</sup> Seegmiller did an extensive study of only B ALL cases with an exhaustive panel of monoclonal antibodies. They determined that over 86% and 9% cases of B ALL express myeloid and T lymphocytes associated antigens respectively<sup>12</sup>. This finding is quite different from our (and other author's) findings where only 26% cases of B ALL express aberrant markers; however, possible reason appears to be an exhaustive antibody panel application. Khawaja et al studied only T ALL and determined that around 10% cases cross-expressed CD13 and CD33<sup>13</sup>. In our study only 1 case (of total 9) aberrantly expressed CD33. Naghmana have also determined that 38% of ALL cases express cross lineage markers

including CD7, CD5, CD3, CD19 and Tdt on myeloid leukemias and CD13, CD33, CD14 and MPO on lymphoid leukemias<sup>14</sup>. Similarly, Anupam et al have found that around 60% cases express aberrant CD markers and CD7 is most commonly aberrantly expressed in myeloid leukemias while CD117 is the commonest aberrant CD marker in lymphoid leukemias<sup>15</sup>. Thus it is determined that a significant percentage (21.2% in our study) of leukemias express aberrant markers like CD13, CD33 and CD7 and those determined by others. The difference in findings is expected due to different panels of monoclonal antibodies used both in primary and secondary panels.

### CONCLUSION

AML especially M2 variant is the dominant kind of leukemia in adult population. Its counterpart in pediatric population is Precursor B ALL. Almost all kinds of leukemic cells may express cross lineage markers termed aberrant CD markers expression. This fact must be kept in mind while performing multiparameter flow cytometric immunophenotyping to avoid inaccurate lineage assignment. Wherever in doubt, monoclonal antibodies panel must be extended with different lineage specific CD markers.

### CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

### REFERENCES

- Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A et al. The 2008 Revision of the World Health Organization (WHO) Classification of Myeloid Neoplasms and Acute Leukemias: Rationale and Important changes. *Blood* 2009; 114(5): 937-51.
- Patel K, Lotodo T, Njuguna F, Emonyi W, Mining S, Buziba N. Use of Flow Cytometry Immunophenotyping for Diagnosis of Acute Leukemia at Moi Teaching and Referral Hospital, Eldoret, Kenya. *American Scientific Research Journal for Engineering, Technology and Sciences* 2015; 13(1): 72-80.
- Jenning CD, Foon KA. Recent Advances in Flow Cytometry: Application to the Diagnosis of Hematologic Malignancy. *Blood* 1997; 90(8): 2863-92.
- Peters JM, Ansari MQ. Multiparameter flow cytometry in the diagnosis and management of acute leukemia. *Arch Pathol Lab Med* 2011; 135(1): 44-54.
- Yuan TT, Liu YR, Chang Y, Hao L, Wang YZ, Jiang B et al. Characteristics of T-cell Lineage Immunophenotype in 95 Patients with Acute Lymphoid Leukemia 2011; 19(5): 1134-40.
- Alhan C, Westers TM, van der Helm LH, Eeltink C, Huls G, Witte BI et al. Absence of Aberrant Myeloid Progenitors by Flow Cytometry is Associated with Favorable Response to Azacitidine in Higher Risk Myelodysplastic Syndromes. *Cytometry Part B (Clinical Cytometry)* 2014; 86B: 207-15.
- Khurram MM, Jafri SA, Mannan A, Nadeem A, Jamal A. Frequency of Aberrant Expression of CD Markers in Cases of Acute Leukemia. *Med J Islamic World Acad Sci* 2010; 18(2): 55-60.
- World Health Organization. Definition of key terms. consolidated ARV guidelines 2013. Available at: <http://www.who.int/hiv/pub/guidelines/arv2013/intro/keyterms/en/>.
- Craig FE, Foon KA. Flow cytometric Immunophenotyping for Hematologic Neoplasms. *Blood* 2008; 111(8): 3941-67.
- Khurram MM, Jafri SA, Mannan A, Nadeem A, Jamal A. Frequency of aberrant expression of cd markers in cases of acute leukemia. *Med J Islamic world Acad Sci* 2010; 18(2): 55-60.
- Koju S, Sachdeva MUS, Bose P, Varma N. Spectrum of acute leukemias diagnosed on flow cytometry: analysis from tertiary care centre from north india. *ACCLM* 2015; 1(1): 12-15.
- Seegmiller AC, Kroft SH, Karandikar J, McKenna RW. Characterization of Immunophenotypic Aberrancies in 200 Cases of Acute Lymphoblastic Leukemia. *Am J Clin Pathol* 2009; 132: 940-9.
- Khawaja MR, Allana SS, Akbarali AN, Adil SN, Khurshid M, Pervez S. Flow cytometric and Demographic Analysis of T Cell Acute Lymphoblastic Leukemia in Pakistani Population *J Ayub Med Coll Abbotabad* 2005; 17(4).
- Mazher N, Malik N, Imran A, Chughtai O, Chughtai AS. Aberrant Expression of CD Markers in Acute Leukemia. *Ann Pak Inst Med Sci* 2013; 9(2): 99-102.
- Sarma A, Hazarika M, Das D, Kumar Rai A, Sharma JD, Bhuyan C et al. Expression of Aberrant CD Markers in Acute Leukemia: A Study of 100 Cases with Immunophenotyping with Multiparameter Flowcytometry. *Cancer Biomarkers* 2015; 15 (4): 501-5.