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Clinical, Morphological and Immunophenotypical Findings in Acute Leukemia: A Study from a Tertiary Care Hospital

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Abstract

Background: Acute leukemias comprise a heterogeneous group of diseases characterized by rapid and uncontrolled clonal expansion of progenitor cells of the hematopoietic system. Immunophenotyping helps subclassify acute leukemias into subgroups with prognostic implications.

Materials and Methods: This descriptive observational cross-sectional study was performed on 80 newly diagnosed cases of acute leukemia in children up to 18 years of age from August 2012 to Febuary 2014.
The immunophenotypic analysis was performed by Beckman Coulter Flow cytometer using monoclonal antibodies against various cell surface / cytoplasmic antigens.

Results: Out of 80 cases, 68 cases had acute lymphocytic leukemia (ALL) and 12 cases had acute myelocytic leukemia (AML). In this study, 68 cases of ALL could be categorized into 53 cases of B-ALL, 13 of T-ALL, and 2 cases of Bi-phenotypic acute leukemia (BAL). Twelve cases of AML comprised of 7 cases of AML with minimal differentiation; 2 of AML with cases of AML with maturation, and 3 cases of acute myelomonocytic leukemia. Hepatosplenomegaly was seen in the majority of cases in both ALL and AML. Most cases had a total leucocyte count between 10,000 and 50,000/µl and platelets <100,000/µl. Hemoglobin levels were < 7.5 g/dl in the majority of them. CD19 and CD79a were 100 % sensitive for B ALL, while cCD3 was 100% sensitive for T-ALL. MPO was positive in all cases of AML.

Conclusion: Immunophenotyping must be done in all AL cases as it helps in risk-stratification and follow up of patients for evaluating minimal residual disease.

Keywords: Acute Leukemia, Acute Lymphoblastic Leukemia, Acute Myeloblastic Leukemia, Paediatric Hematological Malignancies

Introduction

Acute leukemia comprises a heterogeneous group of diseases characterized by rapid and uncontrolled clonal expansion of progenitor cells of the hematopoietic system. They are the most common form of childhood neoplasia; acute lymphoblastic leukemia (ALL) represents 75% of all such cases (1). The diagnosis of leukemia is based upon morphology and immunophenotyping of bone marrow samples and/or peripheral blood. In children, vast majority of ALL cases (80-85%) are precursor B-lineage (1,2).A small number of patients whose blasts simultaneously present antigens of myeloid and lymphoid lineages are characterized as carriers of mixed, hybrid or biphenotypic acute leukemia (3,4).Approximately, 6% of ALL cases express two or more myeloid markers. This subtype of acute leukemia is known to respond well to intensive therapy and should, therefore, be treated according to appropriate risk-based protocols (5,6).
The current World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues incorporate not only immunophenotyping but also cytogenetic and molecular characteristics that contribute towards defining biologically and clinically relevant leukemia subsets (7).The present study was undertaken to characterize the different morphological and immunophenotypic subtypes of acute leukemias in children.

Materials and Methods

In this descriptive observational cross-sectional study, 80 newly diagnosed cases of acute leukemia in children up to 18 years of age, over a period of two years, were included. The study was approved by institutional ethical committee as per guidelines by ICMR (Indian Council of Medical Research). The diagnosis was made based on morphology, cytochemistry, and flow cytometry on peripheral blood and/or bone marrow. A detailed clinical history and physical examination were carried out after taking an informed consent. Complete blood count with peripheral smear and bone marrow aspirated were analyzed. Immunophenotypic analysis was performed on 2 ml of peripheral blood/bone marrow aspirate (collected in Heparinised vacutainers) by Beckman Coulter Flow cytometer (FC 500) using monoclonal antibodies against various myeloid and lymphoid cell surface/ cytoplasmic antigens. The samples were processed within four hours of collection. For the immunophenotypic diagnosis of acute leukemias, a combination of three or four fluorochrome-conjugated Monoclonal antibodies (MoAb) per tube was added to the samples.

The blast gating strategy included dot plots of CD45 expression versus intracellular complexity (side scatter angle, SSC). A negative control tube using CD 45 ECD was run with all samples. A total of 100,000 events were acquired in the target gate. After data acquisition, the data were analyzed using onboard CXP Software (version 2.2).

A positive expression of a marker was considered when it was positive in more than 20% of the gated blast population.

Terminal deoxynucleotidyl transferase (TdT) expression was considered positive when ≥10% cells showed posituve expression.

Lineage identification of the blasts was done based upon the surface and /or cytoplasmic antigens expressed by them. Immunophenotypic classification of all acute leukemia cases was established according to EGIL criteria (8,9). Aberrant phenotypes were defined when at least 20% of blast cells expressed the particular aberrant marker. The diagnosis of biphenotypic acute Leukemia (BAL) was made in cases that showed definitive dual – lineage differentiation and fulfilled the EGIL criteria for the same (8,9).

Statistical analysis

Results obtained were analyzed using Student t-test, Chi-square test, and ANOVA. For all statistical tests, p-value less than 0.05 was taken to indicate a significant difference.

Results

Based on morphology and cytochemistry of peripheral blood film and/or bone marrow aspirate, 80 cases of acute leukemia were classified into 68 cases of ALL and 12 cases of acute myloid leukemia (AML). The final diagnosis was based on immunophenotyping, on which 68 cases of ALL could be categorized into 53 cases of B-ALL, 13 of T-ALL, and 2 cases of BAL. Two cases of BAL were Lymphoblastic on morphology and were positive for both B- and T cell markers on immunophenotyping. Twelve cases of AML comprised 7 cases of AML with minimal differentiation; 2 cases of AML with maturation, and 3 cases of acute myelomonocytic leukemia.

The acute leukemia cases age ranged from 2 months to 18 years with the highest number of patients (33 / 80) in the age group of 3-5 years. There were 4 infants in the present study, one with B and T –ALL and 2 with AML. Almost half of the cases of B-ALL (49.1%) were in the age group of 3 to 5 years. T-ALL involved (46.7%) slightly older age group of 6 to 8 years. AML cases frequently occurred (41.5%) in patients aged 3 to 5 years. The male to female ratio was 3.7:1 (63/17). The distribution of cases within the gender was similar between B-ALL and T-ALL. The most common presenting complaint was fever (81.25%). Pallor was universally

present in all cases. Fever was most commonly seen in T-ALL cases (92.3%); whereas, bleeding manifestations were more frequent (66.7%) in AML cases. Lymph node enlargement was much more frequent in T-ALL cases. Mediastinal widening was seen in 4 cases of T-ALL. None of the patients had testicular or CNS involvement at diagnosis.

Anemia, leukocytosis, and thrombocytopenia were common findings in all patients. The reduction in hemoglobin concentration was more pronounced in AML cases than in ALL. Majority were presented with leukocytosis (70%). Almost all cases of AL ~~were~~ presented with thrombocytopenia. The clinical and laboratory findings are summarized in Table I. Twelve cases of AML were classified into 7 cases of AML- M1, 2 cases of AML-M2, and 3 of AML- M4. All cases of AL showed suppression of erythroid and megakaryocytic series.

Immunophenotyping of acute leukemia was done by flowcytometry. Common B-ALL was the most frequent phenotype. The expression pattern of various antigenic markers in the different phenotypes of AL is shown in Table II (Figure 1).

Immaturity markers were expressed more in B-ALL as compared to T-ALL. TdT expression was seen both in B and T-ALL; more frequently in more immature blasts. In AML, HLA DR and CD34 expression were very high, while TdT expression was quite low. Table III shows the expression of immaturity markers in all cases.

Among B-ALL, common B phenotype was most frequent, accounting for 45/53 (56.3%) of cases. There were 3 cases of pro-B-ALL, which were CD19, CD79a, and HLA DR positive and 2 cases showed positivity for CD22 and CD 34. None of the cases showed any aberrant antigen expression. All cases of common-B-ALL were positive for CD19, CD79a, and CD10. There was aberrant myeloid antigen (CD13, CD33) expression in 2 cases. All 4 cases of pre-B-ALL were positive for CD19, CD79a, and CD10. HLA DR was positive 100% cases, while TdT was positive in 50 % cases. Only one case of Mature B-ALL was noted.

CD5, CD7, and cCD3 were positive in all 6 cases of pre T ALL. There was only 1 male child with age of 12 years with pro-T ALL. It showed positivity for cCD3, CD7, HLA DR, and CD34. There was an aberrant expression of CD13, CD 33, and CD79a. All cases of cortical T ALL were positive for cCD3, CD7, CD5, CD2, CD4, and CD8. Three cases showed aberrant expression of CD13, CD79a, and CD117. Only 2 cases of medullary T ALL were noted, both of them were positive for cCD3 and CD7. CD 4 and CD 8 were positive in one case each, respectively. CD 10 positive acute leukemia deserves a special mention as CD10 positivity is associated with a better prognosis. CD10 expression was seen in 57 out of 80 cases of AL. In the present study, there were 92.5% cases with CALLA positive B-ALL; whereas, 46.2% cases of T-ALL showed CD10 expression. The AML cases were more often negative for CD10 expression and did not show any aberrant marker expression. Table IV shows aberrant marker expression in Acute Leukemia.

Table I: Clinical and laboratory findings in cases of AL

Clinical Presentation	B-ALL (53 cases) N (%)	T-ALL (13 cases) N (%)	AML (12 cases) N (%)	BAL (2 cases) N(%)	Total
Fever	40 (75.4)	12(92.3)	12(100.0)	1(50.0)	65
Pallor	53(100.0)	13(100.0)	12(100.0)	2(100.0)	80
lymphadenopathy	38(71.7)	6(46.1)	5(41.6)	0(0.0)	49
Hepatomegaly	45 (84.9)	11(84.6)	10(83.3)	2(100.0)	45 (84.9)
Splenomegaly	42 (79.2)	11(84.6)	10(83.3)	2(100.0)	42 (79.2)
Bleeding manifestations	30 (56.0)	6(46.1)	8(66.7)	0(0.0)	44
Hemoglobin(mean ±SD) (g/dl)	6.66±1.76	8.24±2.26	8.05±0.35	7.33±2.82	
WBC count(mean ±SD) (10^3/µl)	52.78±.94.39	128.64±.147.61	36.55±34.51	85.39±126.11	
Platelet count(mean ±SD)(10^3/µl)	36.18±.50.26	60.31±.38.28	36.18±50.26	29.52±23.67	

Table II: Immunophenotypic profile of 80 denovo AL patients

	B-ALL 53 cases	T-ALL 13 cases	BAL 2 cases	AML M1 7 cases	AML M2 2 cases	AML M4 3 cases
	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
HLA DR	43(81.1)	1(7.6)	1(50.0)	6(85.7)	1(50.0)	3(100.0)
CD34	25(47.2)	0(0.0)	2(100.0)	5(71.4)	2(100.0)	1(33.3)
TdT	25(47.2)	5(38.5)	0(0.0)	1(14.3)	0(0.0)	0(0.0)
CD19	53(100.0)	1(7.6)	2(100.0)	0(0.0)	0(0.0)	0(0.0)
CD10	49(92.4)	6(46.2)	1(50.0)	1(14.3)	0(0.0)	0(0.0)
CD79a	53(100.0)	4(30.7)	2(100.0)	0(0.0)	0(0.0)	0(0.0)
CD22	26(49.06)	0(0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)
sIgM	2(3.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
cCD3	0(0.0)	13(100.0)	2(100.0)	0(0.0)	0(0.0)	0(0.0)
CD7	0(0.0)	13(100.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)
CD5	0(0.0)	11(84.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
CD2	0(0)	8(61.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
CD8	0(0)	5(38.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
CD4	0(0.0)	5(38.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MPO	0(0.0)	0(0.0)	0(0.0)	7(100.0)	2(100.0)	3(100.0)
CD13	2(3.7)	4(30.7)	0(0.0)	5(71.4)	1(50.0)	3(100.0)
CD33	3(5.6)	1(7.6)	0(0.0)	7(100.0)	2(100.0)	3(100.0)
CD117	1(1.8)	2(15.4)	0(0.0)	5(71.4)	1(50.0)	2(66.6)
CD14	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(66.6)
CD15	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100.0)	3(100.0)
CD11c	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(100.0)

Table 3: Immaturity marker expression in AL

	B-ALL 53 cases	T-ALL 13 Cases	BAL 2 Cases	AML M1 7 cases	AML M2 2 cases	AML M4 3 Cases
	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
HLA DR	43(81.1)	1(7.6)	1(50.0)	6(85.7)	1(50.0)	3(100.0)
CD34	25(47.2)	2(15.4)	0(0.0)	5(71.4)	2(100.0)	1(33.3)
TdT	25(47.2)	5 (38.5)	0(0.0)	1(14.3)	0(0.0)	0(0.0)

Table IV: Aberrant marker expression in AL

	CD13	CD33	CD79a	CD117
Common-ALL 3cases	3	2	3	0
Pro T ALL 1 case	1	1	1	0
Cortical-T ALL 3 cases	2	0	3	0
Medistinal- T ALL 1 case	1	0	0	1
AML M1	Only one case showed CD10 positivity			

Table V: Comparison of the published frequency of various AL phenotypes

Study (Sample size)	B-ALL	T-ALL	AML	BAL
Khalil et al[10],1995* (133 cases AL)	54.13	7.5	21	12
Gujral et al[11], 2009 (1151 cases of paediatric AL)	62.3	15.6	20.7	1.1
Supriyadi et al[12],2011 (498 cases AL)	63.65	12.85	23.2	0.2
Patil et al[13],2012* (61 cases ALL)	67.2	32	-	1
Present study (80 cases AL)	66.3	16.3	15.0	2.5

Discussion

Immunophenotypic characterization of leukemic cells has several goals, including lineage assignment, evaluation of cell maturation, and assessment of phenotypic aberrations. A total of 80 patients with acute leukemia were included in this study. Morphological assessment, cytochemistry, and flowcytometry were carried out on the peripheral blood and/or bone marrow aspirate of all cases. B-lineage ALL was most common, accounting for 66.3% of acute leukemia cases. T-lineage ALL and AML comprised 16.3% and 15% of the cases, respectively. The findings were similar to those by other authors. A comparison of the published frequency of various AL phenotypes is presented in Table V. The incidence of BAL was 2.5 % in the current study, which is similar to other authors except for Khalil et al., (10) who found it to be 12% of all AL. In the present study, the mean age of B-ALL patients was 6.3 years and the maximum number of cases were in the age group of 3-5 years, similar to what described by other authors (11-15). The mean age of T-ALL cases was 6.7 years with the peak incidence at 6-8 years as described by other authors (11-15).

Hemoglobin levels were reduced in all cases of AL. In severely anemic cases (Hb<7g %), there was a statistically significant difference between B-ALL and T-ALL cases. The reduction in hemoglobin concentration was more pronounced in AML cases. Leukocytosis was seen in 70% cases of AL, out of which 37.5% cases showed markedly elevated total leukocyte count (TLC > 50,000/µl). Marked leukocytosis showed a significant association (P- value 0.002) with T-ALL than with B-ALL. Similar results were reported by Bachir et al.,(15) Asgarian et al., (16) and Tong et al., (17) Although marked leucocytosis was also seen in 50% of AML cases in the present study, but there was no significant association between leukocytosis and type of AML.. Marked thrombocytopenia was significantly (P value 0.007) associated with B-ALL as compared to T-ALL.

Immunophenotyping of AL

Common- B ALL was the most frequent phenotype, accounting for 56.3% of total AL cases and 68.2% of ALL cases. Similar results were obtained by other authors (17,19). Immunophenotyping of B-ALL revealed CD79a and CD19 to be most sensitive (100%) marker, while CD19 was more specific (88.9%) than CD79a (74.1%) for B-ALL. CD10 showed 92.5 % sensitivity and 70.4% specificity. The findings were similar to those reported by Bachir et al. (15) and Salem et al. (20) Mature-B-ALL constituted only 1.5% of total ALL cases, while most other authors reported higher figures (18,19). Surface Ig (sIg) was expressed only in mature B-ALL case as described by other authors (15-17).

In the present study, there were 7.6% cases of pro T-ALL, 46.2% of pre T-ALL, 30.8% of cortical-T, and 15.4% of medullary T-ALL among T-ALL subtypes. Most authors have not subclassified T-ALL cases probably because of too few numbers. cCD3 and CD7 were found in all cases of T-ALL and were most sensitive (100%), while CD5 was most specific (100%) for T-ALL, which is in line with findings of the study by Bachir et al., (15) In a study by Yusuf et al., (17) CD5 and CD7 showed 100% positivity in T-ALL, while CD3 was found in 2/3 cases (66.6%). Salem et al., (18) found a much lower CD3 positivity in 38.5% cases with T-ALL.

There were 58.3% (7/12) cases with AML M1; 16.7% (2/12) cases with M2, and 25% (3/12) cases with M4 out of 12 AML cases. These frequencies are quite similar to those reported by Khalil et al., (10) MPO was positive in all cases of AML with 100% specificity. CD33 was 100% sensitive and 94% specific while CD13 showed a sensitivity of 75% and specificity of 91.8%. The most specific marker for myeloid lineage apart from MPO in the present study was CD117 (95.6%). However, its specificity was only 66.7%. Kermani et al.,(22) reported the most common marker in AML M1 to be CD13 and CD33. Yusuf et al., (21) found expression of 100% and 90% of CD33 and CD13, respectively, in their AML cases. Kaleem et al.,(23) found CD33 a much more sensitive marker than CD13 for myeloid lineage, but it was less specific than CD13.

HLA DR and CD34 were more frequently expressed in B-ALL and AML as compared to T-ALL. TdT expression was low in all subtypes of AL, which is similar to findings of other authors (11,17,23). Kermani., (22) reported CD34 expression 20%, 48%, and 38% in AML, B-ALL, and T-ALL cases, respectively. Noronha et al.,[24] found the expression of CD34 and HLA-DR 69.2% and 63% in AML, , 91% and 61.2% in B-ALL ,and 54.5% and 10% in T-ALL , respectively.

Lineage heterogeneity

Aberrant expression of myeloid antigens on ALL cells is a well-documented phenomenon, and has no prognostic or therapeutic implication but can be used for monitoring minimal residual disease (MRD). Eight cases out of 80 cases showed lineage heterogeneity. Aberrant marker expression was more commonly seen with T-ALL (38.46%). CD79a was the most commonly expressed aberrant marker on T-

ALL (38.5%) followed by CD13, CD10, and CD117. Three cases of common B-ALL showed aberrant myeloid markers. CD33 was expressed in all three cases and CD13 in 2 cases. Bachir et al., (15) reported My Ag expression in 52.7% of cases with childhood ALL. The most common marker was CD13. In this study, 3.3% cases expressed aberrant lymphoid antigens, 2 T-ALL cases expressed B cell markers CD19 or CD22, and 7 B-ALL cases expressed T Cell Ag CD7; two of which also showed a ~~third~~ myeloid phenotype expressing CD13 and CD33 also along with CD7. Salem et al., [20] reported aberrant expression of CD33 in 10.5% cases and CD13 in 7.9% cases of B-ALL. Only 1/13 cases (7.7%) of T-ALL cases showed CD33 expression and none expressed CD13. CD10 was expressed by 23.1% of T-ALL cases. **A**berrant expression of lymphoid antigen in AML cases was seen in only one case of AML-M1, which was positive for CD10. In the study by Salem et al.,(20) CD7 was most commonly expressed (23%) followed by CD4 (14.2%) cases. Kaleem et al., (23) also reported CD7 as being the most frequent antigen expressed in AML cases. They reported a very low expression of CD10 (0.9% cases). Gujral et al.,(11) found aberrant expression of CD7, CD4, and CD19 in AML; CD10 and CD13 in T-ALL, and CD23 in B-ALL. Noronha et al.,(24) reported 48.4% B-ALL cases with aberrant phenotypes and 36.4% aberrant phenotypes with T-ALL. CD13 was the most frequent aberrant marker. AML showed aberrant phenotypes in 27% of cases, and the expression of CD7 occurred most frequently. In a study by Patil et al.,(13) 50% of My Ag+ ALL expressed CD13 and CD33. CD14 was expressed in 25% and CD117 in 16% of cases. About 2% of their B-ALL cases co-expressed T lymphoid antigens and 5% of T-ALL cases expressed aberrant B lineage markers.

In the present study, 4 cases of ALL expressed antigens of all three lineages, but did not satisfy the EGIL criteria for the triphenotypic AL. Gujral et al., [11] described a case of triphenotypic AL in their study, which expressed MPO, cCD22, cCD3, CD33, and CD7.

There were two cases of BAL (2.9% of ALL cases), both co-expressing antigens of B and T lymphoid lineages. This case co-expressed myeloid and B-lymphoid antigens. Khalil et al., (10) reported a very high incidence of BAL, 12.0%, probably because they did not strictly follow the EGIL criteria.

Conclusion

Immunophenotyping by FCM must be done in all AL cases as it not only helps in risk-stratification and prognostication but also in planning adequate treatment strategy and follow up of patients especially in evaluating minimal residual disease.

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Conflict of interest

The authors declare no conflict of interest.

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