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Tumor Discovery

ORIGINAL RESEARCH ARTICLE

Clinicohematological profile and immunophenotypic patterns of childhood acute leukemia: Prognostic correlation

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Abstract

Acute leukemia (AL) presents a heterogeneous molecular profile, requiring precise diagnostic categorization and subcategorization. The present study aims to estimate the clinicohematological profile and immunophenotypic pattern of childhood AL while conducting prognostic assessments. This cross-sectional study analyzed a total of 68 samples of AL collected from January 2019 to June 2021. The male-to-female ratio was 4.6:1, with a mean age of 6.6 ± 3.4 years. Total leukocyte count (TLC) was significantly increased in all types of AL (P = 0.03). The median value (interquartile range) of TLC ($\times 10^6$/dL) was 8,450 (4,100 – 27,950), with blast counts in peripheral smears at 59 (24 – 80), and in bone marrow aspirates (BMAs) at 95 (75 – 98). There was a significant association ($P < 0.001$) and a strong association (C = 0.9110) between the morphology of BMA with immunophenotype. Based on immunophenotype, AL was categorized into four groups: B-cell acute lymphoblastic leukemia (B-ALL) (51.5%), T-cell acute lymphoblastic leukemia (T-ALL) (10.3%), AML (22%), and mixed phenotype AL (MPAL) (16.2%). Furthermore, eight subgroups were identified: B lineage, Ia-Common-B-ALL (88.6%) and Ib-Pre-B-ALL (11.4%); T-lineage, IIa-Cortical T-ALL (71.4%) and IIb-Pre-T-ALL (28.6%); AML subgroups, IIIa-M2 (73.93%) and III-M4 (26.7%); and MPAL subgroups, IVa-aberrant expression of myeloid antigens in B-ALL (90.9%), and IVb-aberrant expression of lymphoid markers in AML (9.1%). A poor prognostic immunophenotype (T-ALL, AML) was significantly (P = 0.023) more prevalent in deceased patients with AL. The highest mortality rate was observed in AML (86.4%), followed by T-ALL (57.2%). The most common immunophenotype observed was Common-B-ALL in childhood AL, and a poor prognostic immunophenotype (T-ALL and AML) with the highest mortality rate was found in AML. Thus, knowledge about clinicohematological and immunophenotypic patterns will aid in patient management.

Keywords: Immunophenotype; Aberrant; Acute leukemia; Flow cytometry; Children

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1. Introduction

Childhood acute leukemia (AL), specifically acute lymphoblastic leukemia (ALL), is the most common malignancy observed in children.[1] Various classifications of AL have been introduced over time by literature and the World Health Organization

(WHO).[2] Hematopoietic neoplasm, or AL, exhibits a heterogeneous molecular profile, requiring a more precise diagnostic categorization and subcategorization.[2] Recent classifications of AL are more prognostic-oriented compared to older classification systems, as they are based on immunophenotype, cytogenetics, and molecular typing.[3] Proper classification is instrumental in enhancing patient management.[4]

Immunophenotype-based classifications are more lineage-specific (B-cell ALL [B-ALL], T-cell ALL [T-ALL], and acute myeloid leukemia [AML]) and carry prognostic significance. Therefore, this study has been conducted in specific regions of India. Recently, AL has been confirmed with immunophenotyping using flow cytometry. Occasionally, the asynchronous expression of antigens or antibodies on the cell surface is referred to as an aberrant phenotype, such as the expression of T-cell lineage or B-cell lineage markers in AML or the expression of myeloid lineage markers in T/B ALL.[4] Certain cases cannot be classified as ALL and AML based solely on morphology, cytochemistry, and immunohistochemistry.[5] This phenomenon is due to the co-expression of lymphoid and myeloid immunophenotype markers on the cell surface, or the presence of two distinct cell populations.[6] These cases are diagnosed with the availability of flow cytometry and are labeled as biphenotypic, hybrid, and mixed leukemia.[7]

The aim and objective of the present study are as follows: (i) to determine the immunophenotypic pattern of childhood AL in Delhi-National Capital Region (NCR), (ii) to provide the clinical and hematological profile of the immunophenotype of AL in children, and (iii) to correlate with prognostic outcomes.

2. Methods

2.1. Study design and data acquisition

This retrospective cross-sectional study was performed at a tertiary care institute in Delhi, India. Informed consent was not applicable as the study was retrospective and involved no direct contact with patients. Data were retrieved from the departmental archive.

2.2. Bone marrow aspirate (BMA) and trephine biopsy analysis

Clinical data, BMA, and trephine biopsy slides of pediatric patients diagnosed with AL through bone marrow aspiration, biopsy, and flow cytometry between January 2019 and June 2021 were analyzed. A total of 209 samples of BMA and biopsies were collected from 95 pediatric patients with AL. Of these, 141 remission samples were excluded, and a total of 68 patient samples were analyzed for clinical profile, hematological profile, and immunophenotype. Children with inadequate/suboptimal BMAs and unavailable flow cytometry findings were excluded from the study. All children confirmed with AL through flow cytometry were included in the study.

2.3. Immunophenotyping

Immunotyping through flow cytometry was conducted using the Cytomics FC 500 (Beckman Coulter, USA). Peripheral blood and BMA samples were collected fresh in EDTA (ethylenediaminetetraacetic acid-anticoagulant) vials. The samples were analyzed within 24 h of collection. For most of the cases, a pre-fixed panel of antibodies (CD34, HLA-DR, CD45, CD19, CD20, CD79a, CD10, TdT, CD3, c-CD3, CD4, CD2, CD7, CD8, CD23, CD103, CD38, CD200, CD117, CD13, CD33, MPO, CD64, CD11b, and CD15) was used in conjunction with fluorescein isothiocyanate (FITC [FL1), phycoerythrin (PE [FL2), ECD (FL3), and PC5 (FL5) dyes. The samples were processed as per standard protocols for surface and cytoplasmic antibodies. Results were obtained by gating the blast cells with side scatter (SSC) versus forward scatter followed by SSC versus CD45 gating.

AL, based on immunophenotype, was initially divided into four groups and subsequently into subgroups. Group 1 comprised B-ALL expressing CD19, c-CD79a, and c-CD22, along with variable CD34/HLA-DR, CD10, CD24, and PAX5. Group 2 consisted of T-ALL expressing TdT and variably expressing c-CD3, CD3, CD2, CD5, and CD7. Group 3 included AML, predominantly expressing MPO and variably expressing CD117, CD33, CD13, CD64, and CD15. Group 4 covered mixed phenotypic AL/aberrant immunophenotypic AL antigens. All four groups were correlated with their clinical details, hematological profiles, and prognostic outcomes.

The aberrant immunophenotype was defined as the expression of surface antigen on a leukemic cell that differs from the normal maturation process of the cell lineage.[4] While recent classifications of AL are based on molecular typing, the immunophenotype-based French–America–British (FAB) classification remains robust for patient management. It was feasible for routine practice and cost-effective. The FAB classification includes eight subtypes of AML (M0 – M7) and three subtypes of ALL (L1 – L3) (Table 1).[8]

The 2016 WHO revised classification of leukemia is based on immunophenotypes and molecular characteristics (Table 2).[9]

In addition, demographic profiles, clinical details, hemograms, and flow cytometry analyses of the patients were noted.

Table 1: The French–America–British classification of acute leukemia[8]

AML	ALL
M0 – AML with no Romanowsky or cytochemical evidence of differentiation	L1
M1 – Myeloblastic leukemia with little maturation	L2
M2 – Myeloblastic leukemia with maturation	L3
M3 – APL	
M3h – APL, hypergranular variant	
M3v – APL, microgranular variant	
M4 – AMML	
M4eo – AMML with dysplastic marrow eosinophils	
M5 – AMoL	
M5a – AMoL, poorly differentiated	
M5b – AMoL, differentiated	
M6 – "Erythroleukemia"	
M6a – AML with erythroid dysplasia	
M6b – Erythroleukemia	
M7 – Acute megakaryoblastic leukemia (AMkL)	

Abbreviations: AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; AMML: Acute myelomonocytic leukemia; AMol: Acute monoblastic leukemia; ALL: Acute lymphoblastic leukemia.

2.4. Statistical analysis

Continuous data were reported as mean ± standard deviation (*SD*) for normally distributed variables and as median with an interquartile range (IQR) for skewed variables. Categorical data were reported as percentages. $P < 0.05$ was considered statistically significant. We used STATA 14 software for statistical analysis. All continuous variables were assessed for normal or skewed distribution (*SD* > 40% of mean). For variables with a skewed distribution, the median (IQR) was reported. Comparisons of more than two unpaired groups were performed using the Kruskal–Wallis test, with Dunn's test applied if the Kruskal–Wallis test was significant. All categorical variables (>2 unpaired groups) were assessed using the Pearson Chi-square test to assess significant variability. The contingency coefficient (*C*) was calculated to measure the strength of the relationship between variables, with values ranging from 0 (no association) to 1 (very strong association), where 0 means no association and 1 is a very strong association, which shows the strength of the relationship between variables. SATA 14 software was used for data analysis.

3. Results

Data from a total of 68 patients obtained during the study period were analyzed for clinical profile, hematological

Table 2: 2016 WHO classification of acute leukemia[9]

S. No.	Types of leukemias
1.	AML and related neoplasms
	AML with recurrent genetic abnormalities
	AML with t (8;21) (q22;q22.1); *RUNX1-RUNX1T1*
	AML with in v (16) (p13.1q22) or t (16;16) (p13.1;q22); *CBFB-MYH11*
	APL with *PML-RARA*
	AML with t (9;11) (p21.3;q23.3); *MLLT3-KMT2A*
	AML with t (6;9) (p23;q34.1); *DEK-NUP214*
	AML with inv (3) (q21.3q26.2) or t (3;3) (q21.3;q26.2); *GATA2, MECOM*
	AML (megakaryoblastic) with t (1;22) (p13.3;q13.3); *RBM15-MKL1*
	Provisional entity: AML with BCR-ABL1
	AML with mutated *NPM1*
	AML with biallelic mutations of *CEBPA*
	Provisional entity: AML with mutated RUNX1
	AML with myelodysplasia-related changes
	Therapy-related myeloid neoplasms
	AML NOS
	AML with minimal differentiation
	AML without maturation
	AML with maturation
	Acute myelomonocytic leukemia
	Acute monoblastic/monocytic leukemia
	Pure erythroid leukemia
	Acute megakaryoblastic leukemia
	Acute basophilic leukemia
	Acute panmyelosis with myelofibrosis
	Myeloid sarcoma
	Myeloid proliferations related to Down syndrome
	TAM
	Myeloid leukemia associated with Down syndrome
	Blastic plasmacytoid dendritic cell neoplasm
	Acute leukemias of ambiguous lineage
	Acute undifferentiated leukemia
	MPAL with t (9;22)(q34.1;q11.2); *BCR-ABL1*
	MPAL with t (v; 11q23.3); *KMT2A* rearranged
	MPAL, B/myeloid, NOS
	MPAL, T/myeloid, NOS
2.	B-lymphoblastic leukemia/lymphoma
	B-lymphoblastic leukemia/lymphoma, NOS B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
	B-lymphoblastic leukemia/lymphoma with t (9;22) (q34.1;q11.2); *BCR-ABL1*

(Cont'd...)

Table 2: ***(Continued)***

S. No.	Types of leukemias
	B-lymphoblastic leukemia/lymphoma with t (v; 11q23.3); *KMT2A* rearranged
	B-lymphoblastic leukemia/lymphoma with t (12;21) (p13.2;q22.1); *ETV6-RUNX1*
	B-lymphoblastic leukemia/lymphoma with hyperdiploidy
	B-lymphoblastic leukemia/lymphoma with hypodiploidy
	B-lymphoblastic leukemia/lymphoma with t (5;14) (q31.1;q32.3) *IL3-IGH*
	B-lymphoblastic leukemia/lymphoma with t (1;19) (q23;p13.3); *TCF3-PBX1*
	Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like
	Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
3.	T-lymphoblastic leukemia/lymphoma
	Provisional entity: Early T-cell precursor lymphoblastic leukemia
	Provisional entity: Natural killer cell lymphoblastic leukemia/lymphoma

Abbreviations: WHO: World Health Organization; AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; NOS: Not otherwise specified; TAM: Transient abnormal myelopoiesis; MPAL: Mixed phenotype acute leukemia.

profile, and immunophenotype. The male-to-female patient ratio for AL was 4.6:1. The mean ± *SD* age of affected children was 6.2 ± 2.9 years. Most commonly, patients were referred to the laboratory for a workup of suspected AL (45.6%). The rest of the patients were incidentally diagnosed during the workup with pancytopenia or pyrexia of unknown origin. The clinical presentations of patients ($n = 68$) included anemia (66%), fatigue/weakness (63%), loss of appetite (60%), loss of weight (58%), fever (52%), failure to thrive (42%), hepatosplenomegaly (36%), bone pain (30%), lymphadenopathy (25%), and bleeding from gums/skin rashes (23%). The hematological profiles of these patients revealed mean ± *SD* values of red blood cell count of 2.6 ± 0.9 × 10^9/μL and hemoglobin (Hb) of 7.6 ± 2.3 g/dL. The median value of total leukocyte count (TLC × 10^3/μL) was 8,450 (4100 – 27,950), hematocrit (L/L) 0.22 (0.19 – 0.29), blast in peripheral smear 59 (24 – 80), blasts in BMA 95 (75 – 98), and platelets 31,000 (18,500 – 60,000).

Based on immunophenotype, the frequencies of the various groups of AL were as follows: B-ALL (51.5%; 35/68), T-ALL (10.3%; 07/68), AML (22%; 15/68), and mixed phenotype AL (MPAL) (16.2%; 11/68). These four groups were compared with clinical features and hematological profiles. The median (IQR) age (years) for different AL groups were as follows: B-ALL (5 [4 – 9]), T-ALL (8 [6 – 12]), AML (5 [4.5 – 6]), and MPAL (8 [5 – 10]); the difference was not statistically significant.

Table 3 describes clinical features in different groups of AL. Several clinical definitions in children include:

(i) Hepatomegaly: Defined as liver edge papable 2 – 3.5 cm below the right costal margins in children and newborns
(ii) Splenomegaly: Defined as a palpable splenic edge >2 cm below the left costal margins
(iii) Lymphadenopathy: Defined as any palpable lymph nodes >1 cm in diameter.

No statistically significant difference was observed between the different groups of AL for clinical features. On the other hand, Table 4 illustrates the hematological profile in different groups of AL.

TLC exhibited a significant difference among the different groups of AL. After applying the Dunn test, t*P*-value of TLC showed significant differences ($P = 0.003$) between T-ALL and B-ALL, AML versus T-ALL ($P = 0.038$), and MPAL versus B-ALL ($P = 0.041$).

Furthermore, peripheral blood smear (PS), BMA, and trephine biopsy diagnoses were correlated with four groups of AL determined through flow cytometry. These samples (PS, BMA, and biopsy) diagnosed 60.3% of cases as AL, classified as B-ALL (26 cases), T-ALL (5 cases), AML (1 case), and MPAL (9 cases). In addition, 22.1% of cases were diagnosed as AML, further identified as AML (14 cases) and MPAL (1 case), and 17.6% of cases were diagnosed as ALL, further identified as B-ALL (9 cases), T-ALL (2 cases), and MPAL (1 case). The overall concordance was significant ($P = 0.0001$) between BMA (morphology) and flow cytometry with χ^2 (6) = 58.79, and the contingency coefficient ($C = 0.9110$) indicated a strong association.

The prognostic outcomes (dead and surviving) for the four groups of immunophenotypes were compared in Table 5.

T-ALL (57.2%) and AML (86.7%) were significantly higher in the deceased patient group in comparison to the surviving patient group ($P = 0.023$).

Based on immunophenotype, the highest mortality rate was significantly higher in AML patients, followed by T-ALL and MPAL. Patients with B-ALL (Common-B-ALL) demonstrated the most favorable prognosis among all immunophenotypic groups.

The immunophenotypic groups were, further, subdivided into eight subgroups based on the expression of antigens. The frequency of immunophenotypes of AL in children in Delhi-NCR is shown in Table 6.

Table 3: Clinical manifestations in different groups of AL patients based on immunophenotype between January 2019 and June 2021

Clinical features	B-ALL (%)	T-ALL (%)	AML (%)	MPAL (%)	*p*-value
Fever	80	100	53.3	81.8	0.071
Bone pain	42.9	71.3	40	36.4	0.473
Weight loss	85.7	100	80	81.8	0.647
Loss of appetite	85.7	100	86.6	90.9	0.739
Fatigue/weakness	94.3	100	86.7	90.9	0.675
Paleness/anemia	97.1	100	100	90.9	0.547
Hepatomegaly	57.5	71.4	33.3	54.5	0.316
Splenomegaly	51.4	85.7	33.3	54.5	0.151
Lymphadenopathy	37.1	57.1	13.3	54.5	0.098

Abbreviations: AL: Acute leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; MPAL: Mixed phenotypic acute leukemia; T-ALL: T-cell acute lymphoblastic leukemia.

Table 4: Hematological profiles in different groups of AL patients based on immunophenotype between January 2019 and June 2021

Laboratory parameters	B-ALL	T-ALL	AML	MPAL	*P*-value
TLC (10^3/μL)	5,100 (3,300 – 19,400)	62,000 (9,140 – 94,100)	8,500 (4,200 – 27,500)	17,200 (7,700 – 30,000)	0.031*
RBC (10^9/μL)	2.7 (2.2 – 3.1)	2.7 (2.5 – 3.0)	2.4 (2.2 – 3.1)	2.4 (1.9 – 3.4)	0.725
Hb (g/dL)	7.5 (6.5 – 9.3	7.2 (6.2 – 8.8)	7.2 (6.4 – 9.4)	6.8 (5.8 – 9.2)	0.971
Hematocrit (L/L)	0.22 (0.19 – 0.28)	0.22 (0.21 – 0.29)	0.24 (0.2 – 0.28)	0.22 (0.18 – 0.32)	0.868
Platelets (10^6/μL)	31,000 (20 000 – 60,000)	27,000 (17,000 – 68,000)	38,000 (12,000 – 66,000)	31,000 (16,000 – 49,000)	0.876
PS blast	52 (21 – 80)	82 (37 – 97)	59 (28 – 76)	60 (38 – 80)	0.362
BMA blast	95 (90 – 98)	98 (98 – 98)	93.5 (70 – 98)	72.5 (0 – 98)	0.282

Note: *P<0.05.
Abbreviations: AL: Acute leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; BMA: Bone marrow aspirate; Hb: Hemoglobin; MPAL: Mixed phenotypic acute leukemia; PS: Peripheral blood smears; T-ALL: T-cell acute lymphoblastic leukemia; TLC: Total leukocyte count; TRBC: Total red blood cell count.

Table 5: An immunophenotypic pattern of AL in deceased and surviving patient groups

Immunophenotypic groups	Deceased patient group (%)	Surviving patient group (%)	*P*-value
B-ALL (n=35)	40	60	0.023*
T-ALL (n=7)	57.2	42.8	
AML (n=15)	86.7	13.3	
MPAL (n=11)	45.5	54.5	

Note: *P<0.05.
Abbreviations: AL: Acute leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; MPAL: Mixed phenotypic acute leukemia; T-ALL: T-cell acute lymphoblastic leukemia.

In Group I, B-ALL subgroups were Common-B-ALL in 88.6% and Pre-B-ALL in 11.4%. In Group 2, T-ALL subgroups were Cortical-T-ALL in 71.4% and Pre-T-ALL in 28.6%. In Group 3, AML was predominantly of the M2 subtype in 73.3% and AML-M4 in 26.7%. Group 4 comprised MPAL, with 90.9% showing aberrant expression of myeloid markers with B-ALL and 9.1% showing aberrant expression of lymphoid markers with AML. These eight subgroups of immunophenotypes were also compared with clinical features and hematological profiles, but no significant differences were found.

4. Discussion

Immunophenotypic patterns of childhood AL have been extensively reported in the literature.[10,11] In Indonesia, for instance, 62.8% of cases were classified as ALL (83% of ALL being B-ALL and 17% T-ALL), while 23% were classified as AML, and 7.9% were of unknown origin, with only 0.2% biphenotypic pediatric patients.[10] In this study, we found that T-ALL (57.2%) and AML (86.7%) were significantly higher (P = 0.023) in the deceased patient group compared to the surviving patient group, whereas B-ALL and MPAL were significantly higher in the surviving patient groups. A study from North India reported 81.0% ALL, 15.8% AML, and 3.2% MPAL in pediatric patients.[12] Conversely, a study from South India reported an excess of T-ALL and a paucity of common ALL in children over the past

Table 6: The frequency of immunophenotypes in groups and subgroups of acute leukemia of children in Delhi-NCR, India

S. No.	Groups	Subgroups	Immunophenotype	Type of leukemia	Frequency % (*n*)
1.	B-ALL	Ia	CD34, HLA-DR, CD19, CD20, CD79a, CD10	Common-B-ALL	88.6 (31/35)
2.		Ib	CD19, CD20, CD79a, HLA-DR, CD34	Pre-BALL	11.4 (4/35)
3.	T-ALL	IIa	TdT, c-CD3, CD3, CD2, CD1a, CD5, CD7, CD4, CD10, CD8	Cortical- T-ALL	71.4 (5/7)
4.		IIb	c-CD3, CD2, CD3, CD5, CD7, *TdT	Pre-T-ALL	28.6 (2/7)
5.	AML	IIIa	CD34, HLA-DR, CD13, CD33, MPO, CD117	AML-M2	73.3 (11/15)
6.		IIIb	CD13, CD33, C-MPO, CD117, CD15, CD64, CD11b	AML-M4	26.7 (4/15)
7.	MPAL	Iva	CD34, CD19, CD10, CD79a, HLADR, CD20, CD13, CD33	My+BALL	90.9 (10/11)
8.		IVb	Strong+ (CD33, CD64, cMPO); Weak+ (TdT, CD79a, CD10, CD3)	Ly+AML	9.1 (1/11)

Notes: ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; c: cytoplasmic; Ly+AML: Aberrant expression of lymphoid markers with AML; MPAL: Mixed phenotypic acute leukemia; My+BALL: Aberrant expression of myeloid markers with B-ALL; T-ALL: T-cell acute lymphoblastic leukemia; *TdT (not done); +: Positivity; NCR: National Capital Region.

20 years.[13] These differences might be related to different geographical factors and ethnicities.

Our current study presents an overall epidemiological pattern of immunophenotype observed in Delhi-NCR, the capital region of India, where people from across the country reside. We observed the following overall frequencies of AL: B-ALL (51.5%), T-ALL (10.3%), AML (22%), and MPAL (16.2%). While the overall immunophenotypic pattern is similar to that reported in the literature from around the world and studies from North India, the lower numbers observed in our study might be related to sample size. Notably, this pattern differs from South India, where T-ALL is predominantly observed.

There is no significant difference between all leukemic groups (B-ALL, T-ALL, AML, and MPAL) for age in our study, corroborating the findings from the study by Sharma *et al.*[12]

The most common clinical manifestations of AL patients were anemia, weight loss, loss of appetite, and fever, which were also corroborated by other studies.[14,15] Sharma *et al.*[12] reported physical findings in AL patients: hepatomegaly in 69% of the cases, splenomegaly in 56.5%, and lymphadenopathy in 62.3%. Sharma *et al.*[14] found significant differences between ALL and AML for fever and between MPAL and AML for lymphadenopathy. However, we did not find significant differences in clinical features between different groups of AL, potentially attributed to the small sample size.

The hematology profile of AL evaluated in our study is comparable to other studies.[13-15] We found a significant difference ($P = 0.031$) for TLC between various groups of leukemia. After applying the Dunn test, *P*-value of TLC exhibited a significant difference between T-ALL and B-ALL, between AML and T-ALL, and between MPAL and B-ALL. However, a few studies did not find a significant difference in Hb and TLC between different groups of AL (MPAL vs. ALL/AML).[12,14] Sharma *et al.*[12] noted a significant difference in mean platelets count between MPAL and ALL/AML. Apart from TLC, we did not observe a significant difference in the hematological profile among different AL groups. This variation is represented by the diversity in hematological profiles across different patients.

Supriyadi *et al.*[10] found a very good concordance ($\kappa = 0.82$) between morphology and immunophenotype using a three-color method with a panel of 15 monoclonal antibodies ($n = 387$). Similarly, we also observed a significant association ($P = 0.0001$) between BMA morphology and flow cytometry with $\chi 2$ value of (6) = 58.79 and a C (0.9110) indicating a strong strength of association between both methodologies.

Few studies from Northern India have reported the immunophenotype in 85% of patients as Blineage ALL (PproB- ALL 8%, ccommonB -ALL 74%, and PpreB -ALL 18%), and in 15% of patients as Tlineage ALL (PproT -ALL 29%, PpreT -ALL 11%, cortical- T -ALL 44%, and MmatureT -ALL 16%).[16] The immunophenotypic pattern is similar to the results of our study results (Table 4), but the overall proportion is low in the present study, which might be related attributed to the small sample size.

Rajalekshmy *et al.*[17] from Chennai, India, reported the immunophenotypic pattern of ALL from Chennai, India, indicating T-ALL in 53.6%, B-ALL in 46.4% (precursor B 6.4%, pre-B 5.6%, Common-B-ALL 20.8%,and B- 04%) and unclassified in 5.6%.[17] This study reported a high incidence of T-ALL high in children, which is notably very unusual, and may be it related to different immunophenotypic patterns in across different geographic and ethnic groups. Gupta *et al.* reported immunophenotypic patterns from Kolkata, demonstrating 81.7% B-ALL (Common B-ALL 95.2% and Pro-B-ALL 4.8%), T-ALL comprising

18.3% (Cortical-T -ALL 27.9%, Pro-T- ALL 8.2%, early thymic -T- ALL 9.8%, and medullary-T- ALL 24.6%), AML comprising 32.1% with recurrent cytogenetic abnormalities (11.9% t [8;21], 12.3% t [15;17] that is acute promylocytic leukemia, 2.9% with inversion 16/t [16;16], 3.9% MLL gene rearrangement, and 1.1% with 3q abnormalities), and 2.3% MPAL.[18]

Different studies from North India have reported. The aberrant phenotypes with myeloid antigens at 42.5% and 11% were reported by in ALL cases different studies from North India in ALL cases.[14,16] These findings were different from our study results; we found an overall aberrant antigen expression of 16.2%, and with myeloid antigen expressed most predominantly in 90.8% in of B-ALL (My+B-ALL, 10/11 cases). We found one case (9.1%) expressing aberrant lymphoid antigen in AML (Ly+AML). Other studies have found, which reported the most common aberrant expressing antigen CD13 as the most commonly aberrantly expressed antigen (at 32.2% and 25.6%, respectively).[14,18] Whereas in our study, we found observed mostly a predominance of 14.7% of aberrant expression of myeloid antigens CD13 (70%), CD33 (50%), and both (20% common) aberrant expressing the myeloid antigen. Sharma *et al*.[14] found 2.99% MPAL, out of them, which only seven pediatric patients showed aberrant lymphoid antigen expression in AML.[14] The overall configuration of the immunophenotypic pattern of childhood AL has changed from the maximum proportion that majority of AL cases were being B-ALL to a significant portion of non-B-ALL (T-ALL, AML, and MPAL) (constituting approximately 40 – 45%) of cases in the worldwide and in India. In our study, we found that 48% of AL cases were non-B -ALL proportions of AL, which affects the prognosis of AL.

The present study evaluated the prognostic significance of different immunophenotypes of in AL. We found the maximum highest mortality rate of 86.7% in AML, followed by 57.2% in T-ALL 57.2% and 45.5% in MPAL. B-ALL (subtype-: Common -B -ALL) showed the best most favorable prognosis (with a minimum mortality rate of 40%). Similarly, the world literature worldwide reported a favourable prognosis associated with B-ALL and a poor prognosis related to AML and T-ALL.[19] A study by Santos *et al*.[20] provided the relavance of immunophenotypic markers as independent prognostic factors that could be included integrated into clinical protocols, for risk stratification and therapeutic guidance.[20]

The limitations of our study were included: (i) the sample size was a small, (ii) The lack of further confirmation by molecular techniques and (iii) we were unable to correlate with cytogenetic findings of AL.

5. Conclusion

The immunophenotypic pattern in Delhi-NCR shows B-ALL (51.5%) as the most prevalent, followed by AML (22%), MPAL (16.3%), and T-ALL (10.2%). Based on immunophenotype, the most common childhood AL is B-ALL (Common-B-ALL), in contrast to South India, where T-ALL predominates. However, the mortality rate is highest in AML in comparison to other AL subtypes. Among B-ALL cases, Common-B-ALL demonstrates the most favorable prognosis, consistent with findings in global literature.

The different immunophenotypic patterns observed between Delhi-DCR and South India suggest the presence of underlying factors, warranting further investigation. Additional studies are required to elucidate the reasons behind these differences. Enhanced understanding of immunophenotypic patterns and their prognostic value within specific geographical areas will not only facilitate improved patient management but also aid in the formulation of health-care policies.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Anju Khairwa
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Ethics approval and consent to participate

The University College of Medical Sciences ethics committee approved the study protocol with the number (IECHR-2021-51-15R1). The study was conducted in accordance with the ethical guidelines of the college. This retrospective cross-sectional study was performed at a tertiary care institute in Delhi, India. Informed consent was not applicable as the study was retrospective, and there was no direct contact with patients. Data were retrieved from the departmental archive.

Consent for publication

This retrospective cross-sectional study was performed at

a tertiary care institute in Delhi, India. Informed consent for publication was not applicable as the study was retrospective, and there was no direct contact with patients. Data were retrieved from the departmental archive.

Availability of data

Data used in this work are available from the corresponding author on reasonable request.

Further disclosure

The findings have been presented in a conference DAPCON July 31, 2022, at AIIMS, New Delhi, India as a poster.

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