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Morphological and Immunophenotyping Profile of Acute Leukemia: A Study from a Tertiary Level Hospital in Nepal

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Abstract

Introduction: Acute leukemia is a heterogeneous disorder necessitating a multidisciplinary diagnostic approach. Flow cytometry is essential for lineage assignment and maturation stage identification. This study aims to determine the frequency and immunophenotyping of acute leukemia at Tribhuvan University Teaching Hospital (TUTH).

Methods: Conducted from January 2020, to December 2021, this cross-sectional study analyzed 504 bone marrow aspiration samples. Among these, 97 (19.2%) cases were identified as acute leukemia. Patient demographics, morphology, and immunophenotyping features were recorded and analyzed.

Results: Of the acute leukemia cases, 66% were male and 34% female. The pediatric population comprised 62.9%, while adults made up 37.1%. Cytochemical staining was performed in 92 cases, revealing 67% as acute lymphoblastic leukemia (ALL) and 33% as acute myeloid leukemia (AML). Flow cytometry was conducted on 84 cases, leading to diagnoses of 30 AML, 45 B-ALL, and 9 T-ALL.

Conclusion: Acute leukemia can affect all age groups with male preponderance. ALL is more prevalent in children, while AML is more common in adults. Immunophenotyping is critical for the classification and subtyping of acute leukemia, enhancing diagnostic accuracy and guiding treatment strategies.

Keywords: acute leukemia, immunophenotyping, WHO classification

Introduction

Acute leukemia is a diverse group of blood cancers characterized by the rapid increase of immature cells (blasts) in the blood or bone marrow.[1] Globally, leukemia ranks as the 13th most common cancer and the 10th leading cause of cancer-related deaths. In Nepal, it is the ninth most diagnosed cancer and the eighth leading cause of death.[2]

Acute leukemia is a heterogeneous disease clinically, morphologically, and genetically. Morphological evaluation is the first step in the diagnosis of acute leukemia. They are characterized by ≥20% of blast in peripheral blood or bone marrow. Cytochemical stains like Sudan Black B (SBB) and Periodic Schiff Acid (PAS) are helpful in lineage identification, as SBB positivity excludes the diagnosis of ALL. For the identification and characterization of blasts, immunophenotyping is the widely used technique in acute leukemia. The 2016 World Health Organization classification of acute leukemia has integrated immunophenotyping with morphology, cytogenetics, molecular studies, and clinical manifestations.[3]

In Nepal, access to flow cytometry and molecular studies is limited, resulting in a scarcity of research on the immunophenotypic profiles of acute leukemia. The aim of the study is to analyze the various diagnostic aspects, morphology, cytochemistry, and immunophenotyping to address this research gap.

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Methods

This is an observational, descriptive, cross-sectional study conducted at the hematological unit, Department of Pathology, Tribhuwan University Teaching Hospital, from January 2020 to December 2021. The study was approved by the Institutional Review Committee of TUTH (Ref. Nob 301/ (6-11) E2/076/077). All cases of acute leukemia presented during study period were included. Non-probability, convenient sampling technique was used. All cases of acute leukemia that presented with >20% blasts or <20% blasts with recurrent cytogenetic abnormality in bone marrow aspirated samples were included, whereas cases of acute leukemia under treatment and Chronic Myeloid Leukemia (CML) with blast crisis were excluded.

Written informed consent was obtained from each of the patients before the procedure. Under aseptic measures, bone marrow aspiration was done through the posterior iliac crest. Five hundred and four aspirated bone marrow samples were prepared for smears and sent to the hematology laboratory for Wright stain. The Sudan Black B (SBB) and periodic Acid-Schiff (PAS) staining were employed for diagnosis and preliminary subtyping of leukemia in all cases. Only 97 samples of cases diagnosed with acute leukemia meeting inclusion criteria were sent in heparin vials to referral laboratories for flow cytometry and immunophenotyping as shown in figure 1.

Bone marrow samples collected in heparin vials were processed within 24 hours. For immunophenotyping, various combinations of fluorochrome (fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC) or peridinin chlorophyll protein (PerCP)) and conjugated monoclonal antibodies (MoAbs) were added per tube in the sample.

Data were acquired, and the blast gating strategy included using dot plots of CD45 expression versus intracellular complexity (side scatter angle, SSC), and also a second gate was based on cell forward scatter angle (FSC-A) versus SSC-A. Data were entered into Microsoft Excel and analysed using the software SPSS version 25. The statistical analyses comprised of descriptive statistics, wherein, categorical variables were described using frequency with percentage and by employing tables and different types of charts/diagrams.

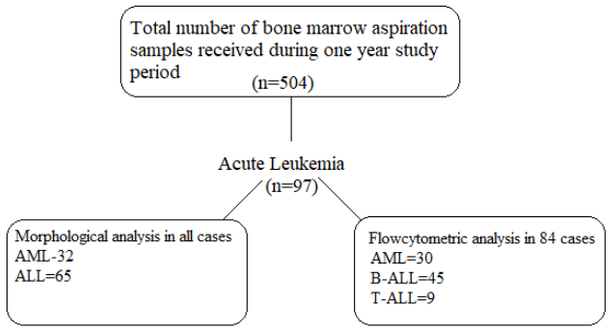

Figure 1: Diagnostic work-up in patients who underwent bone marrow aspiration

Results

Over one year, 97 patients were diagnosed with acute leukemia based on morphology, cytochemistry, and flow cytometry. There were 64 males (n=97, 66%) and 33 (n=97, 34%) females with a male: female ratio of 1.9:1. The mean age of the patient was 17.14 (SD: 18.14, range: 8 months-73 years). A maximum number of cases were seen in the age group 0-10 years (n=47, 48.5%) followed by the age group 11-20 years (n=24, 24.7%) as shown in figure 2.

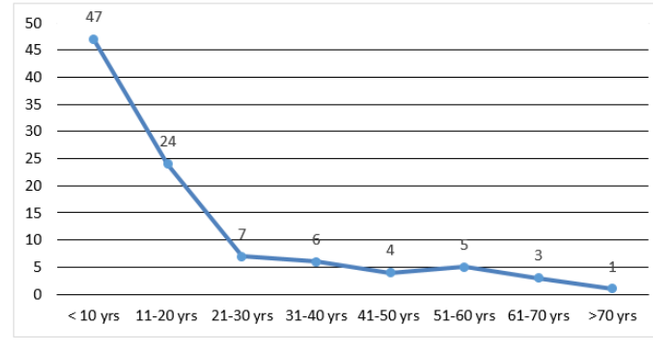

Figure 2: Age-group distribution in acute leukemia

Bone marrow aspiration smears showed a minimum of 20% blasts to a maximum of 96% blasts with a mean of 65% (SD: 22.54) and a median of 68% blasts. Based on the morphology of blasts, 65 cases (67%, n=97) were diagnosed as ALL and 32 (33%, n=97) as AML. Based on morphology, AML was further classified according to French-American-British (FAB) classification. The maximum number of cases (n=23, 71.9%) were AML with maturation (M2), followed by acute promyelocytic leukemia (M3), constituting 15.6% (n=5), as shown in table1.

Table 1: FAB classification of AML

FAB classification	Number (%)
Minimally differentiated AML (M0)	0
AML without maturation (M1)	3 (9.4)
AML with maturation (M2)	23 (71.9)
Acute promyelocytic leukemia (M3)	5(15.6)
Acute myelomonocytic leukemia (M4)	1(3.1)
Acute monoblastic and monocytic leukemia (M5)	0
Acute erythroid leukemia (M6)	0
Acute megakaryoblastic leukemia (M7)	0
Total	32 (100)

Flow cytometric analysis was performed in 84 cases of acute leukemia, comprising 29 cases of morphologically diagnosed AML and 55 cases of morphologically diagnosed ALL. Of 84 cases, 65.5% (n=55) were male and 34.5% (n=33) were female, with a male: female ratio of 1.9:1. Based on Flow cytometric analysis 30 cases (35.7%) were diagnosed as AML and 54 cases (64.3%) as ALL. B-ALL was diagnosed in 53.6% (n=45) of cases. T-ALL was the least common, constituting only 10.7% of acute leukemia.

Table 2: Flow cytometric diagnosis in acute leukemia

ALL		Morphological Diagnosis		
		AML	Total	
Flowcytometric Diagnosis	AML	2 (3.6%)	28 (96.6%)	30 (35.7%)
	B-ALL	44 (80.0%)	1 (3.4%)	45 (53.6%)
	T-ALL	9 (16.4%)	0	9(10.7%)
	Total	55 (100%)	29 (100%)	84 (100%)

Flow cytometric analysis was performed in 52 pediatric cases and 32 adult cases of acute leukemia. AML constituted 43.8% of adult cases and 30.7% of pediatric cases. B-ALL was more common in the pediatric population, which constituted 63.5% of pediatric cases and 37.5% of adult cases. T-ALL constituted 18.7% of adult cases and 5.8% of pediatric cases.

Table 3: Frequency of acute leukemia among pediatric and adult populations

Flowcytometric diagnosis	Frequency (%) (all age groups)	Frequency (%) (adults)	Frequency (%) (pediatric)	Mean Age (years)
AML	30 (35.7)	14 (43.8)	16 (30.7)	29.04
B-ALL	45 (53.6)	12 (37.5)	33 (63.5)	11.48
T-ALL	9 (10.7)	6 (18.7)	3 (5.8)	25.78
Total	84 (100)	32 (100)	52 (100)	-

AML with maturation was the most frequent subtype constituting 15 cases (50%), followed by acute myelomonocytic leukemia (n=6, 20%).

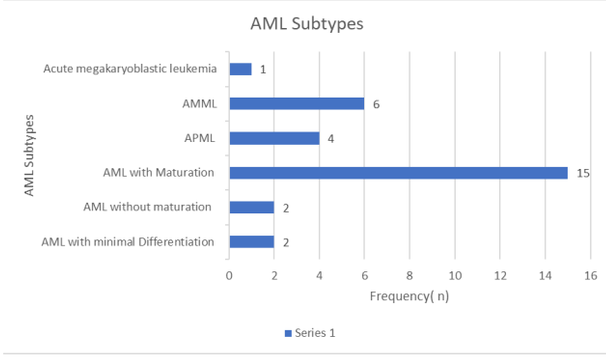

Figure 3: Subtypes of AML by immunophenotyping

Table 4: Immunophenotypic markers in AML

Markers	B-ALL Markers		Total (n=45)
	Common B-ALL (n=40)	Pro B-ALL (n=5)	
CD34	28	3	31
HLA-DR	11	3	14
TdT	26	3	29
CD9	22	0	22
CD10	40	0	40
CD19	40	5	45
CD20	17	4	21
CD22	31	3	34
CD79a	33	5	38
CD38	27	1	28
CD58	16	0	16
CD13	3	0	3
CD33	3	0	3
CD66C	14	0	14

Immunophenotypically, AML was further subclassified into the following subtypes as shown in figure 3.

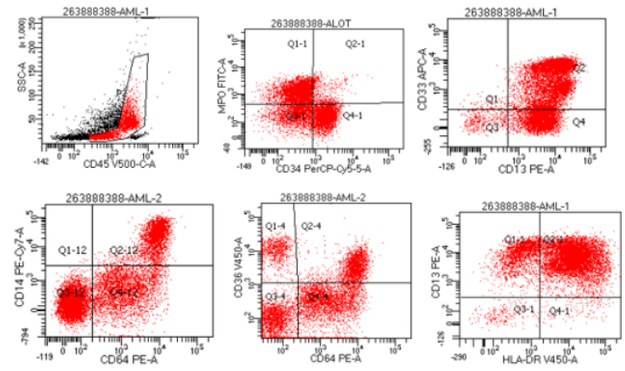

Figure 4: Acute myelomonocytic leukemia, CD45 gated blasts showing HLA-DR, MPO, CD13, CD33, CD14, CD64, and CD36 expression

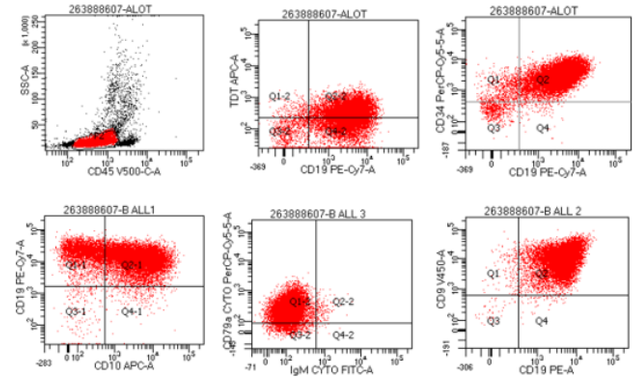

Figure 5: Acute B-lymphoblastic leukemia, CD45 gated blasts showing CD34, CD19, CD79a, CD10, and CD9 expression

Based on flow cytometric findings, 40 cases (88.9%) were categorized as common B-ALL and 5 cases (11.1%) as Pro B-ALL. Immunophenotypical markers in B-ALL are as shown in Table 5.

Table 5: Immunophenotypic markers in B-ALL

Markers	B-ALL Markers		Total (n=45)
	Common B-ALL (n=40)	Pro B-ALL (n=5)	
CD34	28	3	31
HLA-DR	11	3	14
TdT	26	3	29
CD9	22	0	22
CD10	40	0	40
CD19	40	5	45
CD20	17	4	21
CD22	31	3	34
CD79a	33	5	38
CD38	27	1	28
CD58	16	0	16
CD13	3	0	3
CD33	3	0	3
CD66C	14	0	14

Among nine cases of T-ALL, three cases each were classified as ETP ALL, near-ETP ALL, and T-ALL, respectively. Immunophenotypic markers in T-ALL are as shown in Table 6.

Table 6: Immunophenotypic markers in T-ALL

Markers	ETP (n=3)	Near ETP (n=3)	T-ALL (n=3)	Total (n=9)
CD34	2	2	1	5
HLA-DR	0	1	0	1
TdT	1	2	2	5
CD2	0	3	2	5
CD3	3	3	3	9
CD4	0	1	2	3
CD5	0	3	3	6
CD7	3	3	3	9
CD8	0	0	3	3
TCR	0	1	0	1
CD10	1	0	3	4
CD33	1	1	0	2
CD38	0	0	1	1

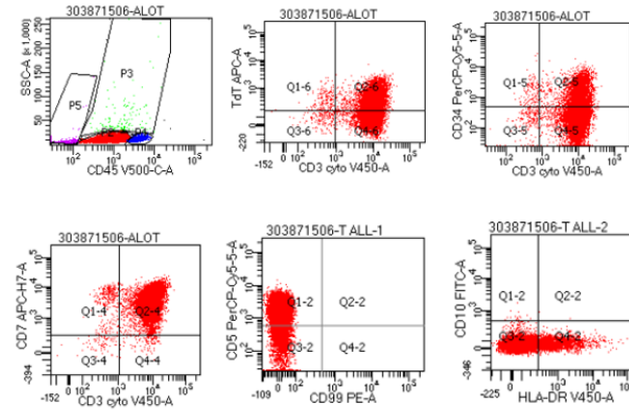

Figure 6: Acute T-lymphoblastic leukemia, CD45 gated blasts showing TdT, CD34, Cy CD3, CD7, and CD5 expression. Blasts are negative for CD10 and CD99.

Cytochemical staining by SBB and PAS was done in 92 cases of acute leukemia, which included 29 cases of AML and 63 cases of ALL. Only 21 cases of acute leukemia showed positivity for SBB; all of them were AML. SBB positivity was seen in 72.4% (21 out of 29 cases) of AML. However, it was negative in three cases (10.4%) and inconclusive in five cases (17.2%) of AML. None of the ALL showed SBB positivity.

Table 7: SBB staining in acute leukemia

SBB	Morphology	
	AML Frequency (%)	ALL Frequency (%)
Positive	21(72.4)	0
Negative	3(10.4%)	51(81.0%)
Inconclusive	5(17.2%)	12(19.0%)
Total	29 (100%)	63(100%)

Twelve cases of acute leukemia showed positive PAS staining, all of which were ALL. PAS positivity was seen in 19% (12 out of 63) of ALL, whereas PAS negativity was seen in 15 cases (23.8%) and inconclusive in 36 cases (57.1%) of ALL. No AML cases showed PAS positivity.

Table 8: PAS staining in acute leukemia

PAS	Morphology	
	AML Frequency (%)	ALL Frequency (%)
Positive	0	12 (19.0 %)
Negative	20(69.0 %)	15(23.9%)
Inconclusive	9(31.0%)	36(57.1%)
Total	29(100%)	63(100%)

Discussion

Acute leukemia (AL) is a clonal hematopoietic stem cell disorder characterized by an increase in immature cells

(≥20%) in peripheral blood and/or bone marrow except, in case of AML with t (8;21), t (15;17), and inv16. Acute leukemia is more prevalent in males than females, with our study showing that 66% of patients were male and 34% female, resulting in a male-to-female ratio of 1.9:1. This finding aligns with other studies, which reported male populations of 73%, 71%, and 59.6%, respectively.[4-6] However, Patel et al. found a slightly higher female prevalence with a ratio of 1:1.1.[7]

The disease affects all age groups, with our study reporting ages ranging from eight months to 73 years, and a mean age of 17.14 years, with a median age of 11 years. In contrast, Gujral et al. noted a range from 2 weeks to 81 years, with a median of 22 years.[5] Our study had 27.1% adult patients (≥ 15 years) and 62.9% pediatric patients (< 15 years), while Gupta et al. reported 58.7% adults.[6] The higher proportion of pediatric cases in our study may be due to sampling from TUTH and Kanti Children's Hospital. Acute myeloid leukemia (AML) is more common in adults, accounting for 43.8% of adult cases in our study, consistent with findings from Gujral et al. and Shrestha et al., which reported 53% and 52.2%, respectively.[4,5] In children under 15, AML comprises 15-20% of cases, with a peak incidence in the first 3-4 years of life;[8] our study showed 30.7% of AML in children. B-ALL represented 63.5% of acute leukemia cases in children, 37.5% in adults, and 53.6% overall, while T-ALL was the least common at 10.7%.

Bone marrow examination is crucial for diagnosis.[9] In our study, 67% of cases were diagnosed as ALL and 33% as AML, which mirrors findings of Shrestha et al. of 59.6% ALL and 38.4% AML.[4] A contrasting study by Abbasi et al. reported a predominance of AML at 51.8%.[10] We found Sudan black B (SBB) positivity in 72.4% of AML cases, with 10.3% negative and 17.2% inconclusive. This is consistent with Gupta et al., who reported 66% positivity[11] although Belurkar et al. noted 91.6% positivity.[12] All ALL cases in our study were SBB negative. PAS positivity, defined by the presence of blocks or coarse granules in 5% or more of the blasts,[13] was observed in 19% of ALL cases. This compares to other studies where PAS positivity rates were higher.[11,12,14] No AML cases showed PAS positivity. Morphologically, AML was diagnosed using the FAB classification, with AML-M2 being the most frequent subtype at 71.9%, followed by M3 at 15.6%, similar to the Arber et al. study.[15] However, predominant subtypes in Harani et al.'s study were AML M4,[16] and Sultan et al.'s was the AML-M1 FAB subtype (23.2%).[17]

Due to financial constraints, flow cytometry analysis was done on 84 of the 97 patients, demonstrating 35.7% AML and 64.3% ALL. Among the ALL, 83.3% were B-ALL, whereas 16.7% were T-ALL. Three cases were reclassified based on the flow cytometry data. The remaining 81 cases (96.4%) in our analysis indicated lineage concordance between flow cytometry and morphology, which is comparable to 95.8% in Kheiri et al.[18] and higher than 86% in Belurkar et al.[12]

In our study, immature marker CD34 was expressed in 80% of cases, which was almost the same as studies done by Shrestha et al. and Rashed et al.[4,24,25] but higher than other literature.[4,19-24] HLA-DR was expressed in 20 cases (66.67%) of AML, which was similar to the findings of Salem and Abd El-Aziz, Bradstock et al. and Shrestha et al. (69.9%, 70%, and 70%, respectively).[23,24,6] CD117 was expressed in 28 cases (93.33%) of AML, similar to Gupta et al.'s and Rashed et al.'s findings [6,25](>90%). CD13 expression was seen in >90% of AML; however, in our study, it was positive in 86.67% (n=26) of cases with expression in all subtypes. CD33 was expressed in 96.67% (n=29) of cases of AML, which was similar to findings in the literature.[4,21,25] MPO was positive in 24 out of 30 cases (80%), but none of the cases of AML with minimal differentiation showed MPO positivity. In our study, AML with maturation was the most frequent subtype, constituting 15 cases (50%), which was similar to Basharat et al.'s study[24] (AML-M2=34%). CD14, CD36, and CD64 were seen in AMML (M4) which was similar to Narang et al.'s findings.[26] APML has a characteristic immunophenotype that facilitates its identification by flow cytometry. By flow cytometry, APML typically displays most of the following immunophenotypic features, including high side scatter, positivity for CD13, CD33, and CD117, and absent expression of CD34 and HLA-DR.[27] All cases of APML (n=4) were HLA-DR negative. AML M7 is a rare type of AML that constituted only one case in our study with CD41a and CD61 expression. The most common aberrant marker expressed in AML was CD7, which was seen in 4 cases (13.33%), which was the same as the above-mentioned studies.

In B-ALL, CD19 is a lineage-specific marker expressed in 100% and other immature marker, CD34 expression was seen in 31 cases (68.89%), which was lower than findings in the literature. TdT expression was noted in 64.44%, which was lower than all above-mentioned studies (>75%) except for Rashed et al. who found it only in 47.6%. HLA-DR expression (31.11%) was lower than in all other studies. CD19 is a lineage-specific marker. In our study, it was expressed in all cases of B-ALL (100%) similar to the above studies.[4,6,24,25] CD10 expression was noted in 88.89% of B-ALL, which was also similar to all studies (>80%) except the study done by Shrestha et al. (75%).[4] Based on CD10 expression, B-ALL was divided into two groups, Pro B-ALL and common B-ALL (CD10 positive). Further classification into pre-B-ALL couldn't be done because a cytoplasmic mu chain marker was not available. CD13 and CD33 are myeloid markers often seen in B-ALL.

CD66c is the most common myeloid antigen expressed in B-ALL and is a predictor of the BCR/ABL1 rearrangement.[28] In our study, CD66c expression was seen in 14 cases (31.11%). Similar to our finding, studies done by Kalina et al. and Guillaume et al. showed CD66c expression in 29% and 40% of cases, respectively.[29,30] In our study, CD34 was expressed in 55.56% of T-ALL, which was similar to Thalhammer-Scherrer et al.'s findings.[22] TdT was seen in 44.44% of cases which were almost similar to Rashed et al.'s findings (50%).[24] In this study, HLA-DR was positive in only 11.11% of T-ALL, likewise in the above-mentioned studies. However, in a study done by Salem and Abd El-Aziz, none of the T-ALL cases were positive for HLA-DR or CD34.[23]

This study is an institution-based study and may not

represent the actual distribution pattern of acute leukemia in the general population. A prospective study is recommended.

Conclusion

Acute leukemia can affect all age groups with male preponderance. ALL is more prevalent in children, while AML is more common in adults. Morphological assessment of peripheral blood smear and/or bone marrow aspiration can diagnose acute leukemia. Immunophenotyping by flow cytometry is required for determining the lineage as well as the degree of maturation of blasts. Therefore, combined morphological examination and immunophenotyping are mandatory for accurate evaluation of acute leukemia.

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