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# Diagnostic Utility of Peripheral Blood Biomarkers in Childhood Leukemia: A Prospective Observational Study

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## ABSTRACT

**Background**: Childhood leukemia is the most common pediatric malignancy and remains a major cause of cancer-related morbidity and mortality worldwide. Early diagnosis is essential for timely initiation of treatment and improved clinical outcomes. Peripheral blood biomarkers may provide valuable diagnostic information before definitive hematological evaluation.
Objective: To evaluate the diagnostic utility of peripheral blood biomarkers in childhood leukemia and determine their effectiveness in differentiating leukemia from non-malignant hematological disorders.
**Methods**: This prospective observational case-control study was conducted in the Department of Paediatrics, ESIC Medical College & PGIMSR, Rajajinagar, Bengaluru, Karnataka, from September 2024 to March 2026. A total of 240 children were enrolled, including 120 newly diagnosed leukemia patients and 120 age- and sex-matched controls with non-malignant hematological disorders. Hematological parameters, including peripheral blast percentage, and biochemical biomarkers such as lactate dehydrogenase (LDH), ferritin, uric acid, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were analyzed. Receiver operating characteristic (ROC) curve analysis, logistic regression, and correlation analyses were performed to assess diagnostic performance.
**Results**: Acute lymphoblastic leukemia accounted for 81.7% of cases, while acute myeloid leukemia constituted 18.3%. Leukemia patients demonstrated significantly lower hemoglobin and platelet counts and significantly higher total leukocyte counts compared with controls ($p<0.001$). Mean LDH (962 ± 312 U/L), ferritin (678 ± 241 ng/mL), uric acid (7.2 ± 2.1 mg/dL), CRP (21.8 ± 8.3 mg/L), ESR (49 ± 18 mm/hr), and peripheral blast percentage (42.8 ± 18.6%) were significantly elevated among leukemia patients ($p<0.001$). LDH exhibited the highest individual diagnostic performance with an AUC of 0.91 (95% CI: 0.87–0.95), sensitivity of 89.2%, and specificity of 85.8% at a cut-off value of ≥620 U/L. Peripheral blast percentage demonstrated an AUC of 0.89 (95% CI: 0.84–0.93). A combined biomarker model incorporating LDH, ferritin, uric acid, and peripheral blast percentage achieved an AUC of 0.94 (95% CI: 0.90–0.97), sensitivity of 94.2%, specificity of 88.3%, and overall diagnostic accuracy of 91.3%. Elevated LDH, ferritin, uric acid, and peripheral blast percentage ≥12% emerged as independent predictors of childhood leukemia.
**Conclusion**: Peripheral blood biomarkers, particularly LDH, peripheral blast percentage, ferritin, and uric acid, demonstrate significant diagnostic utility in childhood leukemia. A combined biomarker approach provides excellent diagnostic accuracy and may serve as a valuable adjunct for early clinical suspicion and prioritization of definitive hematological evaluation. Further multicenter studies are required to validate these findings and establish standardized diagnostic algorithms.

***Keywords**: Childhood leukemia, peripheral blood biomarkers, lactate dehydrogenase, ferritin, uric acid, peripheral blasts, diagnostic accuracy, ROC analysis.*

## INTRODUCTION

Leukemia represents the most common malignancy in childhood and accounts for approximately 30–35% of all pediatric cancers worldwide. Despite remarkable advances in therapeutic approaches and supportive care, early diagnosis remains one of the most important determinants of survival and long-term outcomes. In developing countries, delayed diagnosis continues to contribute significantly to treatment failure, increased morbidity, and mortality.

Acute lymphoblastic leukemia (ALL) constitutes approximately 75–80% of childhood leukemia cases, while acute myeloid leukemia (AML) contributes about 15–20%. The disease originates from malignant transformation and uncontrolled proliferation of hematopoietic precursor cells within the bone marrow. These abnormal cells gradually replace normal hematopoietic elements, resulting in anemia, thrombocytopenia, neutropenia, and systemic manifestations.

Children commonly present with nonspecific symptoms such as prolonged fever, fatigue, pallor, recurrent infections, bruising, bleeding tendencies, bone pain, lymphadenopathy, and hepatosplenomegaly. Such symptoms often overlap with common pediatric illnesses, making early recognition challenging. Definitive diagnosis relies on bone marrow aspiration, immunophenotyping, cytogenetic studies, and molecular testing. However, these investigations may not be immediately available in all healthcare settings and are invasive procedures requiring specialized expertise.

Peripheral blood biomarkers have emerged as promising tools for the early identification of childhood leukemia. Hematological parameters including hemoglobin concentration, total leukocyte count, platelet count, and circulating blast cells provide valuable clues regarding marrow dysfunction. Similarly, biochemical biomarkers such as lactate dehydrogenase (LDH), ferritin, uric acid, ESR, and CRP reflect cellular proliferation, tissue turnover, inflammation, and tumor burden.

LDH is an intracellular enzyme released during rapid cellular destruction and turnover. Elevated serum LDH levels have been associated with leukemia burden, aggressive disease, and poorer prognosis. Serum ferritin functions as an acute-phase reactant and may reflect underlying inflammatory responses associated with malignant proliferation. Hyperuricemia develops due to accelerated nucleic acid degradation resulting from rapid tumor cell turnover. Elevated ESR and CRP have also been observed in children with leukemia due to associated inflammatory responses.

The identification of reliable peripheral blood biomarkers may facilitate earlier suspicion of leukemia, particularly in primary care and resource-limited settings. Such biomarkers could help clinicians prioritize high-risk patients for urgent hematological evaluation and reduce diagnostic delays.

Despite increasing interest in biomarker-based diagnosis, there remains limited prospective evidence from Indian pediatric populations evaluating their diagnostic performance. Therefore, the present study was undertaken to assess the utility of selected peripheral blood biomarkers in childhood leukemia and determine their diagnostic accuracy in comparison with non-malignant hematological disorders.

## MATERIALS AND METHODS

### Study Design

This prospective observational case-control study was conducted to evaluate the diagnostic utility of peripheral blood biomarkers in childhood leukemia and to determine their association with disease characteristics at presentation.

### Study Setting

The study was conducted in the Department of Paediatrics, ESIC Medical College & Post Graduate Institute of Medical Sciences and Research (PGIMSR), Rajajinagar, Bengaluru, Karnataka, India.

### Study Duration

The study was carried out over a period of 19 months from September 2024 to March 2026.

### Study Population

Children presenting to the pediatric outpatient department, inpatient wards, and pediatric oncology services during the study period were screened for eligibility.

The study population comprised:

- **Cases:** Newly diagnosed childhood leukemia patients.
- **Controls:** Age- and sex-matched children with non-malignant hematological disorders attending the same institution.

**Sample Size Calculation**

The sample size was calculated based on diagnostic accuracy methodology using anticipated sensitivity as the primary parameter.

Assuming:

- Expected sensitivity of LDH/peripheral blast percentage = 85%
- Confidence level = 95%
- Power = 80%
- Absolute precision (d) = 7%

The minimum required sample size was estimated using the formula:

$$n = Z^2 \times Se \times (1 - Se) / d^2$$

Where:

- n = required sample size
- Z = 1.96 at 95% confidence interval
- Se = anticipated sensitivity (0.85)
- d = allowable error (0.07)

The calculated minimum sample size was approximately 100 leukemia cases. To compensate for possible exclusions, missing data, and dropouts, 120 confirmed leukemia cases were recruited. An equal number of controls were enrolled, resulting in a final study population of 240 participants.

Sample size estimation was performed using OpenEpi Version 3.01 statistical software.

**Inclusion Criteria**

**Cases**

1. Children aged 1–18 years.
2. Newly diagnosed leukemia confirmed by bone marrow examination and immunophenotyping.
3. No prior chemotherapy or leukemia-specific treatment.
4. Written informed consent obtained from parents or legal guardians.

**Controls**

1. Children aged 1–18 years.
2. Diagnosed with non-malignant hematological conditions such as iron deficiency anemia, nutritional anemia, transient thrombocytopenia, or benign hematological disorders.
3. No evidence of malignancy.
4. Written informed consent obtained from parents or legal guardians.

**Exclusion Criteria**

1. Previously treated leukemia patients.
2. Relapsed leukemia.
3. Chronic inflammatory disorders.
4. Autoimmune diseases.
5. Chronic liver disease.
6. Chronic renal failure.
7. Active tuberculosis.
8. Congenital immunodeficiency disorders.
9. Patients with incomplete laboratory data.

**Data Collection**

A structured case record form was used for data collection.

**Clinical Variables**

The following demographic and clinical information was recorded:

- Age
- Sex
- Presenting complaints
- Duration of symptoms
- Fever
- Pallor
- Fatigue
- Bleeding manifestations
- Bone pain
- Lymphadenopathy

- Hepatosplenomegaly

**Hematological Parameters**
Peripheral venous blood samples were collected at diagnosis before initiation of chemotherapy.
The following hematological parameters were evaluated:
- Hemoglobin concentration (Hb)
- Total leukocyte count (TLC)
- Platelet count
- Peripheral blast percentage

Complete blood counts were performed using an automated hematology analyzer. Peripheral blood smears were independently reviewed by experienced hematopathologists for blast identification and quantification.

**Biochemical Biomarkers**
The following biomarkers were measured:
- Lactate dehydrogenase (LDH)
- Serum ferritin
- Serum uric acid
- C-reactive protein (CRP)
- Erythrocyte sedimentation rate (ESR)

LDH and uric acid levels were measured using automated biochemical analyzers. Serum ferritin was estimated using chemiluminescent immunoassay techniques. CRP was measured by immunoturbidimetric assay, while ESR was determined using the Westergren method.

**Diagnostic Confirmation of Leukemia**
The diagnosis of leukemia was established based on:
1. Peripheral blood smear examination.
2. Bone marrow aspiration and morphology.
3. Flow cytometric immunophenotyping.
4. Cytogenetic and molecular investigations whenever clinically indicated.

Leukemia was classified according to the World Health Organization (WHO) classification of hematopoietic malignancies.

**ROC Curve Analysis and Biomarker Cut-off Determination**
Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of individual biomarkers.

The area under the curve (AUC) was calculated with corresponding 95% confidence intervals (95% CI).
Optimal cut-off values were determined using **Youden's Index**, calculated as:

**Youden's Index = Sensitivity + Specificity − 1**
The cut-off value producing the highest Youden's Index was selected as the optimal threshold for clinical application.

**Biomarker Cut-off Values Evaluated**

| Biomarker | Optimal Cut-off |
|---|---|
| **LDH** | ≥620 U/L |
| **Peripheral Blast Percentage** | ≥12% |
| **Ferritin** | ≥320 ng/mL |
| **Uric Acid** | ≥5.8 mg/dL |
| **CRP** | ≥14 mg/L |
| **ESR** | ≥32 mm/hr |

For each cut-off, the following diagnostic indices were calculated:
- Sensitivity
- Specificity
- Positive Predictive Value (PPV)
- Negative Predictive Value (NPV)
- Diagnostic Accuracy

**Combined Biomarker Model**
A multivariable logistic regression model incorporating LDH, ferritin, uric acid, and peripheral blast percentage was developed to evaluate the diagnostic performance of a combined biomarker approach.

Predicted probabilities generated from the regression model were used to construct a combined ROC curve and estimate overall diagnostic accuracy.

**Statistical Analysis**
Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY, USA).

**Descriptive Statistics**
- Continuous variables were expressed as mean ± standard deviation (SD).
- Categorical variables were expressed as frequencies and percentages.

**Comparative Statistics**
- Student's independent t-test was used to compare continuous variables between groups.
- Chi-square test or Fisher's exact test was used for categorical variables.

**Correlation Analysis**
Pearson correlation coefficient was used to evaluate the association between LDH levels and peripheral blast percentage.

**Multivariate Analysis**
Binary logistic regression analysis was performed to identify independent predictors of childhood leukemia.
Adjusted odds ratios (ORs) with 95% confidence intervals were calculated.

**Statistical Significance**
A two-tailed p-value <0.05 was considered statistically significant.

**Ethical Considerations**
The study protocol was reviewed and approved by the Institutional Ethics Committee (IEC) of ESIC Medical College & PGIMSR, Rajajinagar, Bengaluru.

Written informed consent was obtained from parents or legal guardians before enrollment. Confidentiality of participant information was maintained throughout the study in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

**RESULTS**
During the study period from September 2024 to March 2026, a total of 267 children presenting with clinical suspicion of leukemia or hematological disorders were screened for eligibility. Eighteen children were excluded because of prior treatment, eleven were excluded due to incomplete laboratory investigations, and eighteen declined participation or consent could not be obtained. Finally, 240 children were enrolled, comprising 120 newly diagnosed leukemia patients (cases) and 120 age- and sex-matched children with non-malignant hematological disorders (controls).

The mean age of leukemia patients was 8.7 ± 4.1 years compared with 8.3 ± 3.9 years among controls (p=0.428). Males constituted 60.0% of leukemia cases and 56.7% of controls. There was no statistically significant difference in age or gender distribution between the groups, indicating adequate matching. Fever (80.0%), pallor (73.3%), fatigue (69.2%), hepatosplenomegaly (54.2%), and lymphadenopathy (49.2%) were the most common clinical manifestations among leukemia patients at presentation.

**Table 1. Demographic and Clinical Characteristics of Study Participants**

| Variable | Leukemia (n=120) | Controls (n=120) | p-value |
|---|---|---|---|
| Mean age (years) | 8.7 ± 4.1 | 8.3 ± 3.9 | 0.428 |
| Male sex | 72 (60.0%) | 68 (56.7%) | 0.602 |
| Female sex | 48 (40.0%) | 52 (43.3%) | 0.602 |
| Fever | 96 (80.0%) | 28 (23.3%) | <0.001 |
| Pallor | 88 (73.3%) | 51 (42.5%) | <0.001 |
| Fatigue | 83 (69.2%) | 37 (30.8%) | <0.001 |
| Hepatosplenomegaly | 65 (54.2%) | 8 (6.7%) | <0.001 |
| Lymphadenopathy | 59 (49.2%) | 11 (9.2%) | <0.001 |

**Distribution of Leukemia Subtypes**
Among the 120 leukemia patients, Acute Lymphoblastic Leukemia (ALL) was the predominant subtype, accounting for 98 (81.7%) cases, whereas Acute Myeloid Leukemia (AML) constituted 22 (18.3%) cases. No cases of chronic leukemia were encountered during the study period. The predominance of ALL is consistent with the known epidemiological distribution of childhood leukemia.

**Table 2. Distribution of Leukemia Subtypes**

| Leukemia Type | Frequency (n) | Percentage (%) |
|---|---|---|
| Acute Lymphoblastic Leukemia (ALL) | 98 | 81.7 |
| Acute Myeloid Leukemia (AML) | 22 | 18.3 |
| Total | 120 | 100 |

**Hematological and Biochemical Biomarker Profile**

Comparison of hematological parameters demonstrated marked abnormalities among leukemia patients. Mean hemoglobin concentration was significantly lower in leukemia patients than controls (7.8 ± 1.9 g/dL vs. 10.9 ± 1.6 g/dL, p<0.001). Similarly, platelet counts were substantially reduced, while total leukocyte counts were significantly elevated.

Among biochemical markers, serum LDH showed the greatest difference between groups, with mean levels approximately threefold higher among leukemia patients. Ferritin, uric acid, CRP, and ESR were also significantly elevated in leukemia patients, reflecting increased cellular turnover, inflammatory activity, and tumor burden.

Peripheral blast cells were identified in nearly all leukemia patients, with a mean blast percentage of 42.8 ± 18.6%, whereas no circulating blasts were observed among controls.

**Table 3. Comparison of Hematological and Biochemical Parameters**

| Parameter | Leukemia (n=120) | Controls (n=120) | p-value |
|---|---|---|---|
| Hemoglobin (g/dL) | 7.8 ± 1.9 | 10.9 ± 1.6 | <0.001 |
| TLC (×10⁹/L) | 38.6 ± 27.3 | 9.8 ± 4.2 | <0.001 |
| Platelet Count (×10⁹/L) | 71 ± 42 | 248 ± 67 | <0.001 |
| LDH (U/L) | 962 ± 312 | 318 ± 96 | <0.001 |
| Ferritin (ng/mL) | 678 ± 241 | 132 ± 56 | <0.001 |
| Uric Acid (mg/dL) | 7.2 ± 2.1 | 3.9 ± 1.1 | <0.001 |
| CRP (mg/L) | 21.8 ± 8.3 | 8.4 ± 3.7 | <0.001 |
| ESR (mm/hr) | 49 ± 18 | 17 ± 7 | <0.001 |
| Peripheral Blast Percentage (%) | 42.8 ± 18.6 | 0 | <0.001 |

**Biomarker Variation According to Leukemia Subtype**

Subgroup analysis demonstrated significantly higher LDH, ferritin, uric acid, and peripheral blast percentages among AML patients compared with ALL patients. AML patients exhibited a more pronounced biochemical derangement, suggesting a higher proliferative burden and more aggressive disease biology.

**Table 4. Biomarker Levels According to Leukemia Subtype**

| Biomarker | ALL (n=98) | AML (n=22) | p-value |
|---|---|---|---|
| LDH (U/L) | 918 ± 286 | 1158 ± 374 | 0.004 |
| Ferritin (ng/mL) | 642 ± 218 | 829 ± 273 | 0.006 |
| Uric Acid (mg/dL) | 6.9 ± 1.8 | 8.5 ± 2.2 | 0.003 |
| Peripheral Blast Percentage (%) | 39.8 ± 17.4 | 56.2 ± 19.7 | 0.001 |

**Diagnostic Performance of Individual Biomarkers**

ROC curve analysis was performed to evaluate the diagnostic utility of individual peripheral blood biomarkers (Figure 1). LDH demonstrated the highest diagnostic accuracy with an AUC of 0.91 (95% CI: 0.87–0.95). At an optimal cut-off value of ≥620 U/L, LDH achieved 89.2% sensitivity and 85.8% specificity.

Peripheral blast percentage demonstrated an AUC of 0.89 (95% CI: 0.84–0.93). A threshold of ≥12% blasts provided a sensitivity of 88.3% and specificity of 90.0%. Ferritin and uric acid also showed good discriminatory ability, whereas CRP and ESR demonstrated moderate diagnostic performance.

**Table 5. ROC-Derived Cut-off Values and Diagnostic Performance of Individual Biomarkers**

| Biomarker | Cut-off Value | AUC (95% CI) | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| LDH | ≥620 U/L | 0.91 (0.87–0.95) | 89.2 | 85.8 |
| Peripheral Blast % | ≥12% | 0.89 (0.84–0.93) | 88.3 | 90.0 |
| Ferritin | ≥320 ng/mL | 0.86 (0.81–0.91) | 84.1 | 80.8 |
| Uric Acid | ≥5.8 mg/dL | 0.82 (0.76–0.87) | 80.8 | 78.3 |
| CRP | ≥14 mg/L | 0.79 (0.73–0.85) | 76.7 | 72.5 |
| ESR | ≥32 mm/hr | 0.76 (0.70–0.82) | 73.3 | 70.8 |

**Performance of the Combined Biomarker Model**

To determine whether simultaneous assessment of multiple biomarkers improved diagnostic accuracy, a multivariable logistic regression model incorporating LDH, ferritin, uric acid, and peripheral blast percentage was developed. The combined biomarker model demonstrated excellent discriminatory ability, with an AUC of 0.94 (95% CI: 0.90–0.97) (Figure 2).

At the optimal probability threshold, the combined model achieved a sensitivity of 94.2%, specificity of 88.3%, positive predictive value (PPV) of 89.7%, negative predictive value (NPV) of 93.4%, and overall diagnostic accuracy of 91.3%.

**Table 6. Diagnostic Performance of Combined Biomarker Model**

| Parameter | Value |
|---|---|
| AUC (95% CI) | 0.94 (0.90–0.97) |
| Sensitivity (%) | 94.2 |
| Specificity (%) | 88.3 |
| Positive Predictive Value (%) | 89.7 |
| Negative Predictive Value (%) | 93.4 |
| Diagnostic Accuracy (%) | 91.3 |

**Correlation Analysis and Independent Predictors of Childhood Leukemia**

Correlation analysis demonstrated a strong positive relationship between serum LDH levels and peripheral blast percentage ($r = 0.74$, $p<0.001$), indicating that increasing blast burden was associated with greater cellular turnover and LDH release. Multivariable logistic regression analysis identified elevated LDH, increased ferritin levels, hyperuricemia, and peripheral blast percentage greater than 12% as independent predictors of childhood leukemia. Among these variables, peripheral blast percentage ≥12% emerged as the strongest predictor (Adjusted OR: 8.92; 95% CI: 4.18–18.67), followed by LDH ≥620 U/L (Adjusted OR: 6.84; 95% CI: 3.45–12.76).

**Table 7. Multivariable Logistic Regression Analysis of Independent Predictors of Childhood Leukemia**

| Variable | Adjusted OR | 95% CI | p-value |
|---|---|---|---|
| LDH ≥620 U/L | 6.84 | 3.45–12.76 | <0.001 |
| Ferritin ≥320 ng/mL | 4.12 | 2.31–8.55 | <0.001 |
| Uric Acid ≥5.8 mg/dL | 3.76 | 1.89–7.46 | 0.002 |
| Peripheral Blast Percentage ≥12% | 8.92 | 4.18–18.67 | <0.001 |

Overall, the findings indicate that peripheral blood biomarkers possess substantial diagnostic utility in childhood leukemia. Among individual markers, LDH demonstrated the highest diagnostic accuracy, while the combined biomarker model achieved superior sensitivity and specificity. These biomarkers may therefore serve as valuable adjunctive tools for early clinical suspicion and prioritization of definitive hematological evaluation in pediatric patients suspected of leukemia.

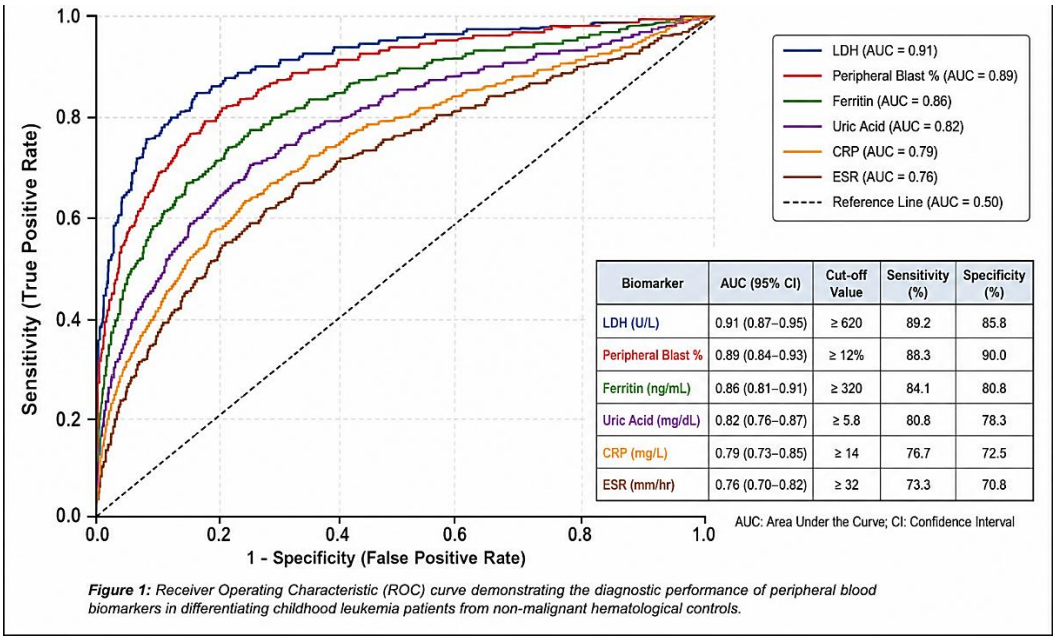


***Figure 1.*** *Receiver Operating Characteristic (ROC) curve demonstrating the diagnostic performance of peripheral blood biomarkers for childhood leukemia. Lactate dehydrogenase (LDH) showed the highest diagnostic accuracy (AUC =*

*0.91), followed by Peripheral Blast Percentage (AUC = 0.89), Ferritin (AUC = 0.86), Uric Acid (AUC = 0.82), C-Reactive Protein (CRP) (AUC = 0.79), and Erythrocyte Sedimentation Rate (ESR) (AUC = 0.76). The diagonal reference line represents a test with no discriminative ability (AUC = 0.50).*

## DISCUSSION

Childhood leukemia remains the most common pediatric malignancy worldwide and continues to be associated with substantial morbidity and mortality, particularly when diagnosis is delayed. Early identification of affected children is critical because prompt initiation of therapy significantly improves survival outcomes. Although definitive diagnosis relies on bone marrow examination, immunophenotyping, cytogenetic analysis, and molecular testing, readily available peripheral blood biomarkers may provide valuable early diagnostic clues in resource-limited settings and during initial clinical evaluation. The present prospective observational study evaluated the diagnostic utility of several peripheral blood biomarkers among children with newly diagnosed leukemia and demonstrated that serum LDH, ferritin, uric acid, inflammatory markers, and peripheral blast percentage possess significant diagnostic value.

The demographic profile observed in the present study is consistent with previous pediatric leukemia studies. The mean age of affected children was 8.7 years, and a male predominance was observed. Similar findings have been reported by Hunger and Mullighan, who demonstrated that childhood acute leukemia most commonly affects school-aged children and occurs more frequently in males than females [1]. Acute lymphoblastic leukemia accounted for more than four-fifths of cases in our cohort, which aligns with global epidemiological data indicating that ALL constitutes approximately 75–85% of childhood leukemia cases [2].

Among clinical manifestations, fever, pallor, fatigue, hepatosplenomegaly, and lymphadenopathy were the most frequent findings. These observations are comparable to those reported by Pui et al., who described bone marrow failure and leukemic infiltration as major contributors to the characteristic clinical presentation of childhood leukemia [3]. The significantly higher prevalence of constitutional symptoms among leukemia patients compared with controls reinforces the importance of maintaining a high index of suspicion in children presenting with unexplained fever, anemia, and organomegaly.

One of the principal findings of this study was the markedly elevated serum LDH level among leukemia patients. LDH demonstrated the highest individual diagnostic performance, achieving an AUC of 0.91 (95% CI: 0.87–0.95) with sensitivity and specificity values of 89.2% and 85.8%, respectively. LDH is released during cellular breakdown and reflects increased tumor burden, rapid cell turnover, and tissue destruction. Elevated LDH has long been recognized as a marker of disease activity and tumor proliferation in hematological malignancies [4]. The strong positive correlation observed between LDH levels and peripheral blast percentage further supports its role as an indirect indicator of leukemic burden. Similar associations have been reported in studies by Kornblau et al. and Tasian et al., which demonstrated that elevated LDH levels correlate with disease severity and adverse biological characteristics in acute leukemia [5,6].

Peripheral blast percentage also exhibited excellent diagnostic performance, with an AUC of 0.89 and specificity of 90%. Although blast identification remains a routine component of peripheral smear examination, quantitative evaluation of blast percentage has received comparatively less attention as a diagnostic biomarker. The present findings suggest that a threshold value of ≥12% blasts may serve as a useful indicator warranting urgent hematological assessment. Moreover, peripheral blast percentage emerged as the strongest independent predictor of leukemia in multivariable analysis, highlighting its central role in disease recognition.

Serum ferritin demonstrated good diagnostic accuracy with an AUC of 0.86. Elevated ferritin levels in leukemia likely reflect a combination of inflammation, increased iron turnover, macrophage activation, and cytokine-mediated immune responses. Previous studies have shown that ferritin concentrations are frequently elevated in hematological malignancies and may correlate with disease burden and inflammatory activity [7,8]. Similarly, hyperuricemia was significantly more common among leukemia patients. Elevated uric acid levels result from increased nucleic acid degradation associated with rapid cellular proliferation and turnover. Hyperuricemia is well recognized as a biochemical hallmark of aggressive leukemia and contributes to the development of tumor lysis syndrome [9].

The inflammatory markers CRP and ESR exhibited moderate diagnostic performance compared with LDH, ferritin, and blast percentage. Although these markers demonstrated statistically significant elevations among leukemia patients, their lower specificity likely reflects their nonspecific response to infection, inflammation, and various non-malignant conditions. Consequently, CRP and ESR should be interpreted as supportive rather than primary diagnostic indicators in the evaluation of suspected childhood leukemia.

A notable strength of the present study is the development of a combined biomarker model integrating LDH, ferritin, uric acid, and peripheral blast percentage. This model achieved an AUC of 0.94 (95% CI: 0.90–0.97), with sensitivity exceeding 94% and specificity approaching 90%. These findings suggest that simultaneous assessment of multiple biomarkers provides superior diagnostic performance compared with individual markers alone. The improved accuracy likely reflects the complementary biological information captured by different biomarkers, including tumor burden, cellular turnover,

inflammatory activation, and hematopoietic disruption. Similar improvements in diagnostic accuracy through multimarker approaches have been demonstrated in other pediatric oncology studies [10].

Nevertheless, the high AUC values observed in this study warrant careful interpretation. The study compared newly diagnosed leukemia patients with children having non-malignant hematological disorders rather than with a broader spectrum of pediatric illnesses. This case-control design may partially enhance apparent diagnostic discrimination. Furthermore, the cut-off values identified through ROC analysis were derived from the current study population and have not yet undergone external validation. Consequently, these thresholds should not be considered universally applicable until confirmed in independent cohorts.

Multivariable logistic regression identified elevated LDH, ferritin, uric acid, and peripheral blast percentage as independent predictors of leukemia. Among these, peripheral blast percentage and LDH demonstrated the strongest associations. These findings emphasize that biomarkers reflecting leukemic cell burden and metabolic activity may provide the greatest diagnostic utility during early clinical assessment.

The present study possesses several strengths. First, it employed a prospective design, minimizing recall bias and improving data quality. Second, all leukemia diagnoses were confirmed through bone marrow examination and immunophenotyping, ensuring diagnostic accuracy. Third, ROC analysis was performed with confidence intervals and clinically applicable cut-off values, thereby enhancing translational relevance. Finally, the combined biomarker model provides a practical framework for integrating laboratory findings in routine pediatric practice.

Certain limitations should be acknowledged. The study was conducted at a single tertiary-care center, which may limit generalizability. The sample size, although adequately powered for diagnostic analysis, remains relatively modest compared with multicenter studies. External validation of the proposed biomarker cut-offs was not performed. In addition, longitudinal follow-up was beyond the scope of the study; therefore, the prognostic significance of these biomarkers could not be evaluated.

Overall, the findings suggest that peripheral blood biomarkers, particularly LDH, ferritin, uric acid, and peripheral blast percentage, possess significant diagnostic value in childhood leukemia. While these markers cannot replace definitive diagnostic procedures such as bone marrow examination and immunophenotyping, they may serve as valuable adjunctive tools for early risk stratification, timely referral, and prioritization of specialized hematological evaluation.

## CONCLUSION

Peripheral blood biomarkers, particularly LDH, peripheral blast percentage, ferritin, and uric acid, demonstrated significant diagnostic utility in childhood leukemia. LDH showed the highest individual diagnostic accuracy, while the combined biomarker model achieved excellent sensitivity and specificity for distinguishing leukemia from non-malignant hematological disorders. Although these biomarkers cannot replace bone marrow examination and immunophenotyping, they serve as valuable adjunctive tools for early clinical suspicion and timely diagnostic evaluation. Further multicenter studies are needed to validate the proposed cut-off values and establish their role in routine pediatric leukemia screening.

### Strengths of the Study

1. **Prospective Study Design:** The prospective observational design minimized recall bias and ensured systematic collection of clinical and laboratory data.
2. **Confirmed Diagnosis:** All leukemia cases were confirmed using bone marrow examination and immunophenotyping, ensuring diagnostic accuracy and reducing misclassification.
3. **Comprehensive Biomarker Assessment:** Multiple routinely available peripheral blood biomarkers, including LDH, ferritin, uric acid, CRP, ESR, and peripheral blast percentage, were evaluated simultaneously.
4. **Clinically Applicable Cut-off Values:** ROC curve analysis provided biomarker-specific cut-off values along with sensitivity, specificity, and AUC estimates, enhancing clinical applicability.
5. **Combined Biomarker Model:** The study demonstrated improved diagnostic performance through a multivariable biomarker model, reflecting real-world clinical decision-making.
6. **Age- and Sex-Matched Controls:** Inclusion of matched controls minimized confounding and improved the validity of comparisons between groups.
7. **Resource-Limited Setting Relevance:** The evaluated biomarkers are inexpensive, widely available, and easily measurable, making the findings particularly relevant for low- and middle-income healthcare settings.

### Limitations

1. **Single-Center Study:** The study was conducted at a single tertiary care institution, which may limit the generalizability of the findings to other populations and healthcare settings.
2. **Moderate Sample Size:** Although adequately powered for diagnostic analysis, the sample size was relatively small compared to large multicenter studies.
3. **Lack of External Validation:** The biomarker cut-off values and combined diagnostic model were derived from the study cohort and were not validated in an independent external population.

4. **Case-Control Design:** Comparison with non-malignant hematological controls may have enhanced diagnostic discrimination and potentially overestimated biomarker performance.
5. **Limited Leukemia Spectrum:** The majority of cases were acute lymphoblastic leukemia, limiting subgroup analysis of less common leukemia subtypes.
6. **Absence of Longitudinal Follow-up:** The study focused on diagnostic utility and did not assess the prognostic significance of biomarkers, treatment response, or survival outcomes.
7. **Potential Laboratory Variability:** Biomarker measurements may vary across laboratories due to differences in assay methods and instrumentation, which could affect the universal applicability of the proposed cut-off values.
8. **Unmeasured Confounders:** Factors such as nutritional status, occult infections, and inflammatory conditions may have influenced biomarker levels despite the exclusion criteria applied.

**Conflict of Interest-** The authors declare no conflict of interest.
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