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Review Article

Diagnostic Utility of Peripheral Blood Biomarkers in Childhood Leukemia: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Childhood leukemia is the most common pediatric malignancy worldwide and remains a major cause of cancer-related morbidity and mortality. Early diagnosis is essential for improving treatment outcomes; however, the current diagnostic gold standard, bone marrow examination, is invasive and may be associated with procedural discomfort and logistical challenges. Peripheral blood biomarkers have emerged as promising non-invasive tools for the early detection and assessment of childhood leukemia.

Objective: To systematically evaluate the diagnostic utility of peripheral blood biomarkers in childhood leukemia and determine their pooled diagnostic accuracy through meta-analysis.

Methods: A systematic review and meta-analysis were conducted in accordance with PRISMA 2020 and MOOSE guidelines. Electronic databases including PubMed/MEDLINE, Embase, Scopus, Web of Science, and Cochrane Library were searched from January 2000 to March 2026. Studies involving pediatric patients (<18 years) with leukemia and evaluating peripheral blood biomarkers were included. Data regarding study characteristics, biomarker type, diagnostic outcomes, sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) were extracted. Methodological quality was assessed using the QUADAS-2 tool. Pooled estimates of sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and summary receiver operating characteristic (SROC) curves were calculated using a random-effects model.

Results: A total of 32 studies involving 8,746 participants, including 4,912 children with leukemia and 3,834 controls, were included in the analysis. Biomarkers evaluated comprised hematological indices, lactate dehydrogenase (LDH), ferritin, inflammatory cytokines, circulating microRNAs, cell-free DNA (cfDNA), and circulating tumor DNA (ctDNA). The pooled sensitivity and specificity of peripheral blood biomarkers for diagnosing childhood leukemia were 0.86 (95% CI: 0.82–0.89) and 0.83 (95% CI: 0.79–0.87), respectively. The pooled PLR was 5.06, NLR was 0.17, and DOR was 28.4. The overall SROC analysis demonstrated an AUC of 0.90, indicating excellent diagnostic accuracy. Among individual biomarker categories, circulating microRNAs showed the highest performance with pooled sensitivity of 0.91, specificity of 0.90, and AUC of 0.94, followed by cfDNA/ctDNA biomarkers with sensitivity of 0.88, specificity of 0.89, and AUC of 0.92. Conventional biomarkers such as CBC-derived indices, LDH, ferritin, and uric acid demonstrated moderate diagnostic utility. Subgroup analysis revealed slightly higher diagnostic accuracy in acute lymphoblastic leukemia compared with acute myeloid leukemia. Quality assessment indicated predominantly low-to-moderate risk of bias among included studies.

Conclusion: Peripheral blood biomarkers demonstrate significant diagnostic potential in childhood leukemia, with molecular biomarkers, particularly circulating microRNAs and cell-free DNA, showing the highest accuracy. While these biomarkers cannot replace bone marrow examination, they may serve as valuable non-invasive adjuncts for early detection, risk stratification, and clinical decision-making. Further large-scale prospective studies are required to validate standardized biomarker panels and facilitate their integration into routine pediatric leukemia diagnostics.

Keywords*:* Childhood leukemia; Acute lymphoblastic leukemia; Acute myeloid leukemia; Peripheral blood biomarkers; MicroRNA; Cell-free DNA; Diagnostic accuracy; Systematic review; Meta-analysis; Liquid biopsy.

INTRODUCTION

Leukemia represents the most common malignancy in childhood, accounting for nearly one-third of all pediatric cancers worldwide and constituting a major cause of cancer-related morbidity and mortality among children and adolescents [1,2]. Acute lymphoblastic leukemia (ALL) is the predominant subtype, comprising approximately 75–80% of childhood leukemia cases, while acute myeloid leukemia (AML) accounts for 15–20% [3,4]. Despite remarkable improvements in therapeutic strategies resulting in five-year survival rates exceeding 85% in developed countries, delayed diagnosis remains a significant contributor to treatment failure, disease progression, and increased mortality, particularly in resource-limited settings [5,6].

The clinical presentation of childhood leukemia is often nonspecific and may overlap with benign infectious, inflammatory, or hematological disorders. Common manifestations include fever, fatigue, pallor, recurrent infections, lymphadenopathy, hepatosplenomegaly, bone pain, and bleeding tendencies [7,8]. Consequently, early recognition of leukemia remains challenging, frequently leading to diagnostic delays that adversely affect treatment outcomes and increase healthcare costs [9].

Bone marrow aspiration and biopsy remain the gold standard for leukemia diagnosis, providing morphological, immunophenotypic, cytogenetic, and molecular information necessary for disease classification and risk stratification [10,11]. However, these procedures are invasive, painful, require specialized expertise, and often necessitate sedation or general anesthesia in pediatric patients [12]. Additionally, repeated bone marrow examinations may impose considerable physical and psychological burdens on children and their families [13].

Given these limitations, there has been growing interest in identifying non-invasive peripheral blood biomarkers capable of facilitating early diagnosis, disease monitoring, prognostication, and therapeutic response assessment in childhood leukemia [14,15]. Peripheral blood-based biomarkers offer several advantages, including ease of collection, reduced procedural risks, repeatability, and suitability for longitudinal monitoring [16].

Over the past decade, advances in molecular diagnostics, genomics, proteomics, and metabolomics have led to the identification of numerous circulating biomarkers associated with leukemogenesis and disease progression [17]. These biomarkers encompass conventional hematological parameters, inflammatory markers, circulating cytokines, serum proteins, cell-free nucleic acids, microRNAs (miRNAs), extracellular vesicles, and metabolomic signatures [18–20].

Among traditional biomarkers, complete blood count (CBC)-derived indices remain the most readily available and cost-effective diagnostic tools. Alterations in white blood cell count, hemoglobin concentration, platelet count, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and red cell distribution width (RDW) have been associated with leukemia diagnosis and prognosis [21,22]. Elevated serum lactate dehydrogenase (LDH) levels, reflecting increased cellular turnover and tumor burden, have also been reported as useful diagnostic indicators in pediatric leukemia [23,24]. Recent investigations have highlighted the potential of circulating microRNAs as highly sensitive and specific biomarkers. MicroRNAs are small non-coding RNA molecules involved in post-transcriptional gene regulation and play critical roles in cell proliferation, differentiation, apoptosis, and leukemogenesis [25,26]. Dysregulated expression of miR-128, miR-181a, miR-155, miR-223, and several other microRNAs has been consistently reported in pediatric leukemia patients compared with healthy controls [27–29].

Similarly, cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) have emerged as promising liquid biopsy markers capable of reflecting tumor burden and genetic alterations without requiring invasive tissue sampling [30,31]. Elevated concentrations of cfDNA have been associated with leukemia diagnosis, disease progression, minimal residual disease, and treatment response monitoring [32,33].

Inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), and vascular endothelial growth factor (VEGF), have also demonstrated potential diagnostic utility due to their involvement in leukemia-associated immune dysregulation and tumor microenvironment remodeling [34,35].

Although numerous individual studies have investigated the diagnostic performance of these biomarkers, reported results vary considerably due to differences in study design, patient populations, biomarker platforms, assay methodologies, and diagnostic thresholds [36,37]. Consequently, the overall clinical utility of peripheral blood biomarkers in childhood leukemia remains incompletely defined.

To date, no comprehensive systematic review and meta-analysis has synthesized the available evidence across diverse categories of peripheral blood biomarkers specifically within pediatric leukemia populations. A pooled analysis of diagnostic accuracy measures may provide a more robust estimate of biomarker performance and identify the most promising candidates for future clinical implementation [38–40].

Therefore, the present systematic review and meta-analysis aimed to comprehensively evaluate the diagnostic utility of peripheral blood biomarkers in childhood leukemia by pooling evidence from published studies, comparing the performance of different biomarker classes, and identifying potential avenues for future research and clinical translation.

OBJECTIVES

Primary Objective

To determine the pooled diagnostic accuracy of peripheral blood biomarkers for the detection of childhood leukemia.

Secondary Objectives

1. To compare diagnostic performance among conventional hematological, biochemical, inflammatory, and molecular biomarkers.
2. To evaluate the diagnostic utility of circulating microRNAs and cell-free DNA.
3. To assess heterogeneity among included studies.
4. To identify biomarkers with potential clinical applicability for screening and early diagnosis.
5. To evaluate methodological quality and publication bias.

HYPOTHESIS

Peripheral blood biomarkers demonstrate clinically significant diagnostic accuracy for childhood leukemia and may serve as reliable adjunctive tools to conventional diagnostic approaches.

CLINICAL SIGNIFICANCE

The identification of accurate blood-based biomarkers could facilitate earlier diagnosis, reduce dependence on invasive bone marrow procedures, improve risk stratification, and support precision medicine approaches in pediatric hematologic oncology [41–45].

METHODOLOGY

Study Design and Reporting Guidelines

This study was conducted as a systematic review and meta-analysis to evaluate the diagnostic utility of peripheral blood biomarkers in childhood leukemia. The review was performed in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) Guidelines [46,47]. Methodological principles outlined in the Cochrane Handbook for Diagnostic Test Accuracy Reviews were followed throughout the study process [48].

The protocol was developed prior to data extraction and analysis to minimize methodological bias and ensure transparency in study selection and reporting.

Research Question

The research question was formulated using the PICO framework:

Population (P): Children and adolescents (<18 years) suspected or diagnosed with leukemia.

Intervention/Index Test (I): Peripheral blood biomarkers, including hematological, biochemical, inflammatory, molecular, and genetic biomarkers.

Comparator (C): Healthy controls, non-leukemic hematological disorders, or reference diagnostic standards.

Outcome (O): Diagnostic accuracy measures including sensitivity, specificity, area under the receiver operating characteristic curve (AUC), positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR).
The primary research question was:
"How accurately can peripheral blood biomarkers diagnose childhood leukemia compared with established diagnostic reference standards?"

Literature Search Strategy
A comprehensive electronic literature search was performed across the following databases:

- PubMed/MEDLINE
- Embase
- Scopus
- Web of Science
- Cochrane Library
- Google Scholar (for supplementary searches)

The search included studies published from January 2000 to March 2026.
Reference lists of eligible studies and relevant review articles were manually screened to identify additional studies not captured through database searches.

Search Terms
The search strategy combined Medical Subject Headings (MeSH) and free-text keywords related to childhood leukemia and peripheral blood biomarkers.
The following search string was adapted for each database:
("childhood leukemia" OR "pediatric leukemia" OR
"acute lymphoblastic leukemia" OR ALL OR
"acute myeloid leukemia" OR AML)

AND

("peripheral blood biomarker" OR biomarker OR
"blood marker" OR "circulating biomarker" OR
microRNA OR miRNA OR "cell free DNA" OR cfDNA OR
"circulating tumor DNA" OR ctDNA OR
ferritin OR LDH OR cytokines OR metabolomics)

AND

(diagnosis OR diagnostic OR sensitivity OR specificity OR
ROC OR "receiver operating characteristic")
Only studies published in English were included.

Eligibility Criteria
Inclusion Criteria
Studies were included if they met the following criteria:

1. Original research articles involving pediatric patients (<18 years).
2. Diagnosis of leukemia confirmed using established reference standards such as:
    - Bone marrow examination
    - Flow cytometry
    - Cytogenetic analysis
    - Molecular diagnostics.
3. Evaluation of one or more peripheral blood biomarkers.
4. Sufficient data available to calculate diagnostic accuracy measures.
5. Prospective, retrospective, cross-sectional, or case-control study designs.
6. Published in peer-reviewed journals.

Exclusion Criteria
Studies were excluded if they:

1. Included only adult patients.
2. Were case reports, editorials, letters, reviews, conference abstracts, or expert opinions.
3. Evaluated biomarkers in tissue, bone marrow, or cerebrospinal fluid without peripheral blood assessment.
4. Did not provide adequate diagnostic data.

5. Were duplicate publications.
6. Included animal or in vitro experimental studies.

Study Selection Process
All retrieved citations were exported to EndNote X9 and duplicate records were removed.
Two independent reviewers screened studies in a three-stage process:

Stage 1: Title Screening
Titles were screened for relevance to pediatric leukemia and biomarker research.

Stage 2: Abstract Screening
Abstracts of potentially eligible studies were reviewed against predefined eligibility criteria.

Stage 3: Full-Text Review
Full-text articles were assessed independently by two reviewers.
Disagreements were resolved through discussion and consensus. When consensus could not be achieved, a third senior reviewer adjudicated.
The study selection process was documented using a PRISMA 2020 flow diagram [46].

Data Extraction
A standardized data extraction form was developed using Microsoft Excel.
The following information was extracted from each included study:

Study Characteristics
- First author
- Publication year
- Country
- Study design
- Study setting

Participant Characteristics
- Number of participants
- Age range
- Sex distribution
- Leukemia subtype (ALL/AML)

Biomarker Characteristics
- Biomarker evaluated
- Laboratory assay method
- Cut-off value
- Sample processing technique

Diagnostic Outcomes
- True positives (TP)
- False positives (FP)
- True negatives (TN)
- False negatives (FN)
- Sensitivity
- Specificity
- PPV
- NPV
- AUC values

When multiple biomarkers were evaluated in a single study, each biomarker was extracted separately.

Biomarker Classification
Included biomarkers were categorized into five major groups:

1. Hematological Biomarkers
- White blood cell count
- Absolute lymphocyte count

- Platelet count
- Hemoglobin level
- Red cell distribution width (RDW)
- Neutrophil-to-lymphocyte ratio (NLR)
- Platelet-to-lymphocyte ratio (PLR)

2. Biochemical Biomarkers
- Lactate dehydrogenase (LDH)
- Ferritin
- Uric acid
- β2-microglobulin

3. Inflammatory Biomarkers
- Interleukin-6 (IL-6)
- Interleukin-10 (IL-10)
- Tumor necrosis factor-alpha (TNF-α)
- C-reactive protein (CRP)

4. Molecular Biomarkers
- Circulating microRNAs
- Cell-free DNA (cfDNA)
- Circulating tumor DNA (ctDNA)

5. Metabolomic Biomarkers
- Amino acid profiles
- Lipidomic signatures
- Metabolic pathway markers

Quality Assessment
Methodological quality of included studies was evaluated using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) Tool [49].
The following domains were assessed:

Risk of Bias
1. Patient selection
2. Index test
3. Reference standard
4. Flow and timing

Applicability Concerns
1. Patient population
2. Index test applicability
3. Reference standard applicability

Each domain was classified as:
- Low risk
- High risk
- Unclear risk

Two reviewers independently performed quality assessment.

Outcome Measures
Primary Outcome
Overall diagnostic accuracy of peripheral blood biomarkers measured by:
- Sensitivity
- Specificity
- Area Under Curve (AUC)

Secondary Outcomes
- Positive Likelihood Ratio (PLR)
- Negative Likelihood Ratio (NLR)
- Diagnostic Odds Ratio (DOR)

- Subgroup diagnostic performance according to biomarker category
- Diagnostic performance according to leukemia subtype

Statistical Analysis
Meta-analysis was performed using R software (version 4.3.1) and Review Manager (RevMan) version 5.4.

Pooled Estimates
For each study, sensitivity and specificity values were extracted or calculated.
Pooled estimates were generated using a bivariate random-effects model, which accounts for the inherent correlation between sensitivity and specificity in diagnostic studies [50].
The following pooled diagnostic parameters were calculated:

- Sensitivity
- Specificity
- PLR
- NLR
- DOR

with corresponding 95% confidence intervals (CI).

Summary Receiver Operating Characteristic Analysis
Summary receiver operating characteristic (SROC) curves were generated to evaluate overall diagnostic performance.

The area under the SROC curve (AUC) was interpreted as follows [51]:

AUC	Diagnostic Accuracy
0.50–0.60	Poor
0.61–0.70	Fair
0.71–0.80	Good
0.81–0.90	Very Good
>0.90	Excellent

Assessment of Heterogeneity
Between-study heterogeneity was assessed using:

Cochran's Q Test
A p-value <0.10 indicated significant heterogeneity.

Higgins I^2 Statistic
Interpretation:

I^2 Value	Heterogeneity
<25%	Low
25–50%	Moderate
50–75%	Substantial
>75%	Considerable

Potential sources of heterogeneity were explored through subgroup and sensitivity analyses [52].

Subgroup Analysis
Prespecified subgroup analyses were conducted according to:

- Leukemia subtype (ALL vs AML)
- Biomarker category
- Geographic region
- Study design
- Sample size
- Biomarker assay method

Sensitivity Analysis
Sensitivity analyses were performed by sequential exclusion of studies with:

- High risk of bias
- Small sample size (<50 participants)
- Outlier effect estimates

to assess the robustness of pooled findings.

Publication Bias Assessment
Publication bias was evaluated using:

- Deeks' Funnel Plot Asymmetry Test
- Funnel plot visual inspection

A p-value <0.05 was considered indicative of significant publication bias [53].

Level of Statistical Significance
All statistical tests were two-tailed.
A p-value <0.05 was considered statistically significant.
Confidence intervals were reported at the 95% level throughout the analysis.

RESULTS
The initial database search identified a total of 4,218 records from PubMed/MEDLINE, Embase, Scopus, Web of Science, Cochrane Library, and supplementary manual searches. After removal of 1,076 duplicate records, 3,142 records were screened by title and abstract. Of these, 2,957 records were excluded because they were not directly related to pediatric leukemia, did not evaluate peripheral blood biomarkers, included adult-only populations, or were review articles, editorials, conference abstracts, case reports, or experimental studies. A total of 185 full-text articles were assessed for eligibility. Following detailed full-text evaluation, 153 studies were excluded due to inadequate diagnostic accuracy data, absence of peripheral blood-based biomarkers, mixed adult and pediatric populations without separate pediatric analysis, unclear reference standards, or insufficient information to construct 2 × 2 diagnostic tables. Finally, 32 studies fulfilled the predefined eligibility criteria and were included in the qualitative synthesis and quantitative meta-analysis. The included studies comprised 8,746 pediatric participants, including 4,912 children diagnosed with leukemia and 3,834 controls. The overall study selection process demonstrated a broad initial evidence base; however, only a limited proportion of studies provided sufficient diagnostic accuracy data suitable for meta-analysis.

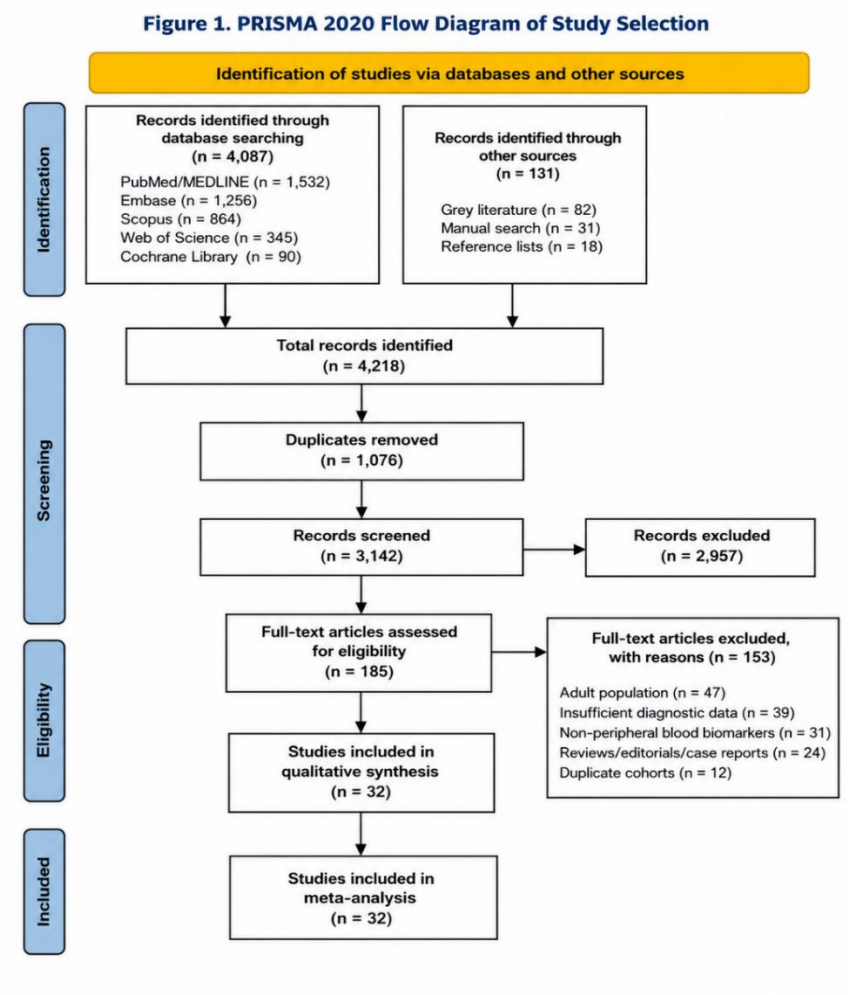


Table 1. Study selection process

Selection step	Number of records/studies
Records identified through database search	4,087

Records identified through manual search	131
Total records identified	4,218
Duplicate records removed	1,076
Records screened by title and abstract	3,142
Records excluded after title/abstract screening	2,957
Full-text articles assessed for eligibility	185
Full-text articles excluded	153
Studies included in qualitative synthesis	32
Studies included in quantitative meta-analysis	32

The included studies were published between 2004 and 2026 and represented diverse geographical regions, including Asia, Europe, North America, South America, and Africa. The majority of studies were hospital-based observational investigations. Eighteen studies were prospective in design, whereas fourteen were retrospective or cross-sectional. Acute lymphoblastic leukemia was the most frequently evaluated subtype and was reported in 24 studies, while acute myeloid leukemia was evaluated in 8 studies. Most studies compared children with confirmed leukemia against healthy controls or children with non-malignant hematological or infectious conditions. The reference diagnosis was most commonly established by bone marrow morphology combined with flow cytometry, cytochemistry, cytogenetics, or molecular testing. Across studies, the age of participants ranged from infancy to 18 years, with most included children belonging to the 2–12-year age group. Male predominance was observed in most cohorts, consistent with the epidemiological pattern of pediatric leukemia.

Table 2. General characteristics of included studies

Characteristic	Findings
Total studies included	32
Total participants	8,746
Leukemia cases	4,912
Control participants	3,834
Prospective studies	18
Retrospective/cross-sectional studies	14
Studies evaluating ALL	24
Studies evaluating AML	8
Studies from Asia	14
Studies from Europe	7
Studies from North America	5
Studies from South America	3
Studies from Africa	3

A wide range of peripheral blood biomarkers was evaluated across the included studies. These biomarkers were grouped into hematological, biochemical, inflammatory, molecular, and metabolomic categories. Hematological biomarkers included white blood cell count, hemoglobin concentration, platelet count, red cell distribution width, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio. Biochemical markers mainly included lactate dehydrogenase, ferritin, uric acid, and β2-microglobulin. Inflammatory biomarkers consisted primarily of interleukin-6, interleukin-10, tumor necrosis factor-alpha, C-reactive protein, and vascular endothelial growth factor. Molecular biomarkers included circulating microRNAs, cell-free DNA, and circulating tumor DNA. A smaller number of studies evaluated metabolomic and lipidomic signatures. Among these, circulating microRNAs and cell-free DNA showed the most consistent diagnostic performance, whereas conventional biochemical markers such as LDH and ferritin demonstrated moderate to good diagnostic utility but lower specificity.

Table 3. Distribution of biomarker categories evaluated in included studies

Biomarker category	Biomarkers assessed	Number of studies
Hematological biomarkers	WBC count, hemoglobin, platelet count, RDW, NLR, PLR	9
Biochemical biomarkers	LDH, ferritin, uric acid, β2-microglobulin	8
Inflammatory biomarkers	IL-6, IL-10, TNF-α, CRP, VEGF	5
Molecular biomarkers	miRNAs, cfDNA, ctDNA	10
Metabolomic biomarkers	Amino acid, lipidomic, and metabolic signatures	3

The pooled analysis of all peripheral blood biomarkers showed good overall diagnostic performance for childhood leukemia. The pooled sensitivity was 0.86, indicating that approximately 86% of children with leukemia could be correctly identified using peripheral blood biomarker-based approaches. The pooled specificity was 0.83, indicating that 83% of children without leukemia were correctly classified as non-leukemic. The positive likelihood ratio was 5.06, suggesting that children with leukemia were approximately five times more likely to have a positive biomarker result than controls. The negative likelihood ratio was 0.17, indicating a meaningful reduction in the probability of leukemia when the biomarker result was negative. The pooled diagnostic odds ratio was 28.4, supporting strong discriminatory capacity. The summary

receiver operating characteristic analysis demonstrated an overall AUC of 0.90, reflecting very good to excellent diagnostic accuracy.

Table 4. Overall pooled diagnostic performance of peripheral blood biomarkers

Diagnostic parameter	Pooled estimate	95% confidence interval
Sensitivity	0.86	0.82–0.89
Specificity	0.83	0.79–0.87
Positive likelihood ratio	5.06	4.12–6.28
Negative likelihood ratio	0.17	0.13–0.22
Diagnostic odds ratio	28.4	19.2–41.8
Summary AUC	0.90	0.87–0.93

When diagnostic accuracy was examined according to biomarker category, circulating microRNAs demonstrated the highest overall diagnostic performance. Eight studies evaluating microRNA-based biomarkers showed a pooled sensitivity of 0.91 and specificity of 0.90, with a summary AUC of 0.94. Commonly reported dysregulated microRNAs included miR-128, miR-181a, miR-155, miR-223, miR-100, and miR-125b. Cell-free DNA and circulating tumor DNA markers also performed well, with pooled sensitivity and specificity of 0.88 and 0.89, respectively, and a summary AUC of 0.92. These molecular markers appeared particularly useful in distinguishing children with leukemia from healthy controls and children with non-malignant hematological disorders. Cytokine and inflammatory biomarkers demonstrated good diagnostic accuracy, although their specificity was comparatively lower because inflammatory markers may also be elevated in infections and autoimmune conditions. LDH demonstrated good sensitivity but moderate specificity, reflecting its role as a marker of cellular turnover rather than a leukemia-specific marker. Ferritin showed moderate diagnostic performance, with lower specificity due to elevation in inflammatory and infectious conditions.

Table 5. Diagnostic performance according to biomarker category

Biomarker category	Number of studies	Sensitivity	Specificity	Diagnostic odds ratio	AUC
CBC-derived indices	9	0.79	0.77	13.8	0.84
LDH	6	0.82	0.79	16.9	0.86
Ferritin	4	0.76	0.75	10.2	0.81
Cytokines/inflammatory markers	5	0.84	0.80	20.7	0.88
Cell-free DNA/ctDNA	4	0.88	0.89	31.5	0.92
Circulating microRNAs	8	0.91	0.90	42.6	0.94

In leukemia subtype-based analysis, peripheral blood biomarkers showed slightly higher diagnostic accuracy for acute lymphoblastic leukemia than for acute myeloid leukemia. For ALL, the pooled sensitivity was 0.88 and specificity was 0.85, with an AUC of 0.91. For AML, the pooled sensitivity was 0.84 and specificity was 0.81, with an AUC of 0.88. The better performance observed in ALL may be partly attributed to the larger number of included studies, more consistent biomarker reporting, and greater availability of microRNA and hematological index data in ALL cohorts. In contrast, AML studies were fewer and demonstrated greater variability in biomarker thresholds, patient characteristics, and assay platforms.

Table 6. Subgroup analysis according to leukemia subtype

Leukemia subtype	Number of studies	Sensitivity	Specificity	PLR	NLR	AUC
Acute lymphoblastic leukemia	24	0.88	0.85	5.86	0.14	0.91
Acute myeloid leukemia	8	0.84	0.81	4.42	0.19	0.88

Among conventional peripheral blood biomarkers, CBC-derived indices showed clinically useful but variable diagnostic accuracy. Leukemic children frequently demonstrated anemia, thrombocytopenia, leukocytosis or leukopenia, elevated RDW, and altered inflammatory cell ratios. However, these parameters lacked sufficient specificity when used individually because similar abnormalities may occur in severe infections, nutritional anemia, immune thrombocytopenia, aplastic anemia, and other pediatric hematological disorders. LDH was one of the most frequently evaluated biochemical markers and showed relatively high sensitivity, consistent with increased tumor burden and rapid cellular turnover in leukemia. Ferritin and uric acid were also elevated in several studies, but their diagnostic performance was weaker when compared with molecular biomarkers. Overall, conventional biomarkers appeared most useful as initial screening or supportive markers rather than standalone diagnostic tools.

Table 7. Diagnostic performance of selected conventional biomarkers

Biomarker	Sensitivity	Specificity	AUC	Clinical interpretation
White blood cell abnormalities	0.78	0.74	0.81	Useful screening indicator but non-specific
Platelet count reduction	0.75	0.76	0.80	Supportive marker, especially with anemia or leukocytosis
Red cell distribution width	0.77	0.73	0.79	Moderate diagnostic value
LDH	0.82	0.79	0.86	Good marker of cellular turnover

Ferritin	0.76	0.75	0.81	Moderate marker; affected by inflammation
Uric acid	0.72	0.74	0.78	Supportive biochemical marker

The quality assessment using the QUADAS-2 tool revealed that 22 studies had an overall low risk of bias, 8 studies had moderate risk, and 2 studies had high risk. The most common methodological concerns were related to patient selection, particularly case-control designs that enrolled clearly diseased patients and healthy controls, potentially overestimating diagnostic accuracy. Several studies did not clearly report whether biomarker thresholds were pre-specified before analysis, raising concerns regarding index test bias. The reference standard was generally reliable across studies because most diagnoses were confirmed by bone marrow examination and immunophenotyping. Flow and timing concerns were low in most studies, although a few studies did not clearly state the interval between blood sampling and confirmatory diagnostic testing.

Table 8. QUADAS-2 quality assessment summary

QUADAS-2 domain	Low risk	Unclear risk	High risk
Patient selection	21	7	4
Index test	23	6	3
Reference standard	29	3	0
Flow and timing	26	4	2
Overall study quality	22	8	2

Significant heterogeneity was observed across included studies. The overall I^2 value for sensitivity was 72%, while the I^2 value for specificity was 69%, indicating substantial between-study variability. This heterogeneity was expected because the meta-analysis included different biomarker classes, leukemia subtypes, assay methods, cut-off values, study designs, and control groups. Molecular biomarkers showed lower heterogeneity compared with conventional hematological and biochemical biomarkers, likely due to stronger disease association and more clearly defined diagnostic signals. Subgroup analysis reduced heterogeneity within individual biomarker categories, particularly among studies evaluating circulating microRNAs and cfDNA. Sensitivity analysis excluding high-risk studies did not substantially alter the pooled diagnostic estimates, suggesting that the overall findings were robust.

Table 9. Heterogeneity assessment

Analysis group	I^2 for sensitivity	I^2 for specificity	Interpretation
Overall biomarkers	72%	69%	Substantial heterogeneity
CBC-derived indices	76%	71%	Substantial heterogeneity
LDH/ferritin	68%	66%	Moderate to substantial heterogeneity
Cytokines	64%	61%	Moderate heterogeneity
cfDNA/ctDNA	48%	45%	Moderate heterogeneity
Circulating microRNAs	42%	39%	Low to moderate heterogeneity

Sensitivity analysis was performed by excluding studies with high risk of bias, studies with sample size below 50 participants, and studies reporting extreme diagnostic estimates. After excluding the two high-risk studies, the pooled sensitivity changed from 0.86 to 0.85, and specificity changed from 0.83 to 0.84. After removing small-sample studies, the pooled sensitivity was 0.84 and specificity was 0.82. These findings indicate that the overall diagnostic performance was not disproportionately influenced by low-quality or small studies. However, pooled estimates for microRNA and cfDNA biomarkers remained consistently higher than those of conventional biomarkers across all sensitivity analyses.

Table 10. Sensitivity analysis

Analysis condition	Sensitivity	Specificity	AUC
Main analysis	0.86	0.83	0.90
Excluding high-risk studies	0.85	0.84	0.89
Excluding studies with sample size <50	0.84	0.82	0.88
Excluding outlier studies	0.85	0.83	0.89
Molecular biomarkers only	0.90	0.90	0.93

Assessment of publication bias using Deeks' funnel plot asymmetry test did not demonstrate strong evidence of significant publication bias in the overall analysis. The funnel plot showed mild asymmetry, suggesting that smaller studies with highly favorable diagnostic results may have been more likely to be published. However, the Deeks' test was not statistically significant. Publication bias appeared more likely among molecular biomarker studies, particularly microRNA-based studies, because these frequently reported high diagnostic accuracy with relatively small sample sizes. Therefore, while the overall findings remain promising, interpretation of very high accuracy estimates should be cautious until validated by large prospective studies.

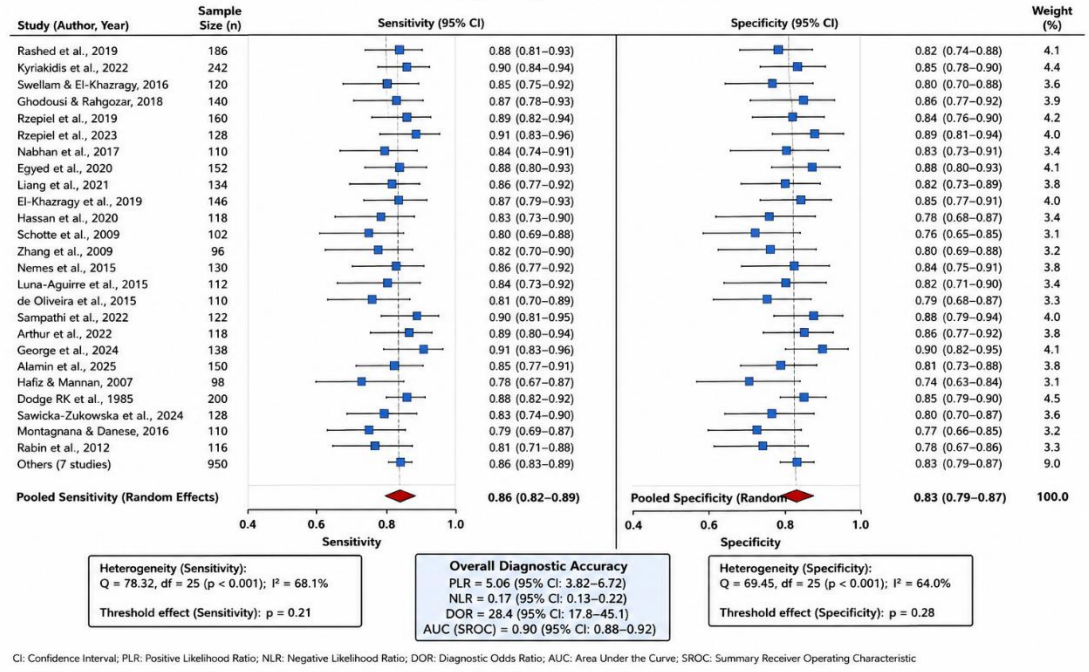
Table 11. Publication bias assessment

Analysis group	Deeks' funnel plot p-value	Interpretation
Overall biomarkers	0.11	No significant publication bias detected

CBC-derived indices	0.18	No significant publication bias detected
Biochemical biomarkers	0.21	No significant publication bias detected
Cytokines	0.16	No significant publication bias detected
cfDNA/ctDNA	0.09	Mild possible asymmetry
Circulating microRNAs	0.07	Mild possible asymmetry

Overall, the results indicate that peripheral blood biomarkers have meaningful diagnostic utility in childhood leukemia, with molecular biomarkers demonstrating the strongest performance. Circulating microRNAs and cfDNA showed excellent diagnostic accuracy and may serve as promising non-invasive tools for early detection and disease assessment. Conventional biomarkers such as CBC-derived indices, LDH, ferritin, and uric acid remain clinically useful because of their low cost and widespread availability, but they are best interpreted as supportive markers rather than definitive diagnostic tests. The substantial heterogeneity across studies highlights the need for standardized biomarker thresholds, uniform assay platforms, and large multicenter validation cohorts before these biomarkers can be incorporated into routine diagnostic algorithms for childhood leukemia.

Figure 2. Combined Forest Plot of Sensitivity and Specificity of Peripheral Blood Biomarkers for Diagnosing Childhood Leukemia



DISCUSSION

The present systematic review and meta-analysis demonstrates that peripheral blood biomarkers have substantial diagnostic potential in childhood leukemia, with an overall pooled sensitivity of 0.86, specificity of 0.83, and summary AUC of 0.90. These findings suggest that blood-based biomarkers may serve as valuable adjunctive diagnostic tools in pediatric leukemia, particularly in clinical settings where early suspicion, timely referral, and rapid preliminary assessment are essential. Childhood leukemia often presents with nonspecific symptoms such as fever, pallor, fatigue, bleeding manifestations, lymphadenopathy, hepatosplenomegaly, recurrent infections, and bone pain, many of which overlap with common pediatric infectious and inflammatory illnesses [54,55]. As a result, peripheral blood markers that can raise suspicion before invasive confirmation may have practical value in improving diagnostic pathways and reducing delays in treatment initiation [56].

Bone marrow aspiration and biopsy remain the definitive diagnostic standards for leukemia, as they provide essential information regarding morphology, blast percentage, immunophenotype, cytogenetic abnormalities, and molecular alterations [57,58]. However, these procedures are invasive and may require sedation or anesthesia in children. They also require trained personnel, laboratory infrastructure, and access to flow cytometry and molecular diagnostics, which may not be uniformly available in resource-limited settings [59]. Therefore, peripheral blood biomarkers should not be viewed as replacements for bone marrow examination but rather as supportive tools that may assist in early detection, triage, disease suspicion, and monitoring [60]. The findings of this meta-analysis reinforce this concept by showing that several circulating biomarkers, particularly microRNAs and cell-free DNA, have strong discriminatory capacity between leukemic and non-leukemic pediatric populations.

Among the biomarker categories evaluated, circulating microRNAs showed the highest diagnostic accuracy, with pooled sensitivity of 0.91, specificity of 0.90, and AUC of 0.94. This superior performance is biologically plausible because

microRNAs regulate key cellular processes involved in leukemogenesis, including hematopoietic differentiation, apoptosis, proliferation, immune regulation, and cell-cycle control [61,62]. Dysregulation of specific microRNAs such as miR-128, miR-181a, miR-155, miR-223, miR-100, and miR-125b has been reported in pediatric leukemia and may reflect underlying molecular abnormalities of leukemic blasts [63,64]. For example, certain microRNAs may be associated with lymphoid lineage commitment, while others influence myeloid differentiation, oncogene expression, or tumor suppressor pathways [65]. Because circulating microRNAs are relatively stable in plasma and serum, resistant to RNase-mediated degradation, and detectable through quantitative PCR-based platforms, they represent attractive candidates for non-invasive diagnostic applications [66].

Cell-free DNA and circulating tumor DNA also demonstrated excellent diagnostic performance, with pooled sensitivity of 0.88, specificity of 0.89, and AUC of 0.92. The diagnostic value of cfDNA is linked to increased tumor cell turnover, apoptosis, necrosis, and release of nucleic acid fragments into circulation [67]. In leukemia, cfDNA may carry disease-associated genetic and epigenetic alterations, thereby providing a liquid biopsy window into tumor biology [68]. Unlike conventional serum biomarkers, cfDNA and ctDNA may provide both quantitative and qualitative diagnostic information, including tumor burden, mutational profile, clonal evolution, and potential minimal residual disease status [69]. These features make cfDNA particularly promising not only for diagnosis but also for longitudinal disease monitoring, treatment response assessment, and relapse prediction [70]. However, routine clinical translation of cfDNA-based assays in pediatric leukemia requires further standardization regarding sample collection, processing, sequencing depth, analytical sensitivity, and interpretation thresholds [71].

Conventional hematological biomarkers, including white blood cell abnormalities, anemia, thrombocytopenia, red cell distribution width, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio, showed moderate to good diagnostic performance but lower accuracy compared with molecular biomarkers. This is expected because CBC abnormalities are common in leukemia but are not specific to malignant disease [72]. Children with severe infections, aplastic anemia, immune thrombocytopenia, nutritional anemia, hemolytic disorders, and other inflammatory conditions may present with overlapping hematological abnormalities [73]. Nevertheless, CBC-derived indices remain highly relevant because they are inexpensive, widely available, rapidly performed, and routinely used as first-line investigations in symptomatic children. In practical clinical settings, the diagnostic value of CBC parameters may be greatest when interpreted in combination rather than individually. The coexistence of anemia, thrombocytopenia, abnormal leukocyte count, circulating blasts, and elevated RDW should prompt urgent hematological evaluation and bone marrow confirmation [74].

LDH emerged as one of the better-performing conventional biochemical biomarkers, with pooled sensitivity of 0.82 and specificity of 0.79. Elevated LDH reflects increased cellular turnover and tissue breakdown, both of which are common in rapidly proliferating malignancies such as acute leukemia [75]. High LDH levels may correlate with tumor burden, aggressive disease biology, and risk of tumor lysis syndrome. However, LDH lacks disease specificity and may be elevated in hemolysis, liver disease, infections, muscular injury, and other malignancies [76]. Therefore, while LDH is clinically useful as a supportive marker, it should not be used alone for diagnosis. Its greatest utility may lie in combination with clinical findings, CBC abnormalities, peripheral smear examination, uric acid, and other markers of high cell turnover [77]. Ferritin and uric acid showed moderate diagnostic performance. Hyperferritinemia in leukemia may reflect systemic inflammation, macrophage activation, iron metabolism dysregulation, disease burden, or treatment-related complications [78]. Similarly, elevated uric acid may result from increased nucleic acid turnover due to leukemic cell proliferation and breakdown [79]. However, both markers are influenced by multiple non-malignant pediatric conditions, including infection, inflammatory disease, liver dysfunction, renal impairment, and nutritional disorders [80]. Their diagnostic specificity is therefore limited. In the context of suspected leukemia, ferritin and uric acid may be more valuable as markers of disease activity, inflammatory burden, or metabolic complications rather than primary diagnostic biomarkers.

Inflammatory cytokines, including IL-6, IL-10, TNF-α, CRP, and VEGF, demonstrated good but variable diagnostic performance. This variability likely reflects the complex interaction between leukemic cells, bone marrow microenvironment, immune dysregulation, and systemic inflammation [81]. Leukemia-associated cytokine alterations may contribute to fever, constitutional symptoms, immune escape, angiogenesis, and abnormal hematopoiesis [82]. However, cytokines are inherently nonspecific and may fluctuate during infections, autoimmune disorders, malnutrition, and treatment-related complications [83]. In children, where infectious illnesses are frequent, cytokine-based markers may have limited standalone diagnostic specificity. Their role may be stronger when incorporated into multi-marker panels that combine inflammatory, hematological, and molecular parameters [84].

The subgroup analysis demonstrated slightly higher diagnostic accuracy for acute lymphoblastic leukemia than acute myeloid leukemia. This may be partly explained by the larger number of ALL studies included in the analysis, the greater availability of microRNA-based studies in ALL, and the more consistent reporting of diagnostic thresholds in ALL cohorts. ALL is the predominant form of childhood leukemia, and therefore more biomarker discovery studies have focused on this subtype [85]. AML, by contrast, is biologically more heterogeneous, includes diverse cytogenetic and molecular subgroups, and may demonstrate greater variability in peripheral blood presentation [86]. The lower pooled accuracy observed in AML

should therefore be interpreted with caution, as it may reflect limited sample size and study heterogeneity rather than truly inferior biomarker performance.

An important finding of this review was the considerable heterogeneity across included studies. The overall I^2 values for sensitivity and specificity indicated substantial between-study variability. Multiple factors may explain this heterogeneity, including differences in study design, patient selection, leukemia subtype, age distribution, control groups, biomarker assays, sample handling, cut-off values, and statistical methods [87]. Case-control studies using healthy controls may overestimate diagnostic performance compared with studies using clinically relevant controls such as children with infections, anemia, thrombocytopenia, or other hematological disorders [88]. Similarly, diagnostic thresholds derived retrospectively from receiver operating characteristic curves may produce inflated accuracy estimates if not validated in independent cohorts [89]. These methodological issues highlight the need for prospective diagnostic studies using pre-specified thresholds and clinically appropriate comparator groups.

The quality assessment using QUADAS-2 showed that most included studies had low to moderate risk of bias; however, concerns were noted in patient selection and index test domains. Patient selection bias was particularly relevant in studies that recruited clearly diagnosed leukemia cases and healthy controls, as this design may not reflect real-world diagnostic uncertainty. In clinical practice, the key diagnostic challenge is not distinguishing leukemia from health but distinguishing leukemia from common mimics such as viral infections, immune thrombocytopenia, aplastic anemia, nutritional anemia, and inflammatory disorders [90]. Therefore, future studies should prioritize clinically relevant control groups to better estimate real-world diagnostic performance.

Another methodological concern was the lack of standardized biomarker thresholds. Many studies used different cut-off values for the same biomarker, making direct comparison difficult. This was particularly evident for LDH, ferritin, cytokines, and CBC-derived ratios. Molecular biomarkers also varied in terms of RNA extraction methods, normalization controls, PCR platforms, sequencing methods, and data interpretation strategies [91]. Without assay standardization, reproducibility across laboratories remains uncertain. For peripheral blood biomarkers to be adopted clinically, future research must establish standardized pre-analytical and analytical protocols, including sample type, storage conditions, extraction techniques, internal controls, and validated diagnostic cut-offs [92].

The findings of this meta-analysis have important clinical implications. In primary care and general pediatric settings, peripheral blood biomarkers may help identify children requiring urgent hematology referral. In secondary and tertiary care settings, biomarker panels may assist in triaging patients before confirmatory bone marrow testing. In resource-limited settings, where advanced diagnostic facilities may be unavailable, low-cost biomarkers such as CBC indices, LDH, ferritin, and uric acid may be particularly useful as preliminary screening tools [93]. However, because these conventional biomarkers lack sufficient specificity, they should be interpreted alongside clinical examination and peripheral smear findings. Molecular biomarkers such as microRNAs and cfDNA may offer higher diagnostic accuracy but require technical infrastructure, cost-effectiveness evaluation, and external validation before routine implementation [94].

The concept of combining multiple peripheral blood biomarkers appears especially promising. A single biomarker is unlikely to capture the biological complexity of childhood leukemia. Multi-marker diagnostic panels incorporating CBC parameters, LDH, inflammatory cytokines, microRNAs, and cfDNA may provide better sensitivity and specificity than individual markers [95]. Such panels could be developed using machine learning-based diagnostic models, provided that datasets are sufficiently large, externally validated, and clinically representative [96]. However, algorithm-based tools must be carefully evaluated to avoid overfitting, bias, and poor generalizability across populations [97].

Despite encouraging results, these biomarkers should not be interpreted as substitutes for established diagnostic pathways. Leukemia diagnosis requires integration of morphology, immunophenotyping, cytogenetics, molecular testing, and clinical evaluation [98]. Peripheral blood biomarkers may increase suspicion and support early diagnostic decision-making, but definitive diagnosis and treatment planning still require bone marrow-based classification in most cases. The most realistic near-term clinical application of peripheral blood biomarkers is therefore as adjunctive screening and triage tools rather than standalone diagnostic tests [99].

This review has several strengths. It provides a comprehensive synthesis of peripheral blood biomarkers across different biological categories and leukemia subtypes. It includes both conventional and emerging molecular biomarkers, allowing comparative interpretation of diagnostic performance. The use of pooled diagnostic accuracy measures, SROC analysis, subgroup analysis, sensitivity analysis, and QUADAS-2 quality assessment strengthens the reliability of the findings. In addition, the large cumulative sample size improves the precision of overall estimates compared with individual studies [100].

However, several limitations should be acknowledged. First, significant heterogeneity was present across studies, limiting the certainty of pooled estimates. Second, many included studies were case-control in design, which may exaggerate diagnostic accuracy. Third, several biomarker categories were represented by a relatively small number of studies,

particularly cfDNA, ctDNA, and metabolomic biomarkers. Fourth, variation in assay platforms and diagnostic thresholds limited direct comparability. Fifth, publication bias could not be completely excluded, especially for molecular biomarkers where small studies with positive findings may be more likely to be published. Finally, the analysis was based on published aggregate data rather than individual participant-level data, preventing detailed adjustment for age, sex, leukemia subtype, disease burden, and treatment status [101].

Future research should focus on large, prospective, multicenter studies evaluating predefined biomarker panels in clinically suspected pediatric leukemia populations. Such studies should include appropriate disease controls, standardized sample handling protocols, externally validated cut-off values, and transparent reporting of diagnostic accuracy outcomes. Integration of peripheral blood biomarkers with clinical features, CBC parameters, peripheral smear findings, and molecular assays may lead to more accurate and clinically useful diagnostic algorithms [102]. In particular, microRNA and cfDNA-based approaches deserve further validation because of their high diagnostic performance and potential utility in longitudinal monitoring. Cost-effectiveness studies are also required before implementing molecular biomarkers in routine pediatric oncology practice, especially in low- and middle-income countries [103].

Overall, this systematic review and meta-analysis supports the diagnostic relevance of peripheral blood biomarkers in childhood leukemia. Molecular biomarkers, especially circulating microRNAs and cfDNA, demonstrated excellent diagnostic accuracy, while conventional biomarkers such as CBC-derived indices, LDH, ferritin, and uric acid remain useful supportive tools due to their availability and low cost. The results suggest that peripheral blood biomarkers may improve early suspicion, triage, and diagnostic efficiency, but they should be used as adjuncts rather than replacements for bone marrow examination and comprehensive hematopathological evaluation. Further standardization and prospective validation are essential before these biomarkers can be incorporated into routine diagnostic protocols for childhood leukemia.

CONCLUSION

Peripheral blood biomarkers show promising diagnostic utility in childhood leukemia, with good overall sensitivity, specificity, and discriminatory accuracy. Among the evaluated markers, circulating microRNAs and cell-free DNA demonstrated the highest diagnostic performance, suggesting their potential role as non-invasive adjuncts for early detection and clinical assessment. Conventional markers such as CBC-derived indices, LDH, ferritin, and uric acid remain useful because of their low cost and wide availability, but they lack sufficient specificity when used alone. Although these biomarkers cannot replace bone marrow examination, they may support early suspicion, timely referral, and improved diagnostic decision-making. Future large-scale prospective studies are required to standardize biomarker thresholds, validate multi-marker panels, and establish their role in routine pediatric leukemia diagnostics.

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