

Original article

Prevalence and diagnostic utility of cytochemical staining versus immunophenotyping in pediatric acute leukemia: a retrospective study at Siriraj Hospital

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Abstract:

Objective: This study evaluated the prevalence of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) among pediatric patients at Siriraj Hospital and compared the diagnostic effectiveness of cytochemical staining and immunophenotyping. **Materials and Methods:** A retrospective review was conducted on 302 pediatric patients (0-18 years) diagnosed with acute leukemia from 2008 to 2021. The prevalence of ALL and AML was determined, and hematologic parameters were analyzed. The sensitivity, specificity and predictive values of cytochemical stains were compared with those of immunophenotyping. **Results:** Among 302 cases (176 males, 126 females), ALL was the predominant diagnosis (76.5%), with 69.2% classified as B-cell ALL (B-ALL) and 7.3% as T-cell ALL (T-ALL). AML accounted for 23.5%. Patients with T-ALL were significantly older ($p < 0.01$) and had higher hemoglobin ($p = 0.044$) and white blood cell counts ($p = 0.012$) compared with patients with AML and B-ALL. Cytochemical stains demonstrated variable utility across leukemia subtypes. Myeloperoxidase (MPO) and Sudan Black B (SBB) showed high diagnostic accuracy for AML, with sensitivities of 74.51% and 85.92% and specificities of 100%. PAS staining had a high PPV (89.94%) for B-ALL but was less sensitive for T-ALL (50%). **Conclusion:** Cytochemical staining serves as a valuable complementary diagnostic tool for acute leukemia. Integrating PAS and MPO staining can enhance preliminary leukemia subtyping, especially in resource-limited settings. This approach facilitates timely and accurate diagnosis, supporting early treatment initiation and improving outcomes for pediatric patients.

Keywords : ● Acute leukemia ● Cytochemical staining ● Immunophenotyping ● Children

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นิพนธ์ต้นฉบับ

การศึกษาความชุกโรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันและเปรียบเทียบประสิทธิภาพการวินิจฉัยระหว่างการย้อมสีไซโตเคมีและอิมมูโนฟิโนไทป์ของผู้ป่วยเด็กในโรงพยาบาลศิริราช

วิทยาการณ อินทรจุกุล สุวิมล ศรีปราชญ์ กลีบสไบ สรรพกิจ และ เจษฎา บัวบุญนำ
สาขาวิชาโลหิตวิทยาและอองโคโลยี ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

บทคัดย่อ

วัตถุประสงค์ ศึกษาความชุกของโรคมะเร็งเม็ดเลือดขาวเฉียบพลันชนิดลิมโฟบลาสติก และชนิดไมอีลอยด์ ในผู้ป่วยเด็กโรงพยาบาลศิริราช และเปรียบเทียบประสิทธิภาพการวินิจฉัยด้วยวิธีย้อมสีทางเซลล์เคมีและอิมมูโนฟิโนไทป์ **วิธีการศึกษา** วิเคราะห์ย้อนหลังผู้ป่วยเด็กอายุ 0-18 ปี จำนวน 302 ราย ที่ได้รับการวินิจฉัยโรคมะเร็งเม็ดเลือดขาวเฉียบพลันปี ค.ศ. 2008-2021 โดยประเมินความชุกของ ALL และ AML พร้อมวิเคราะห์พารามิเตอร์ทางโลหิตวิทยา เปรียบเทียบค่าความไว ความจำเพาะ และค่าทำนายของการย้อมสีทางเซลล์เคมีเทียบกับอิมมูโนฟิโนไทป์ **ผลการศึกษา**: ในผู้ป่วย 302 ราย (ชาย 176 ราย หญิง 126 ราย) พบ ALL มากที่สุด (76.5%) แบ่งเป็น B-ALL ร้อยละ 69.2 และ T-ALL ร้อยละ 7.3 และพบ AML ร้อยละ 23.5 ผู้ป่วย T-ALL มีอายุ ($p < 0.01$) ค่าฮีโมโกลบิน ($p = 0.044$) และจำนวนเม็ดเลือดขาว ($p = 0.012$) สูงกว่ากลุ่ม AML และ B-ALL การย้อม MPO และ SBB มีความถูกต้องสูงในการวินิจฉัย AML โดยมีความไว ร้อยละ 74.51 และ 85.92 ตามลำดับ และความจำเพาะ ร้อยละ 100 การย้อม PAS มีค่าความจำเพาะสูงสำหรับ B-ALL (89.94%) แต่มีความไวต่ำใน T-ALL (50%) **สรุป**: การย้อมสีทางเซลล์เคมีมีประโยชน์ในการวินิจฉัยโรคเม็ดเลือดขาวเฉียบพลัน การย้อม PAS และ MPO สามารถช่วยจำแนกชนิดของโรคได้ โดยเฉพาะในสถานพยาบาลที่มีทรัพยากรจำกัด ทำให้สามารถเริ่มการรักษาได้อย่างทันท่วงทีและเพิ่มโอกาสในการรักษาผู้ป่วยเด็ก

คำสำคัญ : ● มะเร็งเม็ดเลือดขาวเฉียบพลัน ● การย้อมสีทางไซโตเคมี ● อิมมูโนฟิโนไทป์ ● ผู้ป่วยเด็ก

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Introduction

Acute leukemia is a hematologic malignancy characterized by the rapid proliferation of immature white blood cells, known as blast cells, within the bone marrow. This uncontrolled growth disrupts normal hematopoiesis, leading to anemia, thrombocytopenia and neutropenia, which manifest clinically as fatigue, bleeding tendencies and increased susceptibility to infections.¹ Acute leukemia is classified into two main types: acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

In pediatric populations, ALL predominates, accounting for approximately 72% of cases, with B-cell ALL (B-ALL) comprising up to 85% of these. T-cell ALL (T-ALL), while less common, tends to present with higher white blood cell (WBC) counts and occurs among older children compared with B-ALL.² Conversely, AML, though less common, is known for its aggressive clinical course and the need for immediate intervention.³ The accurate differentiation between ALL and AML is crucial, as it directly influences therapeutic strategies and prognostic outcomes.

Advancements in diagnostic methods have significantly enhanced the precision of leukemia classification. Cytochemical staining techniques, such as myeloperoxidase (MPO) and Sudan Black B (SBB), are valuable in distinguishing myeloid from lymphoid lineages.⁴ These stains detect specific enzymatic activities within blast cells, aiding in the identification of leukemia subtypes. For instance, MPO and SBB positivity are indicative of myeloid lineage, thereby facilitating the diagnosis of AML. Studies have demonstrated that cytochemical stains like MPO and SBB achieve sensitivities ranging from 83.33% to 84.44% and a specificity of 100% for diagnosing AML, while Periodic Acid-Schiff (PAS) staining shows a sensitivity of 59.45% and specificity of 98.88% for diagnosing ALL.^{5,6}

Immunophenotyping, particularly using flow cytometry, has emerged as a pivotal tool in leukemia diagnostics. This technique involves the use of monoclonal antibodies to detect specific cell surface antigens, allowing for the

precise classification of leukemia subtypes. Immunophenotyping not only differentiates between ALL and AML but also identifies subcategories within these groups, such as B-ALL and T-ALL, which is essential for risk stratification and treatment planning.

Despite the high accuracy of immunophenotyping, its implementation can be limited by resource constraints, particularly in low- and middle-income countries. In such settings, cytochemical staining remains a valuable diagnostic approach due to its cost-effectiveness and accessibility. However, the sensitivity and specificity of cytochemical methods can vary, underscoring the need for comprehensive studies to evaluate their diagnostic performance relative to immunophenotyping.

This study aims to assess the prevalence of ALL and AML among pediatric patients at Siriraj Hospital and to compare the diagnostic efficacy of cytochemical staining with that of immunophenotyping. By analyzing data spanning 13 years, this research seeks to provide insights into the utility of cytochemical techniques in resource-limited settings and to inform strategies for optimizing leukemia diagnostics in diverse healthcare environments.

Materials and methods

Patients

This study employed a retrospective design, analyzing data from pediatric patients aged 0 to 18 years who were diagnosed with acute leukemia at Siriraj Hospital between January 2008 and September 2021. A total of 302 cases were included, all of which had undergone bone marrow smear examinations using both cytochemical staining and immunophenotyping techniques. Patients included in this study were those who underwent bone marrow aspiration and receiving a diagnosis using both cytochemical staining and immunophenotyping methods. Only cases of ALL and AML were included, while cases of mixed phenotype acute leukemia and those diagnosed solely from peripheral blood samples, including those with hyperleukocytosis, were excluded

from this analysis. Ethical approval for this study was obtained from the Institutional Review Board of the Faculty of Medicine, Siriraj Hospital, Mahidol University (COA number SI 945/2021).

Sample Preparation and Cytochemical Staining Protocols

Bone marrow smears were subjected to various cytochemical staining methods to classify leukemia subtypes based on their cellular composition. The following staining protocols were employed:

Myeloperoxidase (MPO): Slides were fixed in fixative for 30 seconds, rinsed with deionized water and air-dried. They were then incubated with the staining mixture for 30 minutes, rinsed and counterstained with Harris's hematoxylin for 30 minutes. This method was adapted from Graham et al.⁷

Sudan Black B (SBB): Slides were fixed in formalin vapor for 10 minutes, followed by staining with Sudan Black B for 1 hour. The slides were then rinsed with 70% ethanol, air-dried and counterstained with Harris's hematoxylin for 30 minutes, following the method of Hayhoe and Quaglini.⁸

Combined Esterase (CEA) and non-specific esterase (NSE): Slides were fixed with cold buffer formal acetone for 30 seconds, stained with combined esterase reagent for 30 minutes, rinsed and counterstained with Harris's hematoxylin. This technique is based on the work of Lam et al.⁹

Periodic acid-Schiff (PAS): Slides were fixed in 10% formalin and methanol for 10 minutes, treated with 1% periodic acid for 10 minutes and stained with Schiff's reagent for 30 minutes. They were then counterstained with Harris's hematoxylin for 30 minutes. This approach was adapted from Thomson and Hunt.¹⁰

Acid phosphatase (AP): Slides were fixed using cold buffer methanol acetone for 30 seconds, incubated at 37°C with the medium for 1 hour and counterstained with Harris's hematoxylin for 30 minutes. This method was derived from Goldberg and Burka.¹¹

The positivity criteria for cytochemical staining are as follows:

Myeloperoxidase (MPO) or Sudan Black B (SBB): Positive if at least 3% of blast cells exhibit staining (based on a count of 100 blast cells).

CEA, NSE, PAS or AP: Positive if at least 20% of blast cells exhibit staining (based on a count of 100 blast cells).

Immunophenotyping

For comparison and confirmation, immunophenotyping was performed using flow cytometry, where monoclonal antibodies specific to cell surface antigens were employed. This allowed for the precise classification of leukemia subtypes into ALL and AML, including further subclassifications such as B-ALL and T-ALL. The immunophenotyping protocol for diagnosing acute leukemia is as follows: Hematopoietic stem cell markers: Human Leukocyte Antigen (HLA)-DR and cluster of differentiation (CD) 34; Myeloid markers: CD117, CD13-33, CD14, CD41, glycophorin A (GP-A) and MPO; Lymphoid markers: B-ALL: CD20, CD22, CD19, CD10, cytoplasmic μ chain (C μ), surface Immunoglobulin M (sIgM) and terminal deoxynucleotidyl transferase (TDT); T-ALL: CD7, CD2, CD3 and TDT.

Statistical analyses

For intergroup comparisons, the associations between different acute leukemia subtypes and age at diagnosis, platelet count, WBC count and blast cells were determined by the Kruskal-Wallis test, while other clinical variables were analyzed using ANOVA. Prevalence rates for each subtype were calculated as percentages to provide the distribution of acute leukemia cases. All reported *p*-values were two-sided, and values < 0.05 were considered statistically significant. The performance of the diagnostic tests was evaluated by calculating the sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV). These data were analyzed using IBM SPSS Statistics for Windows Software, version 28 (IBM corp., Armonk, NY, USA).

Table 1 Comparison of hematologic parameters among AML, B-ALL and T-ALL among pediatric patients with acute leukemia (total population: 302)

Parameter	AML (n = 71)	B-ALL (n = 209)	T-ALL (n = 22)	p-value
Age (years)	6.00 (0.10,15.00)	5.00 (0.10,17.00)	11.00 (1.00,16.00)	< 0.01
Male sex (%)	45 (25.6)	116 (65.9)	15 (8.5)	0.315
Hemoglobin (g/dL)	8.85±2.03	8.30±2.34	9.40±2.91	0.044
Platelets (x10 ³ /μL)	55.00 (9.00,406.00)	55.00 (0.00,614.00)	65.50 (14.00,721.00)	0.254
White Blood Cells (x10 ³ /μL)	16.85 (0.80,456.68)	10.70 (0.60,783.46)	63.51 (0.80,923.00)	0.012
Blast Cells (%):	35.00 (0.00,99.00)	38.00 (0.00,99.00)	41.50 (0.00,100.00)	0.595

Values are expressed as the mean ± standard deviation or median (min, max).

The p-value represents statistical comparisons between AML, B-ALL and T-ALL.

Results

Patient Demographics and Prevalence of Leukemia

Subtypes

Among 302 pediatric patients (176 males and 126 females), ALL accounted for 76.5% of cases, with 69.2% classified as B-ALL and 7.3% as T-ALL, while AML constituted 23.5%. No significant gender differences were observed in hematologic parameters across the cohort.

Comparison of Clinical and Hematologic Parameters

Clinical and hematologic parameters were compared across the three leukemia subtypes: AML, B-ALL and T-ALL (Table 1). T-ALL patients were significantly older at diagnosis ($p < 0.01$) and exhibited higher hemoglobin levels ($p = 0.044$) and white blood cell counts ($p = 0.012$) compared with those who had AML and B-ALL.

Diagnostic Accuracy of Cytochemical Stains and Predictive Values

The diagnostic performance of cytochemical staining methods was evaluated based on their sensitivity, specificity, PPV and NPV across leukemia subtypes (Table 2). For AML, SBB and MPO demonstrated the highest sensitivity and specificity (85.92% and 100%, respectively), highlighting their diagnostic reliability. MPO and SBB also exhibited the highest PPV (100%) and NPV (92.40% and 95.85%, respectively), further supporting their role in diagnosing AML. For B-ALL, PAS staining showed greater sensitivity (72.73%) and a high PPV (89.94%), reinforcing its utility in identifying B-ALL. However,

PAS was less effective for T-ALL, with a sensitivity of only 50%. In contrast, AP staining provided the highest NPV for T-ALL (96.88%), demonstrating its effectiveness in ruling out this subtype.

Discussion

The study highlights the predominance ALL among pediatric patients, accounting for 76.5% of cases, with B-ALL being the most common subtype (69.2%) followed by T-ALL (7.3%), consistent with a previous report.¹² AML accounted for 23.5% of cases, aligning with the known distribution of leukemia subtypes among children. Comparing hematologic parameters across leukemia subtypes revealed distinct trends. Patients with T-ALL were significantly older at diagnosis compared with those who had AML and B-ALL, which may indicate potential differences in disease biology or progression. Additionally, patients with T-ALL exhibited higher hemoglobin levels and white blood cell counts, possibly reflecting variations in marrow involvement or leukemic burden. This aligns with other studies that have reported similar trends, highlighting the unique clinical and hematologic profiles of T-ALL compared with other leukemia subtypes.¹³

For AML, cytochemical markers such as MPO and SBB showed relatively high sensitivity (74.51% and 85.92%) and specificity (100% for both), along with strong PPV (100%) and acceptable NPV (92.40% and 95.85%,

Table 2 Comparison of sensitivity, specificity, positive predictive value and negative predictive value of cyto-chemical stain methods and immunophenotyping to diagnose acute leukemia subtypes among children

Subtype	Stain/Method	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive Predictive Value (%) (95% CI)	Negative Predictive Value (%) (95% CI)
AML	MPO	74.51 (60.37–85.67)	100 (97.69–100.00)	100	92.40 (88.37–95.11)
	SBB	85.92 (75.62–93.03)	100 (98.42–100.00)	100	95.85 (92.86–97.62)
	CEA	26.76 (16.94–38.59)	100 (98.41–100.00)	100	81.56 (79.35–83.58)
	NSE	22.54 (13.46–34.00)	99.57 (97.61–99.99)	94.12 (68.35–99.16)	80.70 (78.67–82.58)
	PAS	8.45 (3.16–17.49)	29.44 (23.64–35.77)	3.55 (1.68–7.37)	51.13 (45.84–56.39)
	AP	31.43 (20.85–43.63)	90.04 (85.44–93.58)	48.89 (36.26–61.66)	81.25 (78.62–83.63)
ALL	MPO	0.00 (0.00–2.31)	25.49 (14.33–39.63)	0	7.60 (4.89–11.63)
	SBB	0.00 (0.00–1.58)	14.08 (6.97–24.38)	0	4.15 (2.38–7.14)
	CEA	0.00 (0.00–1.59)	73.24 (61.41–83.06)	0	18.44 (16.42–20.65)
	NSE	0.43 (0.01–2.39)	77.46 (66.00–86.54)	5.88 (0.84–31.65)	19.30 (17.42–21.33)
	PAS	70.56 (64.23–76.36)	91.55 (82.51–96.84)	96.45 (92.63–98.32)	48.87 (43.61–54.16)
	AP	9.96 (6.42–14.56)	68.57 (56.37–79.15)	51.11 (38.34–63.74)	18.75 (16.37–21.38)
T-ALL	MPO	0.00 (0.00–20.59)	80.31 (73.99–85.67)	0	90.64 (90.03–91.22)
	SBB	0.00 (0.00–15.44)	78.21 (72.91–82.91)	0	90.87 (90.35–91.37)
	CEA	0.00 (0.00–15.44)	93.19 (89.57–95.85)	0	92.20 (91.97–92.42)
	NSE	4.55 (0.12–22.84)	94.29 (90.89–96.70)	5.88 (0.86–31.01)	92.63 (91.95–93.26)
	PAS	50.00 (28.22–71.78)	43.57 (37.68–49.60)	6.51 (4.33–9.67)	91.73 (87.73–94.50)
	AP	63.64 (40.66–82.80)	88.89 (84.60–92.32)	31.11 (22.22–41.66)	96.88 (94.68–98.18)

Table 2 Comparison of sensitivity, specificity, positive predictive value and negative predictive value of cytochemical stain methods and immunophenotyping to diagnose acute leukemia subtypes among children (continue)

Subtype	Stain/Method	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive Predictive Value (%) (95% CI)	Negative Predictive Value (%) (95% CI)
B-ALL	MPO	0.00 (0.00–2.56)	43.28 (31.22–55.96)	0	16.96 (13.44–21.17)
	SBB	0.00 (0.00–1.75)	34.41 (24.86–44.98)	0	13.28 (10.37–16.85)
	CEA	0.00 (0.00–1.76)	79.57 (69.95–87.23)	0	26.24 (24.30–28.28)
	NSE	0.00 (0.00–1.75)	81.72 (72.35–88.98)	0	26.67 (24.83–28.59)
	PAS	72.73 (66.15–78.64)	81.72 (72.35–88.98)	89.94 (85.23–93.27)	57.14 (51.16–62.93)
	AP	4.31 (1.99–8.02)	60.87 (50.14–70.88)	20.00 (11.16–33.22)	21.88 (19.17–24.85)

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AP, acid phosphatase; CEA combined esterase; MPO, Myeloperoxidase; NSE, nonspecific esterase; PAS, Periodic acid-Schiff; SBB, Sudan black B

respectively). In B-ALL, PAS staining demonstrated notable diagnostic value, with a PPV of 89.94% and a moderate NPV of 57.14%. Additionally, our results were in line with previous studies indicating the utility in identifying myeloblasts and lymphoblasts.^{5,6} For T-ALL, AP staining offered the highest NPV (96.88%), supporting its role in excluding this subtype, though its PPV was relatively lower (31.11%). In cytochemical analysis of AML, CEA analysis employs specific esterase CEA and NSE to differentiate granulocytic and monocytic cell lineages. Granulocytic cells exhibit bright red staining due to CEA activity, while monocytic cells show brown or red-brown staining from NSE. This dual staining technique enables simultaneous visualization and clear distinction between these lineages, aiding in precise AML classification. These findings suggest that a combination of cytochemical stains can improve the distinction of acute leukemia subtypes.

Although immunophenotype is the gold standard for diagnosing acute leukemia,¹⁴ its efficacy can be limited in cases of packed marrow or hypocellular samples due to inadequate specimen quality. In such scenarios,

cytochemistry may provide an alternative, as it requires a smaller sample volume and simpler techniques. However, cytochemical methods also have limitations, including variability in staining quality and reliance on operator expertise, which may affect their consistency and accuracy. For pediatric patients, cytochemistry might play a supportive role in certain settings, particularly where access to advanced methods is limited. This study did not evaluate the time required for each diagnostic method, an important factor in ensuring timely treatment. Future research should further investigate these aspects, including the constraints of cytochemistry, to better understand its role in clinical practice. In conclusion, cytochemistry may provide a supportive role in diagnosing acute leukemia, particularly in settings where flow cytometry is unavailable or in cases of packed marrow or hypocellular samples with compromised specimen quality. The findings emphasize the utility of integrating adaptable diagnostic strategies, such as combining PAS and MPO staining, to support preliminary leukemia subtyping and improve diagnostic accuracy.

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