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Optimizing Leukocyte Analysis for Pediatric Leukemia: A Comparison of Two Advanced Hematology Analyzers

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Abstract

Background: Acute lymphocytic leukemia (ALL) is a hematologic malignancy affecting the lymphoid lineage, characterized by the uncontrolled proliferation of abnormal, immature lymphocytes and their precursors. The routine use of automated hematology analyzers has become a valuable aid for clinicians in diagnosing leukemia. This study aims to examine the correlation between two automated hematology analyzers in patients with leukemia, particularly in pediatric populations.

Materials and Methods: This cross-sectional study involved 57 pediatric patients diagnosed with leukemia who were referred to the Paediatric Hematology Outpatient Clinic at Dr. Soetomo General Academic Hospital, Indonesia. After obtaining informed consent, peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and analyzed using the Sysmex XN-Series and Horiba Yumizen H2500 automated hematology analyzers. Parameters measured included White Blood Cell (WBC) count, Neutrophil Percentage (NEU%), Lymphocyte Percentage (LYM%), Eosinophil Percentage (EOS%), and Monocyte Percentage (MON%). Data were analyzed using SPSS version 21. Statistical tests applied included the Kolmogorov-Smirnov test, Shapiro-Wilk test, paired t-test, Wilcoxon signed-rank test, Spearman's and Pearson's correlation test, Passing-Bablok regression, and Bland-Altman analysis. A p-value of < 0.05 was considered statistically significant.

Results: Statistically significant differences were observed between the two analyzers in the median values of WBC count ($p = 0.001$) and MON% ($p = 0.006$), as well as in the mean value of NEU% ($p = 0.024$). Despite these differences, strong correlations were found across all parameters, including WBC, NEU%, LYM%, MON%, and EOS% ($p < 0.01$). EOS% demonstrated higher variability (S_{res}) = 1.48.

Conclusion: The Sysmex XN-Series and Yumizen H2500 demonstrated a good correlation in the measurement of WBC parameters in pediatric leukemia patients. However, minor variations, particularly in eosinophil percentages, may arise due to differences in measurement techniques, reagent formulations, and interlaboratory variability. Despite these variations, both analyzers are reliable for clinical use.

Keywords: Leukemia, Leukocyte Count, Lymphoid, Pediatrics

Introduction

Acute leukemia is a critical pediatric malignancy that demands early recognition and intervention. Acute lymphoblastic leukemia (ALL), which arises from B- or T-cell lymphoblasts, is marked by the proliferation of abnormal, immature lymphocytes and their precursors (1,2). ALL primarily affects children and adolescents, accounting for approximately 25% of all childhood cancers and up to 75% of childhood leukemias (3,4). Most ALL cases occur in previously healthy individuals, with only a minority linked to genetic syndromes or environmental exposures (5). Globally, the incidence and prevalence of ALL remain substantial. In the United States, the incidence is reported at approximately 3.5 cases per 100,000 individuals per year (6). Indonesia, a 2013

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report from the Ministry of Health estimated that the annual prevalence of cancer in children aged 0 to 14 years is around 16,291 cases (7).

Automated differential leukocyte counts play a vital role in the rapid and accurate evaluation of hematologic conditions. Contemporary analyzers employing flow cytometry technology can precisely quantify neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils, which are critical in diagnosing infections (e.g., neutrophilia in bacterial infections) and hematologic malignancies (8). These automated systems not only reduce human error and processing time but also maintain a strong correlation with manual counts ($r^2 > 0.9$ for major leukocyte subsets), making them ideal for high-throughput laboratories (8). Furthermore, they facilitate standardization across study sites, enhancing reproducibility in large-scale investigations (9).

Automated leukocyte analysis, predominantly based on light scatter or flow cytometry, has become standard practice. This technology uses hydrodynamically focused streams to guide cells through a laser beam within a flow cell, with detection performed by photoreceptors (3,10). Such instruments have largely supplanted manual methods due to improved precision and the capability to analyze additional parameters such as platelet counts (3).

This study aims to assess the correlation between two widely used automated hematology analyzers, Sysmex XN-Series, and Horiba Yumizen H2500, in pediatric leukemia patients. The comparison is expected to inform clinical practice by supporting faster and more accurate diagnostic assessments in children with leukemia.

Materials and Methods

This cross-sectional study involved 57 pediatric patients attending the Pediatric Hematology Outpatient Clinic at Dr. Soetomo General Academic Hospital, Surabaya, from May to October 2023. The sample size was calculated using a formula, $n = \frac{n}{1+\frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2 N}}$, resulting in 45 samples. To accommodate potential outliers, an additional 20% was added, resulting in a total of 57 samples. Sample collection followed a consecutive sampling approach. Diagnoses of ALL, Acute Myeloid Leukemia (AML), and Chronic Myeloid Leukemia (CML) were established based on the French-American-British (FAB) classification. Inclusion criteria were: (1) children aged 1–17 years; (2) patients attending the aforementioned clinic; (3) confirmed diagnosis of leukemia by a clinician based on the FAB criteria; and (4) provision of informed consent by a parent or guardian.

Peripheral blood samples (3 mL each) were collected in K2EDTA tubes (Onemed, Indonesia). Blood analyses were conducted at the Clinical Pathology Laboratory Unit of Dr. Soetomo General Academic Hospital, Surabaya, using the Sysmex XN-3000 and Horiba Yumizen H2500 hematology analyzers. A Complete Blood Count (CBC) with a 5-part differential analysis was performed using the Sysmex XN-3000, whereas the analysis using the Horiba Yumizen H2500 excluded the basophil parameter. Both instruments, Sysmex XN-3000 and Horiba Yumizen H2500 utilize flow cytometry systems, in which leukocytes are fluorescently stained and cellular complexity is assessed using forward light scatter and side fluorescent light detection.

Leukocyte analysis on the Sysmex XN-Series is conducted via the WDF and WNR channels. The WDF channel classifies white blood cells, producing a scattergram that displays clusters of

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lymphocytes, monocytes, eosinophils, basophils, neutrophils, and cellular debris (9). The WNR channel is employed for counting white blood cells and nucleated red blood cells (nRBCs), generating a scattergram that displays clusters of nucleated RBCs, basophils, non-basophil leukocytes, and debris (including lysed red blood cells and platelets). Automated leukocyte analysis on the Horiba Yumizen H2500 is conducted through the LMNE channel, which provides a scattergram showing clusters of lymphocytes, monocytes, neutrophils, eosinophils, large immature cells, erythroblasts, and platelet aggregates (11). Unlike the Sysmex XN-Series, which utilizes two separate channels, the Horiba Yumizen H2500 performs leukocyte analysis using a single channel. As a result, it requires fewer reagents while offering a broader range of leukocyte parameters. However, the interpretation of leukocyte types differs between the two devices, necessitating initial familiarization with the scattergram outputs of the Horiba Yumizen H2500. The Sysmex XN-Series (including the XN-3000) employs an optical system with flow cytometry based on hydrodynamic focusing, which allows individual cells to pass through an aperture, minimizing coincident passage, particle volume distortion, and red blood cell recirculation (3). In contrast, the Horiba Yumizen H2500 adopts a double hydrodynamic focusing approach: the first system measures cell size via electrical impedance, while the second assesses cell complexity (11).

In this study, all samples were processed using both analyzers within 30 minutes of collection. Samples containing clots were excluded and recollected. Before analysis, all samples were maintained at room temperature and then stored at 4°C for a maximum of five days following analysis. Daily quality control checks were conducted on each analyzer before sample processing. This study received ethical approval from the Institutional Review Board of Dr. Soetomo General Academic Hospital (Ethical Clearance No. 2302/121/4/VII/2023).

Data were subjected to normality, difference, and correlation tests. The normality of patient age data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, while patient diagnoses were reported as percentages. If data were normally distributed, the mean and standard deviation (SD) were used for descriptive analysis, the paired t-test for difference testing, and Pearson's correlation for correlation analysis. For non-normally distributed data, the median and range (min-max) were used, alongside the Wilcoxon signed-rank test and Spearman's correlation test.

Statistical analyses were conducted using SPSS software version 21 (IBM, Chicago, USA). Distribution assessments employed the Kolmogorov-Smirnov and Shapiro-Wilk tests. Differences between parameters were evaluated using the paired t-test and Wilcoxon signed-rank test. Correlations between the two analyzers were analyzed using Pearson and Spearman correlation coefficients. Additionally, non-parametric regression methods, including Passing-Bablok regression and Bland-Altman analysis, were utilized to assess the agreement between analyzers. These analyses were performed using web-based tools incorporating the R programming language (RStudio, Boston, USA) (12). A p-value <0.05 was considered statistically significant.

Results

The age range of the subjects was 2–17 years, with a median age of 7 years and a mean age of 7.82 years ($p < 0.005$). The majority of leukemia cases were diagnosed as Acute Lymphoblastic Leukemia (ALL), accounting for 87.7%, followed by

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Chronic Myeloid Leukemia (CML) at 7.0%, Acute Myeloid Leukemia (AML) at 3.5%, and 1.8% of cases which could not be definitively diagnosed due to insufficient data.

The values for each parameter are presented as Mean ± SD, as shown in Table I. The WBC, MON%, and EOS% parameters were not normally distributed ($p < 0.05$), while the NEU% and LYM% parameters followed a normal distribution ($p > 0.05$). Table II presents the results of the correlation and difference tests between parameters measured by the two instruments. Statistically significant differences were observed for WBC, MON%, and EOS% values between the Sysmex XN-3000 and Horiba Yumizen H2500. These differences were noted in the median values for WBC and MON%, and the mean value for NEU%. Correlation tests indicated strong associations between the parameters assessed by both analyzers.

Figures 1-5 illustrate the Passing-Bablok regression and Bland-Altman plots for each parameter. In the correlation tests, the x-axis represents measurements obtained from the Horiba Yumizen H2500, and the y-axis represents those from the Sysmex XN-3000. The diagonal line on the regression plots represents the identity line ($y = x$), indicating perfect agreement between devices. The Bland-Altman plots display the differences between the two analyzers' measurements relative to their mean values, highlighting any systematic bias and limits of agreement, which are denoted by red horizontal lines.

Figures 1-5 demonstrate a strong agreement between the two instruments in measuring total WBC and differential counts of neutrophils, lymphocytes, monocytes, and eosinophils. Notably, the Bland-Altman plot for EOS% (Figure 5) exhibited greater variability than other differential parameters. This may be attributed to the naturally low proportion of eosinophils in peripheral blood, making small measurement variations proportionally more significant. The Spearman correlation coefficients, consistently exceeding 0.9, reflect a high degree of consistency between the two methods. Furthermore, the Bland-Altman plots show that most measurement differences fall within acceptable limits, suggesting minimal systematic bias or significant variability. These findings support the reliability of both analytical methods for clinical and research evaluation of hematological parameters.

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Table I. Normality test for WBC and differential count

Parameter	Mean ± SD		Sig. *
	Sysmex XN-Series	Horiba Yumizen H2500	
WBC(×10^3/µL)	5.9418 ± 4.53844	5.8061 ± 4.46222	0.001
NEU%	51.0667 ± 18.55781	50.1875 ± 19.58818	0.200
LYM%	37.7158 ± 16.98183	37.5750 ± 18.12883	0.200
MON%	8.6561 ± 10.53331	9.5500 ± 11.29151	0.001
EOS%	2.1842 ± 2.38491	2.2214 ± 2.12035	0.001

*. Data are considered not normally distributed if the p-value<0.05.

Table II. Difference and Correlation's Test Result

Parameter	Sig*	Coefficient correlations (r)**
WBC (×10^3/µL)* **	0.001	0.981
NEU%* **	0.024	0.987
LYM%* **	0.869	0.982
MON%* **	0.006	0.923
EOS%* **	0.002	0.960

*. Wilcoxon signed rank test and T-paired test result
**. Correlation is significant at the 0.01 level (2-tailed).
***. WBC (White Blood Cell); NEU% (Neutrophil Percentage); LYM%(Lymphocyte Percentage); MON% (Monocyte Percentage); EOS% (Eosinophil Percentage).

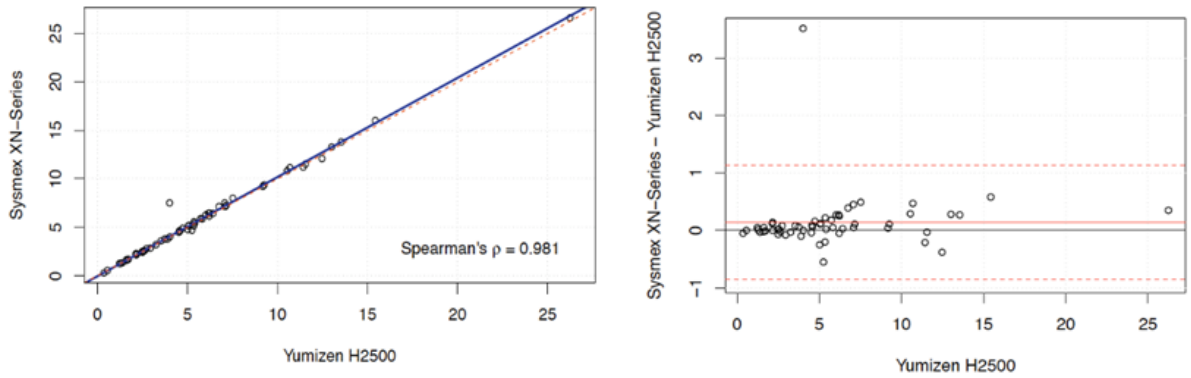

Figure 1. Passing-Bablok and Bland-Altman plot for WBC

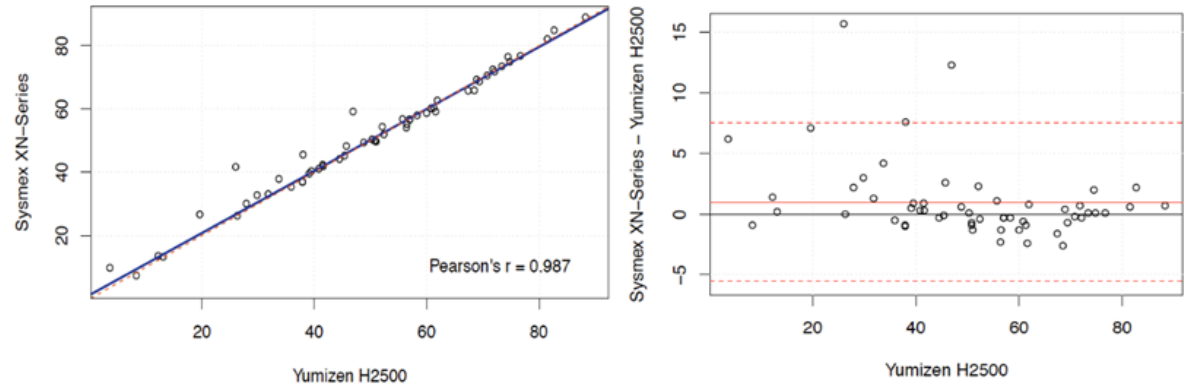

Figure 2. Passing-Bablok and Bland-Altman plot for NEU%

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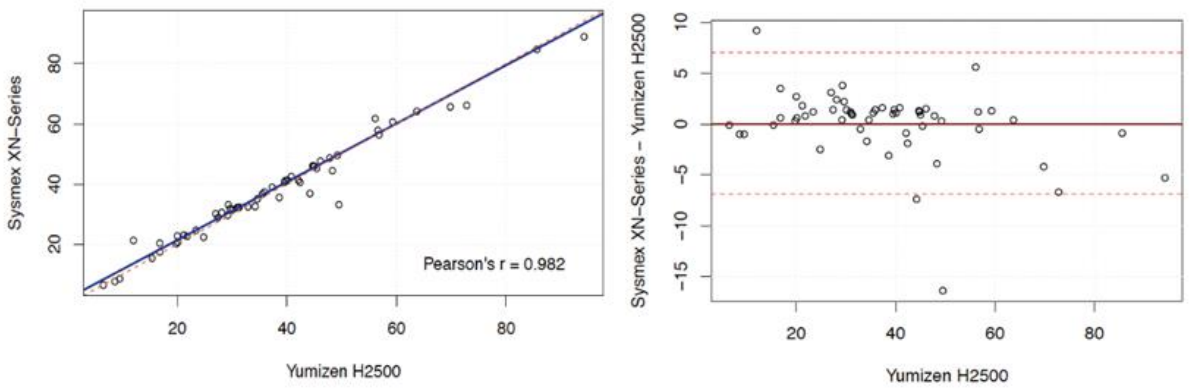

Figure 3. Passing-Bablok and Bland-Altman plot for Lym%

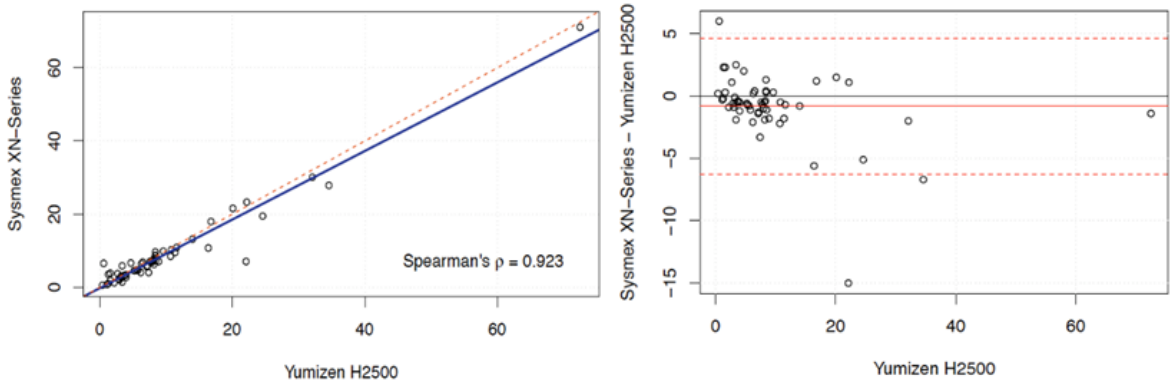

Figure 4. Passing-Bablok and Bland-Altman plot for Mon%

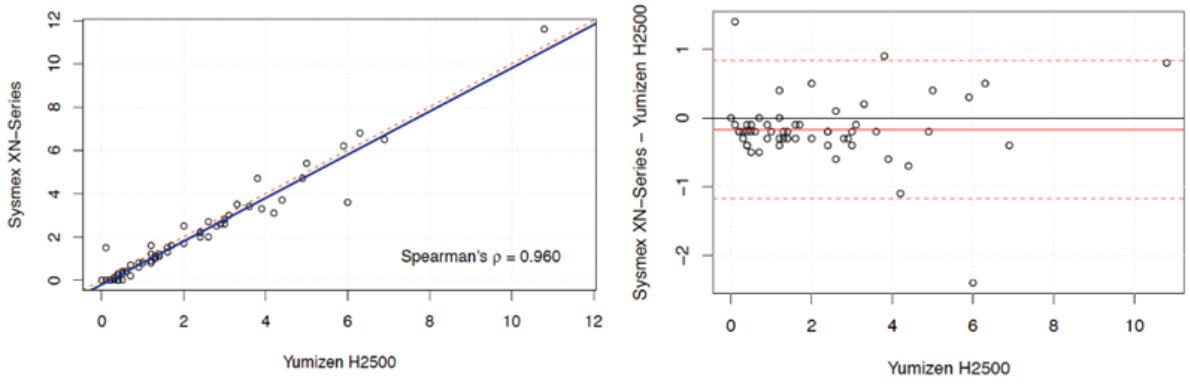

Figure 5. Passing-Bablok and Bland-Altman plot for Eos%

Discussion

Leukaemia is the most prevalent cancer among children and adolescents, accounting for 30% of cancers diagnosed before the age of 15 and 25% of those diagnosed before the age of 20 (13). Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) represent nearly 95% of childhood leukemias, with ALL occurring more frequently than AML during childhood (13). According to research by Garniasih D. et al., the incidence of childhood ALL in Indonesia is 4.32 per 100,000 children, which is higher than reported rates in other countries (5,14). The subjects of this study were pediatric patients, with the majority of leukemia cases being ALL. The correlation graphs demonstrate that most of the measurement results from both devices are close to the identity line (y = x), indicating that both analyzers produce

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similar results. Despite differences in channel nomenclature between the Sysmex XN-Series (WDF and WNR) and the Horiba Yumizen H2500 (LMNE), both instruments utilize optical methods based on flow cytometry for leukocyte counting. The flow cytometry method employed in both devices, known as hydrodynamic focusing, involves directing blood cells into a narrow stream as they pass through an aperture, where they are then detected using a laser beam (9).

For the EOS% parameter, greater measurement variation was observed compared to other parameters. Several factors may influence these variations in automated hematology analyzers. In addition to using optical methods, the Horiba Yumizen H2500 also employs electrical impedance to measure blood cells. According to Malecka et al. in their study using the Sysmex XN-2000 and Horiba Yumizen 2500, the differing measurement techniques and leukocyte staining reagents used by each instrument may contribute to result variability (15). High interlaboratory variability has been associated with elevated absolute eosinophil counts (16). However, factors such as tube agitation and the patient's health status (with or without atopy and eosinophilic asthma) do not appear to affect eosinophil measurements across several automated hematology analyzers, including the Abbott CELL-DYN Sapphire, Beckman Coulter DxH900, Siemens ADVIA 2120i, and Sysmex XN-1000V (17).

The results of this study demonstrate a significant correlation between total WBC levels and differential counts (WBC, NEU%, LYM%, MON%, EOS %). A study conducted by Bhola K. et al. using similar instruments, namely the Sysmex XN-3000 and Horiba Yumizen H2500, on 296 patients of varying ages and genders also reported strong correlations for the WBC, NEU%, LYM%, MON%, and EOS% parameters (18). These findings are consistent with the results of the present study. The main differences are that the current study had a smaller sample size, focused on a specific age range, and did not calculate bias for the parameters investigated.

Conclusion

This study shows that there is generally a good correlation between the Sysmex XN-Series and the Yumizen H2500 in measuring various types of white blood cells (neutrophils, lymphocytes, monocytes, and eosinophils) in pediatric leukemia patients. However, some variation was observed, particularly in the measurement of eosinophil percentages. Overall, both devices are reliable and may be used interchangeably or complementarily in daily clinical practice. The minor discrepancies observed may be attributed to differences in examination techniques, reagent composition, and high interlaboratory variability.

Limitation

The limitations of this study include a relatively small sample size, the use of a specific age group (pediatric subjects), and the absence of bias assessment.

Ethical Considerations

The study involving human participants was reviewed and approved by the Institutional Review Board (IRB) of Dr. Soetomo General Academic Hospital (2302/121/4/VII/2023). Written informed consent was obtained from all patients/participants involved in the study.

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Authors' Contributions

Aryati Aryati contributed ideas and was responsible for designing and developing the concept of the study. Alifferdi Rahman Wiyono interpreted the clinical data and experimental findings, collected the clinical data and blood samples, and prepared the manuscript. Yulia Nadar Indrasari and Mia Ratwita Andarsini critically revised the manuscript for important intellectual content, approved the final version, provided study materials or patient samples, contributed to the analysis of the experimental and microbiological findings, and verified the accuracy of the statistical analysis. Yulia Nadar Indrasari also secured the funding for the study. Alifferdi Rahman Wiyono, Aryati Aryati, Yulia Nadar Indrasari, and Mia Ratwita Andarsini participated in the discussions throughout the research process. All authors contributed to the manuscript and approved the submitted version.

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Conflict of Interest

There are no conflicts of interest among the authors of this study.

References

1. Puckett Y, Chan O. Acute lymphocytic leukemia. Treasure Island (FL): StatPearls Publishing; 2023; 1-7.
2. Szymańska K, Park S. Acute lymphocytic leukemia: Diagnosis and treatment in: Reference module in biomedical sciences. Elsevier; 2018: 1–8.
3. Elaine MK, Catherine NO, Jeanine MW. Rodak's Hematology. 6th Edition. St. Louis, Missouri: Elsevier; 2020.
4. Schüz J, Erdmann F. Environmental exposure and risk of childhood leukemia: An overview. Arch Med Res 2016; 47(8):607–614.
5. Malard F, Mohty M. Acute lymphoblastic leukaemia. The Lancet 2020; 395(10230):1146–1162.
6. Perdana AB, Saputra F, Aisyi M. Update on diagnosis of childhood acute lymphoblastic leukemia (ALL) in Indonesia. Indonesian J Cancer 2020; 14(4):115-119.
7. Siregar OR, Lubis B, Liberty P. Pediatric cancer epidemiology for 2021–2023 based on the cancer registry of Indonesia's national referral hospital. Siriraj Med J 2025; 77(2):108–118.
8. Yamade K, Yamaguchi T, Nagai Y, Kamisako T. Performance evaluation of leukocyte differential on the hematology analyzer Celltac G compared with two hematology analyzers, reference flow cytometry method, and two manual methods. J Clin Lab Anal 2021; 35(8):1–8.
9. Daves M, Roccaforte V, Lombardi F, Panella R, Pastori S, Spreafico M, et al. Modern hematology analyzers: beyond the simple blood cells count (with focus on the red blood cells). J Lab Precis Med 2024; 9: 1–9.
10. Arneth BM, Menschikowki M. Technology and new fluorescence flow cytometry parameters in hematological analyzers. J Clin Lab Anal 2015; 29(3):175–83.
11. Rastogi S. Double hydrodynamic sequential system. Horiba Medical Yumizen Bio 2019; 1–5.
12. Bahar B, Tuncel AF, Holmes EW, Holmes DT. An interactive website for analytical method comparison and bias estimation. Clin Biochem 2017; 50(18): 1025–1029.
13. Hunger SP, Teachey DT, Grupp S, Aplenc R. Childhood leukemia. Clin Oncol 2020; 1748-1764e4.
14. Garniasih D, Susanah S, Sribudiani Y, Hilmanto D. The incidence and mortality of childhood acute lymphoblastic leukemia in Indonesia: A systematic review and meta-analysis. Yassin MA,

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editor. PLoS One 2022; 17(6): e0269706-269709.
15. Małecka M, Ciepiela O. A comparison of Sysmex-XN 2000 and Yumizen H2500 automated hematology analyzers. Pract Lab Med 2020; 22: e00186.-00189
16. Chipps BE, Jarjour N, Calhoun WJ, Iqbal A, Haselkorn T, Yang M, et al. A Comprehensive analysis of the stability of blood eosinophil levels. Ann Am Thorac Soc 2021; 18(12):1978–1987.
17. Åstrand A, Wingren C, Walton C, Mattsson J, Agrawal K, Lindqvist M, et al. A comparative study of blood cell count in four automated hematology analyzers: An evaluation of the impact of preanalytical factors. PLoS One 2024; 19(5): e0301845-0301859.
18. Bhola RK, Fudaly C, Rastogi S. A comparative evaluation of performance of sysmex XN 3000 and horiba Yumizen H2500 automated complete blood count analysers. Indian J Hematol Blood Transfus 2024; 40(2):303–314.

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