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ORIGINAL ARTICLE

Automated haematology analysis and blood smear evaluation in paediatric leukaemia: a method-comparison study

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Running title: Indrasari et al. Analysers in paediatric leukaemia

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ABSTRACT

Aim: To compare complete blood count and leucocyte differential results from the Yumizen H2500 and Sysmex XN-3000 in paediatric leukaemia samples and assess Yumizen H2500 differential counts against manual blood smear evaluation.

Methods: This cross-sectional study included 101 paediatric leukaemia blood samples. Samples were analysed on both automated haematology analysers within one hour of collection, followed by a 200-cell manual differential count. Passing–Bablok regression assessed constant and proportional bias, Lin's concordance correlation coefficient (CCC) agreement, and Spearman's correlation associations between Yumizen H2500 differential parameters and microscopy.

Results: Among 101 leukaemia samples, white blood cells showed the highest concordance (CCC = 0.999), followed by neutrophils (CCC = 0.985), lymphocytes (CCC = 0.982), eosinophils (CCC = 0.977) and platelets (CCC = 0.967), whereas nucleated red blood cells and mean corpuscular haemoglobin concentration showed low concordance (CCC = 0.279 and 0.170, respectively). No constant or proportional bias was detected for 11 parameters. Mean corpuscular volume and platelet distribution width showed proportional bias, eosinophils, monocytes and immature granulocytes constant bias, and mean corpuscular haemoglobin concentration, red cell distribution width standard deviation and platelets both types of bias. Manual counts correlated strongly with eosinophils ($\rho = 0.716$), segmented neutrophils ($\rho = 0.853$) and lymphocytes ($\rho = 0.724$) (all $p < 0.001$).

Conclusion: The analysers showed high concordance for several routine haematological parameters but were not universally interchangeable, supporting manual smear review for discordant or immature-cell findings.

Keywords: blood cell count; leukocyte count; microscopy; reproducibility of results

INTRODUCTION

Paediatric leukaemia, particularly acute lymphoblastic leukaemia (ALL), is the most common haematological malignancy in childhood (1). The complete blood count (CBC), together with differential leucocyte analysis, supports diagnosis, treatment monitoring and assessment of complications such as infection or bone marrow failure (1,2). In paediatric acute care, peripheral leucocyte count also remains a readily available laboratory adjunct to clinical assessment in non-haematological conditions (3).

Automated haematology analysers have largely replaced manual counting for routine blood cell analysis because of their precision, rapid turnaround and broader analytical capabilities. Their performance nevertheless depends on the underlying detection principles, including impedance, hydrodynamic focusing and fluorescence flow cytometry, which may influence results in abnormal samples containing blasts or atypical cells (4).

The Sysmex XN-3000 uses fluorescence flow cytometry and hydrodynamic focusing for white blood cell classification and immature granulocyte detection, whereas the Yumizen H2500 combines impedance with double hydrodynamic focusing and optical measurements. Comparative studies of these platforms have been reported, but evidence in paediatric leukaemia samples remains limited (4–6).

This study aimed to compare CBC and differential parameters measured by the Sysmex XN-3000 and Yumizen H2500 in paediatric leukaemia samples and to assess the correlation of Yumizen H2500 differential parameters with manual peripheral blood smear evaluation.

MATERIAL AND METHODS

Patients and study design

This cross-sectional observational study was conducted at the Clinical Pathology Laboratory of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from September 2023 to August

2024. Paediatric patients attending the haematology outpatient clinic were screened consecutively. The analytical cohort comprised patients aged 1–17 years with leukaemia diagnosed by paediatric haematology-oncology consultants on the basis of clinical and laboratory findings, including CBC, peripheral blood smear and bone marrow evaluation. Samples with insufficient specimen volume were excluded.

Peripheral blood was collected into tubes containing dipotassium ethylenediaminetetraacetic acid (K2EDTA) (Greiner Bio-One Vacuette) and analysed on the Sysmex XN-3000 (Sysmex, Kobe, Japan) and Yumizen H2500 (HORIBA Medical, Marseille, France) within one hour of collection. The paired analyser comparison included CBC and differential parameters. Yumizen H2500 differential results were additionally compared with manual microscopy in patients with ALL, acute myeloid leukaemia (AML) and chronic myeloid leukaemia (CML).

Manual differential cell counting followed the Clinical and Laboratory Standards Institute H20-A2 guideline (7). Two peripheral blood films were prepared by the wedge technique, stained with Wright's eosin methylene blue solution (Sigma-Aldrich, Darmstadt, Germany; lot HX45563583), and examined independently by two experienced clinical laboratory scientists using a 200-cell differential count. Discrepant findings were jointly reviewed, with a third scientist providing adjudication when required. Samples were anonymised before analyser and smear assessment.

Methods

Parents or legal guardians provided written informed consent. The study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital (0742/KEPK/VIII/2023).

Both analysers were operated according to standard laboratory procedures throughout the study. The Sysmex XN-3000 was calibrated using XN CAL and controlled daily with XN Check levels 1–3. The Yumizen H2500 was calibrated using ABX Minocal and controlled daily with

DIFFTROL low, normal and high levels. Laboratory quality-control procedures and instrument performance verification were maintained throughout the study (4).

The Sysmex XN-3000 measures red blood cells (RBCs) and platelets (PLTs) by impedance with hydrodynamic focusing. Haemoglobin (HGB) is measured using the sodium lauryl sulphate method. Haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width standard deviation (RDW-SD) and red cell distribution width coefficient of variation (RDW-CV) are generated from direct measurements and derived erythrocyte indices (4).

White blood cells (WBCs) are analysed by flow cytometry using hydrodynamic focusing and a 633-nm semiconductor laser. Forward scatter reflects cell size, side scatter reflects internal complexity and side fluorescence reflects nucleic-acid staining. Nucleated red blood cells (NRBCs) and differential leucocytes are assessed through the white-cell nucleated (WNR) and white-cell differential (WDF) channels (8).

The Yumizen H2500 combines impedance, optical absorbance and double hydrodynamic focusing. It requires approximately 110 µL of sample and can report up to 55 haematological parameters. Samples are automatically mixed before analysis to ensure homogeneity (5).

For differential leucocyte analysis, the Yumizen H2500 measures total nucleated cells and separates NRBCs from WBC subpopulations. Basophils are counted by impedance after selective lysis, whereas lymphocytes, monocytes, neutrophils, eosinophils and NRBCs are characterised in the lymphocyte-monocyte-neutrophil-eosinophil (LMNE) channel using the Double Hydrodynamic Sequential System and NuceDiff reagent (4,5).

Platelet distribution width (PDW) is derived from the platelet histogram on both analysers.

Immature granulocytes (IMGs) and eosinophils are detected by fluorescence flow cytometry on

the Sysmex XN-3000, whereas the Yumizen H2500 combines impedance and optical flow-based measurements for these cell populations (4,5).

Statistical analysis

Continuous variables were assessed for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Agreement between Sysmex XN-3000 and Yumizen H2500 measurements was evaluated using Passing–Bablok regression and Lin's concordance correlation coefficient (CCC). Constant bias was considered present when the 95% confidence interval (CI) for the intercept excluded 0, and proportional bias when the 95% CI for the slope excluded 1. Associations between Yumizen H2500 differential parameters and manual microscopy were assessed using Spearman's rank correlation because most variables were non-normally distributed. Immature monocytic cell percentages in ALL and CML were compared using the Mann–Whitney U test, with rank-biserial correlation as the effect-size measure. Two-sided p values <0.05 were considered statistically significant.

RESULTS

A total of 159 paediatric patients were screened. Fifty-four non-leukaemia patients were excluded from the analytical cohort, and four of 105 patients with leukaemia were excluded because of insufficient specimen volume, leaving 101 leukaemia samples for paired analyser and smear comparisons (Figure 1). The analytical cohort comprised 91 (90.1%) ALL, one (1.0%) AML and nine (8.9%) CML cases. The mean age was 7.5 years (range 1–17), and 63 (62.4%) participants were male. Among non-leukaemia screening patients, beta-thalassaemia (31.5%) and unclassified tumour (25.9%) were the most frequent diagnoses, whereas ALL predominated in the leukaemia group (90.1%) (Figure S1). The mean age in the non-leukaemia group was 10.0 years (range 2–17), and 25 (46.3%) patients were male.

All 101 leukaemia samples were analysed on both automated platforms within one hour of collection. Median WBC counts were 5.02 × 10⁹/L on the Sysmex XN-3000 and 5.09 × 10⁹/L on the Yumizen H2500, while median platelet counts were 294 × 10⁹/L and 333 × 10⁹/L, respectively (Table 1). RBC, MCV, MCH and neutrophil percentages were normally distributed; the remaining evaluated parameters were non-normally distributed ($p<0.05$).

Passing–Bablok regression showed no significant constant or proportional bias for WBC, RBC, HGB, HCT, MCH, RDW-CV, NRBC percentage, MPV, basophils, neutrophils and lymphocytes. Proportional bias was present for MCV and PDW, constant bias for eosinophils, monocytes and IMG percentage, and both constant and proportional bias for MCHC, RDW-SD and platelets (Table 2). Concordance was highest for WBC (CCC = 0.999), neutrophils (CCC = 0.985), lymphocytes (CCC = 0.982), eosinophils (CCC = 0.977) and platelets (CCC = 0.967). Lower concordance was observed for RDW-SD (CCC = 0.665), PDW (CCC = 0.478), NRBC percentage (CCC = 0.279) and MCHC (CCC = 0.170) (Figure S2).

The Yumizen H2500 immature monocytic cell (IMM) percentage was 0.20% (range 0–2.50%) in ALL and 0.10% (range 0–0.20%) in CML. The difference was statistically significant (Mann–Whitney U = 576, $p = 0.04$), with a rank-biserial correlation of 0.407 (95% CI 0.168–0.626) (Figure 2). Because the CML subgroup contained only nine patients, this comparison was considered exploratory.

Manual differential counts showed strong correlations with Yumizen H2500 eosinophils ($\rho = 0.716$, $p < 0.001$), segmented neutrophils ($\rho = 0.853$, $p < 0.001$) and lymphocytes ($\rho = 0.724$, $p < 0.001$), and weaker correlations with monocytes ($\rho = 0.352$, $p = 0.001$) and myelocytes versus IMG percentage ($\rho = 0.364$, $p = 0.001$). Correlations were not significant for atypical lymphocytes, basophils, band neutrophils, metamyelocytes, promyelocytes or promonocytes/monoblasts (Table 3).

DISCUSSION

This paired comparison showed close agreement between the Sysmex XN-3000 and Yumizen H2500 for WBC and several routine leucocyte differential parameters in paediatric leukaemia samples. However, agreement was not uniform across the full blood count. Selected erythrocyte and platelet indices, NRBCs and immature-cell parameters showed lower concordance or systematic bias, consistent with previous method-comparison studies showing parameter-specific differences between automated haematology platforms (4–6).

Previous comparisons of automated haematology platforms have also shown that agreement varies by analytical principle and parameter (4–6). In the present study, constant or proportional bias affected several indices even when concordance remained relatively high. This distinction is clinically relevant because strong association does not demonstrate numerical interchangeability between methods.

Correlations with manual microscopy were strongest for eosinophils, segmented neutrophils and lymphocytes, whereas monocytes and the myelocyte-to-IMG comparison showed weaker associations. Basophils were not significantly correlated with manual counts, which may partly reflect their low frequency and the limited number of cells examined in a 200-cell manual differential. Automated analysers assess substantially larger cell numbers, but abnormal flags and unexpected immature-cell results still require morphological confirmation (4,5,7).

Modern haematology analysers provide extended differential parameters and abnormal-cell flags that can assist recognition of immature or atypical leucocyte populations (9,10). Such parameters may complement routine differential counting, but their interpretation in leukaemia requires caution because analyser-defined populations do not directly correspond to a complete morphological classification. The weak correlation between myelocytes and IMG in this study

supports retaining peripheral blood smear assessment when immature populations are clinically relevant. Other analyser-derived leucocyte parameters have also shown clinical relevance (11). The difference in IMM percentage between ALL and CML was exploratory. The marked imbalance in subtype numbers and the small CML group preclude conclusions about diagnostic discrimination or treatment monitoring. Because extended differential parameters require instrument-specific interpretation, this observation should be considered hypothesis-generating and requires validation in larger, prospectively defined subtype cohorts (9,10).

Automated differential counts remain valuable for rapid and reproducible screening, while manual smear examination provides morphological information on blasts, atypical lymphocytes and dysplastic changes that automated numerical outputs cannot fully replace (12). Extended differential parameters may complement this assessment, but their clinical value depends on disease context, analyser-specific performance and appropriate morphological review (13,14).

This study has several limitations. It was conducted at a single centre, and the leukaemia cohort was dominated by ALL, with only nine CML and one AML case. Consequently, subtype-specific comparisons were underpowered, and the IMM analysis should not be generalised beyond the present cohort. Manual microscopy was based on a 200-cell differential, which may be less precise for rare cell populations, although this approach follows established reference differential-count procedures (7). In addition, the findings describe analytical agreement in paediatric leukaemia samples and do not establish clinical interchangeability for every reported parameter.

CONCLUSION

Sysmex XN-3000 and Yumizen H2500 showed close agreement for WBC and several routine leucocyte differential parameters in paediatric leukaemia samples, but important disagreement

and systematic bias were present for selected erythrocyte, platelet, NRBC and immature-cell parameters. The two analysers should therefore not be considered universally interchangeable. The Yumizen H2500 can support routine haematological assessment in this setting, provided that parameter-specific limitations are recognised. Manual blood smear review remains necessary for abnormal or immature-cell findings. Larger multicentre studies with more balanced leukaemia subtypes are needed to validate these observations.

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CONFLICTS OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS (CREDIT)

Conceptualisation, Y.N.I.; methodology, Y.N.I. and A.A; supervision, A.A. and I.D.G.U.; validation, Y.N.I. and A.R.W.; resources, A.A. and M.R.A.; investigation, Y.N.I. and M.R.A.; data interpretation, Y.N.I.; visualisation, Y.N.I. and A.R.W.; writing—original draft, Y.N.I.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

ETHICS STATEMENT

The study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital (0742/KEPK/VIII/2023). Written informed consent was obtained from the parents or legal guardians of all participants.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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TABLES AND FIGURES

Table 1. Distribution of complete blood count and differential parameters measured by Sysmex XN-3000 and Yumizen H2500 in paediatric leukaemia samples

Parameter	XN Min	YH Min	XN Median	YH Median	XN Max	YH Max	XN Mean	YH Mean	XN SD	YH SD
WBC (×10⁹/L)	0.61	0.56	5.02	5.09	44.60	44.38	5.76	5.65	4.99	4.94
RBC (×10¹²/L)	2.80	2.74	4.05	3.95	6.19	6.02	4.08	4.02	0.62	0.62
HGB (g/dL)	8.70	8.90	11.30	11.40	15.10	16.20	11.46	11.51	1.33	1.36
HCT (%)	25.60	27.30	33.80	35.60	44.30	50.80	33.97	35.57	4.05	4.28
MCV (fL)	64.70	69.10	83.90	89.60	105.30	108.80	83.86	89.45	7.36	8.09
MCH (pg)	20.70	21.10	28.30	29.00	34.90	35.20	28.33	28.91	2.78	2.81
MCHC (g/dL)	30.60	30.60	33.70	32.30	36.90	33.60	33.77	32.29	1.15	0.59
RDW-CV (%)	11.70	11.60	14.90	16.95	24.50	23.70	15.82	17.38	2.73	2.63
RDW-SD (fL)	26.00	35.90	44.60	54.90	92.40	94.60	47.37	56.17	11.00	12.66
NRBC (%)	0.00	0.00	0.00	0.00	6.50	14.70	0.453	0.923	1.204	2.139
PLT (×10⁹/L)	0.00	0.00	294.00	333.00	1,154.00	1,265.00	289.00	324.19	164.75	183.62
MPV (fL)	7.80	0.80	9.25	9.80	13.10	16.70	9.44	9.96	0.98	1.67
PDW (fL)	6.90	8.60	8.75	12.10	21.10	31.50	9.36	13.21	2.12	3.85
IMG (%)	0.00	0.10	0.55	0.80	13.50	17.30	1.92	1.67	2.86	2.25
Eosinophils (%)	0.00	0.10	1.40	1.55	17.50	17.20	2.50	2.84	3.10	3.15
Basophils (%)	0.00	0.00	0.40	0.40	5.40	5.30	0.52	0.59	0.70	0.80
Neutrophils (%)	1.70	2.10	49.25	49.20	88.90	86.30	49.93	49.37	18.41	18.17
Lymphocytes (%)	6.90	5.97	37.30	36.10	97.80	116	39.20	38.25	18.22	19.81
Monocytes (%)	0.10	0.40	7.10	8.30	33.30	36.90	8.25	9.02	5.08	5.42

HCT, haematocrit; HGB, haemoglobin; IMG, immature granulocytes; max, maximum; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; min, minimum; MPV, mean platelet volume; NRBC, nucleated red blood cells; PDW, platelet distribution width; PLT, platelets; RBC, red blood cells; RDW-CV, red cell distribution width coefficient of variation; RDW-SD, red cell distribution width standard deviation; SD, standard deviation; WBC, white blood cells; XN, Sysmex XN-3000; YH, Yumizen H2500.

Table 2. Passing–Bablok regression and concordance between Sysmex XN-3000 and Yumizen H2500 in paediatric leukaemia samples

Parameter	Passing–Bablok equation	Intercept (95% CI)	Slope (95% CI)	CCC
WBC	y = −0.00237 + 0.992x	−0.002 (−0.050–0.042)	0.992 (0.980–1.000)	0.999
RBC	y = −0.00932 + 0.977x	−0.009 (−0.115–0.112)	0.977 (0.947–1.004)	0.949
HGB	y = −0.1 + 1.000x	−0.100 (−0.100–0.325)	1.000 (0.964–1.000)	0.925
HCT	y = 0.618 + 1.026x	0.618 (−1.461–2.490)	1.026 (0.967–1.088)	0.820
MCV	y = −1.185 + 1.087x	−1.185 (−7.571–4.004)	1.087 (1.023–1.160)	0.759
MCH	y = 0.600 + 1.000x	0.600 (−0.337–1.424)	1.000 (0.970–1.033)	0.968
MCHC	y = 20.064 + 0.364x	20.064 (15.450–23.615)	0.364 (0.259–0.500)	0.170
RDW-CV	y = −0.133 + 1.118x	−0.133 (−2.284–1.700)	1.118 (1.000–1.270)	0.741
RDW-SD	y = −7.752 + 1.338x	−7.752 (−16.453–−0.098)	1.338 (1.168–1.529)	0.665
NRBC (%)	y = 0 + 2.500x	0.000 (0.000–0.000)	2.500 (0.952–4.625)	0.279
PLT	y = 8.633 + 1.089x	8.633 (3.043–16.485)	1.089 (1.059–1.115)	0.967
MPV	y = −0.964 + 1.143x	−0.964 (−2.550–0.300)	1.143 (1.000–1.316)	0.773
PDW	y = −0.600 + 1.400x	−0.600 (−2.071–0.550)	1.400 (1.278–1.571)	0.478
IMG (%)	y = 0.222 + 0.889x	0.222 (0.100–0.256)	0.889 (0.775–1.000)	0.824
Eosinophils (%)	y = 0.312 + 0.967x	0.312 (0.300–0.371)	0.967 (0.922–1.000)	0.977
Basophils (%)	y = −0.050 + 1.000x	−0.050 (−0.138–0.008)	1.000 (0.833–1.250)	0.780
Neutrophils (%)	y = 0.170 + 0.992x	0.170 (−0.774–1.126)	0.992 (0.972–1.012)	0.985
Lymphocytes (%)	y = −0.246 + 0.992x	−0.246 (−1.113–0.766)	0.992 (0.968–1.018)	0.982
Monocytes (%)	y = 0.549 + 1.011x	0.549 (0.083–0.967)	1.011 (0.951–1.096)	0.940

CCC, Lin's concordance correlation coefficient; CI, confidence interval; HCT, haematocrit; HGB, haemoglobin; IMG, immature granulocytes; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; NRBC, nucleated red blood cells; PDW, platelet distribution width; PLT, platelets; RBC, red blood cells; RDW-CV, red cell distribution width coefficient of variation; RDW-SD, red cell distribution width standard deviation; WBC, white blood cells. Constant bias is indicated when the 95% CI for the intercept excludes 0; proportional bias is indicated when the 95% CI for the slope excludes 1.

Table 3. Correlation between manual differential counts and Yumizen H2500 parameters in paediatric leukaemia samples

Manual differential cell type	Yumizen H2500 parameter	Spearman's ρ	p value
Atypical lymphocytes (%)	ALY (%)	0.167	0.130
Eosinophils (%)	EOS (%)	0.716	<0.001
Basophils (%)	BAS (%)	0.129	0.225
Segmented neutrophils (%)	NEU (%)	0.853	<0.001
Lymphocytes (%)	LYM (%)	0.724	<0.001
Monocytes (%)	MON (%)	0.352	0.001
Band neutrophils (%)	IMG (%)	0.157	0.146
Metamyelocytes (%)	IMG (%)	0.168	0.119
Myelocytes (%)	IMG (%)	0.364	0.001
Promyelocytes (%)	IMG (%)	0.168	0.121
Promonocytes and monoblasts (%)	IMM (%)	0.065	0.558

ALY, atypical lymphocytes; BAS, basophils; EOS, eosinophils; IMG, immature granulocytes; IMM, immature monocytic cells; LYM, lymphocytes; MON, monocytes; NEU, neutrophils. Correlations are reported as Spearman's ρ; p values <0.001 are reported as <0.001.

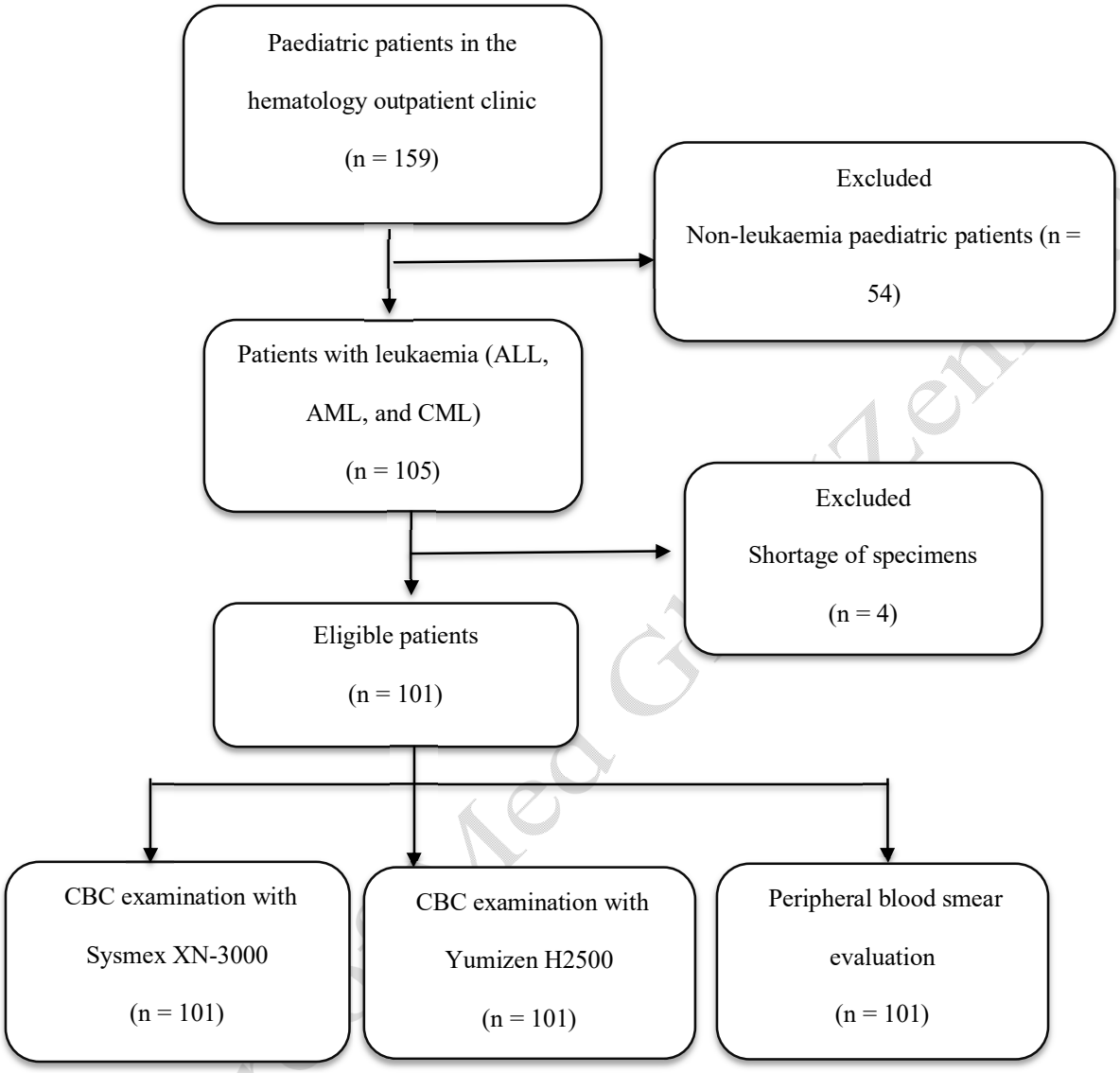

Figure 1. Study flow diagram
ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CBC, complete blood count; CML, chronic myeloid leukaemia.

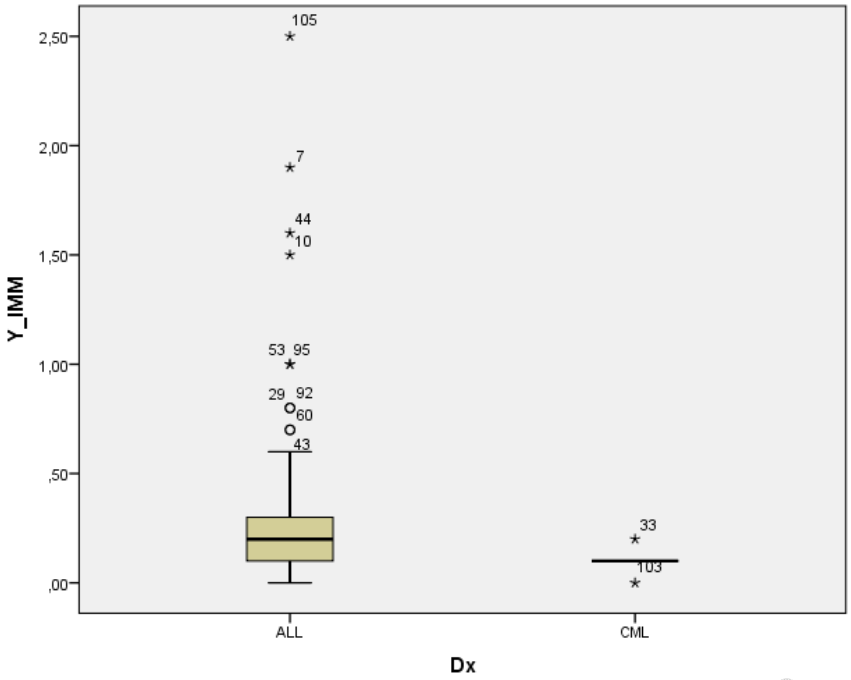

Figure 2. Distribution of immature monocytic cell percentage measured by Yumizen H2500 in patients with acute lymphoblastic leukaemia and chronic myeloid leukaemia

ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; Dx, diagnosis; IMM, immature monocytic cells; Y, Yumizen H2500.