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Performance of the automated digital cell image analyzer UIMD PBIA in white blood cell classification: a comparative study with sysmex DI-60

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Abstract

Objectives: This study aimed to evaluate the performance of PBIA (UIMD, Seoul, Republic of Korea), an automated digital morphology analyzer using deep learning, for white blood cell (WBC) classification in peripheral blood smears and compare it with the widely used DI-60 (Sysmex, Kobe, Japan).

Methods: A total of 461 slides were analyzed using PBIA and DI-60. For each instrument, pre-classification performance was evaluated on the basis of post-classification results verified by users. Pre- and post-classification results were compared with manual WBC differentials, and the ability to identify abnormal cells was assessed.

Results: The pre-classification performance of PBIA was better than that of DI-60 for most cell classes. PBIA had an accuracy of 90.0 % and Cohen's kappa of 0.934, higher than DI-60 (45.5 % accuracy and 0.629 kappa) across all cell classes. The pre-classification performance of both instruments decreased when abnormal cells were observed in manual counts, but PBIA still performed better. PBIA also appeared

to show better correlation with manual WBC differential counts, particularly in pre-classification (Pearson's correlation coefficient: 0.696–0.944 vs. 0.230–0.882 for neutrophils, lymphocytes, monocytes, eosinophils, basophils, and blasts), although the mean differences varied by cell class. For abnormal cells identified in manual counts, PBIA exhibited more false positives for blasts (30.5 vs. 2.3 %), while DI-60 had a higher rate of false negatives (42.1 vs. 6.1 %). Both instruments exhibited high false negative rates for atypical lymphocytes.

Conclusions: PBIA demonstrated better performance than DI-60, highlighting its clinical utility. Further multicenter studies are required for full validation.

Keywords: PBIA; DI-60; digital morphology analyzer; WBC classification; performance

Introduction

In clinical hematology, the examination of peripheral blood smears (PBS) is essential for diagnosing hematologic disorders [1, 2]. When abnormalities are flagged by automated hematology analyzers (approximately 10–15 % of blood samples) or clinical concerns arise, further morphological verification is needed [2–4]. Traditionally, it is performed through a manual microscopic review, which is the gold standard for white blood cell (WBC) differential counts [1, 2, 5]. However, manual review is labor intensive, time consuming, and prone to inter- and intra-observer variability, thus affecting accuracy and consistency [1, 6, 7].

Automated digital morphology analyzers have been developed to address these limitations, thereby providing faster, more consistent results with less reliance on expert labor [6, 8]. Recently, their accuracy in manual count tasks has been improved by artificial intelligence integration [9]. With these features, digital morphology analyzers are increasingly valuable in modern hematology labs; however, their performance must be thoroughly validated before clinical implementation, particularly their ability to identify abnormal findings [1, 7].

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PBIA (UIMD, Seoul, Republic of Korea) is a system designed to automate the pre-classification of WBCs via deep learning algorithms [10, 11]. Similar to the comprehensively studied DI-60 (Sysmex, Kobe, Japan) [6, 8, 12–14], PBIA automates the examination of PBS, thus offering rapid WBC differential results. Despite its potential, studies on the performance of PBIA are limited [10, 11].

In this study, we evaluated the WBC classification performance of PBIA and compared it with DI-60. This comparative study aimed to provide valuable insights into the clinical utility of PBIA.

Materials and methods

Samples

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board/Ethics Committee of Soonchunhyang University College of Medicine, Cheonan, Republic of Korea (approval no. 2024-02-009). The need for informed consent was waived because residual samples were used after the requested complete blood count (CBC) and PBS examinations were completed.

A total of 461 PBS slides were selected from our routine laboratory workload, mostly from patient samples. Venous blood was collected using a K₂-EDTA tube (BD, Franklin Lakes, NJ, USA). CBC for WBC count was performed using XN-9000 (Sysmex, Kobe, Japan) within 4 h of sample collection. PBS slides were prepared and stained by an automated SP-10 slide maker (Sysmex) using Wright–Giemsa stain (Sysmex) and buffer (CellaVision AB, Lund, Sweden), with following protocol: 2 min for Wright pure, 3 min for Wright diluted (1:10), 5 min for Giemsa diluted (1:25), 15 s for rinsing, and 10 min for drying. In each slide, manual WBC differential counts (200 cells) were performed by two well-trained laboratory experts (a skilled medical technician with over 10 years of experience and a laboratory medicine doctor) using the battlement track pattern. If nucleated RBCs (nRBCs) were observed, they were counted per 100 WBCs. Of the 461 PBS slides analyzed, 114 had blasts, 28 had atypical lymphocytes, and 79 had nRBCs. When categorized by WBC count, 53 slides had $<1.00 \times 10^9/L$; 85 had $1.00\text{--}2.00 \times 10^9/L$; 84 had $2.00\text{--}4.00 \times 10^9/L$; 83 had $4.00\text{--}10.00 \times 10^9/L$; and 156 had $>10.00 \times 10^9/L$. Among the $>10.00 \times 10^9/L$ group, 75 (48.1%) contained blasts on manual inspection, whereas none $<1.00 \times 10^9/L$ did. Of the 4,514 blasts identified by manual count, 4,373 (96.9%) were observed in slides with WBC counts $>10.00 \times 10^9/L$.

PBIA

This automated digital image analyzing system is composed of hardware and software components. The hardware component includes 12-slide cassettes, a slide barcode scanner, a precision stage, and a microscope optical system. The software component comprises a user interface, motion control, image processing, and deep learning functionalities. Manual re-classification (verification) is facilitated through a drag-and-drop function on related pages.

For deep learning, the Cell i Network optimized for cell images is utilized. It is based on a database of more than three million slide images, which have been used for training and tuning to achieve optimized classification performance. UIMD's database accounts for variations in staining, patient pathology, and examining professionals' expertise. Additionally, a custom model can be developed by fine-tuning the base model with 200 additional slides (100 normal and 100 abnormal) that have been confirmed by users at the implementing institution. In this study, PBIA was evaluated after it was fine-tuned.

Captured WBC images were pre-classified into 13 types of cells: segmented neutrophils, banded neutrophils, metamyelocytes, myelocytes, promyelocytes, lymphocytes, abnormal lymphocytes, reactive lymphocytes, monocytes, eosinophils, basophils, blasts, and plasma cells. nRBCs, giant platelets, platelet aggregation, malaria, and artifacts were also counted and displayed separately.

DI-60

The DI-60 system is a fully integrated cell image analyzer that combines two Sysmex XN hematology analyzers, namely, the Sysmex SP-10 slide maker/stainer and a CellaVision instrument. It operates using the same software and a modified camera lens system as the CellaVision DM1200 analyzer. Its performance has been thoroughly evaluated in recent studies, which showed that its WBC classification for normal and abnormal samples is reliable, and its accuracy improves after user verification [1, 6, 8].

DI-60 pre-classified 12 WBC classes: segmented neutrophils, banded neutrophils, metamyelocytes, myelocytes, promyelocytes, lymphocytes, atypical lymphocytes, monocytes, eosinophils, basophils, blasts, and plasma cells. It also separately counted and displayed nRBCs, giant platelets, platelet clumps, smudged cells, artifacts, and unidentified cells.

Study design

A total of 461 PBS slides were analyzed using PBIA and DI-60 for pre-classification. Verification was performed by two well-trained experts, yielding post-classification results. The classification process involved 14 different cell classes: segmented neutrophils, banded neutrophils, metamyelocytes, myelocytes, promyelocytes, lymphocytes, abnormal lymphocytes, reactive lymphocytes, monocytes, eosinophils, basophils, blasts, plasma cells, nRBC, and artifacts. We used each instrument's default settings: PBIA attempted to capture about 200 cells per slide, whereas DI-60 attempted to process roughly 300 cells but displayed approximately 100 images for review. In PBIA, abnormal lymphocytes and reactive lymphocytes were categorized as atypical lymphocytes for analysis. In DI-60, smudge cells and unidentified cells were classified as artifacts, and plasma cells were classified as atypical lymphocytes during verification. Unlike PBIA, which verified plasma cells as a distinct category, DI-60 and manual WBC differential counts used our institution's workflow, merging any suspected plasma cells into the 'atypical lymphocyte' unless specific circumstances – such as plasma cell leukemia – required a separate count. Giant platelets, platelet aggregation or clumps, and malaria were excluded from this analysis.

For each instrument, the pre-classification performance, including accuracy, false positive rate (FPR), and false negative rate (FNR), was calculated on the basis of post-classification results, and the agreement between pre- and post-classification was assessed. The pre-classification performance for both instruments was evaluated by categorizing WBC counts into five ranges: $<1.00 \times 10^9/L$, $1.00\text{--}2.00 \times 10^9/L$, $2.00\text{--}4.00 \times 10^9/L$, $4.00\text{--}10.00 \times 10^9/L$, and $>10.00 \times 10^9/L$. The pre-classification performance of both instruments was evaluated when key abnormal cells, such as blasts and atypical lymphocytes, were present in manual counts.

Manual WBC differential counts were used as the reference, and the WBC differentials from pre- and post-classification results were compared. In this study, manual counts did not include plasma cells and artifacts; as such, they were excluded from the analysis when comparing manual counts with the results from each instrument. Therefore, the following cell types were examined: segmented neutrophils, banded neutrophils, metamyelocytes, myelocytes, promyelocytes, lymphocytes, atypical lymphocytes, monocytes, eosinophils, basophils, blasts, and nRBCs. For nRBCs in PBIA and DI-60, the counts were converted to numbers per 100 WBCs to match the units used in the manual count. The mean differences in each cell class were calculated for both instruments. The

correlation between pre- and post-classification with manual counts was determined for each cell type. Differences in the correlation between pre- and post-classification within the same instrument were also examined. Passing–Bablok regression was performed for six WBC types (neutrophils, lymphocytes, monocytes, eosinophils, basophils, and blasts) to compare both pre- and post-classification with manual counts. Results were visualized.

In cases where abnormal cells such as blasts, atypical lymphocytes, and nRBCs were observed in the manual count, the ability of each instrument to detect these cells was evaluated. The accuracy, FPR, and FNR of each instrument were calculated and compared accordingly.

Statistical analysis

Data were analyzed using R (version 4.2.1; R Core Team, 2022). All tests were two sided with $p < 0.05$ considered significant. Categorical data were examined using Fisher's exact test. The agreement between pre- and post-classification was assessed with Cohen's kappa value (κ) interpreted as follows: $\kappa \leq 0.2$, poor; $0.2 < \kappa \leq 0.4$, fair; $0.4 < \kappa \leq 0.6$, moderate; $0.6 < \kappa \leq 0.8$, substantial; and $\kappa > 0.8$: good [15]. The mean differences between WBC differentials from pre-classification, post-classification, and manual counts were calculated via a paired t-test. For correlation analysis, Pearson's correlation coefficients (r) were calculated and interpreted as follows: <0.30 , negligible; $0.30\text{--}0.50$, low; $0.50\text{--}0.70$, moderate; $0.70\text{--}0.90$, high; and $0.90\text{--}1.00$: very high [16]. The Z-difference method was used to compare these correlations. Passing–Bablok regression was performed using the R package "mcr" (version 1.3.3). Results were provided for neutrophils, lymphocytes, monocytes, eosinophils, basophils, and blasts, where the slope was between 0.1 and 100, comparing post-classification WBC differentials with manual counts.

Results

Pre-classification performance

Table 1 shows the pre-classification performance of various cell classes based on post-classification results for PBIA and DI-60. Figure 1 presents the confusion matrices comparing the pre- and post-classification results for PBIA and DI-60. A total of 119,632 and 82,078 cells were analyzed by PBIA and DI-60, respectively. In PBIA, promyelocytes were excluded from the analysis because only two cells were identified. In

Table 1: Performance of WBC pre-classification based on the post-classification for PBIA and DI-60.

Cell class	PBIA (Total cell count=119,632)					DI-60 (Total cell count=82,078)				
	Cell count	Accuracy % (95 % CI)	FPR % (95 % CI)	FNR % (95 % CI)	Kappa (95 % CI)	Cell count	Accuracy % (95 % CI)	FPR % (95 % CI)	FNR % (95 % CI)	Kappa (95 % CI)
NES	42,603	99.0 (99.0–99.1)	1.1 (1.1–1.2)	0.6 (0.6–0.7)	0.979 (0.978–0.980)	19,707	93.3 (93.2–93.5)	3.2 (3.0–3.3)	16.7 (16.2–17.2)	0.822 (0.817–0.826)
NEB	1,898	99.0 (99.0–99.1)	0.5 (0.5–0.6)	30.6 (28.5–32.7)	0.674 (0.657–0.692)	3,738	95.8 (95.7–96)	3.8 (3.7–4.0)	33.9 (31.0–36.9)	0.259 (0.243–0.276)
ME	883	99.6 (99.5–99.6)	0.3 (0.3–0.3)	25.9 (22.7–29)	0.669 (0.643–0.696)	432	99.3 (99.3–99.4)	0.5 (0.4–0.5)	75.7 (70.3–81.2)	0.168 (0.129–0.207)
MY	700	99.7 (99.6–99.7)	0.2 (0.2–0.2)	27.0 (23.6–30.3)	0.709 (0.682–0.737)	1,093	98.7 (98.7–98.8)	1.2 (1.1–1.3)	34.5 (27.3–41.7)	0.172 (0.143–0.200)
PR	2	–	–	–	–	1,387	97.7 (97.6–97.8)	1.4 (1.4–1.5)	77.2 (74.5–79.8)	0.178 (0.156–0.199)
LY	22,499	98.1 (98.1–98.2)	1.3 (1.2–1.4)	4.4 (4.1–4.7)	0.938 (0.936–0.941)	9,405	91.5 (91.3–91.7)	3.6 (3.5–3.8)	39.4 (38.5–40.3)	0.613 (0.605–0.621)
AT	210	98.9 (98.8–98.9)	0.1 (0.1–0.1)	92.1 (90.7–93.6)	0.134 (0.111–0.157)	1,633	97.9 (97.8–98.0)	1.9 (1.8–2.0)	65.0 (59.3–70.8)	0.092 (0.074–0.110)
MO	8,563	98.8 (98.8–98.9)	0.9 (0.8–0.9)	5.6 (5.1–6.1)	0.909 (0.904–0.914)	1,883	96.3 (96.1–96.4)	0.5 (0.4–0.5)	64.0 (62.5–65.4)	0.482 (0.466–0.497)
EO	1,652	100.0 (100.0–100.0)	0.0 (0.0–0.0)	0.4 (0.1–0.7)	0.994 (0.991–0.996)	493	99.6 (99.5–99.6)	0.1 (0.1–0.1)	39.1 (35.4–42.8)	0.700 (0.669–0.730)
BA	776	99.9 (99.9–99.9)	0.1 (0.1–0.1)	4.0 (2.5–5.4)	0.916 (0.901–0.931)	1,390	98.6 (98.5–98.7)	1.4 (1.3–1.4)	12.8 (9.2–16.5)	0.322 (0.293–0.351)
BL	6,881	98.3 (98.3–98.4)	0.4 (0.3–0.4)	19.8 (18.9–20.6)	0.858 (0.852–0.864)	1,548	95.7 (95.5–95.8)	0.4 (0.4–0.5)	72.7 (71.4–74)	0.387 (0.371–0.403)
PC	242	99.8 (99.8–99.9)	0.2 (0.1–0.2)	6.7 (0.4–13)	0.370 (0.301–0.440)	388	–	–	–	–
NR	1,493	99.7 (99.6–99.7)	0.2 (0.2–0.3)	8.1 (6.7–9.6)	0.855 (0.841–0.869)	4,397	96.2 (96.1–96.3)	3.8 (3.7–3.9)	4.3 (3.3–5.4)	0.449 (0.433–0.465)
AR	31,230	99.2 (99.1–99.2)	0.7 (0.6–0.7)	1.3 (1.2–1.4)	0.978 (0.977–0.979)	34,584	85.4 (85.2–85.7)	11.6 (11.3–11.9)	18.4 (18.0–18.8)	0.702 (0.697–0.707)

AR, artifact; AT, atypical lymphocyte; BA, basophil; BL, blast; CI, confidence interval; EO, eosinophil; FNR, false negative rate; FPR, false positive rate; LY, lymphocyte; ME, metamyelocyte; MO, monocyte; MY, myelocyte; NEB, neutrophil banded; NES, neutrophil segmented; NR, nucleated red blood cell; PC, plasma cell; PR, promyelocyte.

DI-60, plasma cells were classified as atypical lymphocytes during verification; consequently, analyzing the pre-classification performance for plasma cells was difficult. Therefore, they were excluded from the analysis.

Overall, the pre-classification performance of PBIA was better than that of DI-60, with higher accuracy, lower FPR and FNR, and stronger agreement. PBIA outperformed DI-60 in most comparisons ($p < 0.001$) except in banded neutrophils ($p = 0.073$) and myelocytes ($p = 0.056$), where no significant differences in FNR were observed. For atypical lymphocytes, PBIA had a significantly higher FNR of 92.1 % (95 % confidence interval: 90.7–93.6) than the FNR of DI-60, which was 65.0 % (59.3–70.8; $p < 0.001$). The pre-classification accuracies based on post-classification results for all cell classes were 90.0 % (95 % confidence interval: 89.8–90.1) for PBIA and 45.5 % (45.2–45.9) for DI-60. All cell classes had κ of 0.934

(0.933–0.936) for PBIA and 0.629 (0.625–0.633) for DI-60. For major cell classes such as segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils, PBIA demonstrated an accuracy of 98.1–100.0 %, FPR of 0.0–1.3 %, and FNR of 0.4–5.6 %. Its κ ranged from 0.909 to 0.994, indicating good agreement. PBIA also outperformed DI-60 for blasts, with κ indicating good agreement. Both instruments showed a generally poor pre-classification performance on immature granulocytes (promyelocytes, myelocytes, and metamyelocytes) and banded neutrophils. For artifacts, PBIA had lower misclassification rates and stronger agreement than DI-60.

For both instruments, when WBC counts were divided into five ranges ($< 1.00 \times 10^9/L$, $1.00–2.00 \times 10^9/L$, $2.00–4.00 \times 10^9/L$, $4.00–10.00 \times 10^9/L$, and $> 10.00 \times 10^9/L$), variability in the pre-classification performance was

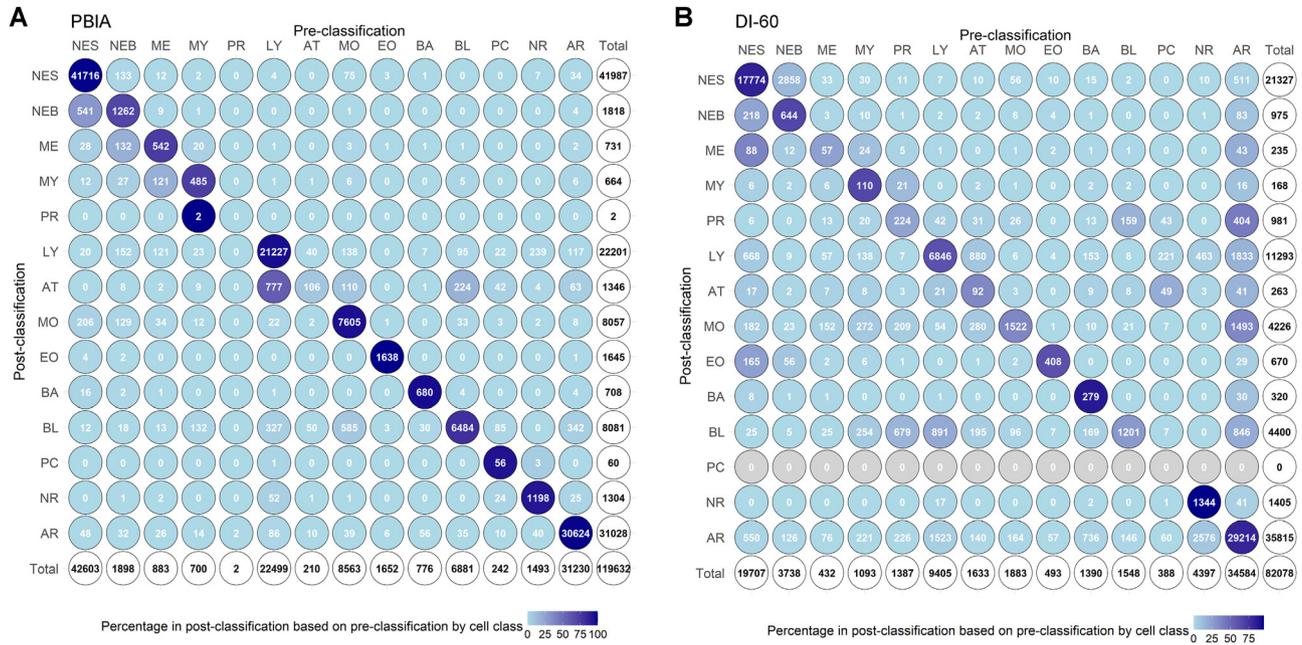


Figure 1: Confusion matrices comparing the pre- and post-classification performance of (A) PBIA and (B) DI-60. Columns and rows represent pre- and post-classification counts, respectively, by cell class. Color indicates the percentage of post-classification counts based on pre-classification counts for each cell class. In DI-60, plasma cells are excluded from post-classification; as a result, all post-classification counts for plasma cells are equivalent to zero. AR, artifact; AT, atypical lymphocyte; BA, basophil; BL, blast; EO, eosinophil; LY, lymphocyte; ME, metamyelocyte; MO, monocyte; MY, myelocyte; NEB, neutrophil banded; NES, neutrophil segmented; NR, nucleated red blood cell; PC, plasma cell; PR, promyelocyte.

observed. The performance was better in leukopenic cases, but tended to decrease as WBC counts increased (Supplemental Table 1). In addition, the pre-classification performance of PBIA and DI-60 was analyzed for 114 PBS slides where blasts were identified in the manual count and for 7 PBS slides where atypical lymphocytes were present in numbers greater than 20 in the manual count; these specimens were from patients previously diagnosed with lymphoma (Supplemental Table 2). The FPR of the cell classes that could potentially be confused with blasts, such as lymphocytes, atypical lymphocytes, monocytes, myelocytes, and artifacts, tended to increase compared with the overall results for both instruments. Specifically, in the 121 slides with abnormal cells, the FPR of lymphocytes (3.1 vs. 1.3 %), atypical lymphocytes (0.2 vs. 0.1 %), monocytes (2.6 vs. 0.9 %), myelocytes (0.5 vs. 0.2 %), and artifacts (2.0 vs. 0.7 %) in PBIA increased compared with that in the overall group. Similarly, the FPR of lymphocytes (8.0 vs. 3.6 %), atypical lymphocytes (3.2 vs. 1.9 %), monocytes (1.5 vs. 0.5 %), myelocytes (2.7 vs. 1.2 %), and artifacts (17.0 vs. 11.6 %) in DI-60 showed an increase. The FPR in PBIA was consistently lower than that in DI-60 across all relevant cell classes. However, for atypical lymphocytes, PBIA showed a significantly higher FNR of 93.0 % (91.2–94.8), while DI-60 showed an FNR of 68.5 % (59.8–77.3; $p < 0.001$).

Comparison with manual WBC differentials

Table 2 presents the median manual WBC differential counts for each cell class and, for each instrument, the mean differences between those manual counts and both the pre- and post-classification WBC differentials. In PBIA, the pre-classification results did not significantly differ from the manual counts for eosinophils and nRBCs. The post-classification results did not significantly vary for atypical lymphocytes, eosinophils, and nRBCs. In DI-60, the pre-classification results for segmented neutrophils did not significantly differ from those for the manual counts. The post-classification results showed no significant differences in segmented neutrophils, myelocytes, lymphocytes, atypical lymphocytes, and eosinophils.

Table 3 presents the comparison of the correlation of pre- and post-classification WBC differentials with manual counts for each instrument. The z-difference was used to assess whether the correlation results significantly differed between the pre- and post-classification. For major cell classes (segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils), PBIA showed high to very high correlation in post-classification; conversely, the correlation between pre- and post-classification for neutrophils, eosinophils, and basophils did not significantly vary. In DI-60, the

Table 2: Comparison of WBC differential counts between manual differential count, PBIA, and DI-60.

Cell class	Median, % [IQR]	Mean difference, % (95 % CI)							
		PBIA				DI-60			
		Manual	Pre-classification vs. Manual	p-Value	Post-classification vs. Manual	p-Value	Pre-classification vs. Manual	p-Value	Post-classification vs. Manual
NES	45.0 [24.0–63.0]	2.8 (1.9–3.6)	<0.001	2.0 (1.2–2.8)	<0.001	–0.3 (–1.4 to 0.8)	0.556	0.8 (–0.2 to 1.7)	0.110
NEB	0.0 [0.0–0.0]	1.3 (1.0–1.7)	<0.001	1.6 (1.1–2.0)	<0.001	7.0 (6.2–7.8)	<0.001	1.5 (1.1–2.0)	<0.001
ME	0.0 [0.0–0.0]	0.8 (0.6–1.0)	<0.001	0.6 (0.4–0.7)	<0.001	0.9 (0.7–1.1)	<0.001	0.3 (0.–0.5)	<0.001
MY	0.0 [0.0–0.0]	0.3 (0.2–0.4)	<0.001	0.3 (0.1–0.4)	<0.001	2.1 (1.8–2.4)	<0.001	–0.1 (–0.3 to 0.1)	0.387
PR	0.0 [0.0–0.0]	0.0 (0.0–0.0)	0.042	0.0 (0.0–0.0)	0.049	2.9 (2.2–3.5)	<0.001	1.5 (0.5–2.4)	0.002
LY	25.0 [9.0–43.0]	–1.4 (–2.3 to –0.4)	0.005	–1.7 (–2.4 to –1.0)	<0.001	–3.7 (–5.2 to –2.2)	<0.001	–0.3 (–1.5 to 0.8)	0.583
AT	0.0 [0.0–0.0]	–1.2 (–2.0 to –0.4)	0.005	0.1 (–0.3 to 0.5)	0.498	2.5 (1.5 to 3.4)	<0.001	–0.7 (–1.6 to 0.2)	0.115
MO	8.0 [3.0–15.0]	–1.3 (–2.0 to –0.6)	<0.001	–1.8 (–2.4 to –1.2)	<0.001	–6.8 (–7.6 to –6.0)	<0.001	–1.9 (–2.5 to –1.3)	<0.001
EO	1.0 [0.0–3.0]	0.0 (–0.1 to 0.2)	0.664	0.0 (–0.1 to 0.2)	0.752	–0.5 (–0.8 to –0.3)	<0.001	–0.1 (–0.4 to 0.2)	0.433
BA	0.0 [0.0–1.0]	0.2 (0.7–1.1)	<0.001	0.2 (0.7–1.0)	0.010	2.8 (2.4–3.1)	<0.001	0.2 (0.0–0.3)	0.023
BL	0.0 [0.0–0.0]	–1.9 (–2.7 to –1.1)	<0.001	–1.0 (–1.5 to –0.5)	<0.001	–6.8 (–8.6 to –5)	<0.001	–2.3 (–3.4 to –1.2)	<0.001
NR	0.0 [0.0–0.0]	–0.5 (–1.2 to 0.1)	0.126	–0.3 (–1.0 to 0.4)	0.387	21.2 (15–27.3)	<0.001	2.4 (0.9–3.9)	0.002

AT, atypical lymphocyte; BA, basophil; BL, blast; CI, confidence interval; EO, eosinophil; IQR, Interquartile range; LY, lymphocyte; ME, metamyelocyte; MO, monocyte; MY, myelocyte; NEB, neutrophil banded; NES, neutrophil segmented; NR, nucleated red blood cell; PR, promyelocyte.

post-classification results showed high to very high correlation for neutrophils, lymphocytes, and monocytes, while eosinophils and basophils showed moderate correlation. In DI-60, the correlation of pre-classification with manual counts was lower than that of post-classification.

For blasts, PBIA showed a very high correlation with manual counts in both pre- ($r=0.905$) and post-classification

($r=0.915$). In DI-60, the correlation coefficients for blasts were 0.322 for pre-classification (low correlation) and 0.842 for post-classification (high correlation). For atypical lymphocytes, PBIA showed a low correlation in pre-classification ($r=0.456$) and a high correlation in post-classification ($r=0.887$). Conversely, DI-60 did not show a significant correlation for atypical lymphocytes. Regarding nRBCs, PBIA

Table 3: Comparison of WBC differential correlation between manual differential count, PBIA, and DI-60.

Cell class	PBIA						DI-60					
	Pre-classification vs. Manual		Post- classification vs. Manual		Z-difference	p-Value	Pre-classification vs. Manual		Post- classification vs. Manual		Z-difference	p-Value
	r	p-Value	r	p-Value			r	p-Value	r	p-Value		
NES	0.944	<0.001	0.940	<0.001	–0.494	0.621	0.882	<0.001	0.925	<0.001	3.568	<0.001
NEB	0.460	<0.001	0.351	<0.001	–1.984	0.047	0.101	0.030	0.484	<0.001	6.445	<0.001
ME	0.532	<0.001	0.526	<0.001	–0.129	0.897	0.168	<0.001	0.371	<0.001	3.321	0.001
MY	0.695	<0.001	0.717	<0.001	0.655	0.512	0.262	<0.001	0.325	<0.001	1.050	0.294
PR	–0.007	0.878	–0.007	0.880	0.002	0.999	0.007	0.879	0.002	0.971	–0.082	0.935
LY	0.886	<0.001	0.935	<0.001	4.449	<0.001	0.692	<0.001	0.840	<0.001	5.582	<0.001
AT	0.456	<0.001	0.887	<0.001	13.823	<0.001	–0.024	0.609	0.037	0.429	0.921	0.357
MO	0.775	<0.001	0.848	<0.001	3.270	0.001	0.681	<0.001	0.842	<0.001	5.993	<0.001
EO	0.875	<0.001	0.872	<0.001	–0.193	0.847	0.624	<0.001	0.660	<0.001	0.923	0.356
BA	0.696	<0.001	0.713	<0.001	0.526	0.599	0.230	<0.001	0.627	<0.001	7.610	<0.001
BL	0.958	<0.001	0.975	<0.001	4.056	<0.001	0.865	<0.001	0.884	<0.001	1.208	0.227
NR	0.905	<0.001	0.915	<0.001	0.930	0.352	0.322	<0.001	0.842	<0.001	13.559	<0.001

AT, atypical lymphocyte; BA, basophil; BL, blast; EO, eosinophil; LY, lymphocyte; ME, metamyelocyte; MO, monocyte; MY, myelocyte; NEB, neutrophil banded; NES, neutrophil segmented; NR, nucleated red blood cell; PR, promyelocyte; r, Pearson's correlation coefficient.

demonstrated a very high correlation in pre- and post-classification ($r=0.905$ and $r=0.915$, respectively). For DI-60, pre- and post-classification had r of 0.865 and 0.884 , respectively. Both PBIA and DI-60 exhibited relatively lower correlation for immature granulocytes compared with major cell classes.

The results of Passing–Bablok regression for the major cell classes and blasts are presented in Figure 2. The scatter plots with r for the remaining cell classes are provided in Supplemental Figure 1.

Ability to identify abnormal cells

We compared the ability of PBIA and DI-60 to identify key abnormal cells when at least one was observed in the manual WBC differential count (Table 4). PBIA produced more false positive results for blasts, with an FPR of 30.5% ($25.9\text{--}35.6$), while DI-60 recorded a lower FPR of 2.3% ($1.2\text{--}4.5$). Conversely, DI-60 had a higher FNR of 42.1% ($33.4\text{--}51.3$) than PBIA (6.1% , $3.0\text{--}12.1$; $p<0.001$ for all). For atypical lymphocytes and nRBCs, PBIA showed higher accuracy and lower FPR than DI-60, although their FNR did not significantly differ. Atypical lymphocytes exhibited high FNRs in both instruments; specifically, PBIA and DI-60 had FNR of 50.0% ($32.6\text{--}67.4$) and 57.1% ($39.1\text{--}73.5$), respectively.

Discussion

DI-60 provides an acceptable WBC classification performance for normal and abnormal samples, with overall pre-classification accuracy reaching $86\text{--}90\%$ and correlates well with manual WBC differential counts after user verification [1, 6, 8, 17]. These previous results differ from our findings possibly because of variations in the sample types analyzed and the potential differences in staining protocols [1, 17, 18]. Notably, a previous study showed that DI-60 performance could depend on the staining method and proportion of abnormal samples [17]. Our use of a different Wright–Giemsa stain and the likely higher proportion of abnormal cases in our real-world sample may both have contributed to the observed discrepancies [8, 17].

To the best of our knowledge, only two studies have reported on the performance of PBIA WBC classification [10, 11]. One study reported a high pre-classification accuracy of 97% for overall WBC counts and approximately 94% for blasts. Its accuracy (81%) for abnormal lymphocytes is lower than that for other cell classes although reactive lymphocytes are separately classified, achieving a higher accuracy

of 94% . For nRBCs, the pre-classification accuracy of PBIA is approximately 96% [10]. The other study demonstrated over 90% accuracy for all cell types except atypical lymphocytes in samples from leukopenic patients [11].

Under the conditions of this study, PBIA outperformed DI-60 in pre-classification and showed stronger correlation with manual counts, resulting in fewer discrepancies between pre- and post-classification. However, for specific cell classes, such as atypical lymphocytes, PBIA showed a lower pre-classification performance than DI-60 did. In addition, the performance of PBIA for immature granulocytes and banded neutrophils remained poor. One possible explanation is the inherent subjectivity in classifying these transitional forms, leading to substantial inter-observer variability [1, 18]. Although verification reduced differences in WBC differentials for some cell classes, improvements varied by class. Therefore, users should understand which cell classes might be prone to misclassification when using PBIA, and they should take additional care during verification.

The presence of abnormal cells may reduce pre-classification performance [8, 18, 19], as our findings also indicated, underscoring the need for verification [6]. However, PBIA performed better under these conditions, potentially helping users verify images more effectively. In DI-60, various cell classes, including blasts, were more frequently misclassified as artifacts, demanding greater vigilance during reclassification.

When evaluating abnormal cell detection, DI-60 showed a better accuracy for blasts and nRBCs in terms of manual count results, whereas PBIA performed better for atypical lymphocytes. Notably, PBIA produced more false positives for blasts, whereas DI-60 had a higher false negative rate. These findings suggested that manual inspection is recommended for PBIA when blasts are observed in post-classification results. This aligns with the International Council for Standardization of Hematology recommendations for digital morphology analyzers, which specify that blasts require manual review [1]. Both instruments showed high false negative rates for atypical lymphocytes. Therefore, digital morphology analyzers alone may be insufficient for detecting these cells.

A previous study demonstrated that digital morphology analyzers can exhibit reduced pre-classification performance in leukopenia. Notably, it showed that leukopenia groups likely contained more abnormal cells [14]. Conversely, more blasts and atypical lymphocytes in the groups with higher WBC counts were observed in our study. This different distribution of abnormal cells, as noted above, potentially influenced the pre-classification results in our study [8, 18, 19].

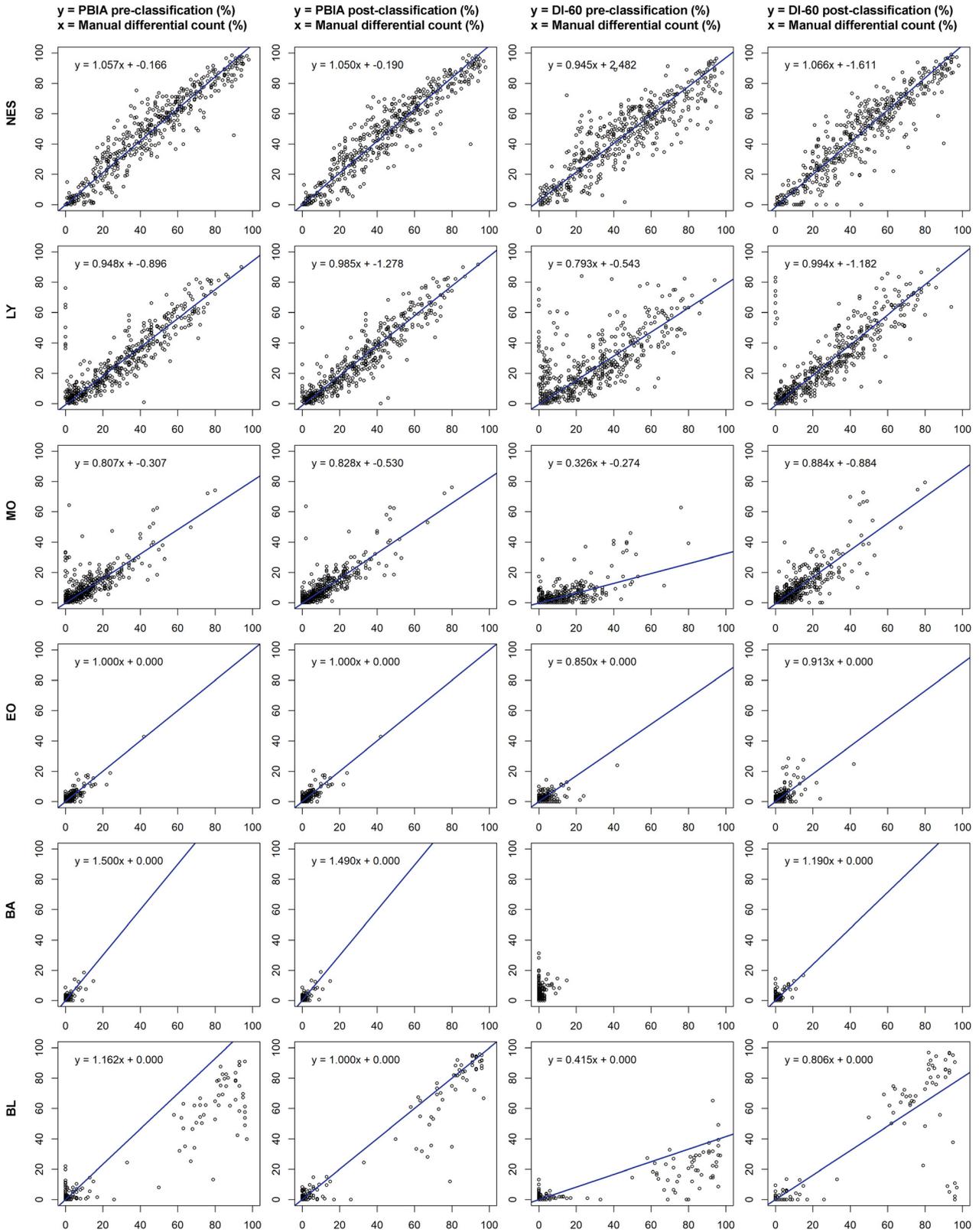


Figure 2: Passing-Bablok regression analysis comparing pre- and post-classification percent counts with manual differential counts. Cell classes are shown on the left side of the figure, and the corresponding variables for the x- and y-axes of each graph are displayed at the top. The Passing-Bablok regression line, the corresponding regression equation, and the correlation coefficient (r) are provided in each graph. The regression for the DI-60 pre-classification of basophils is not provided. BA, basophil; BL, blast; EO, eosinophil; LY, lymphocyte; MO, monocyte; NES, neutrophil segmented.

Table 4: Performance for identifying abnormal cells in post-classification when present in manual differential count.

Cell class	PBIA post-classification			DI-60 post-classification			Fisher's exact test, p-value		
	Accuracy % (95 % CI)	FPR % (95 % CI)	FNR % (95 % CI)	Accuracy % (95 % CI)	FPR % (95 % CI)	FNR % (95 % CI)	Accuracy	FPR	FNR
BL	75.5 (71.4–79.2)	30.5 (25.9–35.6)	6.1 (3.0–12.1)	87.9 (84.6–90.5)	2.3 (1.2–4.5)	42.1 (33.4–51.3)	<0.001	<0.001	<0.001
AT	83.5 (79.9–86.6)	14.3 (11.3–17.9)	50.0 (32.6–67.4)	75.1 (70.9–78.8)	22.9 (19.2–27.0)	57.1 (39.1–73.5)	0.002	0.002	0.789
NR	60.3 (55.8–64.7)	44.8 (39.9–49.8)	15.2 (8.9–24.7)	79.2 (75.2–82.6)	22.8 (18.9–27.2)	11.4 (6.1–20.3)	<0.001	<0.001	0.640

AT, atypical lymphocyte; BL, blast; CI, confidence interval; FNR, false negative rate; FPR, false positive rate; NR, nucleated red blood cell.

PBIA exhibits a strong WBC classification performance possibly because of deep learning algorithms, which are based on a vast WBC image database, and the ability of users to fine-tune the base model. This adjustment may also help compensate for variations in staining methods [1, 9, 20]. Although it improves the alignment between pre- and post-classification, it may introduce a risk of bias [1]. Therefore, after the base model is fine-tuned, a comparison study with manual counts is recommended. Multicenter studies should also be performed to further assess the overall effect on the performance of PBIA.

Morphological identification inherently involves an aspect of subjectivity and requires experienced technologists [1, 9, 18]. We believe that PBIA, with its high accuracy, can assist less experienced users in reducing missed diagnoses of abnormal cells, especially clinically significant blasts [9]. Additionally, PBIA is expected to support morphologists in achieving faster cell classification [9, 11], although this study does not specifically address time efficiency.

This study has several limitations. First, although PBS samples were randomly selected from our routine workload, we neither stratified healthy vs. patient groups nor met the recommendation of Clinical and Laboratory Standards Institute H20-A2 guideline [5]. This may have created a skewed distribution of certain pathologic cells, potentially affecting the overall performance metrics for each analyzer [8, 18, 19]. Nevertheless, we believe that this provides insight into how these instruments perform in everyday practice. Second, although we compared both instruments by using the same set of samples, their performance might still differ because of sample-specific factors, particularly for DI-60, which is influenced by slide quality and staining methods; therefore, performance variations may occur under different conditions [1, 17, 18]. Third, the difference in cell count analyzed may influence performance comparisons. Fourth, this study could not fully separate the effect of abnormal cells on performance across different WBC counts.

Fifth, analyzing the performance for low-count cell types, such as promyelocytes, was challenging. Sixth, comparison of plasma cells was limited. Lastly, we did not evaluate reproducibility or throughput of both instruments.

In conclusion, our study demonstrates that PBIA may offer a better pre-classification performance than DI-60 in this research setting; consequently, users may easily verify results in a clinical laboratory. Since PBIA can be fine-tuned, cell classes with significant differences from manual counts and any changes in overall performance should be evaluated prior to implementation. Multicenter studies should also be conducted to further explore and validate the WBC classification performance of PBIA.

Research ethics: The study was conducted in accordance with the Declaration of Helsinki, and the Institutional Review Board/Ethics Committee of Soonchunhyang University College of Medicine, Cheonan, Republic of Korea (approval no. 2024-02-009).

Informed consent: Not applicable.

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