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Original article

Evaluation of Automated Blood Microscopy System AI100 with Shonit^{TM} in a Tertiary Care Center in Northern India

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Abstract

Background: The microscopic examination of peripheral blood film is labour-intensive, subjective and time-consuming. It also requires trained technical staff. Technological advancements have been made to develop automated morphological analytical systems for the classification of both red blood cells and white blood cells. We aimed to investigate the ability of the automated microscopy system AI100 with the Shonit^{TM} to examine peripheral blood films. ***Methods:*** The study was a prospective study done at the All India Institute of Medical Sciences, New Delhi. We compared the automated morphological analysis system AI100 with Shonit^{TM} with the gold standard of manual microscopy to identify morphological abnormalities in the blood. ***Results:*** A total of 108 cases were studied. Twenty-one cases were excluded due to suboptimal staining and smearing. The male-to-female ratio was 7:5, and the median age was 31.1 Years. The Pearson correlation (r) between % of WBCs between AI100 and manual microscopy was 0.92,0.81, 0.34, 0.94 and 0.25 for neutrophils, lymphocytes, monocytes, eosinophils and basophils, respectively. The concordance of AI100 and manual microscopy for microcytes, macrocytes, tear drop cells, target cells, acanthocytes, and echinocytes was 77%, 86%, 100%, 100%,95% and 97%, respectively. The concordance of AI100 and manual microscopy for platelet count, clumps, and giant platelets was 89%,100% and 89%. ***Conclusions:*** The automated cell analysis system AI100 with Shonit^{TM} is capable of morphological classification of RBC, WBC and platelet in peripheral blood smears.

Keywords: ***Image-Based Analysis of Peripheral Blood Films; automated blood cell imaging; automation in hematology.***

Introduction

Peripheral blood film (PBF) reveals the structure and form of blood cells found in the peripheral blood. It is an essential yet highly informative haematological tool for screening, diagnosing, and monitoring disease progression and therapeutic response [1]. A thorough understanding of the interpretation of peripheral blood is vital for a successful clinical practice. This characteristic makes diagnosing various primary and secondary blood disorders essential. Initiation of a PBF is often a request by the attending clinician based on clinical suspicion or less frequently initiated by the laboratory based on the abnormal findings obtained by the automated haematology analyser [1;2]. The indication of a PBF can be both diagnostic and therapeutic [3;4]. The diagnostic significance of PBF has not been diminished by advancements in haematological automation and molecular techniques [1].

Examining PBF requires well-trained personnel and is prone to sizable statistical variation [1]. Over the past few decades, endeavours have been made to create automated systems for morphological analysis. In 1966, Prewitt and Mendelsohn introduced the concept of automated analysis of cell images [5]. Later; other writers demonstrated that digital image processing could be utilised for the automated identification of white blood cells (WBC) [5]. Despite the significant improvements in haematology analysers, significant progress has yet to be made regarding the automatic examination of peripheral blood cells. Irrespective of the analyser, approximately 15% of the blood samples require manual microscopic observation either because of biological rules or analyser flags [6].

Over the years, several digital analysis systems became commercially available for example, the Hematrak 590 (Geometric data) and the Micro 21 (Cellavision) systems. Ideally, these automated systems should be able to analyse a peripheral blood smear morphologically [5]. Results should be reproducible, faster and with limited analytical error. In addition, these systems should be capable of storing relevant morphological data and distributing images to other workstations for review purposes. However, the existing systems seem to be slow compared with manual differential. Owing to poor automation and cell pattern recognition algorithms, these systems were not truly walk-away systems and required frequent intervention by laboratory physicians. More recently, two new systems, the Cellavision Diffmaster Octavia and the Cellavision

DM96, were introduced in 2001 and 2004, respectively. Evaluations of these systems by Swolin et al. and Kratz et al. showed a good correlation with the manual differentiation of normal cells and blasts. The systems were able to pre-classify 89% and 82%, respectively, of all leukocytes correctly with good reproducibility [7,8]. In this study, we compared the automated morphological analysis system AI100 with Shonit™ and the gold standard manual microscopy to identify morphological abnormalities in the blood.

Objective

To assess the capabilities of the AI100 with the Shonit™ system using PBF and establish equivalence with the reference method (manual microscopy).

Material and Methodology

This study was a pilot study conducted in the Department of Laboratory Medicine (Hematology section) during one year on leftover patient samples. A total of 108 blood samples were examined, of which 21 were excluded from the study because of suboptimal staining and smearing. These blood samples were received in a tube containing K3 EDTA as an anticoagulant. One blood film was prepared and stained manually for each sample using Leishman and Giemsa stains. The slide was then scanned on the AI100 with the Shonit™ system (SigTuple Technologies Private Limited), and the same slide was analyzed via manual microscopy. This study included samples from healthy individuals and patients who gave blood samples for evaluation in the Department of Laboratory Medicine. The study endpoint was a comparison of the ability of AI100 with Shonit™ versus manual microscopy to identify morphological abnormalities in the blood. The technicians and the reviewers were trained on using the AI100 with the Shonit™ system and the Shonit Reporting Platform (Mandara).

Statistical analysis

This section discusses the statistical analysis used to establish the equivalence between the AI100 system result and manual microscopy of PBF slides by qualified reviewers. The system result here refers to the reports generated by the reviewers after reviewing the results of AI100 with the Shonit™ system. We used two metrics for the method comparison study: Pearson correlation with slope and bias and diagnostic parameters, such as sensitivity, specificity and overall agreement. The detailed methodology is discussed in the following subsections. The system-generated results consist of percentages of WBC subclasses, grades for RBC classes and platelets in binary classification such as 'Detected (D)' and 'Not Detected (ND)'.

Pearson correlation with slope and bias

Pearson correlation coefficients were calculated for WBC subclasses, such as neutrophil, lymphocyte, monocyte and eosinophil. As the Pearson correlation coefficient is a direct indicator of the correlation between the cell counter and output of the present system, it can thus be used to compare these two methodologies and establish their essential equivalence.

Calculation of the diagnostic parameters

Our study calculated diagnostic parameters such as sensitivity, specificity, and overall agreement/efficiency to understand the medical significance of the results. The following sub-section details the methodology followed.

WBC thresholds for abnormalities and calculations

A comparison of WBCs concerning different abnormalities has been performed. A 2 x 2 confusion matrix was evaluated for each disease condition by considering the "below" threshold percentage as negative and the "above" threshold percentage as positive (**Table Suppl.1**). The thresholds for calculating the confusion matrix differed for different abnormalities (**Table Suppl.2**).

RBC grades and concordance calculations

In the case of RBC, the 2 x 2 confusion matrix was determined for each class of RBC, considering the Nil and '+' buckets as negative/not detected while the '++' and '+++' buckets as positive/detected. Thus, the results of manual microscopy were compared with the system's output (**Table Suppl.3**).

As we obtained the confusion matrix in Table Suppl.3, we could evaluate the concordance using the equation of the sum of true negative and true positive total divided by the sum of true negative, true positive, false negative and false positive.

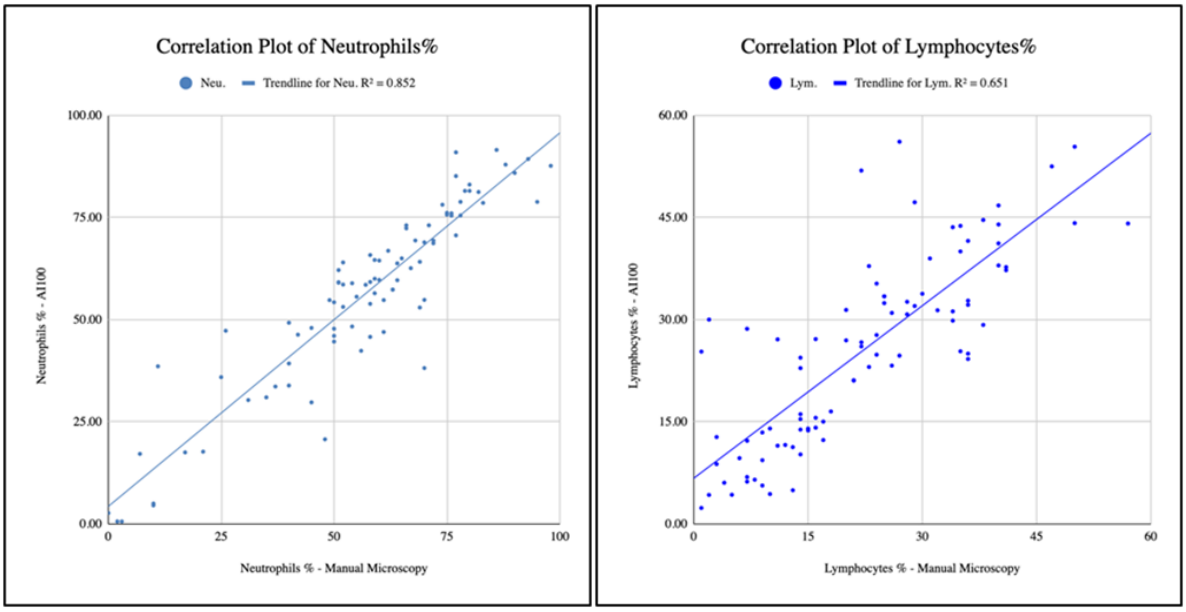
Calculations for platelets

As the data for giant platelets and platelet clumps (aggregates) were already in binary form, we could create a 2 x 2 confusion matrix (table Suppl.4), which further led to the calculation of concordance via the equation mentioned below.

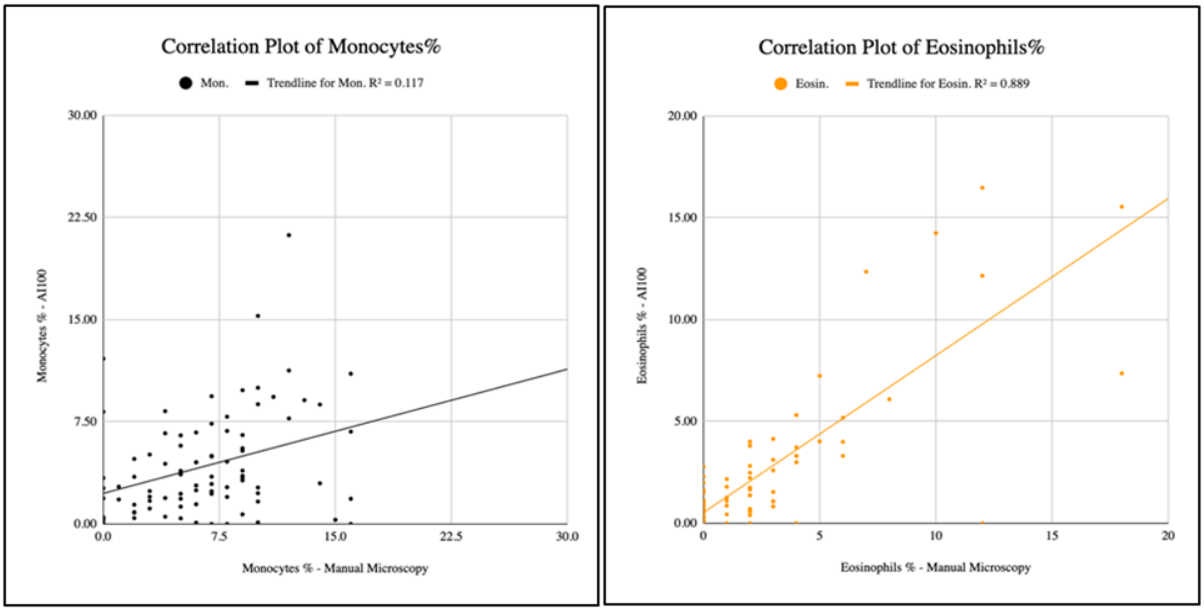
As the total platelet count data has three results, we could create a 3 x 4 confusion matrix (**as shown in Table Suppl.5**), which further led to the calculation of concordance via equation (**formulae Suppl.1**).

Results

A total of 87 samples were studied. After reviewing the report generated by AI100 with visual evidence, the laboratory physician provided the WBC differential counts and morphological findings. These results were compared with manual microscopy findings, and the comparison results are shown in Figure 1(a-d).



(a) (b)



(c) (d)

Figure 1 a-d: The Pearson correlation (r) between the percentage of WBCs between AI100 and manual microscopy was 0.92, 0.81, 0.34 and 0.94 for neutrophils, lymphocytes, monocytes and eosinophils, respectively.

Table 1: Sensitivity and Specificity of AI100 in detecting IGs and Blasts compared to Manual Microscopy

Parameter	IGs#	Blasts
True Positives	11	9
False Negatives	0	0
True Negatives	64	75
False Positives	12	3
Sensitivity	100%	100%
Specificity	84%	96%
Overall agreement	86%	97%

#IGs-immature granulocytes

Table 1 conveys that for the detection of IGs, AI100 is 86% in overall agreement with manual microscopy results at a sensitivity of 100% and a specificity of 84%. For the detection of atypical cells/blasts, the AI100 is 97% in overall agreement with manual microscopy results at a sensitivity of 100% and a specificity of 96%.

Table 2: Concordance of AI100 for RBC and Platelets in comparison to manual microscopy

Cell Category	Element	Concordance (%)
RBC Anisocytosis	Microcytic Blood Picture	77
RBC Anisocytosis	Macrocytic Blood Picture	86

RBC Poikilocytosis	Teardrop cells	100
RBC Poikilocytosis	Target cells	100
RBC Poikilocytosis	Acanthocytes	95
RBC Poikilocytosis	Echinocytes	97
nRBC	nRBCs	89
Platelets	Platelet count	89
Platelets	Platelet clump	100
Platelets	Giant platelets	89

Table 2 shows the concordance of AI100 with manual microscopy for anisocytosis, that is, microcytosis and macrocytosis, which were 77% and 86%, respectively. In contrast, for poikilocytosis, that is, tear drop cells, target cells, acanthocytes and echinocytes, it was 100%, 100%, 95% and 97%, respectively. The concordance of AI100 with manual microscopy for nRBC was 89%. For platelet count, clumps and giant forms, the concordance of AI100 with manual microscopy was 89%, 100% and 89%, respectively.

Discussion

Currently, quantifying and categorizing WBC in laboratories is fully automated. Regularly, a five-part leukocyte differential count is conducted, along with a study of the morphology of various cells. However, despite the availability of automated cell counters, microscopic analysis of blood smears remains a crucial component of laboratory procedures due to its ability to perform detailed morphological examination of blood cells. PBF examination is a labour-intensive process that necessitates the expertise of highly skilled laboratory physicians. Nevertheless, laboratory budgets face ongoing financial constraints, and hiring new experts has become progressively challenging in numerous nations. Investing in the automation of blood smear analysis could thereby enhance laboratory efficiency.

Machine learning-based AI has come of age in recent years [9,10]. Machine-learned models for image identification are now being applied towards detecting objects of interest in medical images [11], and these advances inspire Shonit TM. In this study, we assessed the capabilities of the AI100 with the Shonit™ system using PBF and correlation with the reference method, which is manual microscopy of the peripheral blood smears of the samples. This study aimed to evaluate the performance of Shonit™ on its ability to analyse PBF images and derive quantifiable metrics. Meintker et al. [12] presented an analysis comparing the performance of four different haematology analysers-Abbott Sapphire, Siemens Advia 120, Sysmex XE-2100, and Beckman Coulter DxH 800 regarding CBC, differential count, and efficacy of flagging in 202 samples from patients and normal controls. The analysers exhibited a good correlation for CBC parameters. Neutrophils and eosinophils also correlated well, whereas lymphocytes and monocytes correlated fairly. Flagging for blasts and IG showed moderate sensitivity and specificity. Laboratory physicians must not rely on blast flagging alone to detect leukemic samples with any analyser. Our study also showed that neutrophils, lymphocytes, and eosinophils had a good correlation, while monocytes correlated fairly.In detecting IGs, AI100 is 86% in overall agreement with manual microscopy results at a sensitivity of 100% and a specificity of 84%. In contrast, in detecting atypical cells/blasts, AI100 is 97% in overall agreement with manual microscopy results at a sensitivity of 100% and a specificity of 96%. The concordance of AI100 results with manual microscopy is good for poikilocytes (tear drop cells, target cells, acanthocytes and echinocytes) and platelet clumps, good for anisocytosis (microcytes and macrocytes), nRBCs and giant platelets.

Chari et al. [13] also evaluated a blood smear analysis system based on artificial intelligence, Shonit™ (SigTuple Technologies Private Limited). One hundred samples were taken from normal individuals. PBF slides were prepared using the autostainer integrated with an automated haematology analyser and stained using the May-Grunwald-Giemsa stain. These slides were analysed with Shonit™. Overall, the specificity in WBC classification was greater than 97.90%, and the precision was greater than 93.90% for the differential cell count. The correlation of the WBC differential count between the automated haematology analyser and Shonit™ was within the known inter-cell counter variability.

The automated examination of blood smears offers many advantages. Each WBC image analysed by the system is stored and, therefore, available for re-evaluation. In more complex cases, this vital feature helps the user to discuss the classification with colleagues or experts in the field at any time. The so-called “remote review software” installed on any PC or laptop also allows verification and authorisation of peripheral blood smear analysis results from another location. This option can be attractive for satellite or small laboratories, which depend on expertise from central locations for morphological assessment. In this way, telehematology comes within reach of routine haematological laboratories. In addition, review of these stored images may be used for quality and training purposes [6].

Will these soon completely replace manual differentiation? Peripheral RBCs and WBCs cannot be fully classified using a five-part differential analysis alone. On the other hand, these technologies have great potential for the future when paired with automated morphological assessment of each WBC utilising computerized image analysis software. The outcomes are already compelling, considering how recently these systems have been available.

Future improvement of the image analysis algorithms will undoubtedly increase the number of morphological categories that can be recognized automatically. Combining all information using a decision algorithm could lead to an automated diagnosis of haematological disorders. Other future applications of the automated microscope may include analysis of bone marrow spreads, lymph node preparations, urine sediments and cerebrospinal fluid cell morphology.

Conclusion

To conclude, there was a good correlation between the percentage of WBCs, RBCs, and platelets detected through AI100 and manual microscopy. Moreover, the sensitivity and specificity of AI100 in detecting IGs and blasts compared to manual microscopy were also good.

Limitation

The results may be verified with a more extensive study with a larger sample size.

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This research did not receive a specific grant from any funding agency, commercial or not-for-profit sector.

Conflicts of Interest

None

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2013.

Competing Interests

The author(s) declare none to disclose.

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