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An Automated Image Analysis System Can be Beneficial in Preclassification of Leucocytes in Children With Hematological Disease

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This study was aimed to evaluate the analytical performance of an automated image analysis system (a pilot model of Diff Master™ Octavia) for the preclassification of leucocytes in children with hematological disease. Manual microscopy performed by pediatric hematologists was used as the reference method. Five mature cell class and blasts were evaluated. Diff Master Octavia correctly preclassified 87.4% of all leucocytes with a high reproducibility. The overall accuracy was found to be 93.0%. Clinical sensitivity was 97.7% and specificity was 76.0%. The average time per slide for Diff Master™ Octavia was 2.3 min lower than that of manual method. Our results indicated that the Diff Master™ Octavia can detect and preclassify leucocytes accurately; therefore, it can be used as an efficient and fast method in pediatric hematology routine. J. Clin. Lab. Anal. 25:71–75, 2011.

Key words: preclassification of leucocytes; image processing; artificial neural networks; Diff Master™ Octavia

INTRODUCTION

Accurate leucocytes classification and detection of immature cells are essential for the diagnosis and follow-up treatment of hematological disease. Manual microscopy is accepted as the gold standard; as it depends on the quality of smear and skill of the observer, it lacks a standard accuracy. Automated cell counters provide five-part differential counting of leucocytes as well as total blood cell count, screening of erythrocyte morphology and reticulocyte count based on impedance, light scattering and flow-cytometric methods (1). Those systems are highly precise, reproducible and fast methods containing a flagging system for abnormal cells; however, they cannot accurately recognize immature and atypical cells (2,3). Recently digital imaging systems containing artificial neural network (ANN) software have been introduced to detect immature cells (4). The aim of this study was to evaluate the clinical utility of a digital imaging system, Diff Master™ Octavia (Cellavision AB, Lund, Sweeden) in children with hematological disease.

MATERIAL AND METHODS

Patients and Samples

The study was conducted at Clinical Pathology Laboratory of Hacettepe University Medical School, which is a tertiary care hospital serving a large outpatient population throughout all the country. Four separate laboratories in the hospital area provide 1,200–13,00 total blood cell count and differential counting, and 500 blood smears per day by eight automated cell counters.

Patients were random selected from routine workload of Pediatric Hematology Unit. Venous blood samples were collected into K2-EDTA tubes (Becton Dickinson, NJ) and complete blood count was performed by LH-750 (Beckman Coulter, Miami, FL) in Pediatric Biochemistry Laboratory.

Manual Microscopy

Blood smears were prepared manually from each sample by the wedge pull technique within 2 hr of sampling, and stained manually with Wright's dye according to the standard protocol of Pediatric

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Hematology Unit as follows: Wright pure time: 5 min, Wright dilute time: 5 min, rinse time: 0 min, drying time: 5 min. Blood smears were then evaluated by pediatric hematologists on the light microscope (Olympus, Stuttgart, Germany). Two hundred cells were counted for each blood smear. Samples were then reviewed and confirmed by four pediatric hematologists.

Diff Master™ Octavia

The automated image analysis system that we tested was a pilot model of Diff Master™ Octavia which was then improved by the company to be utilized by everyone. The system comprised a computer-controlled microscope (Olympus BX50WI, Hamburg, Germany, 100 × objective), a video camera (Sony DXC-9100P, Tokyo, Japan), a slide holder, and a motor, supporting by Cytologica 3.0 software program (Lund, Sweeden) which has an ability to store and transfer data. Briefly, cells were located at high magnification (100 ×), images of leucocytes were captured at high magnification (100 ×) and then evaluated by an ANN. At this point, each single cell image was separated into nucleus, cytosol and background based on several transforms. Subsequently, feature extractions of cells were analyzed from color, shape and texture to form a feature vector. According to these feature vectors cells were labeled by some techniques. These preclassified images were stored in a database. This system could preclassify leucocytes as segmented neutrophils, band neutrophils, basophils, eosinophils, monocytes, lymphocytes, promyelocytes, myelocytes, metamyelocytes and blast cells; however, in this study we evaluated only six cell class: segmented neutrophils, basophils, eosinophils, monocytes, lymphocytes and blasts.

Digital Examination

Blood smears were prepared by an automated slide maker of LH-750 within 2 hr of sampling and stained automatically by the same instrument by using Wright's dye. An optimal staining protocol was formed in order to prevent rejection of slides by Diff Master as follows: Wright pure time: 4 min, rinse time: 2 min, rinse time: 2 min, rinse time: 0 min and drying time: 2 min. Eight slides per run were then analyzed by Diff Master Octavia (Cellavision AB, Lund, Sweeden), based on 100 cells, and then examined and approved by an experienced biochemist.

Performance Study

To evaluate the analytical performance of Diff Master Octavia accuracy, sensitivity, specificity reproducibility and timing were determined according to the NCCL H20A guideline (5).

Statistical Analysis

Microsoft excel and software package SPSS for Windows (version 15, Chicago, IL) were used for all statistical studies. Correlations between Diff Master Octavia and reference method were performed by Pearson and Spearman correlation tests. Correlation coefficients were calculated by linear regression analysis.

RESULTS

As Diff Master Octavia requires uniform wedge and staining, all smears in this study were prepared and stained automatically by a slide maker of instrument except three samples that were wedged manually because of inadequate volume. In six samples, Diff Master could not find a monolayer, therefore they were excluded, and left 57 samples were evaluated.

Diff Master Octavia preclassified 88.06% of segmented neutrophils, basophils, eosinophils, monocytes, lymphocytes (mature cells) and 87.42% of overall (Table 1).

Accuracy was evaluated by comparing differential counts of Diff Master Octavia with the manual microscopy (Fig. 1). As segmented neutrophils and lymphocytes showed a normal distribution, the Pearson correlation test was performed, however, in the other cell categories, Spearman correlation test was performed and found that all correlations were significant ($P < 0.001$). By the regression analysis the correlation coefficients were found to be highest for basophils (0.98), eosinophils (0.92), blasts (0.91) and segmented neutrophils (0.88). The lowest correlation coefficient was observed for monocytes (0.79) (Table 2).

Table 3 shows clinical sensitivity, specificity, false positivity and false negativity values according to the reference ranges. The clinical sensitivity of Diff Master was found to be high, whereas the false-negative rate was found to be low.

It is notable that there was a discrepant case, in which Diff Master Octavia preclassified 45% of lymphocytes, 36.4% of segmented neutrophils, whereas reference method preclassifed 39% of lymphocytes and 54% of segmented neutrophils. However, differential leucocytes results obtained by automated cell counter were close to

TABLE 1. Percentages of Cells Preclassified by Diff Master™ Octavia

Cell class	%
Segmented neutrophils	95
Lymphocytes	94
Eosinophils	97
Basophils	79
Monocytes	72
Blasts	84

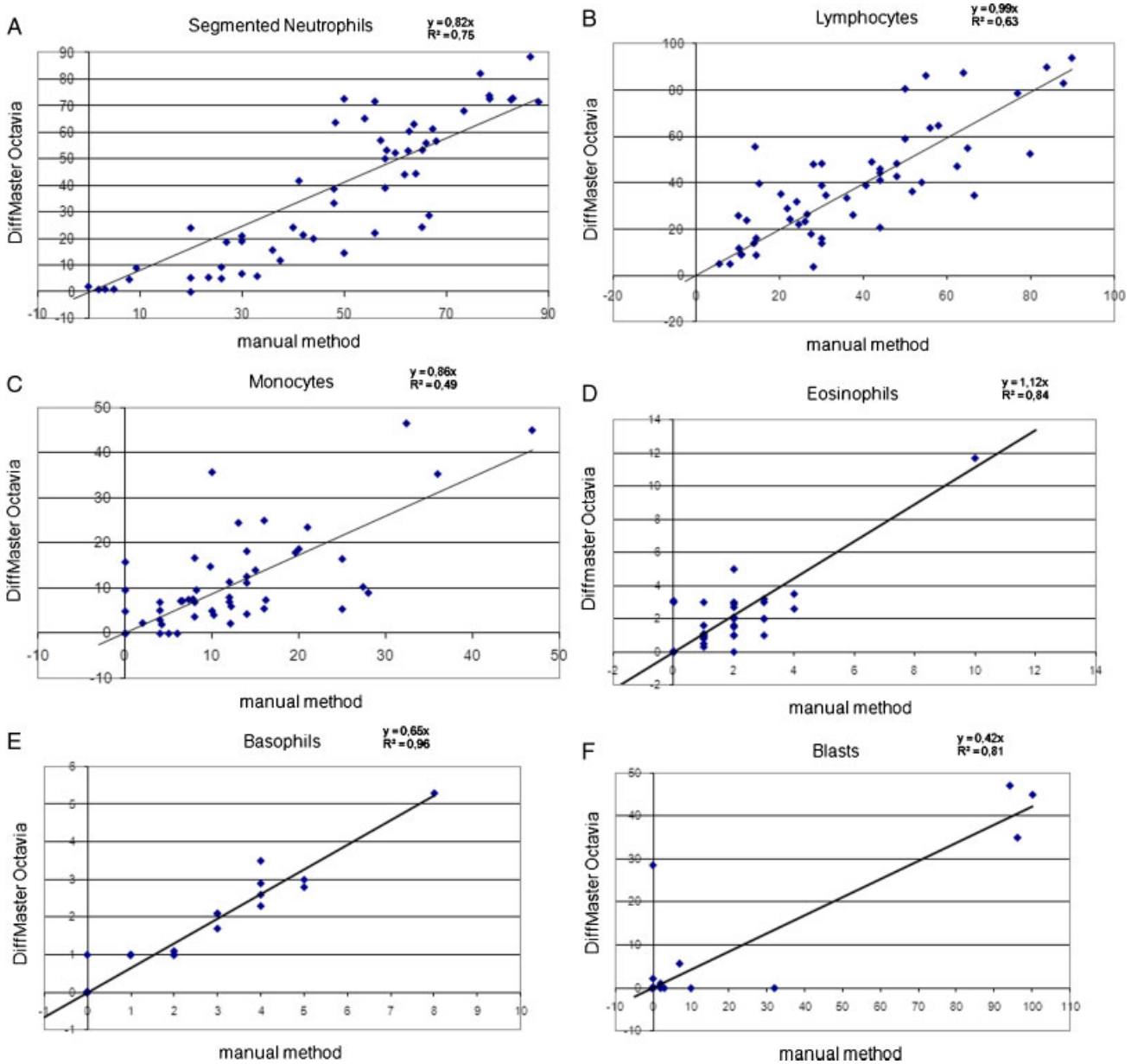

Fig. 1. Accuracy for segmented neutrophils (**A**), lymphocytes (**B**), monocytes (**C**), eosinopils (**D**), basophils (**E**), and blasts (**F**) based on comparison with manual microscopy.

TABLE 2. Accuracy of Diff Master™ Octavia on Six Cell Categories

Cell class	*r*
Segmented neutrophils	0.88
Lymphocytes	0.87
Eosinophils	0.91
Basophils	0.98
Monocytes	0.79
Blasts	0.92

TABLE 3. Clinical Performance of Diff Master™ Octavia

	%
Sensitivity	98
Specificity	76
FPR	25
FNR	2.2

FPR, false-positive rate; FNR, false-negative rate.

TABLE 4. Within Run Imprecision of Diff Master™ Octavia

Cell class	Mean (%)	SD (%)
Segmented neutrophils	50	2.6
Eosinophils	2.8	0.2
Basophils	1.1	0.1
Lymphocytes	32	3.3
Monocytes	11	1.5
Blasts	19	3.4

that of test method (47% lymphocytes, 43% segmented neutrophils). Furthermore, two false-positive results were observed, in which test method revealed myelocytes, metamyelocytes and blasts; however, the results were normal limits of the reference method. There was one false-negative result, in which the test method revealed no segmented neutrophils; however, reference method detected 30% segmented neutrophils.

For reproducibility assay, three blood smears from ten different samples were prepared and examined three times based on counting 100 leucocytes. The reproducibility was found to be high for both mature and blast cells (Table 4).

In addition, the analysis time, starting from loading of sample to Diff Master Octavia or a microscope to reporting of the result, was compared. The average analysis time for eight slides by Diff Master Octavia was found to be 35.8 min (95% CI 28.6–42.8) (approximately 4.5 min per slide), whereas it was approximately 6.8 min per slide for manual method.

DISCUSSION

Pattern recognition and image processing systems have been improved for the last decade owing to the increased requirement in the accurate detection of blood cells. New automated image analysis systems contain ANNs associated with advanced computers, cameras and software. The most important advantage of these systems is accurate and precise detection of blasts and other immature cells. Furthermore, they provide high sample capacity and long walk-away time (6). Diff Master Octavia is an automated imaging system which automatically locates and presents images of blood cells on the stained peripheral blood smears according to the NCCLS scanning procedure, and then analyzes them using ANN's for discrimination of leucocytes. In this study, we evaluated Diff Master Octavia automated system for discrimination of leucocytes in 57 children with hematological disease.

Diff Master Octavia preclassified 87.4% of all cells correctly. Preclassification of leucocytes was more accurate for segmented neutrophils (94.7%), lymphocytes (93.8%) and eosinophils (96.4%). However, there were less accurate results for monocytes (71.9%). Our results were in consistent with the performance criteria for the manual differential leucocytes counting determined by a College of American Pathologist (CAP) survey study (7). Therefore, the improvements to the neural network are required for monocytes and basophils.

A significant concordance was found between Diff Master Octavia and manual microscopy: the correlation ranged between 0.79 and 0.98 for segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils and blasts. The best correlation was observed for basophils, blasts, lymphocytes and segmented neutrophils; however, the correlation was low for monocytes. Previous studies support our results in which the highest correlations between Diff Master Octavia and manual method were observed for lymphocytes and segmented neutrophils, whereas the lowest correlations were observed for monocytes (6,8).

Our results indicated that Diff Master Octavia was a high sensitive (98%) and accurate (93%) method in discrimination of blood cells. Similar results were reported by Swolin et al. who found that the sensitivity and accuracy of Diff Master were 97 and 91.3% (6). The false negativity of the system we tested was very low; however, the false positivity was elevated, but this did not have a significant impact on the clinical outcome of the patients.

Our data also showed that Diff Master Octavia was a high reproducible method for preclassification of leucocytes based on six cell class which was consistent with early studies (6,8).

Duration of preclassification by Diff Master Octavia was found to be shorter (approximately 2.3 min) than that of manual microscopy which may be caused by reexamination of slides by four more hematologists.

Diff Master Octavia provides easy and rapid reclassification of cells by one or more other operators due to its high image quality feature and handy operator interface, thereby offers a high quality differential count.

Evidence of this study demonstrated that Diff Master Octavia was an accurate method to detect and classify blood cells so can be beneficial in clinical practice of hematology units. In large hospital settings, the most important advantage of this method is to share patient information between departments promptly and to facilitate diagnosis and treatment.

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