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ORIGINAL ARTICLE

Comparative assessment of nucleated red blood cells using a fully automated hematology analyzer versus slide examination.

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ABSTRACT… Objective: To determine the sensitivity and specificity of fully automated hematology analyzer for nucleated red blood cells (NRBCs) keeping microscopic slide examination as gold standard. **Study Design:** Cross-sectional study. **Setting:** Department of Hematology, RMI Hospital, Peshawar. **Period:** 1st December 2023 to 29th Feb 2024. **Methods:** A total of 160 blood samples referred for assessment to the hematological department for the suspected abnormal NRBCs were included in the study through consecutive sampling. Fresh Blood samples were analyzed using fully automated hematology analyzer. Manual counting of the NRBCs of the same samples was done using manual blood smear slide examination. Findings of the automated nucleated red blood cell counting methods were statistically analyzed keeping microscopic slide examination as gold standard. Sensitivity, specificity, accuracy and negative and positive predictive values were calculated for diagnosis of NRBC (count $>0.02 \times 10^9$/L). Clinical sensitivity of the test was determined by Receiver Operating Test (ROC). **Results:** The Mean±SD of patient's age in this study was 46.78±14.89 years with an age range of 0.5 to 67 years. The male gender was 53.75% while female gender was 46.25%. Fully automated hematology analyzer detected 30 (18.75%) samples while slide examination detected 31 (19.37%) samples with NRBC count $>0.02 \times 10^9$/L. Fully automated hematology analyzer has shown sensitivity of 93.55 %, specificity 99.23% and diagnostic accuracy by 98.13%. PPV was 96.67% and NPV was 98.46 ($p < 0.000$) for the diagnosis of NRBC. The cutoff value of 0.0175 × 109/L (20/μL) offered the best balance between sensitivity and specificity based on ROC curve. **Conclusion:** Association is present for the NRBC count among the fully automated hematology analyzer and microscopic slide examination.

Key words: Automated Hematology Analyzer, Microscopic Slide Examination, Nucleated Red Blood Cells.

INTRODUCTION

Routine peripheral blood tests, patient's clinical history and symptoms play an important role in diagnosing and treating many conditions, particularly those related to the circulatory system.[1] For instance, hemoglobin levels and platelet counts can tell if a patient needs a blood transfusion, white blood cell counts can tell how infected a patient is, and abnormal cells may be associated with circulatory diseases and tumors.[2,3,4]

Nucleated Red Blood Cells (NRBCs) are found in bone marrow specifically in healthy individuals including newborns after the first week of birth and in non- pregnant women.[5] NRBCs which escape from the bone marrow are immediately washed out from the peripheral circulation by spleen through its sinusoidal and reticuloendothelial functions. NRBCs are closely linked to a variety of serious pathological medical conditions such as asplenia/hyposplenia or ineffective erythropoiesis (severe anemia, megaloblastosis, thalassemia and myelodysplastic syndromes). Primary hematopoietic dysfunctions may also appear like acute or chronic leukemia and myelofibrosis. In thalassemia and some other hematological disorders, the amount of NRBC in peripheral blood has a significant impact on prognosis and treatment decisions.[6] Consequently, techniques that can accurately and effectively assess peripheral blood are of considerable clinical importance in the diagnosis and management of such diseases.[7]

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A recent systemic review to find the diagnostic value and prognostic significance of NRBCs mentioned that in critically sick patients, such as those suffering from sepsis, trauma, ARDS, acute pancreatitis, or severe cardiovascular disease, this diagnosis can be utilized to forecast changes in their clinical state and mortality.[8]

Manual microscopic slide examination has been the established method for visualizing and quantifying abnormalities in blood cells for decades. When performed by a trained hematopathologist, using a 400X light microscope after Romanowski staining on blood smears, it allows for accurate morphological analysis of blood smears, including identification and enumeration of NRBCs. Microscopic review remains the most specific way to differentiate NRBCs from other cell types and therefore traditionally taken as the gold standard. This is a simple method, however, is tedious and requires more experienced human resources making it less practical in clinical settings.[9,10]

Recent fully automated hematology analyzer (FAHA) is equipped with technologies that detect and enumerate NRBCs. These assays measure NRBC and other parameters in the same manner as a standard CBC, without the need for a distinct reagent or programmatic command. Automated analyzers may be more appropriate for clinical applications due to their ability to combine resistance and flow cytometry assays to guarantee the accuracy and reproducibility of results. It can also detect the samples with unusual classifications, numbers and cells.[1,11,12]

Recent advances in instrument technology have significantly enhanced the capabilities of blood cell analyzers, while also introducing a more intricate structure and principles. Studies have been done to guarantee that the performance of an automated blood cell analyzer is in accordance with clinical needs. These analyzers have worked on providing guidance and establishing standards to ensure the assessment of instrument characteristics and evaluation of performance. All these characteristics enable automated NRBC counting to be a cost-effective for use in laboratories. Some studies have mentioned that performance of FAHA is more accurate and in line with the reference slide examination method when the NRBC numbers are <200%.[5,9,12,13]

In our clinical set ups, the manual procedure of slide examination is widely used and believed to be accurate, however, automated hematological analyzers have also increased their share during last few years. This study was therefore planned to evaluate the performance of FAHA keeping microscopic slide examination as gold standard for its specificity, sensitivity and thereby accuracy in assessment of NRBCs even at high counts of $>0.02 \times 10^9$/L. The result of this study will help the hematologists to evaluate the accuracy of FAHA and therefore deciding better option while assessing NRBCs in their patients.

METHODS

This cross-sectional study was conducted at the department of hematology, RMI Hospital, Peshawar from 1st of December 2023 to 29th of Feb. 2024 over a period of 3 months.

The sample size was calculated using sensitivity and specificity calculator.

With Margin of error for both sensitivity and specificity =10%

Prevalence (Samples received to the Hematology department for the suspected raised NRBCs) = 24%.[14]

Sensitivity= 89%, Specificity= 67.77 %.[15]
Sample size =159.

A total of 160 blood samples referred for assessment to the hematological department for the suspected abnormal NRBCs were included in the study through consecutive sampling.

Samples with clear evidence of hemolysis, coagulation, or clots were excluded.

Evaluation of blood samples was completed within 8 hours and samples storage was ensured

between 18–26 ℃. Fresh Blood samples were analyzed using FAHA (Sysmex XN-3000) for NRBCs.

Manual counting of the NRBCs of the same samples was done using manual blood smear slide (Leishman-stained) examination. Patient's data and the results of the findings of both the methods was recorded on arranged format.

Approval of conducting the study was taken from the ethical committee of the hospital (RMI/RMI-REC/Approval/194, Dated November 23, 2023).

Findings of the automated nucleated red blood cell counting method were statistically analyzed keeping microscopic slide examination as gold standard. Sensitivity, specificity, accuracy and negative and positive predictive values were calculated for diagnosis of NRBC (count $>0.02 \times 10^9$/L). Clinical sensitivity of the test was determined by Receiver Operating Test (ROC).

RESULTS

The Mean±SD of patient's age in this study was 46.78±14.89 years with an age range of 0.5 to 67 years. The demographics and the concerning departments referring the patient's blood samples are shown in Table-I.

Demographics		
Age (Mean±SD) years		46.78±14.89
Gender	Male n (%)	86 (53.75)
	Female n (%)	74 (46.25)
Concerned hospital departments		
Oncology Dept. n (%)		32 (20)
Internal Medicine Dept. n (%)		32 (20)
Intensive care Dept. n (%)		24 (15)
Emergency Dept. n (%)		19 (11.88)
Out-patient Dept. n (%)		17 (10.63)
Pulmonary Dept. n (%)		15 (9.34)
Pediatric Dept. n (%)		10 (6.23)
Surgical Dept. n (%)		6 (3.8)
Gyn. Dept. n (%)		5 (3.13)

Table-I. Demographics of patients and concerning hospital departments. n =160

FAHA detected 30 (18.75%) samples while slide examination detected 31 (19.37%) samples with NRBC count of $>0.02 \times 10^9$/L as shown in Table-II.

NRBC (count $>0.02 \times 10^9$/L)	FAHA	Slide Examination
Positive	30 (18.75%)	31 (19.37%)
Negative	130 (81.25%)	129 (80.62%)
Total	160 (100%)	160 (100%)

Table-II. Results of FAHA and slide examination in detection of NRBC n=160

FAHA has shown sensitivity of 93.55%, specificity 99.23% and diagnostic accuracy by 98.13%. PPV was 96.67% and NPV was 98.46% in detecting NRBC count of$>0.02 \times 10^9$/L ($p < 0.000$) as shown in Table-III.

FAHA	Results
Sensitivity	93.55%
Specificity	99.23%
Diagnostic accuracy	98.13%
Positive predictive value	96.67%
Negative predictive value	98.46

Table-III. FAHA sensitivity, specificity and predictive values n=160

Comparison of FAHA versus slide examination for detecting NRBCs count $>0.02 \times 10^9$/L is given in Table-IV.

FAHA	Slide Examination		Total
	Positive	Negative	
Positive	29 (TP)	1 (FP)	30
Negative	2 (FN)	128 (TN)	130
Total	31	129	160

Table-IV. Comparison of FAHA versus slide examination for detecting NRBC n=160

TP=True positive, **FP**=False positive, **FN**=False negative, **TN**=True negative

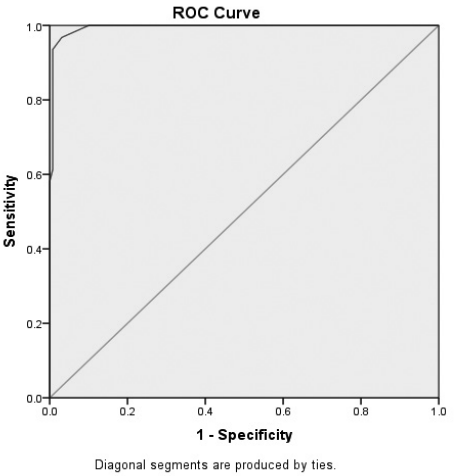

Table-V. Receiver operating curve

The cutoff value of 0.0175 × 109/L (20/μL) offered the best balance between sensitivity and specificity based on ROC curve.

DISCUSSION

Identifying and correctly enumerating peripheral blood NRBCs are important for diagnosing serious diseases. Hence the efforts of developing convenient methods with reliable accuracy are important.

Valina N conducted a study to evaluate the enumeration of NRBCs on Sysmex XN analyzer in comparison to traditional microscopic count. The results of the study reported a good correlation between the automated count and the manual microscopic count as the researchers reported an accurate count and thereby effective performance of automated analyzers.[16]

Wang N evaluated the interpretation of Mindray automatic blood cell analyzer for the NRBC count. The result of 490 blood samples showed high sensitivity and specificity levels for identification of NRBCs and results reached up to satisfaction levels with excellent linearity and reproducibility when compared to blood analyzer.[17]

In a cross-sectional study conducted in Pakistan, Ahmad T et al evaluated the performance of FAHA in counting of NRBC keeping microscopic evaluation as gold standard. Based on results of this data, the NRBC count from FAHA showed a strong correlation with the manual method NRBC count (r2=0.98). The research thereby mentioned that FAHA is quick, precise, and economical for NRBC count.[18]

Tsuchiya K examined the viability of using the FAHA to measure the density of bone marrow cells and total NRBC count in bone marrow samples. The study reported a sensitivity and specificity values of 100% and 88% for hypoplasia while 89% and 86% for hyperplasia. A linear correlation was found for total NRBC count ($R2 = 0.84$, $p < .001$) which supported the conclusion that FAHA provide a good and reliable qualitative assessment.[19]

The Mean±SD of patient's age in this study was 46.78±14.89 years with an age range of 0.5 to 67 years. The male gender was 53.75% while female gender was 46.25% of total study population. Most of the patients samples were referred by oncology department 32 (20%) followed by internal medicine department 32 (20%), intensive care department 24 (15%) and emergency department 19 (11.88%).

The results of our study show that FAHA detected 30 (18.75%) samples while slide examination detected 31 (19.37%) samples with NRBC count $>0.02 \times 10^9$/L. FAHA has shown sensitivity of 93.55 %, specificity 99.23% and diagnostic accuracy by 98.13%. PPV was 96.67% and NPV was 98.46% in detecting NRBC ($p < 0.000$). The cutoff value of 0.0175 × 109/L (20/μL) offered the best balance between sensitivity and specificity based on ROC curve.

These results are in line with results shared by other researchers who have worked on automated analyzers and confirms the utility of FAHA in NRBC count.[16,17,18,19]

The major limitations of our study include the small size. Future study with larger sample sizes will be helpful in providing more evidence for using FAHA in the diagnosis of NRBCs.

CONCLUSION

Association is present for the NRBC count among the FAHA and microscopic slide examination. Being quick and precise, these automated analyzers can be used to reduce the work burden in bigger hematology laboratories.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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3	Maryam Zahra	Manuscript writing, Collection of references, Final checking of manuscript.	
4	Ayesha Ishtiaq	Manuscript writing, Collection of references, Final checking of manuscript.	
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