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Setting Threshold Value for Peripheral Blood Morphology Slide Review of Leucocytes and Lymphocytes at Tikur Anbessa Specialized Hospital in Addis Ababa, Ethiopia

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Abstract

Background: Evaluation of peripheral blood morphology is an important screening tool for many diseases. When abnormalities are detected by the automated hematology analyzer, manual microscopic review of the peripheral smear is necessary to determine the next course of action. The standard practice based on consensus guideline is to review cases with absolute lymphocyte count >5×10^9/L for adults, for children >7×10^9/L and for WBC <4x10^9/L and >30x10^9/L. The aim of this study was to set threshold for blood smear review of peripheral blood leucocytes and lymphocytosis in patients attending at Tikur Anbessa Specialized Hospital.

Method: A hospital based cross sectional study with convenient sampling method was conducted at Tikur Anbessa Specialized Hospital from March to May, 2015 with sample size of 170. No additional samples were collected for our study; we use the left over sample of the patients' blood. Samples were analyzed on two hematology analyzers and by senior laboratory technologists. Outlier samples were run repeatedly to prevent and control bias. Data was entered twice to prevent errors and data analysis was performed using SPSS version 20 with ROC curve.

Result: The threshold value of lymphocytes was found to be >6× 10^9/L for children and >4.5× 10^9/L for adults. For leucocytes, threshold was <2.5×10^9/L and >27.4×10^9/L for children and adults, respectively. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), efficiency and microscopic review rate of Sysmex XT2000i analyzer was 81%, 67%, 79%, 71%, 76%, and 62%, respectively.

Conclusion: There is difference between the peripheral smear review consensus guideline and the peripheral smear review results from patients attending at Tikur Anbessa Specialized Hospital. Each laboratory should verify the criteria for smear review, based on the International Consensus Group for Hematology Review, and optimize them to maximize efficiency. Modifying the standard for leucocytes and lymphocytes smear review threshold through retrospective analysis of institutional data can reduce laboratory workload without compromising quality.

Keywords: Threshold Value; Lymphocytosis; ROC Curve

Introduction

The peripheral blood smear is a laboratory work up that involves examining of peripheral blood cells smeared on a slide. Peripheral blood smear is clinically significance in the investigation and management of anemia, infections and other conditions which produce changes in the appearance of blood cells. The counting and analysis of blood cells allows the evaluation and diagnosis of a vast number of diseases. In particular, the analysis of white blood cells is a topic of great interest to hematologists [1,2].

Leucocytes are heterogeneous group of nucleated cells that are responsible for the body's defenses. The normal WBC count is 4,000 to 10,000/mL. The two groups of leucocytes are; granulocytes include neutrophil, eosinophil and basophils, agranulocytes are lymphocytes monocytes. Lymphocytes are the second most common type of leukocytes in adults constituting 20-45% of the white cells. The average number of lymphocytes in the peripheral blood is 2500/ml. The lymphocyte number is higher in children and also increases with viral infections [3].

The number of leucocytes and lymphocytes increases for different clinical diseases. Lymphocytosis is an increase in lymphocytes and can be found in infections in children [4,5]. It is useful to provide diagnostic information, guiding the selection and monitoring of therapy, indicating adverse effects of treatment. The morphological analysis of blood cells is performed manually by skilled laboratory personnel. However, the speed and accuracy of automated hematology analyzers have revolutionized workflows in the clinical hematology laboratory [6].

In the setting of lymphocytosis, the critical judgment is whether the lymphocytosis represents a benign reactive condition or a neoplastic lympho proliferative disorder that requires additional flow cytometric evaluation [7]. Setting smear review criteria is important for the case of leukemia [8]. For the purposes of proficiency testing, a single abnormal cell cannot be distinguished from a normal by morphology alone [9].

There are currently very few guidelines regarding how the clinical laboratory should deal with leucocytes and lymphocytes. The International Consensus Group for Hematology Review recommends smear reviews for all first time lymphocytosis cases where absolute lymphocyte count is $>5\times10^9$/L in adults ,for children $>7\times10^9$/L and for WBC $<4x10^9$/L and $>30x10^9$/L [10].

For maximal information to be derived from a blood smear, the examination should be performed by an experienced and skilled person. In Europe, only laboratory-trained staff members generally read a blood smear, whereas in the United States, physicians have often done this [11]. In comparison with the procedure for an automated blood count, the examination of a blood smear is a labor-intensive and, therefore, relatively expensive investigation and must be used judiciously [12].

Still now there is no guideline or document in Ethiopia for morphology review of lymphocytes despite the development of automated hematology analyzers for reliable blood count, examining smear under microscope is still indispensable for confounding results when the data the analyzer obtains are qualitatively or quantitatively abnormal. Evaluation of blood smear is also an important screening tool for differentiating between reactive and malignant processes. The significance of smear morphology has not been well studied and there are no consensus guidelines or follow-up recommendations available. The aim of the current study is therefore to set and recommend the threshold value for performing slide review of WBC and lymphocyte.

Materials and Methods

Study Setting and Context

A hospital based cross sectional study was conducted from March to May, 2015 at Tikur Anbessa Specialized Hospital. Tikur Anbessa Specialized Hospital (TASH) is located in the nation's capital Addis Ababa. The hospital is open 24 hours for emergency services. TASH offers diagnosis and treatment for approximately 370,000- 400,000 patients a year. The hospital has 800 beds, with 130 specialists, 50 non-teaching doctors. The emergency department sees greater than 80,000 patients a year. TASH was selected because it is the referral hospital of the country and the felt that the responsibility of the hematology- Immunohematology unit of the Department of Medical Laboratory Sciences to contribute to the quality of laboratory service offered in the hospital, which is also a practical attachment site for students of the department.

Sampling Technique

According to CLSI guideline a minimum of 60 samples are required but we tried to include the samples that were available in the mentioned study period and finally 170 specimens were included conveniently.

Source Population

All specimens that came to Tikur Anbessa Specialized Hospital laboratory from March to May, 2015 were the source population for this study.

Study Population

All blood specimens which came to Tikur Anbessa Specialized Hospital hematology laboratory for hematology investigation were our study population and the blood samples were collected with EDTA. Blood samples that were in acceptable criteria by the laboratory Standard Operating Procedure were included in the study.

Data Collection

The study population is the specimen that come to Tikur Anbessa Specialized Hospital laboratory using Standard operating procedure and transported to the laboratory as early as possible. Patients are informed orally and no additional samples were collected for our study; we use the left over sample of patients.

The collected specimens were analyzed within two hours on Hematology analyzers i.e., Sysmex XT-2000i and Cell Dyn 1800 by senior laboratory technologists that have experience of greater than 20 years in hematology department. Manual differential count was performed on each specimen. Outlier samples were run repeatedly to prevent and control bias.

Quality Assurance

A standard quality control protocol was followed daily. Three level quality control materials i.e. high, normal and low quality control materials run before every work for each analyzer. Extracted data was checked for completeness, consistency, and accuracy and coded by the principal investigator. To obtain reliable analysis we follow the National Committee for Clinical Laboratory Standards for WBC differential count, H20-A2 recommendations [13].

Data Analysis

A checklist that includes information about blood cell count was used to extract hematology data from instrument print out. Smear was reviewed for flagged results that the analyzer flagged for additional testing i.e. reflects a lack of reliability of the WBC count that necessitates the interpretation and validation of the results. Sensitivity and specificity of the existing smear review criteria against the morphological findings of the slide was determined. Finally age specific lymphocyte count thresholds were determined all these are done prospectively. Data entry was done using EPI-Info version 24. Any error identified was corrected. The data analysis was performed by using SPSS version 20. ROC curve and P values less than 0.05 was taken as statistically significant.

Ethical Consideration

Ethical clearance was obtained from the Departmental Research and Review Committee of department of Medical Laboratory Sciences, College of Health Science, and Addis Ababa University before undertaking the research work. Permission letter was written to the hospital management. Leftover blood sample collected for routine examination was utilized Samples were coded and confidentiality of patient data was maintained throughout the study.

Results

From a total of 170 samples used in the study, 79 (46.5%) were collected from female patients the age ranges of patients for adults ranges from 13-80 (median age was 37 years) and from 3-12 (median age was 7) for children. We use 110 samples for adults and 60 samples for children. The smallest WBC count was 1.2×10^9/L and the highest value was 56.3×10^9/L. some of the morphologies observed are changes in blood smear evaluation of leucocytes as hyper segmented neutrophils, granulations toxic, hypo segmented neutrophils, cytoplasmic vacuoles, band neutrophils, and blasts.

Threshold Value for Lymphocytosis of Children

The threshold value of lymphocytes for children was $>6\times10^9$/L. It has Area under the Curve AUC value of 0.728 and 95% CI [0.574, 0.88] (Figure 1).

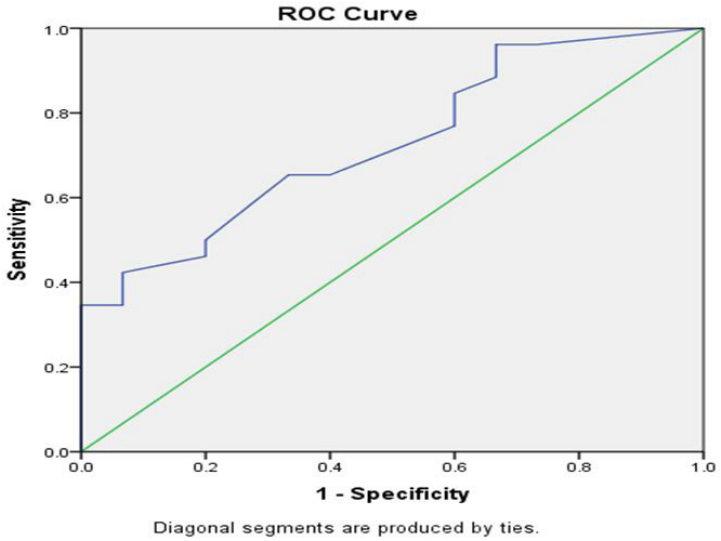

Figure 1: ROC curve for lymphocytes of children

Threshold Value of Adult Lymphocytosis

The threshold value of adult lymphocytosis was $>4.5\times 10^9$/L and it has Area under the Curve AUC value of 0.826 and 95% CI [0.574, 0.88] (Figure 2).

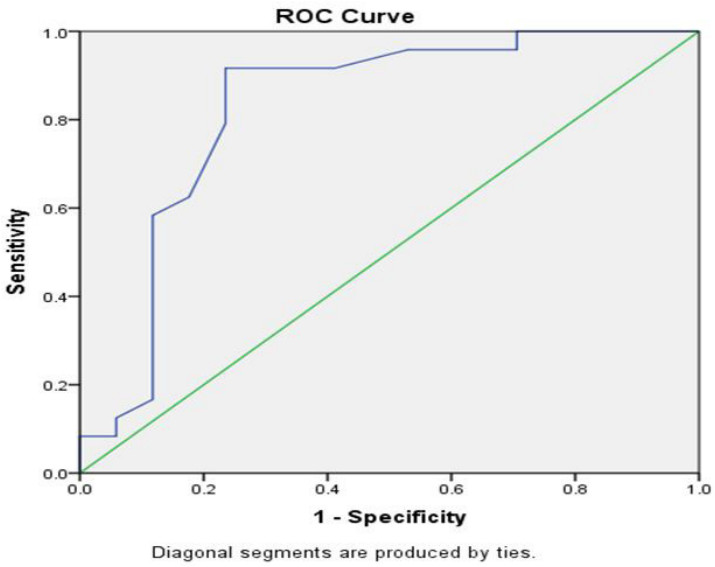

Figure 2: ROC curve of adult lymphocytosis

Threshold Value for Leucocytes

For WBC value <4000 the threshold value for smear review was <2500.With AUC of 0.82 and 95% CI was between [0.67, 0.95] (Figure 3).

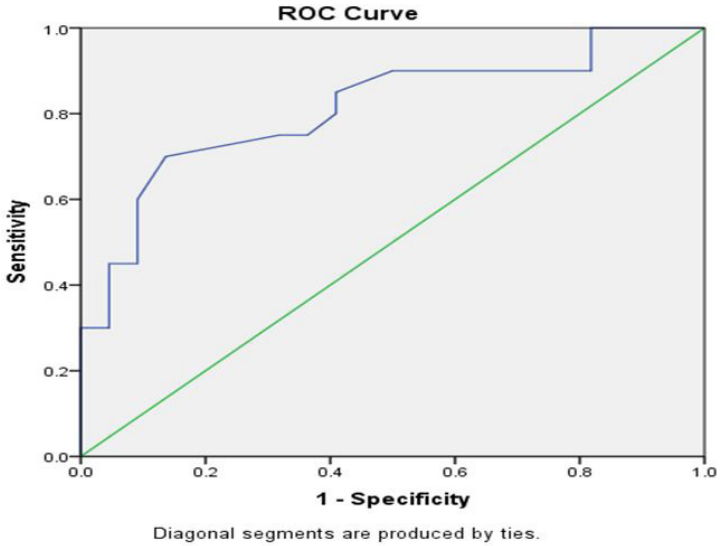

Figure 3: ROC curve for leucocytes leucopenia

We found the threshold value for WBC count smear review to be >27,400. Its AUC is 0.727 with 95% CI was between [0.56, 0.83] (Figure 4).

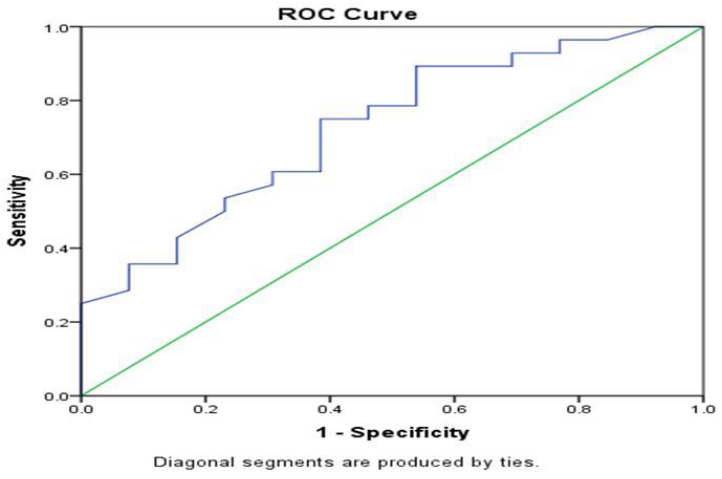

Figure 4: ROC value for Leukocytosis

Flagging Efficiency of Sysmex XT2000i

Sysmex analyzer showed flags for 106 (62%) of the specimens. But peripheral blood smear showed flags for only 22 specimens. Out of the total samples 18 don't show flag while they were actually smear positive. The sensitivity, specificity, PPV, NPV, efficiency and microscopic review rate of SysmexXT200i were 81%, 67%, 79%, 71%, 76% and 62%, respectively. Flagging efficiency was done only for Sysmex XT-2000i.

Discussion

This study aims to set threshold value for peripheral smear review of leucocytes and lymphocytes generated by two automated hematology analyzers Sysmex XT-2000i and Cell Dyn 1800, which are currently in use interchangeably by some health facilitates in Ethiopia. Here, the threshold values for smear review of WBC and lymphocytes are discussed.

In this study the threshold value for peripheral smear review of lymphocytes for adult was found to be $>4.5\times10^9$/L which is lower than the threshold of the consensus group. It shows that this is helpful to have smear review of lymphocytes that would have been ignored when using the consensus group criteria. Similar Tseng *et al.* in USA in 2014 found the absolute lymphocyte count to be between 5×10^9/L and 10×10^9/L AUC value of 0.886 [14].

The current study showed threshold value of $>6\times10^9$/L for children which is lower than the threshold of the consensus group. A study was done by Froom *et al.* in Israel and they explain that adjusting the smear threshold level is important to minimize the smear review rate. They found that setting the threshold level decreases the smear review rate from 39.7% to 5.6% [15] which leads to the proper use of time and man power.

The threshold value for smear review of lymphocyte for adult was $>4.5\times10^9$/L. This result was difference from the study done by Andrew *et al.* which has a value of $>4\times10^9$/L for patients above 67 years and for age between 50-67 a value of $>6.7\times10^9$/L [16]. Another study done by Sun P *et al.* the showed optimal cutoff value to be $>7\times10^9$/L [17]. The different in the threshold level may arise from the different population with having different normal range.

Another study by Gulati *et al.* showed threshold value of $>7\times10^9$/L for age of >14 years $>10\times10^9$/L for age group of 1-14 years and $>14\times10^9$/L for less than one year which is different from our result [18]. Francophone group carried out questioner and found a threshold $>5\times10^9$/L for adults and $>6\times10^9$/L for children [19] which is the same as the consensus which is in agreement with our result. Pratumvinit B *et al.* found the optimized criteria to be $<1.5\times10^9$/L and $>30\times10^9$/L for leukocytes and $>7\times10^9$/L for lymphocytes which is different from our result [3]. 2.8×10^9/L is considered to be normal in adult black male [20]. This agrees with our results because our research is done from black peoples.

Another study done in South Africa by Jobert J *et al.* to investigate the flagging efficiency of Sysmex showed that the instrument flagged for 63.7% as positive 36.3% as negative .False positive flags were found to constitute 18.5% and false negative flags 5.4% having 23.9% incorrect flags, this is consistent with our results [20].

A study done by Pratumvinit B *et a*l. to optimize criteria for manual smear review in Thailand obtained an efficiency of 87.13%, a review rate of 24.22%, and a false-negative rate of 2.98% which is different from our results [3]. In Brazil a study by Comar RS *et al.* to adapt microscopic review criteria showed false negatives rate of 15.5%, false positives10.5%, microscopic review rate 37.3% and efficiency 73.8%, this is partly different with the current study which could be due to differences in sample size and altitude [21].

The clinical sensitivity of the atypical lymphocytes flag showed efficiency, sensitivity and specificity of 92.5%, 65.2% and 94.1% respectively in another study which is different from our results [22]. Which could arises from the variability of the place these machines found like environmental differences, technical skill of the technicians and different underlying disease of the patients.

The reason for this difference in our results and others could be these studies are done in different countries of socio demographic area, the patients that we took samples are with different health status, samples that these researches are from different populations, due to different number of samples they use and in addition these populations have different normal hematological range and the machines that each researches done are different. In addition numbers and experience of professionals working in the laboratory and the different clinical departments in the hospitals.

Conclusion

The peripheral smear morphology review in this study is different from the guidelines of the International Consensus Group for Hematology Review. Therefore, each laboratory should set its own threshold values for peripheral smear review to avoid unnecessary smear review and to increase the quality and reliability of the results.

Competing Interests

Authors declare that they have no conflict of interest associated with the publication of this manuscript.

Authors' contributions

Conceived and designed the experiments: WHK. Performed the experiments: WHK. Analyzed the data: WHK. Contributed reagents/materials/analysis tools: WHK, AT, LH, FH. Wrote the paper: WHK. Assisted with design, analysis, and interpretation of data: AT, FH, LH. Critical review of the manuscript: WHK, AT, FH, and LH. Read and approved the final manuscript: WHK, AT, FH, and LH. Critical appraisal of the manuscript: WHK, AT, LH, and FH.

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