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Validation of criteria for Manual Smear Review following Automated Complete Blood Counts Using the Rules Proposed by International Consensus Group for Hematology Review in St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Belaynesh Tefera, entitled: *Validation of criteria for Manual Smear Review following Automated Complete Blood Counts Using the Rules Proposed by International Consensus Group for Hematology Review in St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopi*a and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
CBC	Complete blood cell count
EDTA	Ethylene diamine tetra acetic acid
FBC	Full Blood Count
ICGHR	International Consensus Group for Hematology Review
ISLH	International Society for Laboratory Hematology
MSR	Manual Smear Review
OIF	oil immersion field
PBF	Peripheral blood Film
PBS	Peripheral blood smear
PLT	Platelet
RBC	Red Blood Cell
SOP	Standard operating procedure
SPHMMC	St. Paul's Hospital Millennium Medical College
WBC	White Blood cell

Abstract

Background: Microscopic assessment of a peripheral blood smear is an essential diagnostic tool in hematology laboratory. It can complement the numerical values on the automated Full Blood Count (FBC) to provide a definite diagnosis or to guide further investigation of a patient. The results of a significant proportion of specimens are flagged by the analyzers, and require confirmation by smear review. Thus, there is a need to validate the rules for smear review proposed by International Consensus Group for Hematology Review (ICGHR).

Objective: To validate criteria for manual smear review following automated complete blood counts (CBC) using the rules proposed by the International Consensus Group for Hematology Review in St.Paul's Hospital Millennium Medical College**.**

Methods: A prospective cross-sectional study was conducted in St.Paul's Hospital Millennium Medical College from September to November 2019. A total of 500 blood samples were collected from patients who are referred to hematology laboratory for CBC evaluation and who fulfilled the eligibility criteria. CBC was performed by DxH 800 Beckman coulter and manual blood smear review was done on Wright's stained blood film. Data was analyzed using SPSS version 25. Crosstabulation was used to determine sensitivity, specificity, Negative and positive predictive values (NPV, PPV), efficiency and review rate.

Result: Out of 500 samples, 100 samples were flagged by the instrument when applying ICGHR rules. Out of these, 87 were positive when confirmed by slide review (true Flags) but 13 were negative on the smear (false positive). Moreover, 142 samples were smear positive; 55/142 smear positives were not flagged as per the ICGHR criteria (38.7%), false negative much higher than the ICGHR acceptable limit of 5% and 345 samples were true negative (normal smear and not flagged).The result showed the ICGHR rule based flagging had sensitivity of 61.3%, specificity of 96.4%, PPV 87%, NPV 86.3%, efficiency of 86.4% and review rate 20%.

Conclusion: Each laboratory wishing to employ ICGHR rules have to validate them for their specific analyzer and sample population. This will ensure an acceptable false negative rate and avoid missing of clinically significant morphological abnormalities

Key words- Complete Blood count, Automated hematology analyzers, DxH 800 Beckman coulter , smear review criteria, Flagging, ICGHR rules

1. Introduction

1.1. Background

Complete blood count (CBC) and white cell differential (diff) are the 2 most frequently performed hematologic tests in the clinical laboratories. Automated hematology analyzers, when appropriately calibrated, generate reliable results for both of these tests on many blood specimens. The results of a significant proportion of specimens are flagged by the analyzers, and require confirmation by other techniques. One of these techniques is the microscopic examination of blood smear [1].

Microscopy and manual differential counting, in most of the automated laboratories, is restricted to cases in which the instrument "flags" the potential presence of abnormal cells or in cases where findings may interfere with analysis (such as overlap in the distribution of different cell types or interference from matrix components). In cases of clinical suspicion of leukemia, review of the peripheral blood smear is mandatory to make the presumptive diagnosis. There are other morphological findings also which may have a clinical significance which cannot be reliably identified by the various automated analyzers. These other findings include the presence of giant platelets, platelet clumps, basophilic stippling, hyper segmented neutrophils, red cell fragments, and Howell-Jolly bodies [2].

Peripheral blood smear review is a useful and economical diagnostic tool which can be used both in adults and children. Despite the advent of automated blood cell analyzers, examination of peripheral smear by the experienced technologist, and qualified hematologists cannot be repudiated. Rapid, reliable access to information about a variety of hematologic disorders is provided; in some cases, review of peripheral smear along with clinical data may be sufficient enough to establish a diagnosis [3].

Reviewing peripheral smear and performing manual differential counts need the expertise of well-trained laboratory staff and leads to under productivity and consumption of time. This has a much greater impact when the automated and manual results are similar leading to decreased working capacity of house staff. In this era of medical advancements and automation, it is

important to reduce the workload and improve turnaround time to combat the continuing pressure on laboratory resources [4].

A standard set of criteria developed by a professional organization such as the college of American pathologists (CAP) is perhaps what the clinical laboratories would like to have. Although feasible, such a set of criteria may not be workable in its entirety for all laboratories. It would have to be based solely on the clinical significance of abnormal CBC and diff findings and could serve only as a recommended guide. Until such a set of criteria becomes available, laboratory professionals need to rely upon their own knowledge, experience, and judgment in developing the list of criteria most suited to the needs of the patient population as well as to the concerns of the clinicians, and to the level of expertise of the technical staff at their institution [1].

In 2005, the International Society for Laboratory Hematology (ISLH) through the International Consensus Group for Hematology Reviews, founded by hematologist Berend Houwen, published a set of 41 rules applicable as criteria for the review of automated CBCs and leukocyte differential results of automated hematology analyzers, i.e., review criteria for automated complete blood counts [5-6].

These guidelines were formulated with the aims of reducing costs and the turnaround time of the results without sacrificing their quality, and justifying the performance and skills of the multiparametric hematology analyzers [6-8].

All hematology laboratories must be encouraged to establish locally valid protocols indicating when a blood smear review (BSR) should be performed. The guidelines suggested by the ISLH can be the starting point as long as they are interpreted in consideration of the experience of the laboratory staff, sophistication of the hematology analyzers and the laboratory's electronic records system. Incidences of abnormalities and variations in reference values of the population being tested are also among factors to be considered when using the guideline [2, 6].

Modern automated blood cell counters are the cornerstone of the hematology laboratory, providing quick, cost-effective, and accurate analysis of the blood cells. Peripheral blood smear review still play an important role to identify some morphologic abnormalities and to complete

the definitive interpretation in some cases. However, it is time consuming, laborious, and demanding the skill of experienced morphologist [9,10].

The microscopic assessment of a peripheral blood smear is an essential diagnostic tool in the hematology laboratory and can complement the numerical values on the Full Blood Count (FBC) to provide a definite diagnosis or to guide further investigation of a patient. Peripheral smear assessments can also be used as a quality control tool for the FBC and provide excellent material for training purposes [11].

Initiation of a peripheral blood film (PBF) is often a clinical request by the attending clinician on account of a clinical suspicion or less frequently initiated by the laboratory. The laboratory may initiate peripheral blood film based on abnormal findings from an automated count or patients clinical information whose diagnosis may be supported by a peripheral blood film. The latter is guided by individual laboratory policies or local regulating guidelines [2, 7].

From the clinical standpoint, blood smear examination serves 3 important objectives. First, it serves as a quality control tool in verifying the results generated by the automated analyzers. Second, it allows for identification of abnormal/immature/atypical cells, if present. Third, it allows for recognition of clinically significant morphologic abnormalities, which the analyzers are incapable of either flagging or detecting and identifying. Currently available automated hematology analyzers do not generate any reportable information about the presence of many of the red cell abnormalities [12].

The examination of the blood smear should include evaluation of the red cell, white cell, and platelet morphology. To evaluate the smear thoroughly the technologist should review at least 8 to 10 oil immersion fields (OIF). The red cell morphology evaluation should include examination for deviations in size, shape, distribution, concentration of hemoglobin, color, and the appearance of inclusions. The white cell morphology evaluation should consist of differentiation of the white blood cells and their overall appearance including nuclear abnormalities, cytoplasmic abnormalities, and the presence of abnormal inclusions that may denote a disease process. Platelet counts should be verified, and in addition the smear should be reviewed for platelet shape and size abnormalities and for clumping [13].

Despite the wide availability of hematological automated analyzers in Ethiopia, there are no published studies validating the criteria for smear review when results are flagged by the automated analyzers.

1.2. Statement of the problem

The diagnostic relevance of a peripheral blood Film (PBF) is enormous. The PBF exposes the morphology of peripheral blood cells, which ensures its place in the morphologic diagnosis of various primary and secondary blood and blood related diseases. Its diagnostic relevance has not been lessened by advances in hematology automation and molecular techniques [12].

Several situations lead to abnormal hemoglobin measurement or to abnormal red blood cell count (RBC), including lipids, agglutinins, cryoglobulins and elevated WBC counts. And also inaccuracies of platelet counts are seen in specimens with a substantial amount of interfering particles, including WBC fragments, RBC fragments, immune complexes, bacteria, lipid droplets, or protein aggregates. WBC fragments can cause the spurious elevation of platelet counts in patients with acute leukemia at diagnosis and during chemotherapy [14].

In many circumstances, several of the measured parameters from the CBC may be altered, and the discovery of a spurious change in one parameter frequently means that the validity of other parameters should be considered. Sensitive flags now allow the identification of several spurious counts, but only the most sophisticated hematology analyzers have optimal flagging and more simple analyzers, especially those without a white cell differential scatter gram, do not possess the same sensitivity for detection of anomalous results. Reticulocytes are integrated now into the CBC in many hematology analyzers, and several situations may lead to abnormal counts [15].

To reduce the rate of manual smear review (MSR), the International Society for Laboratory Hematology (ISLH) through the International Consensus Group for Hematology Review (ICGHR) published a set of rules for peripheral smear review following analysis of samples on AHAs [6].These rules are essentially review criteria for automated blood count analysis and have since been considered an international standard for MSR. The ICGHR has also put forth procedures to follow when complete blood count (CBC) results do not meet the criteria, which specifically include preparation of a peripheral blood smear followed by Manual Smear Review (MSR). These rules take into account gender and age of the patients, whether the sample is sent for the first time or a subsequent sample has been sent to monitor the CBCs and whether there has been a significant difference between the results. Application of these criteria will, in turn, reduce the laboratory cost and turnaround time thus improving productivity [16].

It is therefore advisable for all laboratories to develop their own criteria for smear review. These laboratory criteria can be based on the criteria established by ICGHR but should be verified before adoption or optimized to be suitable for different requirements. Manual microscopic examination of a stained blood film complementing automated analysis can help to validate these established criteria and thus improve the accuracy. However, no published study is available in our country validating the internationally set criteria for smear review in our context.

1.3. Significance of the study

This study aimed to validate the rules proposed by International Consensus Group for Hematology smear review criteria for Saint Paul Hospital Millennium Medical College laboratory. The study will provide information that can help in reducing unnecessary smear reviews which otherwise wastes resource, increase workload on the laboratory professionals and hence compromise the quality. On the other hand, it will help in identifying those clinically significant abnormalities which are not flagged by the analyzer and hence contextualize the set guidelines. This will help to improve the laboratory quality and improves patient management as well as interpretation of data in research. It can also be used as input for future relevant studies that will be conducted in the field.

2. Literature Review

A prospective cross-sectional study was conducted in Brazil by Comar RS *et al* on a total of 1977 consecutive blood samples from the daily workload with the objective to evaluate and establishes appropriate screening criteria for manual blood smear reviews.Three sets of screening criteria were arbitrarily proposed in this study: Group 1 (narrow ranges), Group 2 (intermediate ranges), and Group 3 (wide limits) and one set (Group 4) was adapted from the International Society for Laboratory Hematology. All samples were run on Sysmex hematology analyzers and were investigated using manual blood smear reviews. Diagnostic accuracy and agreement were performed for each set of screening criteria, including an investigation of microscopic review rate and efficiency.

The result showed that the microscopic review rates for Groups 1, 2, 3 and 4 were 73.85%, 54.52%, 46.33% and46.38%, respectively; the false-negative rates were 0.50%, 1.97%, 2.73% and 3.95%, respectively. The efficiency and negative predictive values of Group 3 were 73.04% and 4.91%, respectively [15].

Palur K *et al* in India conducted a prospective cross-sectional comparative study in 2017 on 860 blood samples collected from both inpatients and outpatients from all the departments in the hospital**.** Using ICGHR criteria, sensitivity was 81.58%, specificity was 84.61%, 83.38% positive predictive value, and 82.92% negative predictive value. The microscopic smear review rate was 47.56% and efficiency was 83.14%. Using their laboratory criteria, sensitivity was 98.80%, specificity was 41.40%, positive predictive value of 61.46%, and negative predictive value of 97.34%. The microscopic smear review rate was 78.14% and efficiency 69.30%. They concluded that a significant reduction in the microscopic smear review rates were noted using the ICGHR criteria compared to their laboratory criteria. The ICGHR criteria can thus be adapted to daily laboratory practice provided they are first optimized and locally validated before use[16].

Another cross sectional study was conducted in Thailand from May 2010 to June 2010 by Pratumvinit B *et al*. A total of 2114 samples were collected from 825 males and 1289 females. Of them, 368 (17.40%) had positive smear results according to the definition of the International Consensus Group. Among the positive samples, 230 (62.50%) had RBC abnormalities, 15 (4.08%) had WBC abnormalities, 67 (18.21%) had PLT abnormalities, 34 (9.24%) had both RBC and PLT abnormalities, 17 (4.62%) had RBC and WBC abnormalities, 3 (0.82%) had both

WBC and PLT abnormalities, and 2 (0.54%) had RBC, WBC, and PLT abnormalities. The 3 most common findings of abnormal RBC morphology were microcytic RBC (215 occurrences), anisocytosis (85 occurrences), and hypochromic RBC (70 occurrences). For abnormal WBCs, the 3 most common findings were atypical lymphocytes (18 occurrences), blasts (9 occurrences), and myelocytes (6 occurrences). Platelet clumps (84 occurrences) were found more often than giant PLTs (34 occurrences). They concluded that each laboratory should verify the criteria for smear review, based on the International Consensus Group for Hematology Review, and optimize them to maximize efficiency [18].

A cross-sectional study was conducted in Egypt by Eldanasoury SA *et al* in 2015. This study was aimed to validate and compare the performance of the criteria for smear review suggested by the Consensus Group with the laboratory criteria. A total 800 blood samples were selected randomly from the daily workload. Automated complete blood counts (CBC) and white blood cell (WBCs) differential counts were performed using Beckman Coulter LH750. Blood films were done for all samples and reviewed for positive smear findings as identified by the International Consensus group. The 2 sets of criteria (the Consensus Group and the laboratory criteria) were applied on the samples. Compared with the laboratory criteria, the false negative rate of Consensus Group criteria was higher (9.25% versus 1.62%, $p < 0.05$), the review rate was lower (54.25% versus 71%, $p < 0.05$), the sensitivity was lower (82.13% versus 97.09%, $p > 0.05$), the specificity was higher (78.32% versus 62.04%, $p > 0.05$). In conclusion they found that peripheral smear review rate was significantly reduced by applying the consensus group criteria [10].

In 2012 a cross-sectional study was conducted in South Africa by Joubert J *et al* with the objective to evaluate the flagging efficiency of the Sysmex hematology analyzer and to determine whether this potentially labour-saving technology could assist in safely reducing the number of unnecessary microscopic blood smear assessments. A total of 427 full blood count specimens were collected consecutively over a 24-hour period and were evaluated microscopically and compared with the instruments' abilities to flag potential morphological abnormalities. The result showed that the Sysmex blood cell analyzers flagged 63.7% of specimens as "positive" and 36.3% as "negative". After microscopy, false positive flags were found to constitute 18.5% and false negative flags 5.4% of the total number of smears reviewed, giving a total of 23.9% incorrect assessments. No false negative flag was clinically critical. They

concluded that false negative results occurring with the Sysmex instruments' flagging systems in theirs settings are relevant, although not critical. The potential time and monetary savings of a flagging-based smear review policy may weigh heavier than occasional false negatives. In the African milieu, where laboratories are faced with the challenges posed by staff- and other shortages, relying on instrumentation flagging to guide smear review policy should be considered [11].

Taken together, there is a need to establish contextualized criteria for each laboratory. As far as my knowledge goes there are no published articles comparing automated hematology flagging based on the International Consensus Group for Hematology Review rules in setting smear review criteria in Ethiopia. This is a gap that this study is trying to fill.

3. Objectives

3.1 General Objective

- To validate criteria for manual blood smear review following automated complete blood counts using the International Consensus Group for Hematology Review (ICGHR) criteria in St.Paul's Hospital Millennium Medical College, Addis Ababa Ethiopia from September 2019 to November 2019.

3.2 Specific Objectives

- To determine the rate of smear review when applying ICGHR criteria.
- To assess the false positive rate and false negative rate of ICGHR criteria.

4. Materials and Methods

4.1 Study area

St. Paul's Hospital Millennium Medical College is a referral hospital in Addis Ababa under the Ethiopian Federal Ministry of Health (FMOH). It is the second largest public hospital in the nation, built by the Emperor Haile Selassie in 1961 with the help of the German Evangelical Church. The hospital was established to serve the economically under privileged population, providing services free of charge to about 75% of its patients. In 2007 it became a medical college and its core services include the provision of medical care, teaching and research. It has 800 clinical and non-clinical staff members that provide medical specialty services to an estimated 110,000 people annually who are referred from all over the country [19].

St Paul's Hospital Laboratory has different departments and the hematology department is one of them. The Hematology Laboratory is newly equipped with Beckman DxH 800 Hematology analyzer a five part Diff analyzer. The hematology laboratory receives on average 450-700 samples per day.

4.2 Study design and period

A prospective cross-sectional study was conducted in St.Paul'sHospital Millennium Medical College from September to November 2019

4.3 Source population

Samples from all patients referred to St. Paul's Hospital Millennium Medical College hematology laboratory were the source samples.

4.3.1 Study population

The source samples were all blood samples of patients that were collected for CBC analysis in 5ml EDTA tube

4.3.2. Inclusion and exclusion Criteria

Inclusion criteria

- Non hemolyzed Blood sample
- those whose laboratory request is complete

Exclusion criteria

- Overfilled blood samples
- Clotted blood samples
- Improperly labeled samples

4.4 Variables

4.4.1 Dependent Variables

- Frequency of smear review
- Sensitivity, Specificity, Negative and positive predictive values, efficiency, error rate

4.4.2 Independent Variables

- Sex
- Age
- Clinical diagnosis
- WBC morphological abnormalities
- RBC morphological abnormalities
- Platelets morphological abnormalities

4.5 Sample size

A total of 500patient samples that was sent to St. Paul's Hospital Millennium Medical College hematology laboratory during the study period and fulfill the eligibility criteria were selected.

4.6 Sampling Technique

Non probable convenient sampling technique was used.

4.7 Data collection and laboratory analysis

4.7.1. Data collection procedure

Fresh whole blood samples were collected from patients of any age and gender with any diagnosis at hematology laboratory reception for their own routine care. Peripheral blood smear was done from leftover samples. Data of age, sex and clinical diagnosis of patients was taken from the request forms.

4.7.2. Laboratory analysis

About 3-4 ml whole blood was collected in EDTA tube as part of the routine care. CBC was performed using DxH 800 Beckman coulter Hematology analyzer. Peripheral blood smear was

done and stained with Wright's stain. The standard operating procedures are found in **Annex I and II.**

Automated CBC and white blood cell differential counts

The UniCelDxH 800 Analyzer is a quantitative, automated hematology analyzer for in vitro Diagnostic use in screening patient populations found in clinical laboratories. The UniCel DxH 800 analyzer provides a Complete Blood Count (CBC), Leukocyte 5 Part Differential (Diff), Reticulocyte (Retic) and Nucleated Red Blood Cell (NRBC) on whole blood.

In hematology, the complete blood count, the CBC, is the fundamental analytical test that evaluates the three main cellular components: white blood cells, red blood cells and platelets. Those cells are counted by the Coulter Principle by UniCel DxH 800 analyzer.

All Diff, NRBC, and Retic counting is based on VCSn principle (volume, conductivity and scatter). The VCSn module is responsible for controlled sample preparation and delivery of the prepared sample to the flow cell for analysis of the WBC differential, reticulocytes and NRBC. The VCSn module includes the Air Mix and Temperature Control (AMTC) Module and the Multi-transducer Module (MTM).

The lytic reagent used for the WBC prepares the blood so the system can count leukocytes and measure the amount of hemoglobin. The lytic reagent rapidly and simultaneously destroys the erythrocytes and converts a substantial proportion of the hemoglobin to a stable pigment while it leaves leukocyte nuclei intact. The absorbance of the pigment is directly proportional to the hemoglobin concentration of the sample [20].

Manual blood smear review

Blood films were smeared manually from all randomly selected samples. The smears were stained with wright stain. Wright's stain is a polychromatic stain consisting of a mixture of eosin and methylene blue. When applied to blood cells, the dyes produce multiple colors based on the ionic charge of the stain and the various components of the cell. The eosin ions are negatively charged and stain basic cell components giving them an orange to pink color. The methylene blue ions are positively charged and stain the acid cell components in varying shades of blue.

The neutral components of the cell are stained by both components of the dye producing variable colors [21].

The microscopic smear review focused on the morphology of blood cells. The positive smear results were reviewed by experienced medical laboratory technologists. If the film contained a positive finding, the sample was labeled positive. The criteria for a positive smear were applied as recommended by the International Consensus Group, which included abnormality of RBC morphology, that is, abnormality at 2 + or greater, presence of malaria, giant Platelets at moderate or greater, Platelets clumps at greater than rare/occasional, Döhle bodies/toxic granulation/vacuoles at moderate or greater, blasts at 1 or greater, metamyelocytes at greater than 2, myelocytes/promyelocytes at 1 or greater, atypical lymphocytes at greater than 5, normoblast at 1 or greater, or plasma cell at 1 or greater per 100x objective [6].Only those flags generated by the automated analyzers which met these internationally recommended criteria for smear review were selected and evaluated against the manual smear review findings (**annex III**).

4.8 Quality assurance

Blood samples quality was ensured by collecting and processing according to the standard operating procedures. High, normal and low quality control materials were run every morning for checking the analyzer before patient samples were analyzed. Quality of the Wright's stain was checked by preparing one differential slide daily using a patient sample with a normal MCV, MCH, MCHC and total white cell count. The stained slides were reviewed for meeting color specifications of the different white cells, red cells and platelets under normal conditions. It was also being checked for the presence of any precipitation and/or contamination. All analyses was done in duplicates by two senior professionals independently and discrepant results checked by a third person. Data was recorded and documented properly by using instrument print out for the automation and careful transcription for the manual methods.

4.9 Statistical analysis

Data was collected, entered, cleaned and analyzed using SPSS version 25 software according to the study objectives. If a rule shows a flag and the smear result is positive, the sample was graded as a ‘‘true positive.’’ If a rule has shown a flag and the smear did not have any positive findings, the sample was graded as a ‘‘false positive.’’ If a rule did not show a flag and the smear result is negative, the sample will be graded as a ‘‘true negative.’’ If a rule did not show a flag but the smear contained a positive finding, the sample was graded as a ‘‘false negative.’’ Sensitivity, specificity, positive predictive value, negative predictive value, efficiency and smear review rate was determined.

4.10. Ethical Consideration

The study was conducted after obtaining ethical clearance from the Department Research and Ethics Review Committee (DRERC) of Department of Laboratory Sciences of College of Health Sciences, Addis Ababa University. After getting permission from Saint Paul hospital left over samples were used for the study. No additional samples were collected for the purpose of this study. All information collected in this study was given code numbers and no name was recorded. The key to this code numbers was kept in a locked file and were accessible to the authorized staff only.

4.11. Dissemination of results

The findings of the study will be presented to the Department of Medical Laboratory Sciences of Addis Ababa University and will be communicated to St. Paul's Hospital Millennium Medical College. The document will be submitted to Addis Ababa University Library as reference. The paper will be presented to seminars or workshops to disseminate the findings and finally it will be published in peer reviewed journals.

Operational definitions

Acceptable criteria of samples – proper amount of sample with right anticoagulant, volume, labeling, at the right time of collection and non - hemolyzed.

Flags- Written or displayed output intended to signal or attract attention. Flags are generated by the instrument to alert the Operator to instrument malfunctions that occur during sample processing, or to data abnormalities detected during data analysis.

Sensitivity- is the ability of the ICGHR criteria to correctly classify a sample as a flag (Flag Positive and Smear Positive).

Specificity - The ability of the ICGHR criteria to correctly classify a sample as it does not have a flag (Flag Negative and Smear Negative).

Positive predictive value- is the percentage of patients with a positive smear finding who actually have the flag (Smear Positive and Flag Positive)**.**

Negative predictive value- is the percentage of patients with a negative smear finding who do not also have the flag (Smear Negative and Flag Negative).

False Positive-Flag as per the ICGHR criteria Positive but Smear Negative

False Negative-Flag as per the ICGHR criteria Negative but Smear Positive

5. Result

5.1 Socio-demographic and Clinical characteristics of the study population

A total of 500 samples were collected from 233(46.6%) males and 267 (53.4) females. The mean age of the study population was 36 years and the range was 2-70years.

Table1: Socio-demographic characteristics of the study population at St. Paul's Hospital Millennium Medical College, Sep-Nov, 2019 (n=500)

Variables	Frequency	Percent (%)
Sex		
Male	233	46.6
Female	267	53.4
Age (years)		
Under 14	43	8.6%
14-24	102	20.4%
24-34	104	20.8%
34-44	88	17.6%
44-54	63	12.6
54 and older	100	20%

About 17 clinical conditions were identified in the patients from whom blood samples were obtained for this study. The majorities were diagnosed with diabetes mellitus (15.6%) followed by UTI (11.8%) and cervical cancer (8.8%) (Table 2).

Table 2: Percentage and frequency of clinical diagnosis among patients at St. Paul's Hospital Millennium Medical College, Sep-Nov, 2019 (n=500)

Clinical diagnosis	Frequency	Percent
DIABETICS MELITES	78	15.6
HTN	34	6.8
CERVICAL CANCER	44	8.8
AFI	39	7.8
BREAST CANCER	41	8.2
PTB	19	3.8
UTI	59	11.8
ANEMIA	34	6.8
TONSILITES	30	6.0
SEPSIS	23	4.6
CKD	21	4.2
CLD	9	1.8
CML	10	2.0
CHD	21	4.2
BLADDER CANCER	11	2.2
ALL	11	2.2
AML	16	3.2

*ALL=Acute lymphocytic leukemia; AML=Acute myelocytic leukemia; CHD=chronic heart disease; CLD=chronic liver disease; CLL=chronic lymphocytic leukemia; CML=chronic myelocytic leukemia; HTN= Hypertension; PTB= Pulmonary tuberculosis; UTI=Urinary tract infection CKD= Chronic kidney disease.

5.2 Peripheral blood smear review Findings

Manual review of the wright's stained peripheral blood film reveled that, out of the 500 samples 142(28.4%) had positive findings and 358 (71.6%) had negative smear findings according to the definition of the International Consensus Group. Among the positive smear results, as shown in Figure 1, most of the abnormalities57(11.4%) were WBC abnormalities, 34 (6.8%) had RBC abnormalities, and 17 (3.4) had platelet abnormalities. Abnormalities in the three blood cell populations (WBC, RBC and platelet) were seen in 8 (1.6%) of them.

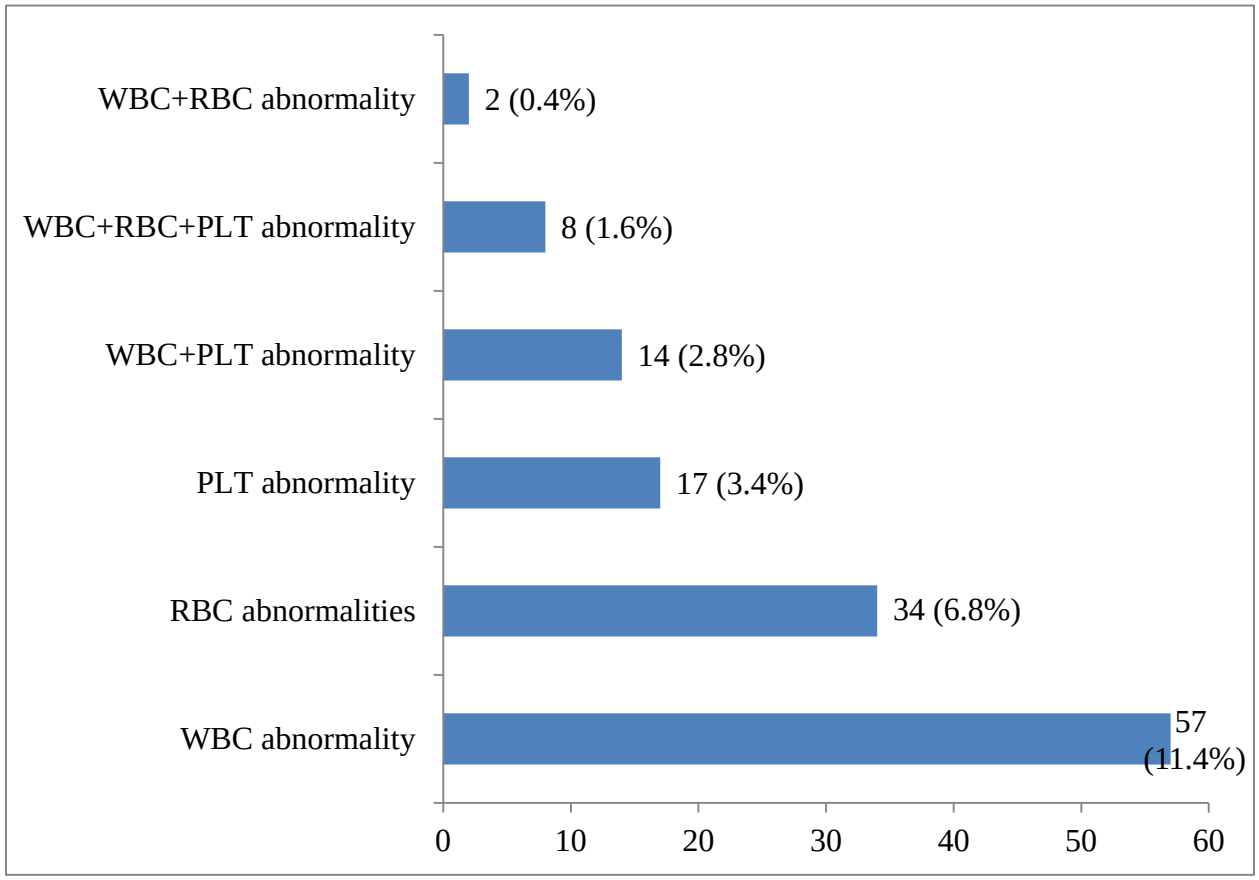

Figure1.Peripheral blood smear review Findings among patients at St. Paul's Hospital Millennium Medical College, Sep-Nov, 2019 (n=500)

The three most common findings in WBC abnormalities were bands (15 occurrence), blast and Immature Granulocyte (IG) (13 occurrence each), and myelocyte, metamyelocyte and toxic granulation (9 occurrences each) (Figure 2).The three most common findings in RBC

morphology were microcytrosis (13 occurrences), anisocytosis (12 occurrences) and macrocytosis (7 occurrences). Platelet morphology abnormalities were giant platelet (30 occurrences) and platelet clumps (20 occurrences).

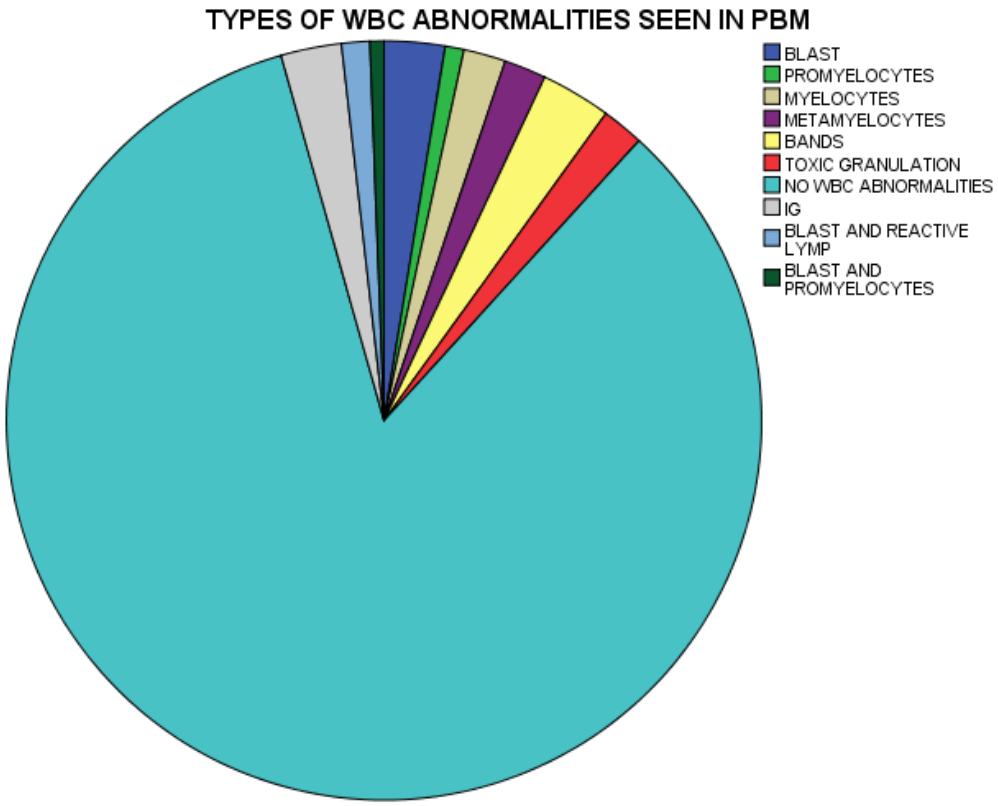

Figure 2 Types of WBC abnormalities seen in peripheral blood morphology of patients at St. Paul's Hospital Millennium Medical College, Sep-Nov, 2019 (n=500)

5.3Frequency of Flags triggered by Beckman Coulter 800 machine

Out of 500 samples analyzed, 100 (20%) positive flags that were triggered by the machine met the ICGHR criteria the rest 400 (80%) had negative result. The most frequent flag encountered by the machine were left shift in 16 samples (3.2%), blast and IG in 11samples (2.2%) each, followed by RBC fragments in 10 (2.0%) samples and atypical lymphocyte in 5 samples (1%). Dimorphic RBC flags were occurred in 2 cases (0.4%). Flags related to platelets triggered by the rules in the machine were platelet clumping in 12 samples (0.4%) and giant platelet in 10 sample (0.2%) (Table3).

Table 3.Types of flags encountered by the Beckman Coulter 800 machine as per the ICGHR criteria

Types of flags	Frequency	Percent
ATYPICAL LYMP	5	1.0
BLAST	11	2.2
LEFT SHIFT	16	3.2
IG	11	2.2
RBC FRAGMENTS	10	2.0
PLT CLUMPS	2	0.4
GIANT PLT	5	0.2
BLAST AND PLT CLUMPS	5	1.0
IG AND GIANT PLT	6	1.2
VARIANT LYMP	5	1.0
BLAST AND VARIANT LYMP	2	0.4
LEFT SHIFT AND VARIANT LYMP	4	0.2
DIMORPHIC RBC	2	0.4
IG AND BLAST	3	0.6

*IG= Immature Granulocyte

Using the suggested rules for action by the International Consensus Group for Hematology Review (ICGHR), 100 samples were triggered by the machine. Out of these, 87 were positive when confirmed by slide review (true positive) and 13 were negative (false positive). There were 55 samples (55/142=38.7%) which were not flagged but smear review showed abnormality (false negative) and 345 samples were not flagged and confirmed by slide review (true negative). The performance of flagging by the machine as per the ICGHR criteria is summarized in Table 4. Accordingly, the Sensitivity, Specificity, PPV, and NPV were 61.3%, 96.4%, 87% and 86.3%, respectively (Table 4).

Table4 Summary of results based on review criteria of International Consensus Group for Hematology Review

Sensitivity TP/(TP+FN)	61.3%
Specificity TN/(FP+TN)	96.4%
Positive predictive value TP/(TP+FP)	87%
Negative predictive value TN/(FN+TN)	86.3%
Error rate (FP + FN)/Total	13.6%
Review rate (TP+FP)/Total	20%
Efficiency (TP+TN)/Total	86.4%

6. Discussion

Blood smear review is defined as a microscopic examination of an appropriately prepared and stained blood smear by a qualified hematomorphologist. After verifying the quality of the smear and stain, the reviewer will examine the smear for clinically significant findings. All abnormalities should be noted, suspected as well as unsuspected, blood cells-related or otherwise. A complete examination should include observation under both low (x100) and high magnification (x500 and/or x1000) [3, 21].

Besides meeting accreditation requirement, review of blood smears by a well-trained and experienced hematomorphologist serves several functions that are essential to patient care. It serves as a quality control/quality assurance (QC/QA) tool for CBC, diff, and reticulocyte count results. It can be used to assess competency of the technical staff performing manual diffs. Blood smear review allows appropriate interpretation of CBC and manual diff data with other available laboratory findings and clinical information [12, 13].

The study reported herein aimed at determining the performance of the International Consensus Group for Hematology Review criteria following automated complete blood counts at St.Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Considering the actual demands and capabilities of each laboratory, this initial validation clarifies whether such criteria require optimization or not.

Out of 500 samples analyzed in the current study, 100 (20%) positive flags that were triggered by the machine met the ICGHR smear review criteria while the rest 400 (80%) had negative result. The most frequent flag encountered by the machine were left shift in 16 samples (3.2%), blast and Immature Granulocyte (IG) in 11 samples (2.2%) each followed by atypical lymphocyte in 5 samples (1%). Dimorphic RBC flags were occurred in 2 cases (0.4%). Flags related to platelets triggered by the rules in the machine were platelet clumping in 2 samples (0.4%) and giant platelet in 1 sample (0.2%).Blast and PLT clumps 5(1.0%), IG and giant platelet6(1.2%), variant lymph 5(1.0%), Blast and variant lymph 2(0.4%).The microscopic smear review rate of 20% which is observed in the current study is comparable with a result of a study conducted by Kim S *et al* [22] who studied three analyzers and reported a rate of 28.6% by

applying Consensus Group criteria on results obtained from Sysmex XE 2100, They also reported rates of 22.8% for DxH800 and 20.2% for Advia 2120 analyzer

On the other hand, others reported a higher rate of smear review. For example, Eldanasoury AS *et al,* [10] reported a review rate of 46.06% which is comparable with a study reported by Comar*et al,* [15].Equipment type is less likely to contribute for the observed differences between the current study and the studies by both Eldanasoury AS *et a l*[10] and Comar*et al*[15] as demonstrated by the consistent findings by Kim *et al*who investigated three types of equipment and found comparable smear review rates [22]. Perhaps, the most probable explanation could be technical differences as well as equipment status which might depend on competency of professionals and equipment management.

In this study, based on the positive criteria suggested by the International Consensus Group for Hematology Review, the result showed sensitivity of 61.3%, specificity of 96.4% and efficiency of 86.4%.Higher sensitivity (81.5%),lower specificity (84.61%) and slightly lower efficiency (83.14%) was observed in a study conducted byPalur K *et al* in India [16] compared to the current study. This is different with the current study which could be due to differences in sample size and clinical status of the study population.

The consensus group considered 5% as the maximum acceptable false negative rate to ensure patient safety [6]. In the present study the false negative rate was 38.7%. Comar*et al* , and Pratumvinit B *et al* reported a false negative rate of 6.73% and 2.22% which is not comparable to the current study result. The reason for the discrepancy in the results of the current study with published literatures may be due to smaller sample size in this study as well as inherent population differences. These differences stress the need for each laboratory to validate ICGH rules and optimize accordingly. On the other hand, the higher false negative rate in the current study also indicated some abnormal blood cells findings will be missed unnoticed and hence suggests the need for close communication between clinicians and laboratory people. Such close communication will help to perform smear reviews and look for any abnormalities even though patients' results are not flagged by the automated analyzer.

The current literature offers a wide variety of suggestions and useful panel rules of procedure on blood smear review criteria following CBC analysis [18, 22-24].ICGH guidelines have been

introduced in 2005 and limited studies have been conducted to determine their performance. However, data have often showed a great variability of results on ICGHR rules performances when applied on different hematology analyzers, even on those based on similar technology [13, 23-26].No single published study is available in Ethiopia to determine the performance of the International Consensus Group for Hematology Review rules against manual smear review. Despite the extensive capabilities of the latest generation multi parametric hematology analyzers, microscopic review of blood smears still plays an important role in hematology laboratories [23].

Establishing and assessing specific review criteria for a particular laboratory means that the ISLH screening and positive smear criteria must first be validated, especially because these criteria are international standards [15]. For instance, Palur*et al* evaluated the effectiveness of the International Consensus Group Criteria for manual peripheral smear review. Using ICGHR criteria*versus* their own laboratory set criteria, sensitivity was 81.58%*vs* 98.80%, specificity was 84.61%*vs*41.40%, positive predictive value 83.38%*vs* 61.46%, and negative predictive value 82.92%*vs* 97.34% indicating their laboratory criteria was highly sensitive but less specific. The microscopic smear review rate was 47.56%vs 78.14% and efficiency was 83.14%*vs*69.30%indicating the significant reduction in the microscopic smear review rates when using the ICGHR criteria compared to their laboratory criteria [16]. In the current study, such comparison between ICGHR criteria and a laboratory set criteria was not possible as the hospital where this study was carried out did not establish its own criteria.

7. Strength and limitations

7.1 strength

- Two slides were prepared for each sample and Peripheral blood morphology was examined by two experienced medical laboratory technologists independently and discrepancies checked by a third senior laboratory professional.

7.2. Limitations

- Due to the lack of standardized smear review criteria at St. Paul hospital Millenium Medical College (SPHMMC), this study could not compare the ICGHR rules with SPHMMC review criteria.
- The samples included in this study were only analyzed on Beckman Coulter DxH 800 machine. Thus, flags triggered by different analyzers have not been assessed.

8. Conclusion and recommendation

8.1 Conclusions

The capabilities of the modern automated analyzers are well known and valued but despite the massive improvements in technology there remains a need for manual smear review. Each laboratory wishing to employ ICGHR rules will need to validate the rules for their specific analyzer and sample population. This will ensure an acceptable false negative rate and avoid missing clinically significant morphological abnormalities.

8.2 Recommendation

- The finding of this study indicates local validation and optimization the ICGH rules using a variety of analyzers is required before applying the ICGHR rules.
- Absence of flagging by the instrument when actually the blood smear shows abnormal finding indicates the need for close communication between clinicians and laboratory professionals about the clinical condition of the patient not to miss abnormal findings and request a blood smear review though the result was not flagged by the machine
- There were 38.7% false negatives in the current study which is much higher than the ICGHR acceptable limit of 5%. Thus, St Paul Hospital should establish its own criteria for smear review in order to minimize unnecessary reviews (there were 13 false positive flags) and also identify those which actually need to be reviewed.

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Annexes

Annex I: Standard operating procedure and reagents for DxH 800 Beckman Coulter

Specimen requirements

About 3-4 ml of venous blood collected into EDTA tubes.

Procedure

1. Turn ON the power switch on the front side of the analyzer.
2. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
3. Slide each sample firmly into the cassette. Ensure the bar-codes are facing up within the cassette window
4. Place the cassettes into the input buffer to the right of the SPM. The SPM automatically begins cycling the cassettes.
5. After the SPM cycles the samples, review the sample results at the System Manager

Reagents of DxH 800 Beckman Coulter

COULTER® DxH Diluent

Is a cyanide-free, isotonic buffered saline solution. COULTER DxH Diluent dilutes the specimen, is used for rinsing SPM components between sample analyses, and provides a sheath stream to transport the sample through the flow cell.

COULTER DxH Cell Lyse

Is a cyanide-free CBC lytic reagent that lyses red blood cells for the white blood cell count, and works in conjunction with COULTER DxH Diluent to generate a stable hemoglobin measurement. And also used to lyse the red blood cells and discriminates nucleated red blood cells from white blood cells

DxH Diff Pack

Consists of the Erythrolyse Lytic reagent and StabiLyse preservative reagent. The Erythrolyse Lytic Reagent is a cyanide-free lytic reagent that dilutes theblood sample, and lyses red blood cells in preparation for white blood cell measurement in the flowcell. The StabiLyse Preservative reagent neutralizes the Diff lytic reagent and preserves the whiteblood cells for measurement in the flow cell. Together, Erythrolyse and StabiLyse provide the five partdifferential.

The DxHRetic Pack

Consists of a reticulocyte stain and a reticulocyte-clearing reagent. The reticulocyte stain reagent is a cyanide-free reagent that uses a dye to stain reticulocytes. The reticulocyte-clearing reagent is a cyanide-free reagent that stabilizes the dye-reticulum complex to enhance discrimination of reticulocytes from mature red blood cells utilizing the VCSn technology.

DxH Cleaner

Is a cyanide-free, aldehyde-free cleaning agent that degrades residual materials so thatthey may be flushed from the system with the diluent

Annex II:Standard operating procedure for blood smear wright staining

Specimen requirements

About 3-4 ml of venous blood collected into EDTA tubes

Procedure

1. Place a drop of blood, about 2 mm in diameter approximately inch from the frosted area of the slide.
2. Place the slide on a flat surface, and hold the narrow side of the non frosted edge between your left thumb and forefinger.
3. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.
4. Hold the spreader slide at a 30 angle, and draw it back against the drop of blood.
5. Allow the blood to spread almost to the edges of the slide.
6. Push the spread forward with one light, smooth, and fluid motion. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.
7. Label the frosted edge with patient name, ID# and date.
8. Allow the blood film to air-dry completely before staining.
9. Place the air dried smear film side up on the staining rack
10. Cover the smear with Wright stain and leave for 2 minutes
11. Dilute with buffer for three minutes.
12. Wash the smear with tap water
13. Air dry the smear

Annexes III: Criteria for a positive smear recommended by the International consensus group for hematology Review

RBC morphology

2+/moderate or greater.

Platelets

Giant platelets at 2+/ moderate or greater

Platelet clumps at rare or occasional

WBC

Döhle bodies at either 2+ /moderate or greater

Toxic granulation at either 2+/moderate or greater

Vacuoles at either 2+/ moderate or greater

Abnormal cell types

Blasts ≥ 1%

Myelocytes/promyelocytes ≥1%

Metamyelocytes >2%

Atypical lymphocytes>5%

Nucleated RBC≥1%

Bands > 10%

Plasma cells ≥1% per 100x oil immersion field.

Declaration

Assurance of Principal Investigator

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged. **Name of the student: Belaynesh Tefera,(BSc,MSc)**

Date _____ Signature _____

Approval of Advisors:

Aster Tsegaye, MSc, PhD

Date_____ Signature _____

Afework Hagos, MD, Hematologist

Date_____ Signature _____