

**UTILITY OF THE INTERNATIONAL CONSENSUS GROUP  
CRITERIA FOR MANUAL PERIPHERAL SMEAR REVIEW  
FOLLOWING AUTOMATED BLOOD CELL ANALYSIS**

By

**DR. KATYAYANI PALUR**

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**DOCTOR OF MEDICINE**

**IN**

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Under the Guidance of

**DR. SUREKHA U. ARAKERI**

Professor, Department of Pathology

**B.L.D.E. UNIVERSITY'S, SHRI B.M. PATIL MEDICAL COLLEGE,  
HOSPITAL & RESEARCH CENTRE, VIJAYAPUR, KARNATAKA.**

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Date:

**Dr. KATYAYANI PALUR**

Place: Vijayapur

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Date:

Place: Vijayapur

**Dr. SUREKHA U. ARAKERI**

Professor  
Department of Pathology,  
B.L.D.E.U's Shri B. M. Patil  
Medical College, Hospital &  
Research Centre, Vijayapur,  
Karnataka

**B.L.D.E. UNIVERSITY'S  
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& RESEARCH CENTRE, VIJAYAPUR**

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This is to certify that the dissertation entitled **“UTILITY OF THE INTERNATIONAL CONSENSUS GROUP CRITERIA FOR MANUAL PERIPHERAL SMEAR REVIEW FOLLOWING AUTOMATED BLOOD CELL ANALYSIS”** is a bonafide research work done by **Dr. KATYAYANI PALUR** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology)**.

Date:

**Dr. B. R. YELIKAR**

Place: Vijayapur

Professor and H.O.D,  
Department of Pathology,  
B.L.D.E.U's Shri B.M.  
Patil Medical College,  
Hospital & Research  
Centre, Vijayapur,  
Karnataka.

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& RESEARCH CENTRE, VIJAYAPUR**

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This is to certify that the dissertation entitled **“UTILITY OF THE INTERNATIONAL CONSENSUS GROUP CRITERIA FOR MANUAL PERIPHERAL SMEAR REVIEW FOLLOWING AUTOMATED BLOOD CELL ANALYSIS”** is a bonafide research work done by **Dr. KATYAYANI PALUR** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology)**.

Date:

**Dr. S. P. GUGGARIGUDAR**

Place: Vijayapur

Principal,  
B.L.D.E.U's Shri B.M. Patil  
Medical College, Hospital &  
Research Centre, Vijayapur,  
Karnataka.

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Date:

**Dr. KATYAYANI PALUR**

Place: Vijayapur

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**Date:**

**Dr. KATYAYANI PALUR**

**Place: Vijayapur**



## **LIST OF ABBREVIATIONS USED**

AHA	Automated Haematology Analyser
MSR	Manual peripheral Smear Review
WBC	White Blood Cells
RBC	Red Blood Cell
ILSH	International Society for Laboratory Hematology
ICGHR	International Consensus Group for Hematology Review
CBC	Complete Blood Count
DLC	Differential Leukocyte Count
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
IG	Immature Granulocyte
K3 EDTA	Tripotassium salt Of Ethylene Diamine Tetraacetic Acid
HB	Haemoglobin
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW-CV	Red cell Distribution Width – Coefficient of Variation
nRBC	Nucleated RBC
TC	Total WBC count
AN	Absolute Neutrophil count
AL	Absolute Lymphocyte count
AM	Absolute Monocyte count
AE	Absolute Eosinophil count
AB	Absolute Basophil count

MPV	Mean Platelet Volume
Abn Lympho/ Blast	Abnormal Lymphocyte/ Blast
ICSH	International Committee for Standardisation in Hematology
TP	True Positive
TN	True Negative
FP	False Positive
FN	False Negative
PPV	Positive Predictive Value
NPV	Negative Predictive Value
MRR	Microscopic Review Rate
WDF	White Cell Differential Channel
WNR	White Cell Nucleated Channel
IPF	Immature Platelet Fraction
PLT-F	Fluorescent Platelet Channel
Ret-He	Reticulocyte Haemoglobin Concentration
BSS	Blood Smear Scan
BSE	Blood Smear Examination
BSR	Blood Smear Review
CAP	College of American Pathologists
PLT	Platelet
Atypical Lympho	Atypical lymphocyte
Turb/HB interf	Turbidity/ Hemoglobin interference
HB defect	Hemoglobin defect
Dimorphic pop	Dimorphic Population

## **ABSTRACT**

### **BACKGROUND**

Automated haematology analysers have become an integral part of the present day clinical laboratory as they have reduced the number of manual hematology procedures and increased the speed of reporting without sacrificing the quality of the results. Manual smear reviews (MSR), however, still play an important role in identifying morphological abnormalities and to confirm the results of the analysers. It is thus important to make a decision on whether manual smears are necessary for each and every sample.

In 2005, the International Society for Laboratory Hematology (ISLH) through the International Consensus Group for Hematology Review (ICGHR), published 41 rules for peripheral smear review after analysis of samples in AHAs, which were review criteria for automated blood count analysis in order to reduce the number of manual smear reviews.

### **OBJECTIVE**

The objective of this study was to evaluate the effectiveness of the ICGHR criteria for MSR by performing manual peripheral smears for all the samples in the study group following automated blood cell analysis.

### **MATERIALS AND METHODS**

The study was performed on whole blood samples sent for complete blood count testing to the central laboratory of the Department of Pathology in B.L.D.E.U.'s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapur. The study period was from 1<sup>st</sup> December 2014 to 30<sup>th</sup> June 2016.

Analysis of the blood samples was done on the 6 part differential automated haematology analyser Sysmex XN-1000 and manual peripheral smear review was performed along with a 100-cell manual differential count. Each sample was reviewed according to the adapted ICGHR criteria and the laboratory criteria. Truth tables were prepared for each set of criteria.

## **RESULTS**

Using the ICGHR criteria, 39.65% samples were true positive, 43.49% were true negative, 7.91% false positive and 8.95% samples were false negative. Accordingly the 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% with an efficiency of 83.14%.

Our laboratory criteria revealed a true positivity of 48.02%, true negativity of 21.28%, false positivity of 30.12% and a false negativity of 0.58%. The sensitivity was 98.80%, specificity 41.40%, positive predictive value of 61.46% and, negative predictive value of 97.34%. The microscopic smear review rate was 78.14% with an efficiency of 69.30%.

## **CONCLUSION**

There was a significant reduction in the microscopic review rates with the application of the ICGHR criteria. However, the false negative rate was higher than the recommended level. Thus the ICGHR criteria can be adapted in laboratories but must be optimized and locally validated for manual smear review before use.

**KEY WORDS:** Automated hematology analysers, Manual peripheral smear review, International Consensus Group for Hematology Review criteria

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## **INTRODUCTION**

Automated haematology analysers (AHA) have reduced the number of manual hematology procedures without sacrificing the quality of the results. Likewise, they have increased the speed of reporting even in the presence of shortages of trained and skilled personnel. Therefore now, in the era of sophisticated AHAs, it is not necessary to perform a manual peripheral smear review (MSR) or manual differential count for each and every hematology sample.<sup>1</sup>

Automated hematology analysers are superior to manual methods for the count of white blood cells (WBC), red blood cells (RBC) and platelets and for differential counting of mature WBC. Despite great precision, high accuracy and expandability of AHAs, MSR still plays an important role in identifying morphological abnormalities, immature cells and certain sample characteristics such as platelet clumps. MSRs are also used to confirm the results produced by the analyser.<sup>1-4</sup> In order to reduce the rate of MSR, it is important to make a decision on whether manual smears are necessary for each and every sample. This, in turn, will reduce the laboratory cost and turnaround time.<sup>5</sup>

There has been little uniformity regarding criteria for MSR after analysis of blood samples on AHAs. The International Society for Laboratory Hematology (ISLH) through the International Consensus Group for Hematology Review (ICGHR), in the year 2005, published 41 rules for peripheral smear review after analysis of samples on AHAs.<sup>1</sup> These rules are essentially review criteria for automated blood count analysis and have since been considered the 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 MSR. The rules take into account the 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. This is based on the screening thresholds for individual AHAs and suspect flags.<sup>2,4</sup>

Comar *et al*<sup>2</sup> stated that all hematology laboratories must, therefore, be encouraged to optimise and validate the ICGHR criteria and further establish locally valid criteria for MSR. These locally valid criteria must be established by taking into consideration features such as the experience of the laboratory staff, the sophistication of the AHAs in the laboratory, the sophistication of the hospital electronic records system and population being tested with respect to normal reference values and incidences of abnormalities and variations.

The objective of this study was to evaluate the effectiveness of the ICGHR criteria for MSR by performing manual peripheral smears for all the samples in the study group following automated blood cell analysis and thus differentiate the samples that have a high probability of containing relevant morphological abnormalities from those that do not. This will further aid in prompt diagnosis and appropriate treatment of patients.

## **OBJECTIVES OF THE STUDY**

- 1) To evaluate the efficacy of the International Consensus Group for Hematology Review criteria for manual peripheral smear review following automated blood cell analysis on Sysmex XN-1000.
- 2) To compare the efficacy of our laboratory criteria for manual peripheral smear review with that of the International Consensus Group for Hematology Review criteria following automated blood cell analysis on Sysmex XN-1000.

## **REVIEW OF LITERATURE**

The most commonly requested hematology laboratory test is the CBC, also known as the Hemogram. The differential leukocyte count (DLC) is another important aspect amongst various other hematology laboratory tests.<sup>6</sup> These tests not only provide insight into conditions that involve blood elements and related bone marrow elements but also give information on the general condition of the patient. Several automated hematology technologies have been developed since the past century for performing CBC and DLC, in order to reduce manual time taking procedures, cost and turnaround time in the laboratory.<sup>7</sup>

The world of hematology had first moved toward automation with the Moldavan capillary method in 1934.<sup>8</sup> Prior to the establishment of AHAs, cell counts for RBCs, WBCs and platelets were performed manually in volume-calibrated chambers along with a microscopic examination of the peripheral blood smear for morphology and DLC.<sup>1,9</sup> In 1956 the first single channel AHA, developed by Wallace Coulter, transformed the practice of cell counting into a more acceptable system.<sup>1</sup> This not only reduced the time of cell counting but also improved its precision thereby reducing counting errors.<sup>10</sup> Multichannel CBC analysers were developed in the 1960s. In the 1970s cytochemical techniques were used for WBC differential count. In the 1980s multichannel CBC analysers were combined with flow WBC differential methods to create the present day AHAs.<sup>1</sup>

AHAs have undergone impressive development over the past 3 decades. Changes in software along with the introduction of new principles in cell analysis have been foremost in this regard. AHAs are now considered superior to the MSR for counting WBCs, RBCs, and platelets. While MSR is preferred for identification and characterization of immature cells, AHAs are favoured for differential counting of

mature cells. For the above reasons, AHAs are now the preferred method for CBC and WBC differentials thereby sidestepping the MSR without sacrificing the quality of results. Thus, while MSRs were used in the past alongside AHAs as complementary procedures, their rates have declined over the years as the performance of the analysers has improved.<sup>1</sup>

AHAs may be semi-automated or fully automated. Semi-automated instruments require a few steps to be carried out manually by the operator. They measure only a few parameters at a time. The fully automated instruments, on the other hand, require merely a properly collected blood sample of appropriate amount to be presented to the instrument. They are usually multichannel multi-parameter instruments which also include parameters that cannot be measured manually.<sup>11</sup>

In either case, the sample is first aspirated into the analyser, ideally by piercing the cap of a closed tube. This ensures maximum safety especially while handling infectious samples. This aspirated sample is then separated into different streams and mixed with various buffers in modified flow cytometers to facilitate accurate and specific analysis of the various cell types.<sup>12</sup>

The principles used in various AHAs include light scatter, electrical impedance and conductivity, fluorescence flow cytometry and light absorption of cells stained in flow. In light scatter, the diluted cell suspension passes through a tiny aperture allowing the cells to pass one cell at a time in a single file in front of a light source. Each cell scatters the light at various angles which is detected by a photodiode or a photomultiplier. The photodiode then converts the scattered light into electrical impulses which are counted. This principle yields information about cell size, nuclear lobulation and granularity of the cytoplasm.<sup>12</sup>

The principle of electrical impedance was first introduced by Wallace Coulter in 1956.<sup>13</sup> It is the most commonly used principle in hematology analysers. In this principle, cells are considered non-conductive with no resistance. Current is passed between two chambers filled with a conductive buffered electrolyte solution separated by a tiny aperture (sensing zone) which is responsible for most of the impedance.<sup>14</sup> When a cell passes through the aperture, it displaces a volume of diluent which in turn increases resistance and produces a voltage pulse. The number of pulses indicates the number of cells and the height of the impulse indicates the volume of the cell. Using this information, histograms are plotted as frequency and size distribution curves. Based on the size of the cells (RBCs, WBCs, and platelets) threshold values are established which help to separate the individual cells.<sup>13</sup>

Coincidence and non-identification are two main problems with the electrical impedance method. If more than one cell passes through the sensing zone at the same time instead of the cells passing one by one it is known as coincidence and the error is known as coincidence error. As the concentration of the cells in the electrolyte suspension increases, the coincidence error increases. This error can be corrected by a correction formula that may be integrated into the analyser's computer.<sup>14</sup>

In 1986, the method of hydrodynamic focussing was developed to circumvent the problem of coincidence. In this method, a steady flow of diluent is focussed toward the aperture and the cell suspension passes through this diluent in a fine stream. The major advantage of this method was the ability to distinguish clearly between RBCs and platelet particles and to recognize multiple RBC populations thus improving the accuracy of these cell counts. However, large platelets cannot be distinguished from microcytic cells or fragmented RBCs in this method. This distinction is better achieved by light scattering.<sup>14</sup>

The present day hematology analysers use a combination of various principles which also include the method of fluorescence flow cytometry. This allows for both qualitative and quantitative estimation of the blood cells with an increase in precision and more importantly, allows for detection of immature cells. In this method, fluorescently labelled conjugates are incubated with the sample which helps in labelling each cell. The blood cells are then analysed by flow cytometry where they are analysed by monochromatic light using a semiconductor laser. A characteristic wavelength of light is detected which has a longer wavelength than the original light. Based on the scatter angle, scattergrams are plotted from which various cell populations can be determined. The fluorescent dyes also stain nucleic acids of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) within the cell. The concentration of nucleic acids in the cell is directly proportional to the intensity of the fluorescent light detected.<sup>15</sup>

Over the years software upgrades have been introduced which provide reliable automated counting of immature granulocytes (IG) in the differential channel.<sup>16</sup> The white cell differential channel (WDF) classifies WBC and IG cell characteristics based on their side scatter and fluorescent intensity.<sup>16,17</sup> The white cell nucleated channel (WNR) differentiates nucleated RBCs (nRBC) from basophils and other leukocytes. The process of analysis includes the lysis of the cytoplasmic membranes of nRBCs and perforation of the cytoplasmic membranes of leukocytes with the help of a lysing reagent. Polymethine, a fluorescent dye, then binds to the bare nuclei of the nRBCs, nucleic acids and cytoplasmic organelles of the leukocytes. On analysis by fluorescent flow cytometry, each cell's forward scatter which determines the cell size and fluorescent intensity are determined. This results in a very clear distinction

between the nRBCs and leukocytes. Hence, there is no need for the correction of the total WBC count in the presence of nRBCs.<sup>17</sup>

A new automated method was developed in order to quantitate reticulated platelets by using flow cytometry with the use of a nucleic acid specific dye in the reticulocyte/optical platelet channel. This is expressed as the immature platelet fraction (IPF).<sup>16</sup> A separate channel known as the fluorescent platelet channel or PLT-F is useful to determine the platelet count along with the immature platelet fraction (IPF). The procedure for analysis includes dilution of the blood sample and addition of oxazine, a fluorescent dye. The fluorescent dye binds to the nucleic acids present in the platelet organelles and reticulocytes and is subsequently analysed by fluorescent flow cytometry for each cell's forward scatter and fluorescent intensity. As the fluorescent dye diffusely binds to reticulocytes, a clear distinction can be made between platelets and reticulocytes. IPF is determined by analysis of cells with high fluorescent intensities. The PLT-F channel provides an accurate platelet count especially when the platelet counts are low or when there are RBC fragments or leukocyte fragments.<sup>17</sup> The IPF has been very useful clinically especially for the laboratory diagnoses of thrombocytopenia due to increased peripheral platelet destruction, especially autoimmune thrombocytopenic purpura, and thrombotic thrombocytopenic purpura. It is also very useful as a predictor of platelet recovery following haematopoietic progenitor cell transplantation.<sup>16</sup>

Another recent development for the measurement of reticulocyte haemoglobin concentration is Ret-He. Ret-He measures the forward scatter characteristics of stained reticulocytes and in turn provides an early measure of the response to iron therapy. It also provides an indirect measure of the iron available for new red blood cell production over the previous 3–4 days.<sup>16</sup>



Automated hematology analysers also provide information on the distribution of cells based on their sizes in the form of a Gaussian curve. This is especially useful for anisocytosis in RBCs and platelets. Histograms provide a means to compare the sizes of a patient's cells with normal populations. Shifts in one direction or the other can be of diagnostic importance.<sup>18</sup>

Apart from the CBC and WBC differential counts, AHAs also provide details of qualitative abnormalities which include abnormal cells such as immature granulocytes, blasts and atypical lymphocytes and quantitative abnormalities in blood cell counts such as platelet clumps. These abnormalities are detected by electronic or printed alerts displayed by the automated instrument by a process termed "flagging".<sup>1,19-21</sup> The specific abnormalities noted by the analyser are termed "flags".<sup>19</sup> Flagging alerts the operator of increased probability of an error or an abnormality that the instrument may be unable to assess. This usually calls for MSR in order to rule out or confirm these findings.<sup>19,21</sup>

AHAs provide rapid analysis of the blood samples, they are more precise for cell counts, efficient, reliable, and cost-effective and preferred for DLC of mature forms. But the results produced by the AHAs require validation while analysing cells with morphological abnormalities as the results are not confirmatory.<sup>4,5,9</sup> To ensure accuracy and reproducibility of the results obtained from the AHAs proper calibration of the instruments is of utmost importance. AHAs have a coefficient of variation of 1 to 2% accounting for its reproducibility which is not achievable by most manual peripheral smears.<sup>12</sup>

Although the number of blood samples that require peripheral blood smear has diminished over the past decade or so, 10-15% or less in some clinical settings, it still remains a crucial diagnostic aid.<sup>22</sup> Manual peripheral smears may be in the form of a

blood smear scan (BSS) or may include a complete blood smear examination (BSE) with a DLC.<sup>6</sup>

BSS is useful to confirm the platelet counts given by the AHAs. Hence this method is also known as platelet scan or platelet estimate. As it does not include a DLC it is also called blood smear examination without a DLC. The uses of this method of examination of a manual peripheral smear include verification of automated platelet count, especially in the presence of a platelet flag or if the platelet count is below the lower limit of the normal reference range, verification of other CBC results flagged by the analyser, to verify if the automated DLC results are reliable and to determine if the smear and stain are suitable for a manual DLC and to determine the area for performing a DLC.<sup>6</sup>

Most laboratories verify platelet counts by BSS if the count is below  $100 \times 10^9/L$  on the AHA, as it helps to rule out pseudo-thrombocytopenia which will further prevent postponement of a surgical procedure or platelet transfusions. This, in turn, will help to improve laboratory turnaround time and reduce the cost for the healthcare system. BSS is performed by scanning the entire smear under 100x magnification looking for platelet clumps. Smear scanned under 400x and 1000x magnification will help to pick up smaller platelet clumps which may not be visible under 100x. The presence of giant platelets, organisms, and red cell fragments is also noted simultaneously as they may alter the platelet count given by the AHA.<sup>6</sup>

Gulati *et al*<sup>6</sup>, based on their own experience and a review of literature, suggested criteria for BSS which include: for verification of CBC and/or DLC results when flagged by the AHA, when the initial platelet count is below  $100 \times 10^9/L$  whether or not flagged by the analyser, when any of the following flags are generated by the AHA: platelet clumps, giant or large platelets, red cell fragments and qualitative white

cells-associated flags (morphologic or suspect or interpretive flags). These include blasts, atypical lymphocytes, immature granulocytes and left shift. If these cells are present on the BSS then a manual DLC must be performed.

The BSE includes a 100-cell WBC differential count along with an evaluation of the morphology of the various cell types on the peripheral smear. Hence the BSE is also known as Manual DIFF. BSE is first performed with an acceptable smear as done in BSS examination. Then under 100x magnification, the smear is scanned for clumps of platelets, WBCs or RBCs along with any extracellular organisms, cryoprecipitates, and rouleaux formation. These findings are further confirmed under 400x and 1000x magnifications. Additionally, a 100-cell WBC differential count is performed using manual counters. Absolute counts are more significant as compared to the differential counts as they reflect the true increase or decrease of each individual cell type. After completion of the differential leukocyte count, the morphology of RBC, WBC and platelets are evaluated.<sup>6</sup>

The objectives of performing a BSE, as explained by Gulati *et al*<sup>6</sup>, include identification of abnormal or immature or atypical cells, recognition of morphological abnormalities that the AHAs may not be able to flag or identify (for example, elliptocytes, sickle cells, tear drop cells, RBC inclusions, Auer rods, Dohle bodies, platelet satellitism), and as a tool for quality control to verify the results of the AHA.<sup>6</sup>

The Blood Smear Review (BSR) is also known as blood smear interpretation or physician review of blood smear. It can be requested by the clinician or performed by the laboratory staff. For the clinician, the indications are usually unexplained anemia or suspicion of microangiopathic haemolytic anemia, hemoglobinopathy, red cell membranopathy, lymphoproliferative disorder, myeloproliferative disorder, myelodysplastic syndrome, parasitic infection, inherited leukocyte or platelet disorder

to name a few. However, for the laboratory staff, a BSR is either a good laboratory practice or a requirement by regulatory or professional accreditation agencies.<sup>6</sup>

Criteria for smear review are usually developed by individual laboratories with suggestions from pathologists, clinicians, and the hematology supervisory staff. Even though the clinical significance of the abnormal CBC and DIFF findings is the major determining factor in deciding which blood smears need review a number of other factors may also influence such a decision. These include patient population and concerns clinicians may have with regard to certain patient populations, training and experience of blood smear reviewers, workload of the laboratory, quality control / quality assurance consideration, and teaching / educational considerations.<sup>6</sup>

The major functions of a BSR are:

- (1) It can serve as a quality assurance tool for the CBC, especially manual DLC, as there is no other commercially available quality control for the daily use.
- (2) To provide a definite diagnosis or to provide information for additional work-up of the case.
- (3) To differentiate cells based on their morphological features as for immature granulocytes, blasts and atypical lymphocytes and estimation of platelet count.
- (4) As an exceptional teaching resource for training of students, residents, fellows and newly hired staff, and for continuing education of the technical staff.<sup>6,19</sup>

The BSR is inclusive of the steps followed for BSS and BSE except that the reviewer may decide whether or not to perform the manual differential leukocyte count. The manual count has to be performed only for the purpose of quality control

or to assess the competency of the staff. It is also performed in case the reviewer suspects that the differential count is inaccurate, incomplete or contains unidentifiable cells.<sup>6</sup>

The MSR is a gold standard tool to confirm the morphological assessment of abnormal cells especially immature granulocytes, blasts, atypical lymphocytes or cell numbers when the AHAs detect abnormalities. It can also detect or identify cells that the AHA may not be able to classify. On the other hand, examination of the manual smear is tedious, imprecise and time-consuming, labour intensive and has higher overall laboratory costs when compared to the AHAs. It is more demanding with an increased turnaround time. Nevertheless, MSR is still an essential diagnostic tool in the hematology laboratory. Despite the latest generation multi-parametric hematology analysers MSRs provide a definitive diagnosis and thus aid in the treatment of a patient.<sup>5,7,9</sup>

In 2002 Dr. Berend Houwen invited 20 experts to generate internationally acceptable guidelines (“rules”) as there were no uniform criteria applied to AHAs for MSR. Dr. Houwen founded the ICGHR and published a set of 41 rules as review criteria for peripheral blood smear review. Out of the 41 rules, 15 rules were related to CBC parameters, 7 to differential parameters, 7 related to instrument suspect flags for RBC and platelet, 10 to WBC suspect flags and 2 for reticulocytes. These criteria take into account the age and gender of the patient, whether the request is initial or subsequent (delta checks) to monitor the results, CBC parameters, absolute count for 5 white cell types, reticulocytes and instrument flag for RBC, WBC and platelets. The review criteria allow for the release of results of the AHAs without a MSR. These rules also guide the operator in situations where the results of the AHAs would trigger a review requiring a manual peripheral smear examination.<sup>1</sup>

These rules, however, are not standardised for use. It has been found that many laboratories have adopted these criteria without validation and optimisation. To maximize efficiency, the review criteria must first be validated before use, taking into consideration the following points: the type of facility, laboratory budget, laboratory requirements, instrument model and characteristics, workload and sample volume, number of staff members capable of operating the AHA and carrying out the MSR, rate of review of MSRs, turnaround time and type of patient population in that area.<sup>2,4</sup> Failure to consider the above points before validation and implementation of these criteria may, in fact, lead to an increase in false results or an unnecessary MSR thus leading to an increase in the workload and turnaround time.<sup>4</sup> Various studies have been performed since the introduction of the ICGHR criteria.

Comar *et al*<sup>2</sup> initially used the ICGHR criteria and adapted the screening criteria due to limitations in their electronic hospital records and interfacing systems. Using the XE-2100D and XT-2000i hematology analysers, they found a high false negative rate of 6.73% and a microscopic smear review rate of 46.03%. The same screening criteria together with positive smear findings of their institution also showed high false negative rates of 15.5% with microscopic smear review rates of 37.3%. The authors have recommended developing and validating institution-specific review criteria in order to decrease false negative results to an acceptable and safe rate for patients.

In an attempt to develop personalised criteria for microscopic smear review, Wei *et al*<sup>4</sup> first verified the criteria suggested by the ICGHR, for 4 series of hematology analysers, Siemens Advia 2120, Sysmex XE-2100, Sysmex XT-1800i and Sysmex XS-8000i, in order to meet their requirement. Out of the samples analysed for, false negative rates for all their analysers was <3% and the microscopic

smear review rates were between 30 and 40%. Based on these results they adjusted their rules to reduce false negative rates and microscopic smear review rates. The authors suggested that all laboratories should have their own optimised criteria for the smear review and that these criteria can be based on the criteria established by ICGHR. They also suggested that the criteria should be improved continuously once or more times a year depending on the laboratory's requirements.

Eldanasoury AS *et al*<sup>9</sup> performed a similar study on the Beckman Coulter LH750 analyser to validate the ICGHR criteria and performed the study alongside their own laboratory criteria. They found that the peripheral smear review rate was significantly reduced by applying the ICGHR criteria. Recommendations given by the authors were to optimize the review criteria keeping in mind the population served and the type of analysers used in the laboratory with the aim to reduce MSR without overlooking important diagnostic information. This will, in turn, improve the efficiency and reliability of the CBC results directly released without a smear review.

Pratumvinit *et al*<sup>3</sup> also performed a study for validation and optimisation of criteria for MSR with Coulter LH750 and Sysmex XE-5000 hematology analysers. The false negative rate was 2.22% with the ICGHR criteria and 8.09% with their laboratory criteria. Their review rate was 29.33% with the ICGHR criteria and 22.37% with their laboratory criteria. The authors recommend each laboratory to first verify the criteria for smear review, based on the ICGHR, and to further optimize them to maximize efficiency.

Kim *et al*<sup>5</sup> performed a comparative study of the rates of MSR using the ICGHR guidelines with 3 different AHAs, the Unicel DxH 800, ADVIA 2120i, and XE 2100. Their false-negative rates were higher than the recommended cut-offs. The authors found that slide review rates had distinct characteristics among different

analysers and suggested that each individual laboratory should select the most appropriate analyser for analysis based on clinical characteristics. Analysers with high sensitivity may be more beneficial for screening patients in outpatient setting and analysers with high specificity can be helpful in inpatient settings for efficient patient care.

Despite the various problems associated with manual peripheral smears, most clinicians still consider the manual WBC differential count effective at least as a source of collateral information even though the 100-cell differential count is often criticized for its statistical shortcomings. With the advent of the AHAs, the number of blood smear examinations performed by each laboratory is now influenced by additional specific criteria developed by each individual laboratory, the reliability of the automated flagging system, the daily workload and the number of trained staff. The ICGHR suggest that each individual laboratory develop their own set of criteria which may include numerical results and qualitative flags generated by the AHA.<sup>1,6,23</sup>



## **MATERIAL AND METHODS**

### **Study Samples:**

A prospective cross-sectional comparative study was performed on whole blood samples sent for complete blood count testing in the central laboratory of the Department of Pathology in B.L.D.E.U.'s Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapur. The study included blood samples collected by systematic random sampling. The samples collected included both inpatients and outpatients from all the departments in the hospital. The study period was from 1<sup>st</sup> December 2014 to 30<sup>th</sup> June 2016.

### **Method of collection of data:**

Under aseptic precautions, 2ml of venous blood was collected from the antecubital vein into K3 EDTA (tripotassium salt of ethylene diamine tetraacetic acid) vacutainers and mixed well by gentle inversion. Using systematic random sampling, every day first 10 samples were collected from the daily workload, keeping in mind the inclusion criteria and exclusion criteria for this study, to evaluate the effectiveness of the ICGHR criteria for MSR. The samples were analysed within 1 hour of collection. Analysis of all the blood samples was done using the 6 part differential automated haematology analyser, Sysmex XN-1000. Procedures for quality control and quality assurance were followed during the entire period of this study.

Thin blood smears were then prepared for all the samples included in the study. The smears were stained with Leishman stain and MSR was performed to identify morphological abnormalities, immature cells and to confirm the results produced by the analyser. A 100-cell manual differential count was also done for all the samples in the study. Each sample was reviewed according to the adapted ICGHR

criteria and the laboratory criteria. A rule in the criteria would be triggered when the result was beyond the specified range and/or a specified flag appeared.

**Inclusion Criteria:**

Randomly collected blood samples from both inpatients and outpatients sent for CBC testing.

**Exclusion Criteria:**

Samples showing sampling errors such as inappropriate blood to anticoagulant proportion, tiny clots or inadequate blood sample. Samples from the paediatric population were also excluded.

**Review Criteria and Positive Smear Criteria:**

**A) Review Criteria:**

Adaptations were made to the ICGHR criteria as per Comar *et al*<sup>2</sup> (Table 1 and 2) because of limitations in the hospital's instrument and record systems. The main adaptations made were with regard to delta check rules and rules for reticulocytes. Comar *et al*<sup>2</sup>, who also had limitations in their hospital electronic record system and interfacing systems, adapted their review criteria from the ICGHR criteria itself. The instrument results were also reviewed according to our hospital's laboratory criteria.

**Table 1: Adapted ICGHR review criteria and our laboratory criteria for automated complete blood counts – Screening criteria**

S. No.	Parameter	Adapted ICGHR Criteria	Laboratory Criteria
<b>SCREENING CRITERIA</b>			
<b>RBC Parameters</b>			
1	Haemoglobin (HB)	<7 or >18.5 g/dL	<11 or >17 g/dL
2	Mean Corpuscular Volume (MCV)	<75 or >105 fL	<80 or >100 fL
3	Mean Corpuscular Hemoglobin Concentration (MCHC)	<30 or >36.5 g/dL	<31.5 or >35.5 g/dL
4	RDW-CV (Red cell Distribution Width – Coefficient of Variation)	>22%	>14%
5	Nucleated RBC (nRBC)	Any value	Any value
<b>WBC Parameters</b>			
6	Total WBC count (TC)	<4 or >30 x 10 <sup>3</sup> /μL	<4 or >11 x 10 <sup>3</sup> /μL
7	Absolute Neutrophil count (AN)	<1 or >20 x 10 <sup>3</sup> /μL	<2 or >7 x 10 <sup>3</sup> /μL
8	Absolute Lymphocyte count (AL)	>5 x 10 <sup>3</sup> /μL	<1 or >3 x 10 <sup>3</sup> /μL
9	Absolute Monocyte count (AM)	>1.5 x 10 <sup>3</sup> /μL	>1 x 10 <sup>3</sup> /μL
10	Absolute Eosinophil count (AE)	>2 x 10 <sup>3</sup> /μL	>0.50 x 10 <sup>3</sup> /μL
11	Absolute Basophil count (AB)	>0.5 x 10 <sup>3</sup> /μL	>0.1 x 10 <sup>3</sup> /μL
<b>Platelet Parameters</b>			
12	Platelet count	<100 or >1000 x 10 <sup>3</sup> /μL	<150 or >400 x 10 <sup>3</sup> /μL
13	Mean Platelet Volume (MPV)	<5 or ≥12.5 fL	<7.4 or >11.4 fL

**Table 2: Adapted ICGHR review criteria and our laboratory criteria for automated complete blood counts – Suspect flags**

S. No.	Parameter	Adapted ICGHR Criteria	Laboratory Criteria
<b>SUSPECT FLAGS</b>			
<b>WBC Suspect flags</b>			
14	Immature Granulocyte (IG)	Flag	Flag
15	Left Shift	Flag	Flag
16	Atypical Lymphocyte	Flag	Flag
17	Abnormal Lympho/ Blast	Flag	Flag
18	nRBC	Flag	Flag
<b>RBC Suspect flags</b>			
19	Dimorphic Population	Flag	Flag
20	Fragments?	Flag	Flag
21	Turbidity/ Hemoglobin interference?	Flag	Flag
22	Hemoglobin defect?	Flag	Flag
<b>Platelet Suspect flags</b>			
23	Platelet clumps	Flag	Flag
24	Platelet flags (except platelet clumps)	Flag	Flag
25	(*)	This symbol beside the counts on the read out indicates that automated counts are not reliable.	
26	---	This symbol beside the counts on the read out indicates that automated counts are not available for the sample in question.	

The laboratory criteria have been followed as per the normal reference ranges specified in Dacie<sup>24</sup> and Wintrobe<sup>25</sup>.

**B) Positive Smear criteria:**

Criteria for positive smear followed in this study are as shown in Table 3 and 4.

**Table 3: Criteria for a positive smear – Based on morphology.**

<b>BASED ON MORPHOLOGY</b>	
<b>RBC Morphology</b>	Anisocytosis $\geq 2+$
	Hypochromia $\geq 2+$
	Macrocytes $\geq 2+$
	Microcytes $\geq 2+$
	Elliptocytes $\geq 3+$
	Stomatocytes $\geq 3+$
	Codocytes $\geq 2+$
	Dacrocytes $\geq 2+$
	Schistocytes $\geq 1+$
	Acanthocytes $\geq 2+$
	Dreopanocytes present
	Spherocytes $\geq 1+$
	Howell-Jolly present
	Cabot ring present
	Basophilic stippling $\geq 1+$
	Rouleaux present
	Polychromatophilia $\geq 2+$
	RBC agglutination present
Hemoglobin C crystal present	
Hematozoa present	
<b>Platelet Morphology</b>	Giant Platelets $\geq 1+$
	Degranulated platelets present
	Gray platelets present
	Platelet aggregates present
<b>WBC Morphology</b>	Dohle bodies $\geq 1+$
	Toxic granulations $\geq 1+$
	Cytoplasmic Vacuoles $\geq 1+$
	Hypersegmented neutrophils
	Hyposegmented neutrophils $\geq 2+$
	Neutrophil hypo/ degranulation present
	Auer rod present
	Pseudo-pelgerhuet present
Dysplastic cells present	

**Table 4: Criteria for a positive smear – Based on counts of abnormal cell types**

<b>BASED ON COUNTS OF ABNORMAL CELL TYPES</b>	
Blasts	$\geq 1$
Metamyelocytes	$\geq 2$
Myelocytes/ Promyelocytes	$\geq 1$
Band forms	$> 8$
Atypical Lymphocytes	$\geq 5$
Prolymphocytes	$\geq 1$
Nucleated RBCs	$\geq 1/100$ leukocytes
Plasma cells	$\geq 1$

Adaptations to certain rules such as suspect flags for RBC lyse resistance were made in this study. Taking into consideration the recommendations made by the International Committee for Standardisation in Hematology (ICSH) in 2015 on the nomenclature and grading of peripheral blood cells, certain modifications were made to the positive smear criteria which included poikilocytosis $\geq 2+$ , polylobocytes $\geq 1+$ , microplatelets and platelet anisocytosis.<sup>26</sup>

**Sample classification criteria:**

The results from the Sysmex XN-1000 automated hematology analyser were compared with the findings on the peripheral smear for each sample. The samples were then classified as follows<sup>2</sup>:

A sample was classified as true positive (TP) if it was positive for the screening criteria with positive findings on the peripheral smear.

A sample was classified as false positive (FP) if it was positive for screening criteria with no abnormal findings on the peripheral smear.

A sample was classified as false negative (FN) if it was negative for screening criteria but with abnormal findings on the peripheral smear.

A sample was classified as true negative (TN) if it was negative for both screening criteria and MSR.

### **Statistical Analysis:**

Tabulation of data was done using Microsoft Excel software. True positive, true negative, false positive and false negative rates, efficiency, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and microscopic smear review rates (MRR) for both the adapted ICGHR criteria and the laboratory criteria were calculated and truth tables were prepared accordingly. The various parameters that were calculated are as follows<sup>2</sup>:

1. Sensitivity (%) =  $[\text{TP}/(\text{TP}+\text{FN})] \times 100$ ,
2. Specificity (%) =  $[\text{TN}/(\text{TN}+\text{FP})] \times 100$ ,
3. Positive predictive value (%) =  $[\text{TP}/(\text{TP}+\text{FP})] \times 100$ ,
4. Negative predictive value (%) =  $[\text{TN}/(\text{TN}+\text{FN})] \times 100$ ,
5. Efficiency (%) =  $[(\text{TP}+\text{TN})/(\text{TP}+\text{FP}+\text{FN}+\text{TN})] \times 100$  and
6. Microscopic review rate (%) =  $[(\text{TP}+\text{FP})/(\text{TP}+\text{FP}+\text{FN}+\text{TN})] \times 100$ .

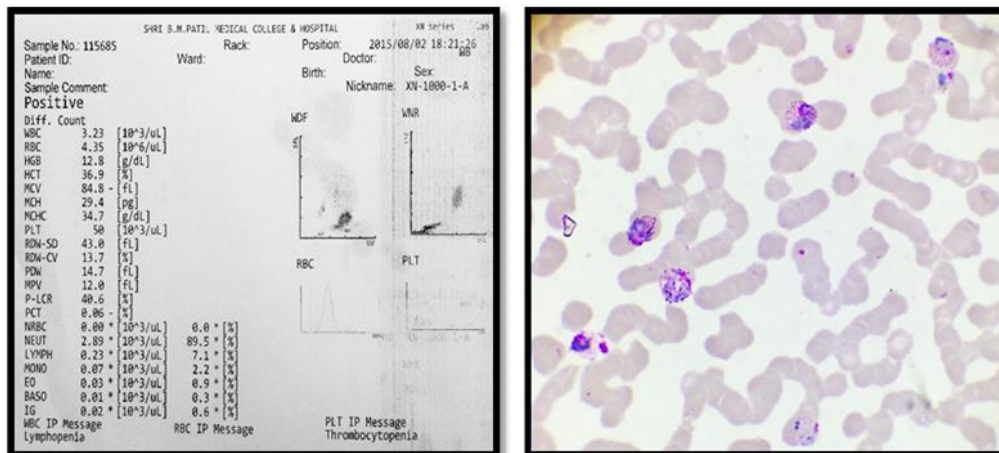
Further statistical analysis was performed using tests of proportion to compare the different performance specifications between both sets of criteria. A p value of <0.05 was considered statistically significant.



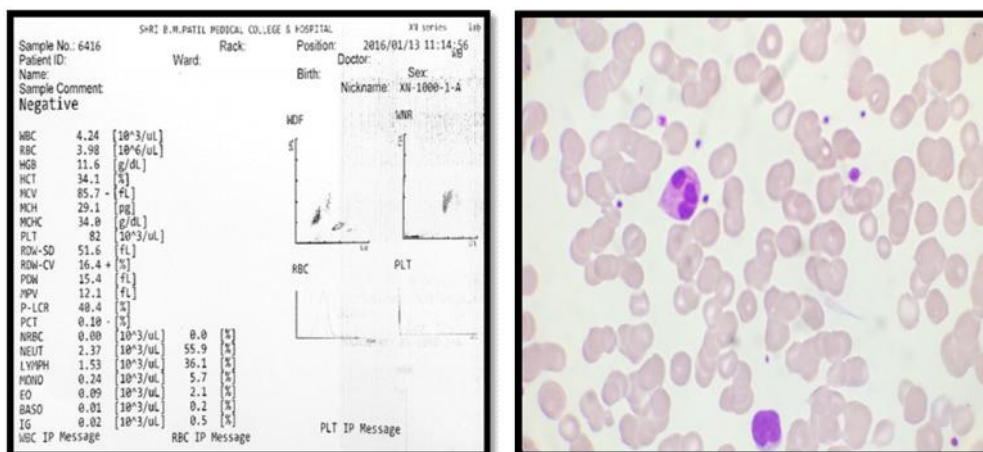
Sysmex XN-1000 automated hematology analyser in laboratory.



## PHOTOMICROGRAPHS

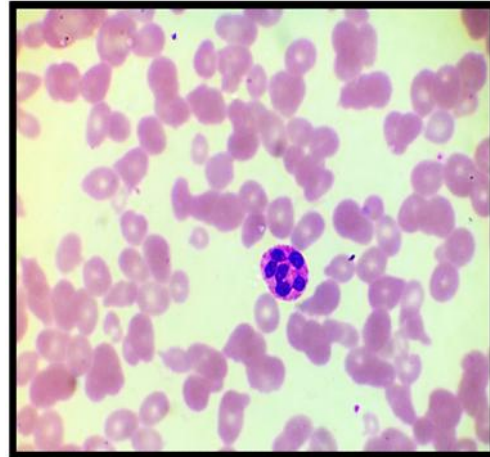
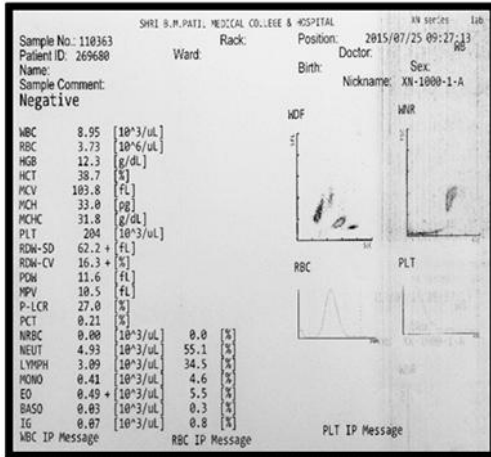


A sample showing trophozoites of plasmodium vivax - True positive case for both our laboratory and the ICGHR criteria (1000x).

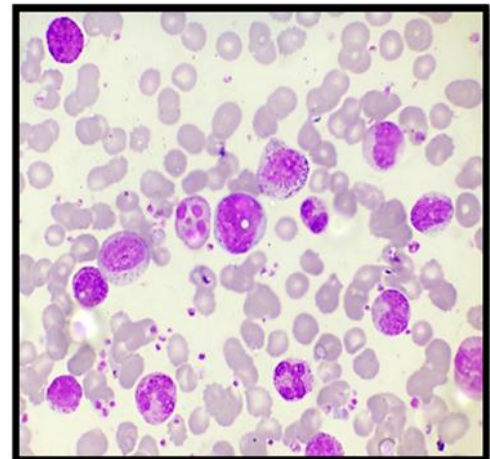
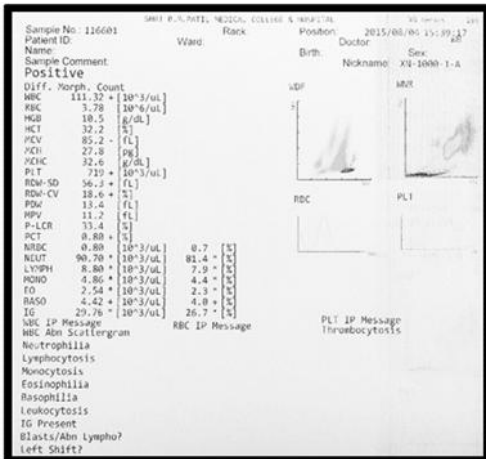


A sample with platelet count  $<100 \times 10^3/\mu\text{L}$  on the automated analyser with no morphological abnormalities on smear review (manual platelet count was  $150 \times 10^3/\mu\text{L}$ ) – False positive for both our laboratory and the ICGHR criteria (1000x).

## PHOTOMICROGRAPHS



A sample showing hyper-segmented neutrophils – a true positive case for our laboratory criteria but false negative for the ICGHR criteria (1000x).

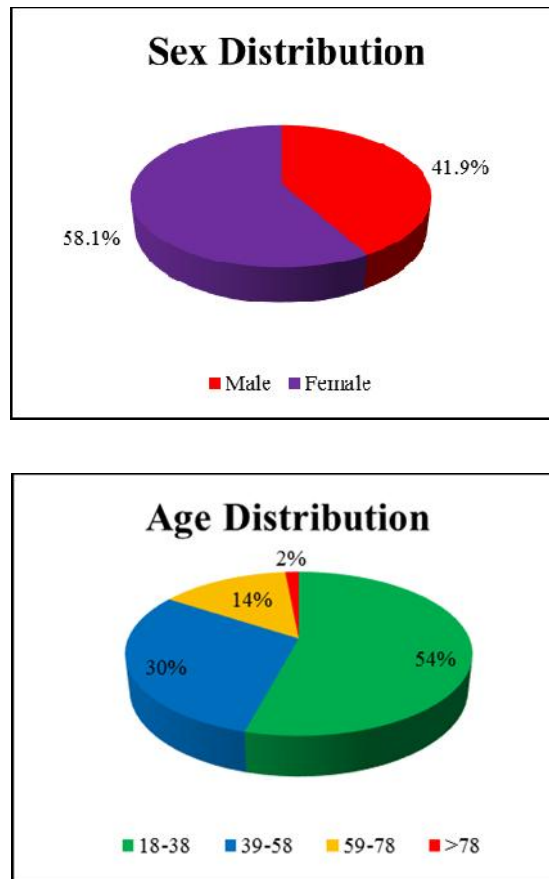


A sample of chronic myelogenous leukemia - True positive case for both our laboratory and the ICGHR criteria (1000x).

## RESULTS

A total of 860 samples were analysed using the Sysmex XN-1000 hematology analyser in the present study. Of these 360 were male (41.86%) and 500 were female (58.14%) with a male to female ratio of 0.72. The age ranged from 18 to 98 years and majority of the patients were between 18 and 38 years.

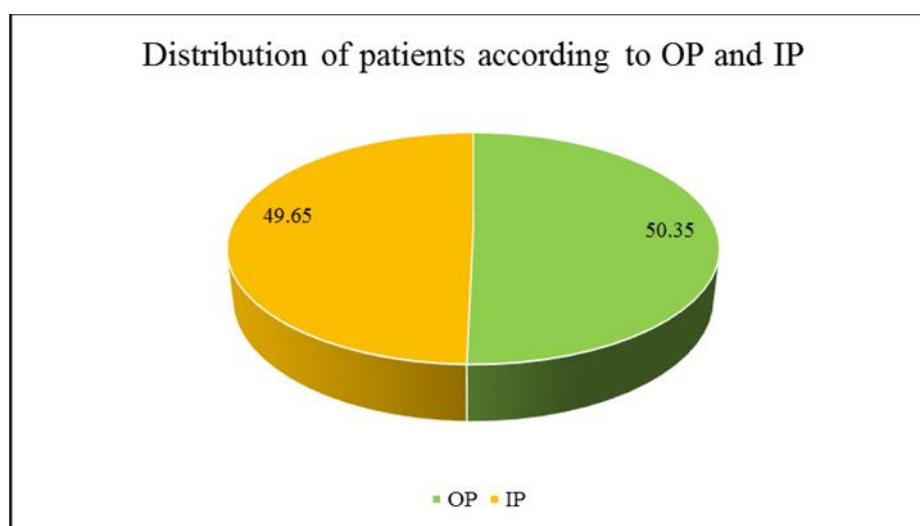
**Figure 1: Distribution of cases according to age and sex of the patients**



**Table 5: Distribution of cases according to age and sex in years**

Age (in years)	Male		Female	
	N	%	N	%
18-38	141	39.17%	325	65%
39-58	138	38.33%	120	24%
59-78	72	20%	51	10.2%
>78	09	2.50%	04	0.8%
Total	360	100.0%	500	100.0%

**Figure 2: Distribution of cases according to Outpatients (OP) and Inpatients (IP)**



Majority of the patients were from the department of Medicine (41.7%), followed by Obstetrics and Gynaecology (22%) and Surgery (11.2%). The number of samples from outpatients (433 samples; 50.35%) were slightly higher when compared with the inpatients (427 samples; 49.65%).

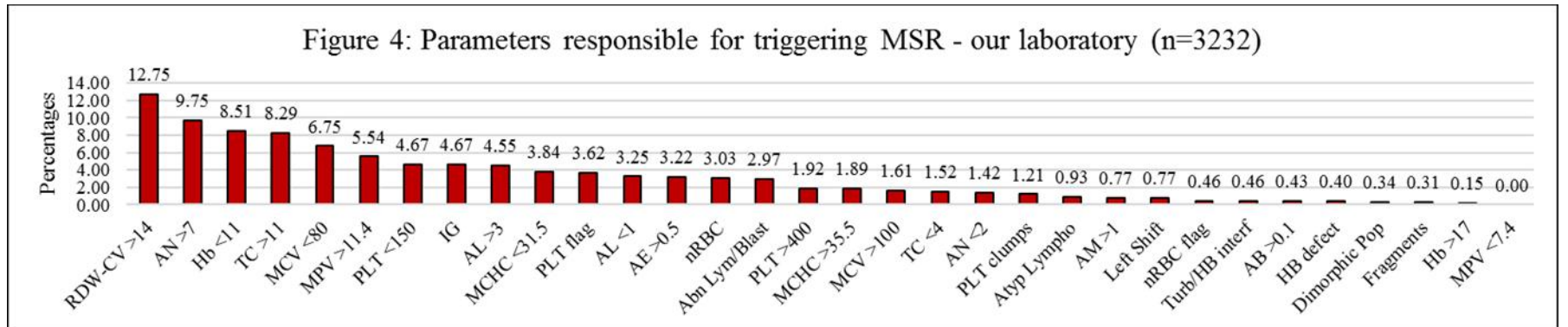
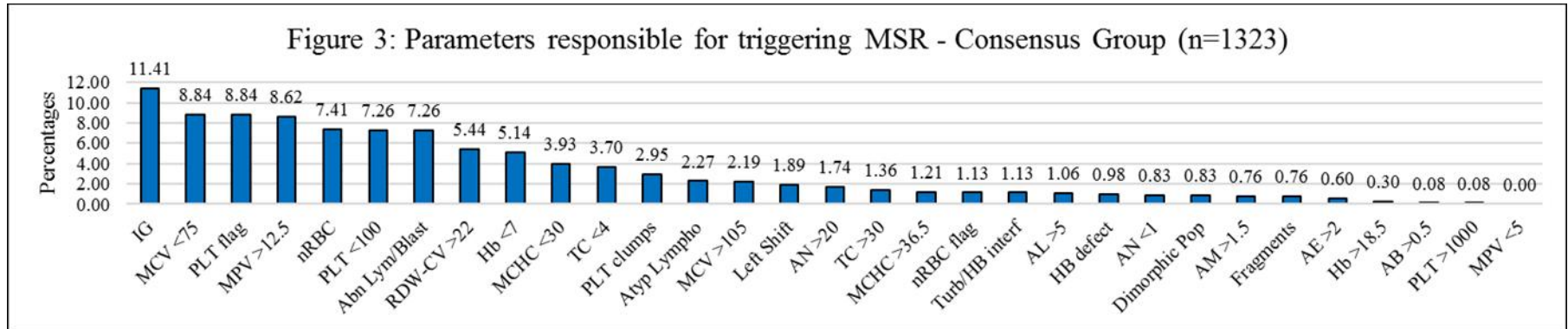
**Table 6: Number of positive and negative samples according to the ICGHR and our laboratory criteria**

		REVIEW CRITERIA					
		Adapted ICGHR Criteria			Laboratory Criteria		
		Positive	Negative	Total	Positive	Negative	Total
<b>SMEAR</b>	Positive	341	77	418	413	5	418
<b>FINDINGS</b>	Negative	68	374	442	259	183	442
	Total	409	451	<b>860</b>	672	188	<b>860</b>

Out of 860 samples analysed in this study, 409 samples (47.56%) were positive and 451 samples (52.44%) were negative for the adapted ICGHR review criteria. Out of the total number of samples positive for the review criteria, 341 samples out of 409 (83.37%) had positive smear findings and 68 samples (16.63%) were negative for smear findings. Among the samples that did not trigger any review criteria (451 samples), 374 (82.92%) were truly negative on peripheral smear examination but 77 out of 451 (17.07%) had positive smear findings. A MRR of 47.56% was observed using ICGHR review criteria.

Using our laboratory review criteria, 672 out of 860 were positive (78.14%) and 188 were negative (21.86%). Of all the positive samples, 413 samples (61.46%) showed positive smear findings and 259 samples out of 672 (38.54%) were negative for smear findings. Five samples (2.66%) out of the 188 samples negative for the laboratory review criteria showed positive smear findings, i.e., morphological abnormalities and 183 samples (97.34%) were negative for both our laboratory's review criteria and positive smear criteria. A higher MRR of 78.14% was observed with the use of the laboratory criteria.

**Figures 3 and 4: Percentages of all the parameters that triggered MSR using the adapted ICGHR and laboratory criteria**



The samples that required review were further analysed according to the criteria triggered, i.e. the total number of triggers for each parameter was analysed regardless of the other parameters triggered in that sample, in order to analyse most common reasons for MSR. With the ICGHR criteria, the suspect flags (522 samples; 39.46%) were most commonly triggered followed by RBC (456 samples; 34.46%), platelet (211 samples; 15.97%) and WBC parameters (134 samples; 10.11%). With our laboratory criteria, the RBC parameters (1245 samples; 38.52%) were the most common causes for positive samples followed by WBC parameters (1073 samples; 33.20%), suspect flags (522 samples; 16.15%) and platelet parameters (392 samples; 12.13%).

The three most common flags triggered by the adapted ICGHR criteria contributing to MSR included IG suspect flag (151 samples; 11.41%) followed by MCV <75fL (117 samples; 8.84%) and platelet flags excluding platelet clumps (117 samples; 8.84%).

The three most common flags triggered by our laboratory criteria included RDW-CV >14% (412 samples; 12.75%), AN >7 x 10<sup>3</sup>/μL (315 samples; 9.75%) and HB <11gm/dL (275 samples; 8.51%). The percentages indicated represent the proportion of total number positive occurrences for the ICGHR criteria (1323 positive occurrences) and our laboratory criteria (3232 positive occurrences) respectively.

**Table 7: RBC parameters requiring MSR by each of the triggering criteria of the ICGHR and our laboratory**

<b>RBC Parameters</b>	<b>Adapted ICGHR Criteria (total number of positive occurrences =1323)</b>			<b>Laboratory criteria (total number of positive occurrences =3232)</b>		
	<b>Rule triggered</b>	<b>N</b>	<b>%</b>	<b>Rule triggered</b>	<b>N</b>	<b>%</b>
<b>Hb</b>	<7 g/dL	68	5.14	<11 g/dL	275	8.51
	>18.5 g/dL	04	0.30	>17 g/dL	05	0.15
<b>MCV</b>	<75 fL	117	8.84	<80 fL	218	6.75
	>105 fL	29	2.19	>100 fL	52	1.61
<b>MCHC</b>	<30 g/dL	52	3.93	<31.5 g/dL	124	3.84
	>36.5 g/dL	16	1.21	>35.5 g/dL	61	1.89
<b>RDW-CV</b>	>22%	72	5.44	>14%	412	12.75
<b>nRBC</b>	Any value	98	7.41	Any value	98	3.03
<b>Total</b>		<b>456</b>	<b>34.46</b>		<b>1245</b>	<b>38.52</b>

The most common triggers for positive MSR among the RBC parameters include MCV <75fL (117 samples; 8.84%), nRBC (98 samples; 7.41%) and RDW-CV >22% (72 samples; 5.44%) with the ICGHR criteria. With our laboratory criteria, the most common triggers were RDW-CV >14% (412 samples; 12.75%) followed by HB <11 g/dL (275 samples; 8.51%) and MCV <80 fL (218 samples; 6.75%).



**Table 8: WBC and platelet parameters requiring MSR by each of the triggering criteria of the ICGHR and our laboratory**

WBC and Platelet Parameters	Adapted ICGHR Criteria (total number of positive occurrences =1323)			Laboratory criteria (total number of positive occurrences =3232)		
	Rule triggered	N	%	Rule triggered	N	%
TC	<4 x 10 <sup>3</sup> /μL	49	3.70	<4 x 10 <sup>3</sup> /μL	49	1.52
	>30 x 10 <sup>3</sup> /μL	18	1.36	>11 x 10 <sup>3</sup> /μL	268	8.29
AN	<1 x 10 <sup>3</sup> /μL	11	0.83	<2 x 10 <sup>3</sup> /μL	46	1.42
	>20 x 10 <sup>3</sup> /μL	23	1.74	>7 x 10 <sup>3</sup> /μL	315	9.75
AL	-	-	-	<1 x 10 <sup>3</sup> /μL	105	3.25
	>5 x 10 <sup>3</sup> /μL	14	1.04	>3 x 10 <sup>3</sup> /μL	147	4.55
AM	>1.5 x 10 <sup>3</sup> /μL	10	0.76	>1 x 10 <sup>3</sup> /μL	25	0.77
AE	>2 x 10 <sup>3</sup> /μL	8	0.60	>0.5 x 10 <sup>3</sup> /μL	104	3.22
AB	>0.5 x 10 <sup>3</sup> /μL	1	0.08	>0.1 x 10 <sup>3</sup> /μL	14	0.43
Platelet count	<100 x 10 <sup>3</sup> /μL	96	7.26	<150 x 10 <sup>3</sup> /μL	151	4.67
	>1000 x 10 <sup>3</sup> /μL	1	0.08	>400 x 10 <sup>3</sup> /μL	62	1.92
MPV	<5 fL	0	0	<7.4 fL	0	0
	≥12.5 fL	114	8.63	>11.4 fL	179	5.54
<b>Total</b>		<b>345</b>	<b>26.08</b>		<b>1465</b>	<b>45.33</b>

The most common trigger for MSR among the WBC parameters included total count of  $<4 \times 10^3/\mu\text{L}$  (49 samples; 3.70%), absolute neutrophil count  $>20 \times 10^3/\mu\text{L}$  (23 samples; 1.74%) and total count  $>30 \times 10^3/\mu\text{L}$  (18 samples; 1.36%) using the ICGHR criteria. The most common trigger for MSR with our laboratory criteria on the other hand was absolute neutrophil count  $>7 \times 10^3/\mu\text{L}$  (315 samples; 9.75%). Total count of  $>11 \times 10^3/\mu\text{L}$  (268 samples; 8.29%) and absolute lymphocyte count  $>3 \times 10^3/\mu\text{L}$  (147 samples; 4.55%) were also other common triggers with our laboratory criteria.

Among the platelet parameters, high mean platelet volume was most frequently triggered for both the ICGHR criteria ( $\text{MPV} \geq 12.5 \text{ fL}$ ) and our laboratory criteria ( $\text{MPV} > 11.4 \text{ fL}$ ) with 114 samples (8.62%) and 179 samples (5.54%) respectively.

**Table 9: Suspect flags requiring MSR by each of the triggering criteria of the ICGHR and our laboratory**

<b>Suspect flags</b>	<b>N</b>	<b>% (ICGHR; total number of positive occurrences =1323)</b>	<b>% (Laboratory; total number of positive occurrences =3232)</b>
IG	151	11.41	4.67
Left Shift	25	1.89	0.77
Atypical Lymphocyte	30	2.27	0.93
Abnormal Lympho/ Blast?	96	7.26	2.97
nRBC flag	15	1.13	0.46
Dimorphic Population	11	0.83	0.34
Fragments?	10	0.76	0.31
Turbidity/ Hemoglobin interference?	15	1.13	0.46
Hemoglobin defect?	13	0.98	0.40
Platelet clumps	39	2.95	1.21
Platelet flags (except platelet clumps)	117	8.85	3.63
<b>Total</b>	<b>522</b>	<b>39.46</b>	<b>16.15</b>

The suspect flags for both the ICGHR and our laboratory criteria were the same but as the total number of positive occurrences in either criteria was different, the proportion of each of the suspect flags responsible for triggering MSR was varied.

The most common suspect flag triggered for MSR was the immature granulocyte flag (151 samples) followed by the platelet flag excluding the platelet clumps flag (117 samples) and the abnormal lympho/blast flag (96 samples).

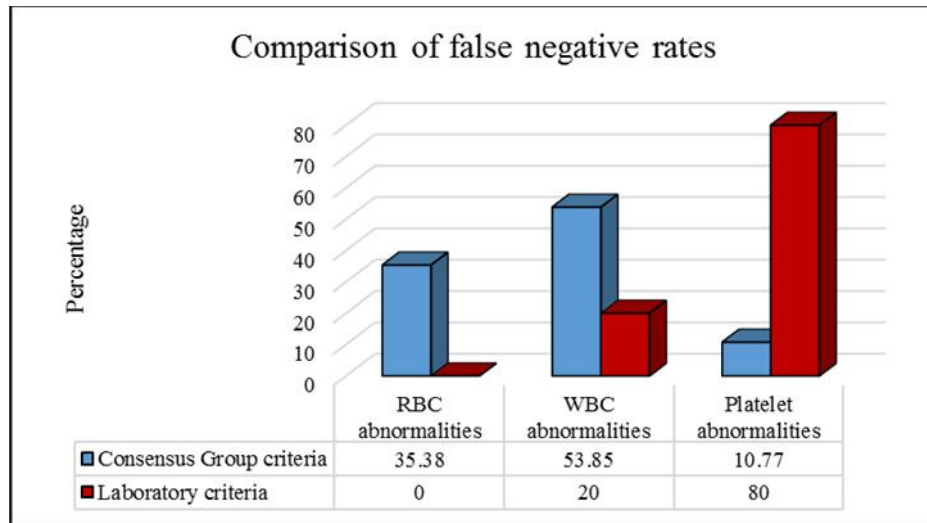
**Table 10: Top five causes for false positive results with the ICGHR and our laboratory criteria**

Criteria	Rate of occurrence	
	N	%
<b>ICGHR criteria (total number of false positive occurrences=88)</b>		
Abnormal lymphocyte/Blast flag	23	26.14
Immature granulocyte flag	15	17.05
MPV <5.0fL or $\geq$ 12.5fL	7	7.95
Platelet flag (except platelet clump)	5	5.68
Total count <4.0 x 10 <sup>3</sup> / $\mu$ L or >30.0 x 10 <sup>3</sup> / $\mu$ L	5	5.68
<b>Laboratory criteria (total number of false positive occurrences=742)</b>		
AN <2 or >7 x 10 <sup>3</sup> / $\mu$ L	125	16.85
TC <4 or >11 x 10 <sup>3</sup> / $\mu$ L	100	13.48
RDW-CV >14%	96	12.94
AL <1 or >3 x 10 <sup>3</sup> / $\mu$ L	94	12.67
AE >0.5 x 10 <sup>3</sup> / $\mu$ L	64	8.63

The abnormal lympho/blast flag was the most common cause for false positive results (23 samples; 26.14%) followed by the immature granulocyte flag (15 samples; 17.05%) with the ICGHR criteria. The suspect flags alone were responsible for 59.09% of false positive results. With our laboratory criteria, the most common cause

for false positivity was absolute neutrophil count  $<2$  or  $>7 \times 10^3/\mu\text{L}$  (125 samples; 16.85%) followed closely by total count  $<4$  or  $>11 \times 10^3/\mu\text{L}$  (100 samples; 13.48%).

**Figure 5: Comparison between the false negative rates of the ICGHR and our laboratory criteria**



**Table 11: Causes for false negative occurrences with ICGHR and our  
laboratory criteria**

Smear findings	ICGHR Group criteria		Laboratory criteria	
	N	%	N	%
<b>RBC abnormalities</b>	<b>46</b>	<b>35.38</b>	<b>0</b>	<b>0</b>
Anisocytosis	11	8.46	0	0
Microcytosis	6	4.62	0	0
Hypochromia	20	15.38	0	0
Macrocytosis	8	6.15	0	0
Codocytes	1	0.77	0	0
<b>WBC abnormalities</b>	<b>70</b>	<b>53.85</b>	<b>1</b>	<b>20</b>
Toxic changes (Toxic granules, toxic vacuolations, Dohle bodies)	50	38.46	0	0
Hypersegmented neutrophils	5	3.85	0	0
Myelocytes, metamyelocytes, band forms	14	10.77	0	0
Atypical lymphocytes	1	0.77	1	20
<b>Platelet abnormalities</b>	<b>14</b>	<b>10.77</b>	<b>4</b>	<b>80</b>
Platelet clumps	14	10.77	4	80
<b>Total number of false negative occurrences</b>	<b>130</b>	<b>100</b>	<b>5</b>	<b>100</b>
<b>Total number of false negative samples</b>	<b>77</b>	<b>8.95</b>	<b>5</b>	<b>0.58</b>

False negative analysis revealed that WBC morphology was the most frequent cause of false negativity with the ICGHR criteria followed by RBC morphology but platelet morphology was the most common cause of false negativity in our laboratory criteria. Toxic changes inclusive of toxic granules, toxic vacuolations and dohle bodies were the most commonly missed with the ICGHR criteria whereas our laboratory criteria did not miss any of these cases. No cases with blasts were missed by either criteria.

Our laboratory criteria missed 4 samples with platelet clumps out of a total of 5 false negative samples while the ICGHR criteria missed 14 samples with platelet clumps. With regard to RBC morphology, samples with hypochromia (20 samples) were most commonly missed using the ICGHR criteria followed by anisocytosis (11 samples). One sample with atypical lymphocytes was missed by our laboratory criteria.

The performance of the ICGHR criteria for MSR was compared with that of our laboratory criteria in the form of the “Truth Table”.

**Table 12: The ‘Truth Table’ comparing of performance of the ICGHR criteria with our laboratory criteria**

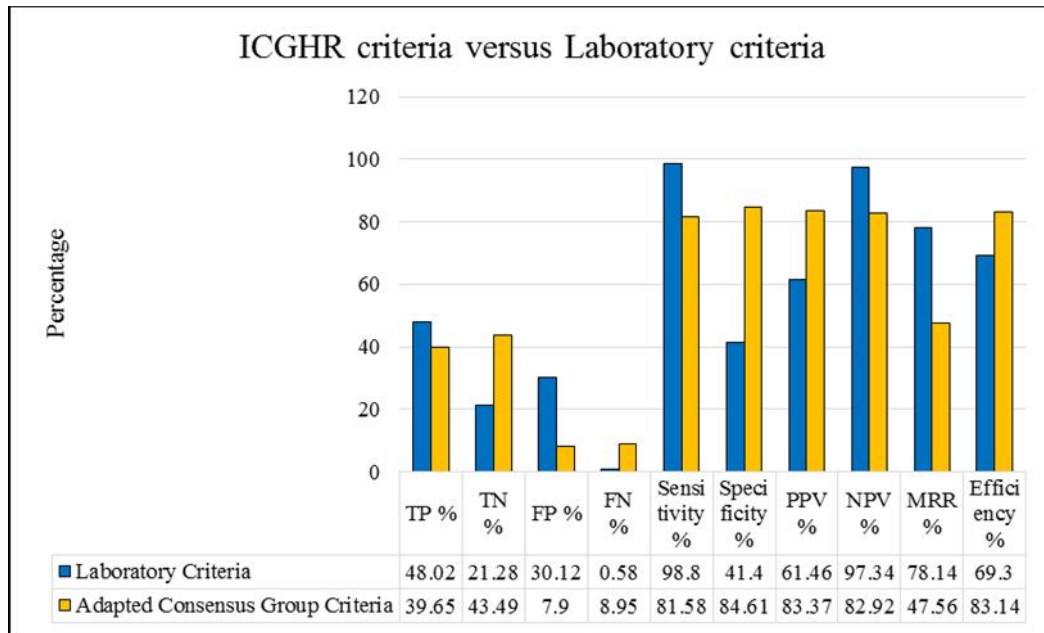
<b>Parameters</b>	<b>Laboratory Criteria</b>	<b>Adapted ICGHR Criteria</b>	<b>p value</b>
True Positive (TP) %	48.02	39.65	<0.001*
True Negative (TN) %	21.28	43.49	<0.001*
False Positive (FP) %	30.12	7.91	<0.001*
False Negative (FN) %	0.58	8.95	<0.001*
Sensitivity (%)	98.80	81.58	<0.001*
Specificity (%)	41.40	84.61	<0.001*
Positive Predictive Value (PPV) (%)	61.46	83.37	<0.001*
Negative Predictive Value (NPV) (%)	97.34	82.92	<0.001*
Microscopic Review Rate (MRR) (%)	78.14	47.56	<0.001*
Efficiency (%)	69.30	83.14	0.0203*

\*significantly different at 5% level of significance



All these parameters obtained from our laboratory criteria were significantly different from the ICGHR criteria with a p value <0.001.

**Figure 6: Comparison of the truth table values between the ICGHR and our laboratory criteria**



The true positive and false positive rates, sensitivity, negative predictive value and MRR were higher with our laboratory criteria than the ICGHR criteria. The false negative and true negative rates, specificity, positive predictive value and efficiency were higher with the ICGHR criteria when compared with our laboratory criteria.

## **DISCUSSION**

The commission on laboratory accreditation of the College of American Pathologists (CAP) recommends that each laboratory must establish certain criteria to determine when to perform MSR following automated blood count analysis.<sup>27</sup> The ICGHR presented a total of 41 rules to be used as guidelines for MSR following automated hematology analysis.<sup>1</sup> They also recommended the validation of these rules first, before implementing them for use on patient samples.<sup>1,3</sup> Our laboratory has a set of criteria which, however, has not been validated.

In the present study, apart from analysing the effectiveness of the adapted ICGHR criteria, these criteria were also compared with our current laboratory criteria. The use of the ICGHR criteria in the present study, generated a MRR of 47.56%. This MRR is comparable to the overall MRR of 46.06% as reported by Comar *et al*<sup>2</sup>, who applied the ICGHR criteria to results obtained by using the XE-2100D and XT-2000i hematology analysers. In the study done by Eldanasoury *et al*<sup>9</sup>, a MRR of 54.25% was obtained by applying the ICGHR criteria to blood sample analysis using the Beckman Coulter LH750.

Other investigators who have also applied the ICGHR criteria, however, have reported lower MRRs. Froom *et al*<sup>28</sup>, had a MRR of 13.9% on using ICGHR criteria with the Advia120 or Advia2120 analyser. Pratumvinit *et al*<sup>3</sup>, reported a MRR of 29.33% on applying ICGHR criteria on results obtained from the Sysmex XE-5000 and Coulter LH750 analysers. Kim *et al*<sup>5</sup> also applied the ICGHR criteria to 3 different automated analysers – Unicel DxH 800, ADVIA 2120i, and XE 2100. These analysers showed MRRs of 22.8%, 20.2%, and 28.6% respectively. Wei *et al*<sup>4</sup> had

MRRs of 37.94%, 37.94%, 35.56% and 33.44% for SIEMENS ADVIA 2120, Sysmex XE-2100, Sysmex XT-1800i and Sysmex XS-800i automated analysers respectively.

**Table 13: Comparison of the “Truth Tables” amongst different studies using the ICGHR criteria**

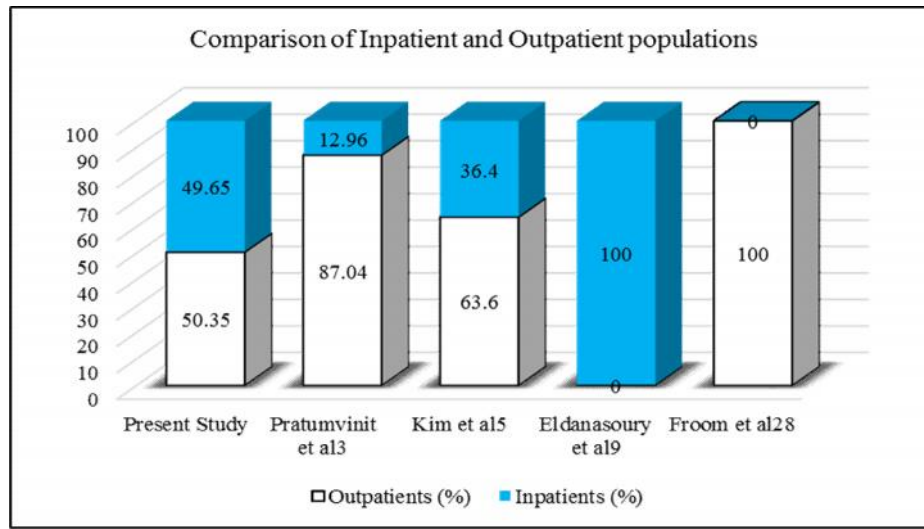
Parameters	Present study	Comar <i>et al.</i> <sup>2</sup>	Barnes <i>et al.</i> <sup>1</sup>	Eldanasoury <i>et al.</i> <sup>9</sup>	Pratumvinit <i>et al.</i> <sup>3</sup>	Kim <i>et al.</i> <sup>5</sup> (XE2100)	Wei <i>et al.</i> <sup>4</sup> (XE2100)
TP %	39.65	22.76	11.20	44.25	15.18	-	14.23
TN %	43.49	47.24	67.30	36.12	68.45	-	59.61
FP %	7.91	23.27	18.60	10.00	14.14	17.3	23.71
FN %	8.95	6.73	2.90	9.25	2.22	9.7	2.45
Sensitivity %	81.58	77.19	-	82.13	-	60.3	-
Specificity %	84.61	67.00	-	78.32	-	77.1	-
PPV %	83.38	49.45	-	81.56	-	-	-
NPV %	82.92	87.54	-	78.96	-	-	-
MRR %	47.56	46.03	-	54.25	29.33	-	37.94
Efficiency %	83.14	70.00	-	80.37	83.63	-	-

A MRR of 30% has been recommended by the American College of Pathologists.<sup>27</sup> The present study had a high MRR (47.56%) with the ICGHR criteria adapted as per Comar *et al.*<sup>2</sup>. The type of hospital along with the population attending for health care services varies from place to place. This can lead to differing results with the ICGHR’s smear review criteria if they have been introduced without validation.<sup>4</sup> Comar *et al.*<sup>2</sup>, stated that local peculiarities must be taken into account during the analysis of samples with positive smear findings so as not to overlook them.

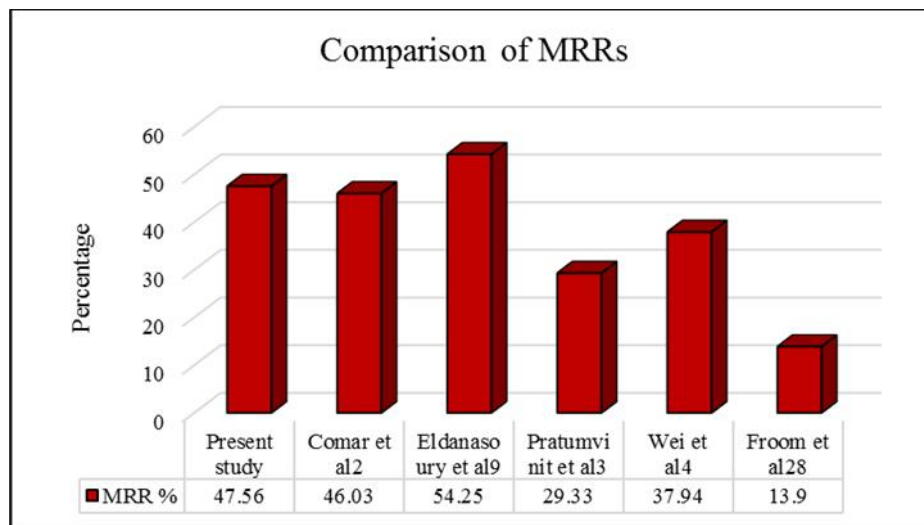
Our hospital serves both urban and rural population, a majority from rural background. Although our hospital provides a variety of clinical and laboratory services, most patients tend to present clinically in the late stage possibly due to low socioeconomic status. We have hence found a higher percentage of positive samples which in turn has led to high MRR. Out of the total number of positive samples in this study, 83.37% were true positive and only 16.63% were false positive samples using the ICGHR criteria. This indicates that most of the samples contributing to MRRs had morphologically abnormal cells which were picked up by both the automated analyser using the review criteria and further confirmed on MSR.

The MRR also depends on the patient composition, i.e., inpatients versus outpatients. This study was conducted on both inpatients and outpatients. Pratumvinit *et al*<sup>3</sup>, Kim *et al*<sup>5</sup>, Comar *et al*<sup>2</sup> and Wei *et al*<sup>4</sup> also conducted their study on inpatients and outpatients. Eldanasoury *et al*<sup>9</sup> conducted their study only on inpatients while Froom *et al*<sup>28</sup> conducted their study on outpatients. Out of all these studies Froom *et al*<sup>28</sup> had the lowest MRR of 13.9% and Eldanasoury *et al*<sup>9</sup> had the highest of 54.25% which can be explained by the patient composition included in their studies, i.e., only outpatient population and only inpatient population respectively.

**Figure 7: Comparison of inpatient and outpatient populations studied in various studies which may influence MRR**



**Figure 8: Comparison of MRRs among various studies using the ICGHR criteria**



Application of the delta check rules is another criterion that can lower the MRR. Due to limitations in the hospital's instrument and record systems, rules for delta check could not be applied in this study and all the patient's samples were considered first time samples. Studies done by Comar *et al*<sup>2</sup> also did not include delta

check rules. Their MRR correlated with that of our study. Froom *et al*<sup>28</sup> observed that the use of delta check lowered the MRR from 13.9% to 2.6%.

On applying our laboratory criteria, the MRR was 78.14%. This is much higher than the MRR of the ICGHR criteria (47.56%). Eldanasoury *et al*<sup>9</sup> reported similar results with their laboratory criteria with an MRR of 71% as compared to 54.25% with the ICGHR criteria. In their study, Eldanasoury *et al*<sup>9</sup> pointed out that their laboratory criteria were not validated and consequently could not be compared to the MRR reported by different authors who applied their own optimized laboratory criteria which was also the case in the present study. The higher MRR in this study using the laboratory criteria were due to a greater number of positive samples (672 samples) out of which 413 samples (61.46%) were true positive and 259 (38.54%) were false positive.

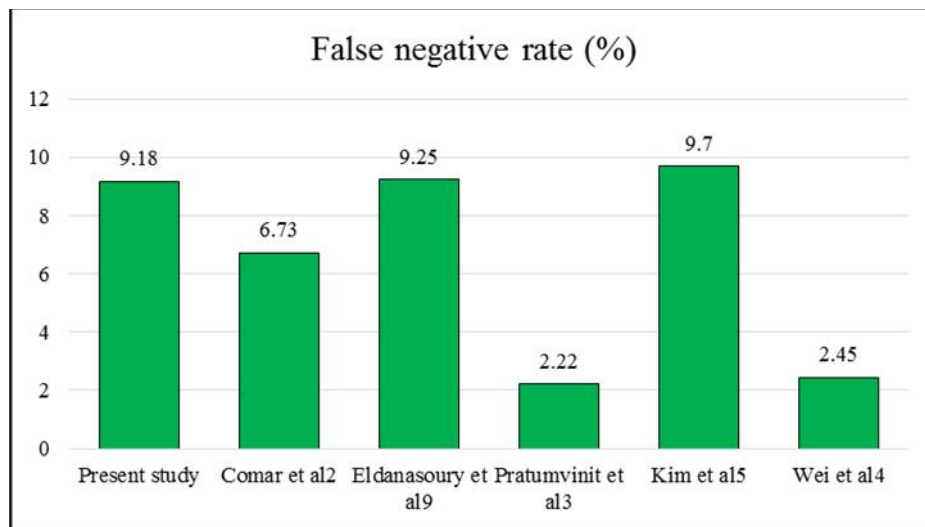
In order to obtain a comprehensive and effective evaluation of the review criteria, the false negative rate is of paramount importance. The false negative rate reveals the effectiveness with which the review criteria can screen samples with positive smear findings, i.e., samples with morphological abnormalities. Barnes *et al*<sup>1</sup> found that that false negative rates were 2.9% and 3.8% in laboratories using the ICGHR criteria and their own laboratory criteria respectively and recommended a maximum acceptable false negative rate of 5% in order to ensure patient safety.

The present study had a false negative rate of 8.95% using the ICGHR criteria and 0.58% using our laboratory criteria. The low false negative rate with our laboratory criteria, however, came at the expense of a high MRR. On analysis of the false negatives, platelet clump were the most common cause (4 samples) and only one sample with atypical lymphocytes was missed using our laboratory criteria. The

threshold cut offs for our laboratory criteria were more sensitive than the ICGHR criteria which would probably explain the low false negativity of the laboratory criteria.

With the ICGHR criteria, most of the false negative rates were contributed by WBC abnormalities which accounted for 53.85% of the false negative rate. Toxic changes (38.46%) followed by band forms, metamyelocytes and myelocytes (10.77%) were the most common causes for false negativity. No samples with WBC morphologic abnormalities were missed on using our laboratory criteria.

**Figure 9: Comparison of false negative rates among various studies using the ICGHR criteria.**



Comar *et al*<sup>2</sup> reported high false negative rate of 6.73% and also reported WBC abnormalities as the most common cause using the adapted ICGHR criteria considering both the ICGHR criteria for positive smear review and the Hospital de Clínicas da Universidade Federal do Paraná criteria for a positive smear, which incidentally, was followed in this study as the adapted ICGHR criteria. The WBC

abnormalities included most commonly left shift followed by atypical lymphocytes and toxic granulation with their hospital's positive smear criteria. In the study by the Barnes *et al*<sup>1</sup> also, the most common cause of false-negative results was the presence of immature granulocytes.

The ISLH recommends that the use of band cell counts should be in accordance to laboratory standard operating procedures.<sup>1</sup> In the present study, a band count of >8% was considered as positive. The same was followed by Comar *et al*<sup>2</sup> in their study. The clinical utility of the band count in patients greater than 3 months of age is debatable. Cornbleet<sup>29</sup> stated that “the band count is a nonspecific, inaccurate, and imprecise laboratory test”. Ward *et al*<sup>30</sup> also had similar views stating that there was no association between band level and inpatient mortality in those with sepsis. Anne Mare *et al*<sup>31</sup>, however, stated that increased number of band forms have diagnostic significance for sepsis, provided that measurements are not confined to patients with normal WBC counts.

False negative analysis of band counts in the present study revealed that band forms of >8% accounted for 5.38% of these samples. Comar *et al*<sup>2</sup> caution that technical limitations of the manual differential leukocyte count must be taken into account when interpreting results regarding band counts and variations occurring with age, gender, and conditions such as pregnancy and physical exercise must be kept in mind.

Eldanasoury *et al*<sup>9</sup> reported high false negative rates of 9.25% respectively and reported that their false negative rates were mainly related to RBCs abnormalities in both ICGHR and their laboratory criteria. Since they did not include band cell counts



as positive smear findings, it would possibly explain why majority of their false negative rates were due to RBC and not WBC abnormalities.

Kim SJ *et al*<sup>5</sup> also obtained high false-negative rates of 14.3%, 14.3%, and 9.7% for Unicel DxH 800, ADVIA 2120i, and XE 2100 automated analysers respectively. They reported that RBC morphology was the most common cause followed by platelet morphology. RBC morphology contributed to 35.38% of all the false negative samples in the present study using the ICGHR criteria. No samples with abnormal RBC morphology went undetected with our laboratory criteria.

Pratumvinit *et al*<sup>3</sup> had a lower false negative rate of 2.22% with the ICGHR criteria than with their own laboratory criteria (8.09%). They reported that platelet morphology was the most common cause of their false negative rates using the ICGHR criteria and RBC morphology was the most common using their laboratory criteria. A false negative rate of <3% was reported by Wei *et al*<sup>4</sup> by applying both the ICGHR to results obtained from 4 different analysers (Siemens Advia 2120, Sysmex XE-2100, Sysmex XT-1800i and Sysmex XS-800i).

Pratumvinit *et al*<sup>3</sup>, Kim SJ *et al*<sup>5</sup>, and Eldanasoury *et al*<sup>9</sup> found that no case of blast was missed by the ICGHR criteria or their own laboratory criteria. There were 5 cases of hematologic malignancy in the present study and none were missed by either the ICGHR criteria or our laboratory criteria. Conversely, in the study done by Wei *et al*<sup>4</sup>, two cases of acute leukemia on chemotherapy were missed on Sysmex XE-2100 and XT-1800i and one of them was missed on Sysmex XS-800i. False negative analysis by Comar *et al*<sup>2</sup> in their study revealed that one false negative sample contained blasts in a case of acute leukemia. Both authors stated that it was unacceptable to miss a case of serious hematology disease, whether on treatment or

undiagnosed. They recommended that each institution evaluate the need to perform MSR in all patients in the hematology unit even at the expense of an increased MRR. In order to achieve this, Wei *et al*<sup>4</sup> added a rule of “hematology department source” in the criteria of Sysmex series analysers so as to smear review all samples from hematology department. No serious hematology disease was missed after this addition. Consequently, there was a rise in the MRR with a reduction in the false negative rate.

The false negative rate is the inversely related to the negative predictive value. In the present study the negative predictive value was 82.92%. This means that in all the samples where the ICGHR criteria did not indicate the need for a MSR, 82.92% of those samples truly did not contain any positive smear finding. The negative predictive value on using our laboratory criteria was 97.34%.

False positive samples are those that trigger review criteria but are negative for any morphological abnormalities on the smear. These samples are thus responsible for increasing the MRRs and decreasing the specificity. The contribution of the individual rules to false positives was different on applying the ICGHR and our laboratory criteria. The difference in the false positivity can possibly be explained by the fact that the threshold cut offs for all the parameters were not the same for both sets of criteria. The cut offs for our laboratory criteria were more sensitive than the ICGHR criteria. This alone was responsible for a greater number of MSRs for RBC, WBC and platelet parameters.

In the current study, the triggers for suspect flags were the same for our laboratory criteria and the ICGHR criteria. However, as the total number of positive

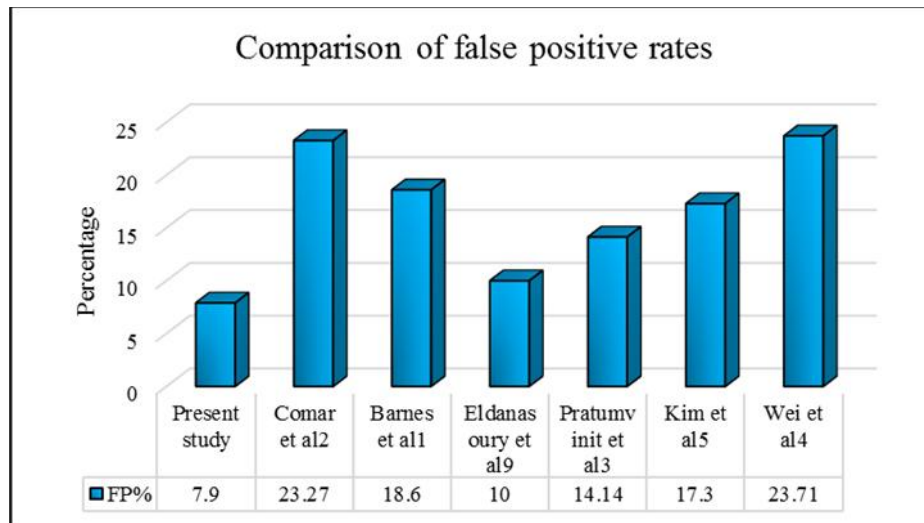
smears in either criteria was different, the proportion of each of the suspect flags responsible for triggering MSR was different in both criteria.

The parameters responsible for false positivity using the ICGHR criteria included most commonly the abnormal lymphocyte/blast flag (23 samples) followed by the immature granulocyte flag (15 samples) and MPV  $<5.0\text{fL}$  or  $\geq 12.5\text{fL}$  (7 samples). However, the commonly triggered parameters which led to false positivity with our laboratory criteria included absolute neutrophil count  $<2$  or  $>7 \times 10^3/\mu\text{L}$  (125 samples), total leukocyte count  $<4$  or  $>11 \times 10^3/\mu\text{L}$  (100 samples) and RDW-CV  $>14\%$  (samples). The main rules producing false positive results in the study done by Comar *et al*<sup>2</sup> using the ICGHR criteria was most commonly total leukocyte count  $<4.0 \times 10^3/\mu\text{L}$  followed by platelets  $<100 \times 10^3/\mu\text{L}$  and suspect flags.

After false-positive analysis, MCV  $<75\text{fL}$  or  $>105\text{fL}$ , using the ICGHR criteria, caused the most number of false positive smear reviews for the study done by Pratumvinit *et al*<sup>3</sup>. The study done by Eldanasoury *et al*<sup>9</sup> revealed that the platelet clump and giant platelet flags were the most common cause for false positivity followed by platelet  $<100$  or  $>1000 \times 10^3/\mu\text{L}$  and total count  $<4$  or  $>30 \times 10^3/\mu\text{L}$  using the ICGHR criteria.

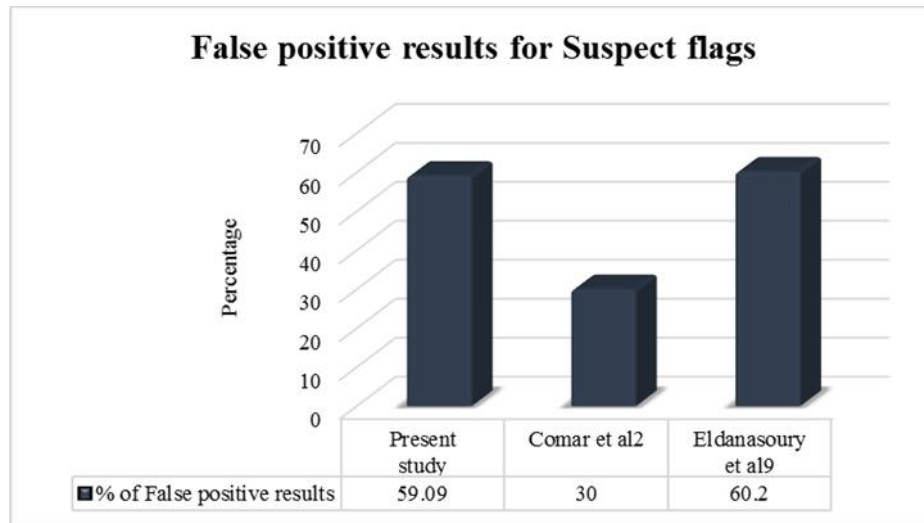
The proportion of samples accounting for a platelet count of  $<100 \times 10^3/\mu\text{L}$  was 11.16% with the ICGHR criteria in the present study and 13.3% in the study done by Comar *et al*<sup>2</sup>. They stated that this proportion was quite high and the same was true with the present study. Comar *et al*<sup>2</sup> therefore recommended the performance of microscope estimate of the platelet count on samples with this profile in order to verify how well it complies with automated counting and to search for platelet aggregates and giant platelets, both of which are factors that produce underestimates.

**Figure 10: Comparison of false positive rates among various studies using the ICGHR criteria.**



Hematology analysers use suspect flags to notify the user that the automated differential WBC count may not be correct and requires review. In the study done by Comar *et al*<sup>2</sup>, 30% of the false positive results were of samples with suspect flags. This indicated that the hematology analysers used were guilty of over-flagging, i.e., they gave more warnings than necessary. Eldanasoury *et al*<sup>9</sup> also showed that suspect flags were responsible for 60.2% of the false positive results. The same was the case with the present study where the suspect flags alone were responsible for 59.09% of false positive results with the ICGHR criteria. Over-flagging is thus responsible for an increase in unnecessary MSR.

**Figure 11: Comparison of suspect flags responsible for false positive results in different studies using the ICGHR criteria**



With our laboratory criteria, however, only 7.01% of the false positive results were due to suspect flags indicating that triggers from the other parameters were responsible for majority of the false positive samples. The same was the case with Eldanasoury *et al*<sup>9</sup> with suspect flags accounting for 18% of false positive results. This point again emphasises the downside of having strict threshold cut offs, as with our laboratory criteria, which of course have greater higher sensitivity but at the cost of a higher MRR.

As the sensitivity of the suspect flags are adjusted by technicians of the hematology analyser's manufacturer, Comar *et al*<sup>2</sup> suggested that each laboratory should first evaluate the efficiency of each suspect flag from the analysers and then make proper adjustments to the sensitivity of the hematology analyser or define whether a suspect flag is actually useful as a screening criterion.

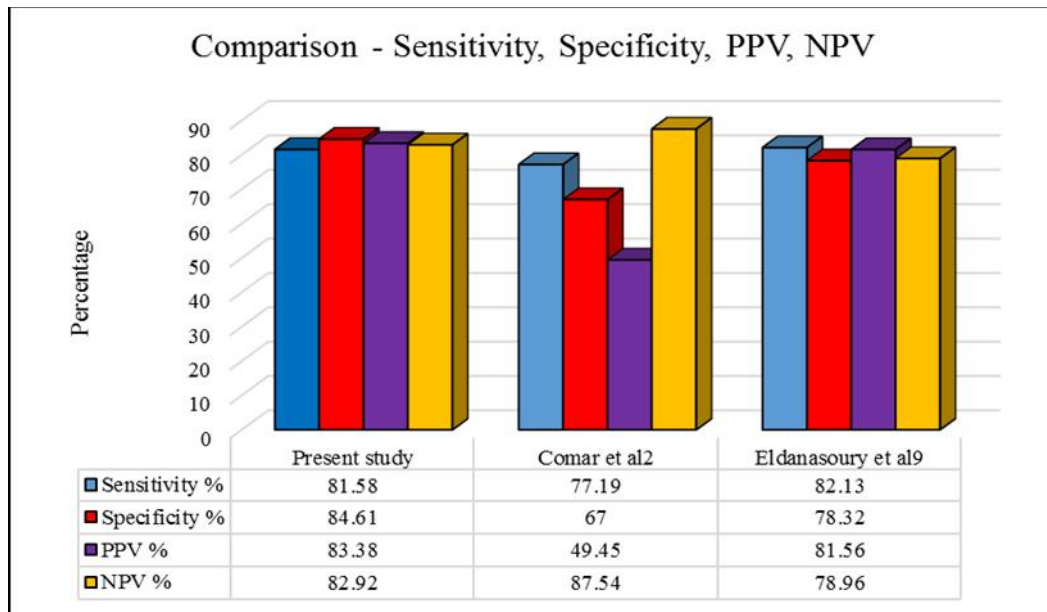
In the same line of thought, Kim *et al*<sup>5</sup> had an interesting point about using different analysers for their study. They stated that the rates of slide review have distinct characteristics among the studied analysers and that individual laboratories should consider selecting the most appropriate analyser in accordance with clinical

characteristics including clinic size and patient population. In the present study however, as only one analyser was used, the above aspect could not be reviewed.

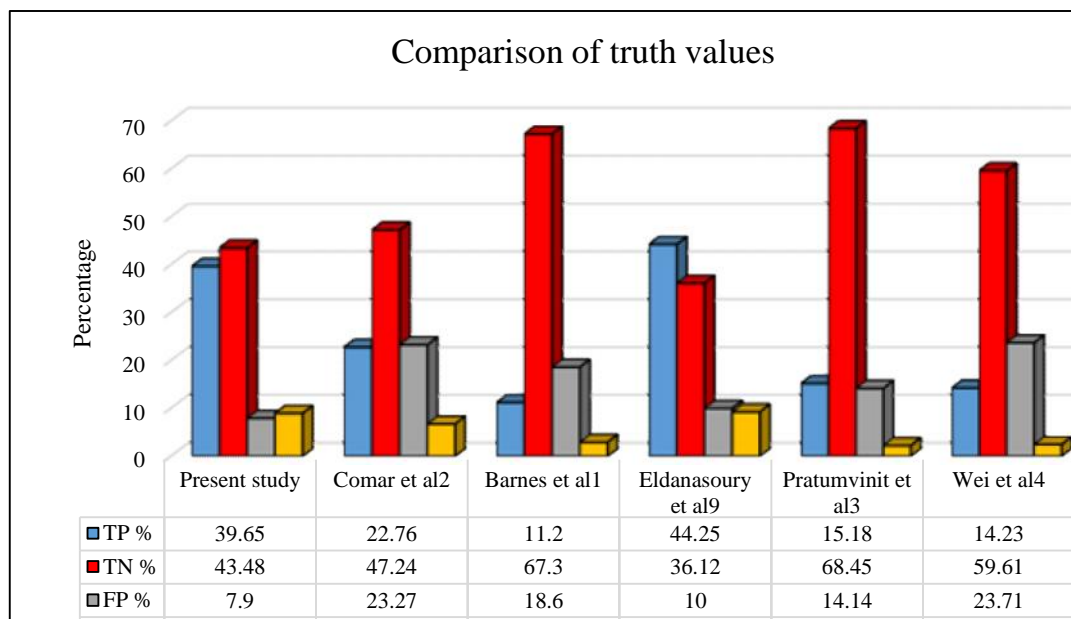
A sensitivity of 81.58% was observed in the present study by applying the ICGHR criteria. This means that 341 samples out of a total of 418 samples that had positive findings on smear review were truly positive. The sensitivity was higher by applying our laboratory criteria (98.80%). The specificity of ICGHR criteria (84.61%) was higher than that of our laboratory criteria (41.40%). Both sensitivity and specificity showed statistically significant difference between the laboratory and ICGHR criteria.

The sensitivity using our laboratory criteria (98.80%) was much higher than the specificity (41.40%). This in view of the high MRR of 78.13% using our laboratory criteria. Eldanasoury *et al*<sup>9</sup> also reported that the sensitivity (77.19%) was higher than specificity (67%) with their laboratory criteria again in accordance with their high MRR. The sensitivity using the ICGHR criteria in their study was however lower than the specificity and accordingly, the MRR was lower (47.56%).

**Figure 12: Comparison of the sensitivity, specificity, positive and negative predictive values between various studies using the ICGHR criteria**



**Figure 13: Comparison of the true positive, true negative, false positive and false negative values among various studies using the ICGHR criteria**



The greatest modification made to the adapted ICGHR criteria in the present study was regarding the delta check rules. These rules are important for the efficiency and reliability of the CBC results directly released without a MSR. Due to high software development costs, many clinical laboratories cannot implement the delta check rules in their electronic records or interfacing systems.

The ICGHR criteria are ideal to decrease the number of MSRs in the clinical laboratory. Although our laboratory criteria performed better with regard to sensitivity and negative predictive value, it came at the expense of a very high MRR which in turn may lead to decrease in laboratory productivity and increase in the turnaround time. Therefore, development of optimised criteria based on the ICGHR criteria followed by their validation will improve turnaround time and efficiency in our laboratory.



## **CONCLUSION**

In the present day and scene where the sophistication of the automated hematology analysers is only improving with the launch of every new analyser, it is important for clinical laboratories to consider methods for reducing the number of manual peripheral smear reviews in order to improve their productivity and efficiency.

The ICGHR criteria are essentially guidelines for manual peripheral smear review following automated CBC results. In the present study, the ICGHR criteria had higher efficiency (83.14%) with a lower microscopic smear review rate (47.56%) when compared with the laboratory criteria. Our laboratory criteria had a higher number of positive samples (78.14%) which accounted for a higher smear review rate (78.14%) and lower efficiency (69.30%). The rate of false negative samples was higher with the ICGHR criteria (8.95%) when compared with our laboratory criteria (0.58%). The sensitivity (98.80%) was more than the specificity (41.40%) for our laboratory criteria while the specificity was higher (84.61%) than the sensitivity (81.58%) with the adapted ICGHR criteria.

Therefore, it is 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.

**Limitations of the present study:**

- Delta check rules were not analysed due to limitations in the hospital's instrument and record systems.
- The paediatric population was not included in the study.

## **SUMMARY**

Two sets of criteria for peripheral smear review were compared in this study - the adapted ICGHR criteria and our laboratory criteria from samples sent to the central laboratory, Department of Pathology, B.L.D.E. University's Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapur over a period of 19 months.

Under aseptic precautions, 2ml of venous blood was collected in EDTA vacutainers. Samples were collected from the daily workload by systematic random sampling and analysed using the 6 part differential automated haematology analyser Sysmex XN-1000. Thin blood smears were then prepared for all the samples included in the study and stained with Leishman stain. Microscopic smear review was then performed for identification of morphological abnormalities and a 100-cell manual differential count was done. Each sample was reviewed according to the adapted ICGHR criteria and the laboratory criteria. A rule in the criteria would be triggered when the result was beyond the specified range and/or a specified flag appeared.

Majority of the patients were female (58.14%) with a male to female ratio of 0.72. The age ranged from 18 to 98 years. The number of samples from outpatients (50.35%) were higher than the inpatients (49.65%). With ICGHR criteria, 39.65% samples were true positive, 43.49% true negative, 7.91% false positive and 8.95% samples were false negative. The sensitivity was 81.58%, specificity 84.61%, positive predictive value 83.38% and, 82.92% negative predictive value. The microscopic smear review rate was 47.56% with an efficiency of 83.14%. The results from our laboratory criteria revealed a true positivity of 48.02%, true negativity of 21.28%, false positivity of 30.12% and a false negativity of 0.58%. The sensitivity was

98.80%, specificity 41.40%, positive predictive value of 61.46% and, negative predictive value of 97.34%. The microscopic smear review rate was 78.14% with an efficiency of 69.30%.

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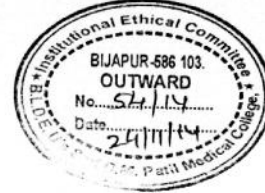
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**ANNEXURE – I**

**INSTITUTIONAL ETHICAL COMMITTEE CLEARANCE CERTIFICATE**



**B.L.D.E. UNIVERSITY'S  
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103  
INSTITUTIONAL ETHICAL COMMITTEE**

***INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE***

The Ethical Committee of this college met on 22-11-2014 at 3-30pm.  
to scrutinize the Synopsis of Postgraduate Students of this college from Ethical  
Clearance point of view. After scrutiny the following original/corrected &  
revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Utility of The International Consensus Group  
criteria. For Manual. Peripheral Smear Review Following  
Automated Blood cell. Analysis"

Name of P.G. student Dr. Katyayani Palur.  
Dept of Pathology

Name of Guide/Co-investigator Dr. Suresh U. Aakeri. Professor.

for

**DR. TEJASWINI VALLABHA  
CHAIRMAN  
INSTITUTIONAL ETHICAL COMMITTEE  
BLDEU'S, SHRI.B.M.PATIL  
MEDICAL COLLEGE, BIJAPUR.**

Following documents were placed before E.C. for Scrutinization

- 1) Copy of Synopsis/Research project.
- 2) Copy of informed consent form
- 3) Any other relevant documents.

**ANNEXURE II**

**B.L.D.E.U's SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND**

**RESEARCH CENTER, VIJAYAPUR - 586103**

**RESEARCH INFORMED CONSENT FORM**

I, the undersigned, \_\_\_\_\_, S/O D/O W/O \_\_\_\_\_, aged \_\_\_\_\_ years, ordinarily resident of \_\_\_\_\_ do hereby state/declare that Dr \_\_\_\_\_ of \_\_\_\_\_ Hospital has examined me thoroughly on \_\_\_\_\_ at \_\_\_\_\_ (place) and it has been explained to me in my own language that I am suffering from \_\_\_\_\_ disease (condition) and this disease/condition mimic following diseases . Further the Doctor has informed me that he/she is conducting dissertation/research titled \_\_\_\_\_ under the guidance of Dr \_\_\_\_\_ requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

The Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further the Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt \_\_\_\_\_ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place

**ANNEXURE-III**

**PROFORMA FOR STUDY**

NAME : OP/IP No. :

AGE :

SEX : D.O.A :

RELIGION : D.O.D :

OCCUPATION :

RESIDENCE :

**Presenting Complaints** :

**Past history** :

**Personal history** :

**Family history** :

**Treatment history** :

**General physical examination:**

Pallor present/absent

Icterus present/absent

Clubbing present/absent

Lymphadenopathy present/absent

Edema present/absent

Built poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

**SYSTEMIC EXAMINATION:**

Cardiovascular system:

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

**INVESTIGATIONS - HEMATOLOGICAL PARAMETERS:**

**1. Automated hematology analyzer (Sysmex XN-1000) findings.**

A) Screening criteria – Adapted ICGHR review criteria and our laboratory criteria

followed for automated complete blood counts.

S. No.	Parameter	Adapted ICGHR Criteria	Laboratory Criteria
<b>SCREENING CRITERIA</b>			
<b>RBC Parameters</b>			
1	Haemoglobin (HB)	<7 or >18.5 g/dL	<11 or >17 g/dL
2	Mean Corpuscular Volume (MCV)	<75 or > 105 fL	<80 or >100 fL
3	Mean Corpuscular Hemoglobin	<30 or >36.5 g/dL	<31.5 or >35.5 g/dL

	Concentration (MCHC)		
4	RDW-CV (Red cell Distribution Width – Coefficient of variation)	>22%	>14%
5	Nucleated RBC (nRBC)	Any value	Any value
<b>WBC Parameters</b>			
6	Total WBC count (TC)	<4 or >30 x 10 <sup>3</sup> /μL	<4 or >11 x 10 <sup>3</sup> /μL
7	Absolute Neutrophil count (AN)	<1 or >20 x 10 <sup>3</sup> /μL	<2 or >7 x 10 <sup>3</sup> /μL
8	Absolute Lymphocyte count (AL)	>5 x 10 <sup>3</sup> /μL	<1 or >3 x 10 <sup>3</sup> /μL
9	Absolute Monocyte count (AM)	>1.5 x 10 <sup>3</sup> /μL	>1 x 10 <sup>3</sup> /μL
10	Absolute Eosinophil count (AE)	>2 x 10 <sup>3</sup> /μL	>0.50 x 10 <sup>3</sup> /μL
11	Absolute Basophil count (AB)	>0.5 x 10 <sup>3</sup> /μL	>0.1 x 10 <sup>3</sup> /μL
<b>Platelet Parameters</b>			
12	Platelet count	<100 or >1000 x 10 <sup>3</sup> /μL	<150 or >400 x 10 <sup>3</sup> /μL
13	Mean Platelet Volume (MPV)	<5 or ≥12.5 fL	<7.4 or >11.4 fL

B) Adapted ICGHR review criteria and our laboratory criteria for suspect flags followed for automated complete blood counts

S. No.	Parameter	Adapted ICGHR Criteria	Laboratory Criteria
<b>SUSPECT FLAGS</b>			
<b>WBC Suspect flags</b>			
14	Immature Granulocyte (IG)	Flag	Flag
15	Left Shift	Flag	Flag
16	Atypical Lymphocyte	Flag	Flag
17	Abnormal Lympho/ Blast	Flag	Flag
18	nRBC	Flag	Flag
<b>RBC Suspect flags</b>			
19	Dimorphic Population	Flag	Flag
20	Fragments?	Flag	Flag
21	Turbidity/ Hemoglobin interference?	Flag	Flag
22	Hemoglobin defect?	Flag	Flag
<b>Platelet Suspect flags</b>			
23	Platelet clumps	Flag	Flag
24	Platelet flags (except platelet clumps)	Flag	Flag
25	(*)	This symbol beside the counts on the read out indicates that automated counts are not reliable.	
26	(---)	This symbol beside the counts on the read out indicates that automated counts are not available for the sample in question.	



**2. Peripheral smear review findings:**

- a. RBC
- b. WBC
- c. Platelets
- d. Abnormal cell types

**ANNEXURE-IV**

**KEY TO MASTERCHART**

<b>Abbreviation Full Form</b>	<b>Full form</b>
S. No.	Serial Number
HB	Hemoglobin
TC	Total Count
AN	Absolute Neutrophil Count
Ab. IG	Absolute Immature Granulocyte Count
AL	Absolute Lymphocyte Count
AM	Absolute Monocyte Count
AE	Absolute Eosinophil Count
AB	Absolute Basophil Count
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW-CV	Red Cell Distribution Width – Coefficient Of Variation
PLT count	Platelet Count
MPV	Mean Platelet Volume
Ab. nRBC	Absolute nRBC count
WBC	White Blood Cell flags
RBC	Red Blood Cell flags
PLT	Platelet flags

IG	Immature Granulocyte flag
LS	Left Shift flag
Atyp L	Atypical Lymphocytes
Ab L/B	Absolute Lymphocyte/ Blasts flag
nRBC	Nucleated RBC flag
Frag	Fragments flag
Dimorphic	Dimorphic Population flag
Turb/ HB interf	Turbidity/ Hemoglobin Interference flag
HB def	Hemoglobin Defect flag
PLT clumps	Platelet Clumps flag
Abn PLT distribution	Abnormal Platelet Distribution flag
LAB Rev	Positives Or Negatives For Our Laboratory Review Criteria
LAB MSR	Positives Or Negatives For Laboratory Manual Smear Review
Truth LAB	Truth For Laboratory Criteria
ICGHR Rev	Positives Or Negatives For The ICGHR Criteria
ICGHR MSR	Positives Or Negatives For ICGHR Manual Smear Review
Truth ICGHR	Truth For ICGHR Criteria
ICGHR	International Consensus Group For Hematology Review
p	Positive

n	Negative
TP	Truth Positive
FP	False Positive
FN	False Negative
FP	False Positive





















Table with 27 columns and rows numbered 665 to 739. Columns include numerical values and categorical labels such as P, N, TP, TN, FN. Some cells contain the letter 'p'. The final three columns of each row contain categorical labels in red text: TP, TN, FN.



