



Comparison of Automated Hematology Analyzer (XN 1000) Flags & Peripheral Blood Smear Examination: An Experience from a Tertiary Care Hospital

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ABSTRACT

Background: In the era of new automated hematology analysers, they analyze blood samples and highlight the abnormal results of complete blood abnormal count with flags. If these abnormal flags are accurate and reliable, the workload of a hematopathologist would decrease, and turnaround time would reduce significantly, besides aiding in the early diagnosis of certain disorders. **Objective:** To correlate abnormal flags of a six-part differential automated hematology analyzer with a peripheral smear study by a hematologist/pathologist. This study aims to determine the diagnostic accuracy of peripheral blood smears and automated hematology analyzers with abnormal flagging. **Methodology:** This retrospective cross-sectional study was conducted in the hematology department of CHK Central Laboratory Dr Ruth KM Pfau Civil Hospital Karachi, from July 2022 to December 2022. Samples of 158 patients were included which showed flagging messages by automated hematology analyzer XN-1000. The flagging messages were analyzed with their respective PBS findings. Pearson's Chi-square test was used for the statistical analysis. Venous blood samples were collected in an EDTA tube for Complete blood count and were processed by the six-part differential automated hematology analyzer-Sysmex XN 1000. All age groups and genders were included. **Results:** Peripheral smears were reviewed in 158 samples from different departments; 112 were inpatient, and 46 were outpatient samples. In 129/158, the findings of the XN 1000 analyser correlated with the peripheral smear review. Flags identified included Anemia in 97(61%), leukocytosis 27(17%) immature white blood Cell/blast (WBC) 16(10.1%), thrombocytopenia 73(46.2%), and malaria 20(12.6%). Peripheral smear examination: Anemia in 102, Leukocytosis in 28, thrombocytopenia in 91, and malaria in 29 cases. Comparison of XN-1000 with peripheral blood smear findings shown in table 1. 17 False positive cases of platelet clumps were detected on XN analyzer, and smear detected only 07 cases of platelet clumps. Peripheral smear examination was found to be a more accurate and sensitive technique for the detection of morphological abnormalities. In 10.7% of the cases, the analyzer missed important findings. **Conclusion:** This study depicts that the flagging of abnormal findings is accurate but it should always be correlated with the PBS verified by a hematopathologist. We should analyze the suspect flags and use it as a screening tool to pick up pathological samples quickly.

INTRODUCTION

Peripheral blood smear examination gives us important information about the morphology of different cells which are not explored by automated hematology analyzers. Peripheral smear examination, in most of the fully automated laboratories, is only for cases in which the abnormal flags generated by an automated analyzer due to the potential presence of blast cells and other abnormal findings regarding the counts of red cells, white cells and platelets. It can decrease the workload and turnaround

time in laboratory setups².

An automated hematology analyzer has a major benefit in analyzing the differential white cell count and decreases the requirement of blood smear preparation for microscopic review¹.

An examination of the peripheral smear is necessary in cases of suspected leukemia to make the presumptive diagnosis. There is a need for expert and well-trained pathologists and laboratory staff to review the blood smears, but it can be time-consuming and under-

productive.

However, if we completely rely on diagnostic information by automated hematology analyzer, keep an eye on important diagnostic information that must not be missed, as an automated analyzer does not give you complete information that is needed by the hematologist or physician³ or sometimes the flag maybe false positive too. In this period, automated hematology analyzers are advanced day by day and they have become an essential part of hematology laboratories with accurate and efficient results.

This impact significantly improves the efficiency and quality of blood tests in clinical laboratories, the ability of hematology analyzers to detect and flag abnormal cells and malaria has played a pivotal role in the diagnosis⁴.

Automated hematology analyzers explore the information regarding red cell parameters and white cell count along with differential count, platelet count and platelet clumping. Newer hematology analyzers also give us information by generating abnormal flags by detecting abnormal morphologic features.

Peripheral smear examination is often used as the gold standard for the diagnosis of Malaria, numerous RBC, white blood cell (WBC), and PLT disorders⁵.

Flagging messages are generated by a system of signaling in the XN 1000 automated hematology analyzer. In the hematology laboratory, a flag is a sign or signal to the laboratory staff and pathologist that the analysed blood sample may have abnormal cells or other abnormalities. So, it is necessary to undergo a microscopic examination of the peripheral smears of samples with flag messages generated by an automated analyzer^{6, 7}.

The present study was conducted to check the flagging messages generated by the automated analyser and to correlate them with peripheral blood smear findings.

MATERIALS AND METHODS

This cross-sectional retrospective study was conducted in the hematology department of the CHK central laboratory, Dr Ruth KM Pfau Hospital Karachi, over 6 months from July 2022 to December 2022, following approval from the Institutional Review Board (IRB-2624/DUHS/EXEMPTION/2022/945). This study was conducted in accordance with the principles of the Declaration of Helsinki. 158 samples from the pediatrics, gynecology, medicine, and surgery departments were included, and the automated analyzer displayed flagging messages. These flags were noted, and peripheral smears of the respective samples were prepared and stained with Leishman's staining method for microscopic examination of smear.

Blood samples for complete blood count (CBC) were collected in an EDTA tube for Complete blood count and processed by the six-part differential automated hematology analyzer-Sysmex XN 1000. All age groups and genders were included.

The flagging messages were generated for RBC (Anemia, fragmented red cells, anisocytosis), WBC (blast, IGs, left shift, eosinophilia, leukocytosis, and leukopenia), and Platelet (thrombocytopenia, thrombocytosis, giant PLT, and PLT clumps). These were followed with their respective peripheral smear examination prepared

according to the standard operating procedures and stained by Leishman stain. The microscopic examination of the peripheral smears was done to correlate and confirm the flagging messages and also demonstrate hemoparasites if any.

Analyzer reports were studied based on the flag messages generated for WBC (MO blast, IGs, left shift, eosinophilia, leukocytosis, and leukopenia) and PLT (thrombocytopenia, thrombocytosis, large and giant Platelet, and Platelet clumps). These were followed with their respective.

peripheral smear examination prepared according to the standard operating procedures and stained by Leishman stain. The count, morphology, and presence of red cell morphology for anemia, any immature cells were noted for WBCs. Platelet were examined for the count, large and giant platelet, and platelet clumps.

Statistical analysis was done using Pearson's Chi-square test. *P* value (*P* < 0.05) was considered statistically significant.

Inclusion Criteria

All CBC samples with flagging on the XN 1000 analyzer were included.

Exclusion Criteria

CBC samples with no flagging messages on the analyzer are excluded.

RESULTS

The analyzer reports and peripheral smears of 158 patients were analyzed. The age group of patients ranged from 1 to 77 years. Most patients were below 20 years of age (56.9%). The male and female ratio was 1:1. The detailed distribution of Red cell, WBC and platelet abnormalities, both by analyzer and peripheral smear, is shown in Figure 1.

Peripheral smears were reviewed in 158 samples from different departments; 112 were inpatient, and 46 were outpatient samples. In 129/158, the findings of the XN 1000 analyser correlated with the peripheral smear review. Flags identified included Anemia in 97(61%), leukocytosis 27(17%) immature white blood Cell/blast (WBC) 16(10.1%), thrombocytopenia 73(46.2%), and malaria 20(12.6%). Peripheral smear examination: Anemia in 102, Leukocytosis in 28, thrombocytopenia in 91, and malaria in 29 cases. Comparison of XN-1000 with peripheral blood smear findings shown in table 1. 17 False positive cases of platelet clumps were detected on XN analyzer, and smear detected only 07 cases of platelet clumps. Peripheral smear examination was found to be a more accurate and sensitive technique for the detection of morphological abnormalities. In 10.7% of the cases, the analyzer missed important findings.

Table 1

Comparison of XN-1000 with Peripheral Blood Smear Findings.

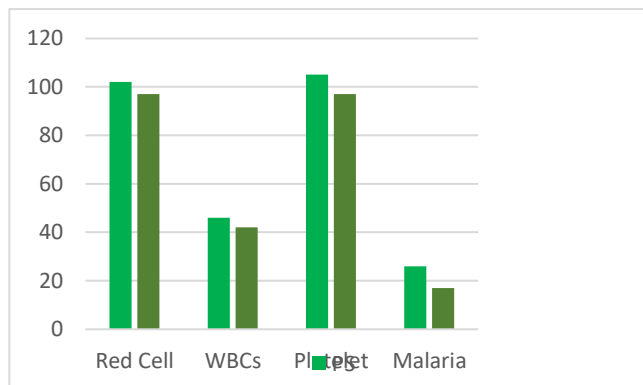
Methods	Anemia	Leukocytosis	Blast cells	Malaria	Platelet clumps
XN-1000	97 (61.3%)	27 (17.1%)	16 (10.1%)	20 (12.6%)	24 (15.1%)
Peripheral smear	102 (64.5%)	28 (17.7%)	18 (11.3%)	26 (16.4%)	07 (4.4%)

In our study, the sensitivity of abnormal flags was 81.6%, while the sensitivity of abnormal parameters was 100% as shown in table 2.

Table 2
Diagnostic Accuracy

Variables	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV)	Negative predictive value (NPV)	Accuracy
Abnormal flag	81.6%	63%	90.2%	18.3%	81.6%
Abnormal parameters	100	-	-	-	-

Figure 1
Comparison of Analyzer Flags Versus Peripheral Blood Smear Abnormalities



DISCUSSION

In the current study, we compared the flagging messages generated by the automated hematology analyzer with their respective peripheral blood smears to correlate the accuracy and findings of the automated analyzer. In the present study, we included 158 patient samples based on the peripheral blood smear examination, 158 cases were found to have abnormalities on smear examination.

The minor differences could have been due to the cases which showed a very mild increased or

Correlation between the WBC histograms and their peripheral smears was found to be statistically significant ($P = 0.006$). Thus, the efficiency of automated analyzers in differentiating normal from abnormal specimens was well established.

In our study, microcytic anemia was found 42 and 28 on peripheral smear and XN flag, respectively. Macrocytic anemia diagnosed on peripheral smear was 09, and XN analyzer generated flags in 06 cases of macrocytic anemia. White cell abnormalities were detected on peripheral smear was 46 smears and XN flag was present in 43 cases of white cell disorders. A similar study from Pakistan conducted by Sidra et al. shows the findings of the hematology analyzer correlated with peripheral smear review. Flags identified included nucleated red blood cells (NRBCs) in 155 (40%), immature white blood cells (WBC) in 129 (34%), and atypical lymphocytes 100 (26%). In 23% of the cases, the analyzer missed important findings¹³.

In our study, peripheral smear examination of platelets showed abnormalities in 121 cases compared to the 106

cases diagnosed by suspect flags. Thrombocytopenia was seen in 91 as compared to 73 cases on XN 1000, and thrombocytosis was observed in 15 instead of 07 as per the analyzer reports. Platelet clumps were found in 15 instead of 24 cases as per the suspect flags on XN. There are 17 false positive cases for platelet clumps detected on the analyzer. This difference can be explained by the fact that the presence of platelet aggregates and cases of pseudothrombocytopenia were misinterpreted by the analyzer. These cases are purely diagnosed on PBS. Platelet flags are produced rarely and only in extreme cases of thrombocytopenia and thrombocytosis. Therefore, to diagnose diseases of platelet, PBS examination should be considered the gold standard.

Reporting prompt and accurate blood cell counts with clearly defined turnaround time is essential because these results often have an impact on medical decision-making. One study from Cmar et al. reported the review criteria for blood counts generated by automated hematology analyzer and this study showed that microscopic review rate of 46.03% with false negatives of 6.73%, false positives of 23.27%, and efficiency equivalent to 70.0%. After adopting the review criteria as per the International Society of laboratory hematology, the microscopic review rate dropped to 37.3% with false negatives reaching 15.5%, false positives at 10.5%, and efficiency at 73.8%⁸. Another study by Avi Nahar was conducted in 2017, they compared the coulter analyzer's flag messages and blood smear examination. After conducting this study, they concluded that the manual smear examination is still needed because it can give some additional information regarding the abnormal morphology of blood cells, especially blast cell morphology⁹. Hawkins et al. reported and assessed the accuracy and reliability of the sysmex XE 5000 automated hematology analyzer in detecting platelet clumps. They correlate the two platelet flags, the platelet size distribution (PAD) flag, and the platelet clumps (CLP) flag. They found the CLP flag is more reliable and superior to the PAD flag¹⁰.

The International Society for Laboratory Hematology reported that the efficacy of the microscopic review rate is 73.8%, with 10.5% false positives and 15.5% false negatives.¹¹ Another similar study conducted by Pratumvinit et al. reported that manual peripheral smear review had 87.13% efficacy accuracy with 2.98% false negatives.¹²

These automated CBC instruments are standardized to show the presence of interfering particles as 'flags' it needs expert manual review to check for its authenticity. The flags generated in the automated analyzer are useful in warning of abnormal blood results, it can be either not sensitive enough (false negative) or sometimes misidentify the cellular flags (false positive)

CONCLUSION

The present study confirms and assures that the automated hematology analyzer is as good as a standard manual method in giving accurate results. However, the flags in the automated instruments should always proceed with the examination of peripheral blood smears to avoid errors. We should analyze the suspect flags and use it as a screening tool to pick up pathological samples quickly.

These should then be followed up with a thorough Peripheral blood examination for a definitive diagnosis. We should analyze the suspect flags and use it as a screening tool to pick up pathological samples quickly. These should then be followed up with a thorough PBS

examination for a definitive diagnosis.

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