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UTILITY OF PERIPHERAL BLOOD SMEAR AND ITS CORRELATION WITH AUTOMATED HAEMATOLOGY ANALYZER

Pathology

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ABSTRACT

Background And Objective:
Blood smear examination serves as a quality control tool in verifying the results generated by the automated analyzer and identification of abnormal or immature cells. With the advent of automated hematology analyzer, the use of traditional microscopy of blood film has become limited. The objective of our study was to determine the percentage of peripheral blood smear review in our institution in the era of automation and to identify reasons of manual review.
Materials And Methods: This was a retrospective study from november 1, 2019, to november 15, 2019. Samples for complete blood count (CBC) and peripheral smear comment are included in the study. All age groups and genders were included. The variables to be analyzed included inpatient and outpatient samples, frequency of peripheral blood smear review, identifying reasons of smear review, and addition of information missed by the automated analyzer.
Results: We analyzed 1400 CBC samples. Peripheral smear was reviewed in 650 (46.4%) of which, 410 were inpatient, and 240 were outpatient samples. In 545/650, the findings of hematology analyzer correlated with peripheral smear review. Flags identified included nucleated red blood cells (NRBCs) in 220 (40%), immature white blood cell in 155 (28%), and atypical lymphocytes 125 (22%). In 16 % of the cases, the analyzer missed important findings. The sensitivity of abnormal histogram in our study was 91.8%, while the sensitivity of abnormal parameters was 99.4%.
Conclusion: Peripheral smear review was performed in 46 % of the cases. The analyzer identified NRBC, immature WBC precursors, and atypical lymphocytes as the most common abnormality. The information correlated in 84 % of the cases.

KEYWORDS

Automated analyzer, complete blood count, peripheral blood smear

INTRODUCTION

Peripheral Blood Smear (PBS) is a highly informative hematological tool that can be used for the screening, diagnosis and monitoring of disease progression, and for therapeutic response [1]. The microscopic blood smear examination may be limited to a blood smear scan or may include complete blood smear examination with manual differential count and or a blood smear review [2].

Blood smear examination serves as a quality control tool in verifying the results generated by the automated analyzers, identification of abnormal or immature or atypical cells and recognition of clinically significant morphological abnormalities, which the analyzers are incapable of either flagging or detecting and identifying [2].

It has been reported that the common clinical indications for peripheral blood film analysis include unexplained cytopenia such as anemia, leucopenia or thrombocytopenia; unexplained leucocytosis such as lymphocytosis, monocytosis; suspected chronic or acute myeloproliferative disease such as chronic myeloid leukaemia; suspected cases of nutritional anemia; suspected chronic lympho-proliferative disease such as chronic lymphocytic leukemia; suspected organ failure such as liver disease, renal disease; several bacterial sepsis and parasitic infections and evaluation of therapeutic response amongst other conditions [3-5].

Other conditions in which the peripheral smear findings can be diagnostic include microangiopathic hemolytic anemia, hemoglobinopathy, myeloproliferative disorders, and parasitic infections (especially malaria)[3].

With the advent hematology analyzers, microscopic review of peripheral blood film is declining. Sophistication of analyzers has increased to the point that they are able to provide cells counts, differentials, plots, and histograms[3].

Microscopy and manual differential counting, in most of the automated laboratories, is restricted to cases in which the instrument "flags" the potential presence of abnormal cells or in cases where findings may interfere with analysis (such as overlap in the distribution of different cell types or interference from matrix components). In cases of clinical suspicion of leukemia, review of the peripheral blood smear is mandatory to make the presumptive diagnosis. There are other morphological findings also which may have a clinical significance which cannot be reliably identified by the various automated analyzers[3].

These other findings include the presence of giant platelets, platelet clumps, basophilic stippling, hypersegmented neutrophils, red cell fragments, and Howell-Jolly bodies.[3]

Since the inception of automated differential counting methods, manual blood smear review is recommended as a validation, rather than as a replacement of automated methods.[6] The Colleges of American Pathologists (CAP) have conducted numerous studies though Q-probes program to determine performance benchmarks in Pathology. Novis *et al*. in 2006 have reported peripheral smear review frequency of 26.7%.[7]

In our studt, the objective was to determine the frequency of peripheral smear review in our institution and compare it with CAP standards. We also wanted to correlate the findings of peripheral smear and analyzer flags and to identify information which was missed by the analyzer.

MATERIALS AND METHODS

A retrospective study conducted in the central pathology laboratory(CPL) , Department of Pathology, Shyam Shah medical college, Rewa(M.P.). Peripheral blood film is made of only those blood samples which shows abnormal counts, thrombocytopenia or flagging given by the hematology analyzer.

For the study, data for 2 weeks (Npvember 1,2019–november 15, 2019) were collected. All age groups and genders were included.

Specimens with clots, obvious hemolysis , insufficient amount of sample or wrong vacutainer were excluded.

CBCs were performed on Automated hematology analyzer Mindray Bc 2800 using impedance method.

Each blood sample collected in EDTA container was used for the preparation of thin blood film or PBS and for full blood count determination. The thin blood films were stained by Leishman`s method for the peripheral smear examination, determination of estimated platelet and WBC counts.

PBS was examined where the red cells were not overlapping or showing platelet clumps and platelet counts were determined by multiplying the average numbers of platelets per 10, 100X oil immersion fields by 20.0 x109/L and 15.0 x109/L respectively [8-12] while WBC count was determined by multiplying the average number of WBCs counted in 10, 40X high power fields by2.0 x109/L (WBC count multiplication factor) [13].

RESULT

During the study, we analyzed 1400 CBC samples. Peripheral smear was reviewed in 650 (46%) of the cases using random sampling technique. Of these 650 samples, 410 were inpatient and 240 were outpatient samples. In 545/650, the findings of hematology analyzer correlated with peripheral smear review.

Flags which were identified included nucleated red blood cells (NRBCs) in 220 (40%), immature white blood cell in 155 (28%), and atypical lymphocytes 125 (22%). In 16 % of the cases, the analyzer missed important findings.

These included abnormalities in hemoglobin indices in 25(24%), WBCs differential counts in 22 (21%) , large platelets in 28 (26%) and platelet clump in 30(28%). Furthermore, the sensitivity of abnormal histogram in our study was 91.8%, while the specificity of the same was 8.2%. Accordingly, the sensitivity of abnormal parameters was 99.4%, and the specificity of the same was 0.6 %.

DISCUSSION

Despite the advances in haematology automation and application of molecular techniques, the PBS remains a very important diagnostic test to the haematologist [1]. Microscopic examination and morphological assessment are an essential part of CBC reporting that provides crucial information apart from the cell counts. Review of peripheral blood smear serves to ensure that no clinically significant finding is missed, besides providing a clue to the diagnosis, when interpreted by a physician.[2]

This information is now provided by automated analyzers with more sensitivity reducing the need of manual examination.

The purpose of automation is to provide faster results, to reduce the technologist hands - on time, in addition to providing high quality and precision.[14]

Defining acceptable and safe rates for microscopic examination of the blood smear is crucial to ensure the quality of the results, but reported rates are highly variable.[15]

Comar *et al*. verified the review criteria for automated blood counts suggested by the International Society for Laboratory Haematology and their results showed microscopic review rate of 46.03% with false negatives of 6.73%, false positives of 23.27%, and efficiency equivalent to 70.0%. After adapting the review criteria, the microscopic review rate dropped to 37.3% with false negatives reaching 15.5%, false positives of 10.5%, and efficiency of 73.8%.[16]

Pratumvinit and his colleagues evaluated their criteria for manual smear review and after optimization, their review rate was found to be 24.2% with an efficiency of 87.13% and false negative rate of 2.98%.[17]

In our study, the sensitivity of abnormal histogram was 91.8%, abnormal parameter was 99.4%, and the sensitivity for flagging was 96.6%.

Our manual smear review rate was 46%, which is approximately twice as compared to CAP standards.

CONCLUSION

It is concluded that the frequency of peripheral blood smear review in our setup was 46%. In 84% of cases, the findings of peripheral blood smears review correlated with that of the analyzer.

It is recommended that each hematology department or medical laboratory should determine the multiplication factors for platelet and WBCs using PBS in order to estimate platelet and WBC counts since there may be possibility of automated cell counters having different sensitivities.

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