



Evaluation and Correlation of Abnormal Cell Flagging of Automated Haematology Analyzer with Peripheral Blood Film at a Hematology Laboratory in Tertiary Care Oncology Centre

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ABSTRACT

Objectives: Automated hematology analyzer generates flags for white blood cells abnormalities, indicating associated manual peripheral blood film examination. This study was aimed at evaluation and correlation of abnormal cell flagging with manual blood film reviews to improve performance in a hematology laboratory. In this study, an automated slide maker SC-120 was used to study the performance of fully automated haematology analyzer Mindray BC-6800.

Methods: Total 500 routine samples were obtained over a period of seven consecutive days, and run through analyzer which displayed flags for white blood cells. Thereby, results from flags were evaluated and correlated with smear findings through careful statistical analysis of the observed parameters.

Results: Flags for white blood cells and Nucleated Red Blood Cells (NRBC) were generated by BC-6800 for 211 samples out of total 500 run samples, from which 46.45% patients were diagnosed with haematological disorder and 53.55% with solid neoplasms. Sensitivity, specificity and Youden's Index observed against total 500 samples were 100%, 99.31% and 0.99 respectively. Efficiencies for blasts, abnormal lymphocytes and atypical lymphocytes were 95.64%, 95.20% and 97.60% respectively, whereas sensitivity for blasts was 75.81%. Further, sensitivity and specificity of NRBC were 86.76% and 100% respectively. Youden's index observed for various flags was close to 1, indicating near to satisfactory performance of analyzer.

Conclusion: The present study confirms that performance of an automated analyzer aligns with standard manual methodology. However, to avoid false-negative results by analyzer, peripheral smears should be examined manually.

Key Words: Automated haematology analyzer, Flagging, Peripheral blood film, Evaluation and Correlation

Abbreviation: NRBC - Nucleated Red Blood Cells

INTRODUCTION

The complete blood count and differential cell count performed in hematology laboratories play a vital role in the diagnosis of blood disorders. Enhancement in technologies of laboratory equipment and advancement in the automation of cell counters not only helps in generating precise and accurate results but also reduce the test time enormously [1].

On other contrast, manual peripheral blood film examination has its own importance since morphological abnormalities of cells determined by flags are largely dependent on the validation, verification and calibration of analyzer which may differ from individual laboratory's criteria and standards.

However, after sufficient evaluation and correlation, the reliability on flags reduces the extra burden in the form of laboratory cost, labour cost and turnaround time as it avoids the

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need of manual peripheral blood film examination. This way, pathologists can focus more on reviewing smears against flags displayed by analyzing critical in the diagnosis of patients.

In this study, evaluation and correlation of abnormal cell flagging performance of Mindray BC-6800 automated hematology analyzer was done with peripheral blood films prepared and stained by the SC- 120. The Mindray SC- 120 is an automatic slide maker that is a standalone or integrated into the CAL 8000 cellular analysis line. The BC-6800 is a fully automated hematology analyzer works on two methodologies to perform complete blood count and differential counts: Light scattering at two angles and fluorescence signals of flow cytometry and thus generates flags of abnormal cells [2].

The aim and objective of this study are following:

1. To correlate the abnormal cell flagging performance of Mindray BC-6800 automated hematology analyzer with peripheral blood film findings.
2. To evaluate the reliability of flags generated by Mindray BC-6800 automated hematology analyzer by comparing them with the results of peripheral smear examinations.

MATERIALS AND METHODS

This study presents prospective evaluation and correlation of results generated by BC- 6800 with peripheral blood films at a hematology laboratory in a tertiary care oncology centre which is National Accreditation Board for Testing and Calibration Laboratories accredited by ISO 15189 since year 2010.

The data used in this study have been compiled over a period of seven consecutive days from blood samples of patients who needed a peripheral blood film examination by physician's recommendation and from patients who had routine check-ups. A total five-hundred samples were obtained in order to evaluate flagging performance for this study.

Peripheral blood samples were collected in K2EDTA vacutainers and analysed within the span of two hours of collection.

Various flags of different parameters were obtained by Mindray BC- 6800 automated hematology analyzer on which quality controls are performed on daily basis and abnormal parameters were shown by various flags. The base value of flags was optimized in the laboratory by altering the cut-off provided by the manufacturer as default settings and matching with peripheral blood films. The results produced by Mindray BC- 6800 were analysed with microscopic examination of peripheral blood films prepared and Wright stained by an automatic slide maker SC- 120 without carry over, in-

tegrated into CAL 8000 cellular analysis line. Two qualified examiners verified morphology of cells and 200 cells differential count on peripheral blood films.

During the smear findings for blasts, immature granulocytes and nucleated red blood cells(NRBCs), the positive result for abnormality is considered when at least one or more than one cell shows abnormality out of 100 white blood cells. For immature granulocytes, presence of promyelocyte/myelocyte ($\geq 1\%$) or metamyelocyte ($\geq 2\%$) is considered as positive smear findings. Similarly, for atypical lymphocytes, positive smear findings are to be considered when more than five abnormal cells found out of 100 white blood cells following the consensus guidelines provided by International consensus group of hematology review [3].

Further, in order to the evaluation of flags generated by BC-6800 Microsoft excel was used to compare the findings of flags with the results produced from peripheral blood film examination.

Sensitivity, Specificity, Efficiency, Positive predictive value, negative predictive value and Youden's index of flag performance by BC- 6800 were determined based on peripheral blood film examination. To determine all these parameters of flag performance, cases were classified as 'true and false positives and negatives'.

RESULTS

Total 500 blood samples were obtained. Among them, 56% samples were of male and 44% were of females. Considering age factor, a total 29% samples were from people less than 18 years of age and 71% were from age greater than 18 years. Among total 500 samples, 61% of patients were clinically diagnosed with the hematological disorder and 39% were diagnosed with solid tumors or neoplasms.

All samples were run through Mindray BC-6800 automated hematology analyzer, out of which analyzer displayed flags for white blood cells and NRBC against 211 samples and no flags against 289 samples. Thereafter results obtained from peripheral blood films for all those 500 samples were evaluated and correlated with that of automated analyzer. The values of important parameters evolved through this study is shown in Table 1.

The age and sex-wise distribution of 211 flagged samples between hematological disorder and solid neoplasms depicts results as shown in Table 2. Among rest of the 289 samples for which analyzer did not display white blood cell and/or NRBC flag, 80 samples were from patients diagnosed with solid neoplasms and 209 were of hematological disorder.

Specificity and efficiency observed for blasts flag was 98.4% and 95.6% with relatively low sensitivity of 78.81%. Sensi-

tivity, specificity and efficiency of Mindray BC-6800 was found to be 100%, 94.5% and 95.2% for abnormal lymphocyte flags and 100%, 97.5% and 97.6% for atypical lymphocytes respectively.

Out of 500 samples, analyzer displayed true flags for NRBCs in 59 samples while false negative flags were 9 out of total 500 samples. Sensitivity, specificity and efficiency was found to be 90.35%, 99.48% and 97.40% for immature granulocytes and 87.50%, 99.57% and 98.60% for shift to left respectively. Table 3 depicts the above result.

Table 4 shows a satisfactory good correlation between differential white blood cell flags obtained from the automated analyzer and manual examination of peripheral blood film.

DISCUSSION

Currently, results generated for complete blood count by automated analyzer has been satisfactorily enough to replace the manual standard methods for haematological abnormalities [4]. Over the period of time they are proved to be a reliable source and hence immensely helped to reduce laboratory manual errors and time taken to accomplish the test. However, despite sophisticated results given by the analyzer, inevitable errors were observed for various flags displayed. The automated analyzer is standardized to display the values for interfacing parameters as flags, it eventually needs an expert manual review to confirm its authenticity.

Flags indeed are helpful to warn against abnormalities but sometimes either they misidentify other cellular types displaying as false positive or they lack sufficient identification and displaying false-negative results [5]. Hence, there is a definite need for manual evaluation against all the displayed parameters of flags by automated analyzer in order to confirm abnormal findings and to maintain a high level of quality control.

In this study, analyzer displayed flag for blast against 54 samples, out of which 7 samples were found negative for blast on observation through peripheral blood film findings. Further out of those 7 samples, 6 were diagnosed with hematological disorder and they were on chemotherapy treatment while 1 was diagnosed with non-Hodgkin's lymphoma.

Apart from this, the analyzer displayed a flag for abnormal lymphocytes for 88 samples, among which 24 samples were found negative on manual review. Out of those 24 samples, 17 were diagnosed with hematological disorder and 7 with solid neoplasms. All patients were on chemotherapy.

The analyzer also displayed flag for atypical lymphocytes against 32 samples, among them 12 samples found to be negative on manual review. Out of those 12 samples, 11 were

found with haematological disorder and 1 found with solid neoplasms. Hence, there is false positive results for blast, abnormal lymphocytes and atypical lymphocytes in post chemotherapy patients.

In order to determine the sensitivity and specificity of flags generated by hematology analyzer, the cut-off values are tuned to generate more false-positive results, since the end result of errors due to deep analysis of peripheral findings are less dramatic than missing of particular information related to patient's diagnosis and follow-ups [6].

Analyzer was unable to display flags for immature granulocytes for 11 samples. Identification of immature granulocytes plays a critical role in case of neonatal septicemia in order to differentiate neutrophils and band cells. This is in lines with studies which proved that although time taking, manual checking of granulocytes differential count is the gold standard in giving band cell count and total to immature granulocyte cell ratio [7].

Comparison of this study with the results of another study done elsewhere, according to Mostafa et al., wherein they took 75 cases and observed sensitivity for blast, atypical lymphocytes, immature granulocytes and NRBC detection on NS-hema21t as 0%. Similarly, specificity observed for blast, atypical lymphocytes, immature granulocytes and NRBC was 1%, 33.3%, 90% and 1%. Efficiency of the same flags was 60%, 28.5%, 66.6%, 66.6% [8]. According to a study done by AviNahar et al., out of total 500 samples, specificity, sensitivity and efficiency of five-part differential automated hematology analyser - Beckman Coulter Ac. T was found to be 77.83%, 94.83 % and 70.60 % respectively for white blood cells [4]. In present study sensitivity, specificity and efficiency is comparatively high than observed values by Mostafa et al. The difference can be due to specific profile of patients and particular haematology analyzer used in the study.

Hence it is proposed by few studies that in order to maximize the flag's efficiency, the cut off values could be set in automated analyzer through the development of a probability rate, which may help in minimal occurrence of false negative and false positive results [9,10].

The following limitations of the present study should be considered: (i) The study was not blinded; the observers could access the findings of analyzer which might have led to over-reporting of suspected flags. (ii) The data accumulated is collected consecutively in seven days from a single population having a specific profile in a tertiary care hospital that uses particular type of hematology analyzer. According to these factors, the results that we have shown in this study may not be as accurate in other laboratories.

CONCLUSION

In a conclusion, this study provides sufficient evidence against the performance of automated analyzer's alignment with that of standard manual methods. However, manual peripheral blood film review plays a critical role to avoid false-negative results and thus ensuring the final results. Hence necessary to prosper appropriate, timely and high-quality outputs.

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Table 1: Results obtained from comparison of flags generated by Mindray BC-6800 with peripheral blood film for total 500 samples

Parameter	Value	95% Confidence Interval
True Positive (no.)	209	-
False Positive (no.)	2	-
False Negative (no.)	0	-
True Negative (no.)	289	-
Sensitivity (%)	100.00%	98.25% to 100.00%
Specificity (%)	99.31%	97.54% to 99.92%
Positive Predictive Value (%)	99.05%	96.33% to 99.76%
Negative Predictive Value (%)	100.00%	-
Efficiency (%)	99.60%	98.56% to 99.95%
Youden's Index	0.99	-

Table 2: Age and sex wise distribution of 211 flagged samples between haematological disorder and solid neoplasms

Description	Haematological Disorder	Solid Neoplasms	Total
	Number of Patients (%)	Number of Patients	
Number of flags	98 (46.45%)	113 (53.55%)	211
<18 years of age	42 (42.86%)	11 (9.73%)	53
>18 years of age	56 (57.14%)	102 (90.27%)	158
Male	58 (59.18%)	51 (45.13%)	109
Female	40 (40.82%)	62 (54.87%)	102

Table 3: Results obtained from analysis of flags for blast, abnormal lymphocytes, atypical lymphocytes, immature granulocytes and shift to left

Parameter	Blasts	Abnormal Lymphocytes	Atypical Lymphocytes	Immature granulocytes	Shift to left	NRBC
True Positive (no.)	47	64	20	103	35	59
True Negative (no.)	431	412	468	384	458	432
False Positive (no.)	7	24	12	2	2	0
False Negative (no.)	15	0	0	11	5	9
Sensitivity (%)	75.81	100	100	90.5	87.5	86.76%
(95% Confidence Interval)	(63.26% to 85.78%)	(94.40% to 100.00%)	(83.16% to 100.00%)	(83.39% to 95.08%)	(73.20% to 95.81%)	(76.36% to 93.77%)
Specificity (%)	98.4	94.5	97.5	99.48	99.57	100%
(95% Confidence Interval)	(96.73% to 99.36%)	(91.92% to 96.44%)	(95.67% to 98.70%)	(98.14% to 99.94%)	(98.44% to 99.95%)	(99.15% to 100.00%)
Positive Predictive Value (%)	87.04	72.73	62.5	98.1	94.59	100.00%
(95% Confidence Interval)	(76.06% to 93.42%)	(64.38% to 79.73%)	(48.80% to 74.45%)	(92.81% to 99.52%)	(81.37% to 98.59%)	-
Negative Predictive Value (%)	96.64	100	100	97.22	98.92	97.96%
(95% Confidence Interval)	(94.87% to 97.81%)	-	-	(95.22% to 98.39%)	(97.58% to 99.52%)	(96.31% to 98.88%)
Efficiency (%)	95.6	95.2	97.6	97.4	98.6	98.2
(95% Confidence Interval)	(93.41% to 97.22%)	(92.94% to 96.90%)	(95.85% to 98.75%)	(95.59% to 98.61%)	(97.14% to 99.44%)	(96.61% to 99.17%)
Youden's Index	0.74	0.94	0.98	0.97	0.87	0.87

Table 4: WBC differential flags analysis

Parameter	Neutrophilia	Neutropenia	Lymphocytosis	Lymphopenia	Mono cytosis	Eosinophilia	Basophilia
True Positive (no.)	35	83	33	98	19	22	9
True Negative (no.)	1	0	0	0	0	1	0
False Positive (no.)	0	0	0	0	0	3	1
False Negative (no.)	381	381	369	369	480	490	490
Sensitivity (%)	100.00%	100.00%	100.00%	100.00%	100.00%	88.00%	90.00%
Specificity (%)	99.74%	100.00%	100.00%	100.00%	100.00%	99.80%	100.00%
Positive Predictive Value (%)	97.22%	100.00%	100.00%	100.00%	100.00%	95.65%	100.00%
Negative Predictive Value (%)	100.00%	100.00%	100.00%	100.00%	100.00%	99.39%	99.80%
Efficiency (%)	99.76%	100.00%	100.00%	100.00%	100.00%	99.22%	99.80%
Youden's Index	0.997	1	1	1	1	0.88	0.90