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Utilizing WBC Differential Channel and Abnormal WBC Flags for Diagnosing Hematological Malignancies Using Sysmex XN-9000 Analyzer

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Abstract

Objective: The objective of the study is to determine the predictive role of abnormal flags (monocytosis; blast/abnormal lymphocytes; atypical lymphocytes) along with variation of WDF scatter plot and comparing them with peripheral blood smears for diagnosing and differentiation of leukaemia.

Methodology: This study was performed at Chughtai Lab, Lahore from July, 2023 till October, 2023**.** A total of 160 EDTA samples were run on Sysmex NX 9000. Parameters that were studied and compared were WBC count, absolute monocyte count, monocyte percentage, abnormal flags and WDF scattergram. The results were confirmed by microscopy. Data was analyzed by SPSS version 23.00. Mean±SD was calculated for the continuous variable. Frequency and percentage were calculated for categorical variables. Pearson coefficient was performed to assess the correlation between various hematological parameters. Logistic regression was performed to determine the predictive role of abnormal flags and WDF scatter plot patterns in diagnosing leukemia. A p-value of <0.05 was taken as significant

Results: The CBC analysis by the instrument shows a good ability to detect true positive cases (sensitivity of 57.5%). Specifically, it has zero specificity and negative predictive value (NPV), meaning it cannot accurately identify true negatives, leading to a high false positive rate. Consequently, the instrument test cannot reliably rule out the condition when it gives a negative result, necessitating confirmatory testing with the gold standard i.e. Microscopic examination of the peripheral smear.

Conclusion: Relying solely on the flags generated by Sysmex XN 9000 does not warranty accurate diagnosis. Further, examining of the peripheral blood smear should be mandatory for the detection of suspicious cells whenever an abnormal flag is generated.

Key words: Automated hematology analyzer, Abnormal flags, Sysmex XN, Monocytosis, WBC Flags

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Introduction

Haematological malignancies are a group of heterogenous disorders arising from bone marrow or the lymphatic system. These disorders are broadly divided into leukaemia, lymphoma and plasma cell neoplasm.[1] . Haematological malignancies are the 4thmost commonly diagnosed malignancies in the world and constitute around 6.5% of all the cancers. Haematological malignancies are more prevalent in adults as compared to the children[2].

Automated haematology analysers have become an integral part of a haematology laboratory. To diagnose a haematological disorder both cellular and morphological analysis is important. These automated analysers apart from giving cell counts and differential counts also provide additional information in form of abnormal flags and scattergrams[3]. Sysmex XN-9000 is one such latest haematology analyser which uses principles of impedance, conductivity and optical detection to obtain different parameters of peripheral blood. Through its white blood cell channel, it can differentiate between abnormal lymphocytes and blast cells using fluorescent dyes and lysing agents. This instrument also uses cluster analysis algorithms to identify and flag the abnormal white blood cells[4].

Diagnosing haematological malignancies has been a very challenging task in areas where a haematologist is not available specially in the rural areas of Pakistan. Because of this many a times, a lot of cases go undiagnosed and lead to a fatal outcome owing to no proper referral and further management. Thus, a need arises for a method of diagnosing or suspecting a haematological malignancy so that proper referral can be made on time. Therefore, the objective of this study is to determine the predictive role of abnormal flags (monocytosis; blast/abnormal

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lymphocytes; atypical lymphocytes) along with variation of WDF scatter plot and comparing them to blood morphology done through microscopy for diagnosing and differentiation of leukemic subjects.

Methodology

This observational cross-sectional study that was performed at Chughtai Lab, Lahore from July, 2023 till October, 2023**,** after taking approval from Institutional Review Board (IRB), vide reference number CIP/IRB/1116. After a thorough literature search, we calculated a sample size of 160 via the WHO calculator, keeping the margin of error at 5%, a confidence level at 95%. Sampling was done using a non-probability consecutive sampling technique.

Inclusion Criteria: Routine complete blood counts (CBCs) of adult patients (age > 15 years) with TLC ≥ 15x103/µL showing abnormal WDF scattergram and abnormal flags (monocytosis; blast/abnormal lymphocytes; atypical lymphocytes). Written consent was obtained before enrolling all patients, and their confidentiality was ensured at all levels. 2 ml of venous blood sample was taken from each patient in Ethylenediamine tetra acetic acid (EDTA) tube following standard sampling procedures. EDTA samples were run on Sysmex XN-9000 haematology analyser. Following parameters were studied and compared: WBC count, absolute monocyte count, monocyte percentage, abnormal flags and WDF scattergram. The results were confirmed by microscopy. Peripheral smears were stained properly using Giemsa stain and were examined. The samples were then divided into two groups as infection/reactive changes and haematological malignancy (acute or chronic) based on the presence of blast/atypical cells. Data were entered in the excel work sheets, checked manually and logically corrected where needed.

Data was analyzed by using Statistical Package for the Social Sciences (SPSS) version 23.00. Mean±SD was calculated for the continuous variable. Frequency and percentage were calculated for categorical variables. Pearson coefficient was performed to assess the correlation between various hematological parameters. Logistic regression was performed to determine the predictive role of abnormal flags (Monocytosis, Blast/Abnormal lymphocytes, Atypical Lymphocytes) and WDF scatter plot patterns in diagnosing leukemic subjects (Acute malignancy and chronic malignancy). Validation of blast presence given by the CBC was performed in comparison to the gold standard (Atypical cells or blast cells on peripheral smear). A p-value of <0.05 was taken as significant.

Results

The total of one hundred and sixty patients were included. All patients were aged more than 15 years. TLC count of all patients was ≥ $15x10^3$/µL with a mean TLC count of 53.47 $x10^3$/ µL. Other peripheral blood parameters recorded for the patients included absolute monocyte count, monocytes percentage, absolute lymphocyte count, lymphocyte count percentage, Blast/Abnormal Lymphocytes flag, Atypical Lymphocytes flag, monocytosis flag, WDF Scattergram Correlation and presence of blast cells on Peripheral Smear (%). The mean values of all these parameters are presented in Table I. The parameters recorded in the form of presence or absence as given by the CBC analyzer are presented in Table II.

Table I: Mean ± SD of continuous blood parameters. (n=160)

Parameter	Min.	Max.	Mean ± SD
TLC	10.05	389.63	*53.4716± 77.0*
Absolute Monocyte count	0.08	224.41	16.4996±35.4
Monocytes percentage	0.0	78.0	13.763±11.4
Absolute Lymphocyte count	0.29	293.80	23.4418±50.2
Lymphocytes percentage	1.0	90.0	23.744±17.4
Blast Atypical Cells on Peripheral Smear (%)	0.0	98.0	32.981±37.1

Table II: Frequency & percentage of categorical parameters. (n=160)

Parameter	N	%
Monocytosis Flag	143	89.4
Blast/Abnormal Lymphocytes Flag	126	78.8
Atypical Lymphocytes Flag	69	43.1
WDF Scattergram Correlation	54	33.8
Chronic Malignancy	12	7.5
Acute Malignancy	67	41.9
Infection/Reactive Changes on microscopy	80	50.0
Blast/ Atypical Cells on microscopy	80	50.0

Pearson coefficient was performed to assess the correlation between the different hematological parameters. Table III represents the values of correlation coefficients. In association with Total Leukocyte Count (TLC), a strong positive correlation was seen with

Table III: Correlation between various hematological parameters (n=160)

Correlation of	Correlation with	Correlation	p-value
TLC	Absolute Monocyte Count	Negative	p < 0.01
	Monocytes	Negative	p < 0.01
	Absolute Lymphocyte Count	Negative	p < 0.01
	Lymphocytes	Negative	p < 0.01
	Blast/Atypical Cells on Peripheral Smear	Negative	p < 0.01
Absolute monocyte count	Monocytes	Negative	p < 0.01
	Absolute Monocyte Count	Negative	p < 0.01
	Absolute lymphocytes	Negative	p < 0.01
	Lymphocytes	Negative	p < 0.01
	Blast/Atypical Cells on Peripheral Smear	Negative	p < 0.01
Monocytes	TLC	Negative	p < 0.01
	Absolute monocyte	Negative	p < 0.01
	Blast/Atypical Cells on PS	Negative	p < 0.01
	Absolute Lymphocytes	Negative	p < 0.01
	Lymphocytes	Positive	p < 0.01
Absolute Lymphocyte Count	TLC	Positive	p < 0.01
	Blast/atypical cells on PS	Positive	p < 0.01
	Absolute monocyte	Positive	p < 0.01
	Monocytes	Negative	p < 0.01
	Lymphocyte	Negative	p < 0.01
Lymphocytes	TLC	Negative	p < 0.01
	Absolute monocyte	Negative	p < 0.01
	Blast atypical cells on PS	Negative	p < 0.01
	Absolute lymphocytes	Negative	p < 0.01
	Monocyte	Positive	p < 0.01
Blast/Atypical Cells on PS	TLC	Positive	p < 0.01
	Absolute monocyte	Positive	p < 0.01
	Absolute lymphocyte	Positive	p < 0.01
	Monocyte	Negative	p < 0.01
	Lymphocyte	Negative	p < 0.01

absolute monocyte count (AMC) and absolute lymphocyte count (ALC), a Moderate positive correlation with presence of blast cells on peripheral smear (PS), and a moderate negative correlation with monocytes and lymphocytes percentages. In association of AMC, a strong positive correlation was observed with TLC and a Moderate positive correlation with Blast/Atypical Cells on PS while a Weak positive correlation with ALC and a Weak negative correlation with Monocytes and Lymphocytes percentages. In association of Monocytes, a moderate negative correlation was seen with TLC, AML, and Blast/Atypical Cells on PS, a moderate positive correlation with Lymphocytes, and a Weak negative

Table IV: Regression analysis of CBC parameters (n=160)

Disease	Parameter	p-value	Significance
Acute malignancy	Absolute lymphocytes count	0.035	Significant
	Monocytosis Flag	0.998	Non-sig
	Blast/Abnormal Lymphocytes Flag	0.820	Non-sig
	Atypical Lymphocytes Flag	0.537	Non-sig
	Absolute Monocytes count	0.451	Non-sig
	Monocytes percentage	0.291	Non-sig
	Lymphocytes percentage	0.159	Non-sig
	WDF Scattergram Correlation	0.063	Non-sig
	Blast Atypical Cells on Peripheral Smear (%)	0.323	Non-sig
	Blast/ Atypical Cells on PS (Yes/No)	0.996	Non-sig
Chronic malignancy	Absolute lymphocytes	0.010	Significant
	Monocytosis Flag	0.997	Non-sig
	Blast Abnormal Lymphocytes Flag	0.694	Non-sig
	Atypical Lymphocytes Flag	0.745	Non-sig
	Absolute Monocytes	0.364	Non-sig
	Monocytes %	0.837	Non-sig
	Lymphocytes %	0.170	Non-sig
	WDFScattergramCorrelation	0.131	Non-sig
	Blast Atypical Cells on Peripheral Smear (%)	0.231	Non-sig
	Blast Atypical Cells (Yes/No) on PS	0.995	Non-sig
Infective/reactive changes	Absolute lymphocytes	<0.001	Significant
	Monocytosis flag	0.996	Non-sig
	Blast/ Abnormal Lymphocytes Flag	0.002	Significant
	Atypical Lymphocytes Flag	0.013	Significant
	Absolute Monocytes	0.011	Significant
	Monocytes	0.001	Significant
	Lymphocytes	<0.001	Significant
	WDF Scattergram Correlation	0.995	Non-sig
	Blast Atypical Cells on Peripheral Smear (%)	0.896	Non-sig
	Blast Atypical Cells (Yes/No)	0.786	Non-sig

Table V: Comparison of machine CBC parameters with Peripheral smear examination (n=160)			
	Peripheral smear-positive (80)	Peripheral smear-negative (80)	Total: 160
Instrument Test Positive (126)	TP: 46	FP: 80	
Instrument Test negative (34)	FN: 34	TN: 0	
Total: 160			

correlation with ALC. In association with Absolute Lymphocyte Count (ALC), a Strong positive correlation was deduced with TLC and a Moderate positive correlation with Blast/Atypical Cells on PS. While with AML count, a Weak positive correlation was seen and with Monocytes and Lymphocytes percentages, a weak negative correlation was observed. While performing association of Lymphocytes, a Moderate negative correlation with TLC, AML, and Blast/Atypical Cells on PS was seen, a Weak negative correlation with ALC and a Moderate positive correlation with monocytes percentage. In association of Blast/Atypical Cells on PS, a Moderate positive correlation was seen with TLC, AML, and ALC and a Moderate negative correlation with Monocytes and Lymphocytes percentages.

Table IV represents the summary of Logistic regression analysis performed to determine the predictive role of abnormal flags (Monocytosis, Blast/Abnormal Lymphocytes, Atypical Lymphocytes) and WDF scatter plot patterns in diagnosing leukemic subjects (acute malignancy and chronic malignancy) and for infective/reactive changes. For acute malignancy, only ALC showed a significant p-value (0.035), indicating it is a significant predictor of acute malignancy, while monocytosis flag, Blast/ Abnormal Lymphocytes flag, Atypical Lymphocytes flag, AML, Monocytes percentage, Lymphocytes percentage, WDF Scattergram Correlation, Blast/Atypical Cellson PS, were observed to be non-significant predictors of acute malignancy.

Logistic regression of predictors for chronic malignancy also showed. Only ALC (absolute lymphocyte count) to be a significant predictor with a p-value of 0.010, while Monocytosis flag, Blast/Abnormal Lymphocytes, Atypical Lymphocytes, AML, Monocytes percentage, Lymphocytes percentage, WDF Scattergram Correlation, Blast/Atypical Cellson PS were not statistically significant predictors.

In infective/reactive changes, AML, Monocytes percentage, Blast/Abnormal Lymphocyte flag, Atypical Lymphocyte flag, ALC, and Lymphocyte percentage were found to be statistically significant predictors of the disease.

The CBC analysis by the instrument shows a good ability to detect true positive cases (sensitivity of 57.5%), but it has significant limitations. Specifically, it has zero specificity and negative predictive value (NPV), meaning it cannot accurately identify true negatives, leading to a high false positive rate. This results in many healthy individuals being incorrectly labeled as positive. Consequently, the instrument test cannot reliably rule out the condition when it gives a negative result, necessitating confirmatory testing with the gold standard peripheral smear.

True Positives (TP): Cases where both the instrument test and the peripheral smear are positive. False Positives (FP): Cases where the instrument test is positive, but the peripheral smear is negative. True Negatives (TN): Cases where both the instrument test and the peripheral smear are negative. False Negatives (FN): Cases where the instrument test is negative, but the peripheral smear is positive. Diagnostic Performance showed a Sensitivity of 57.5%, Specificity: 0%, PPV: 36.5% and a NPV of 0%.

Discussion

It has been observed that in most myeloid neoplasms, blast population moves higher along the SFL axis and to the right on the SSC axis. This leads to scattering of the blast population in between the areas of monocytes and neutrophils. And because of this, abnormal cell population is counted in monocytes and a flag of monocytosis is raised if the population exceeds 10%. Similarly, for lymphoid neoplasms, blast population moves higher along the SFL axis but to the left on the SSC axis and scattering is observed between the lymphocyte and monocyte area raising a flag of abnormal lymphocytes/blasts. In a study conducted it was observed that 18 samples generated Atypical Lymphocytes flag but it was microscopically confirmed in 6 cases only. In 11 samples, the presence of atypical lymphocytes could not be demonstrated and one sample even contained blasts and therefore was considered false negative for the Blasts/Abnormal Lymphocytes

flag([5]). In our study also a significant number of samples were giving false negative results.

The usefulness of the flags generated by the automated hematological analyzers depends upon their sensitivity and specificity and are an indication that some abnormality has been detected in the blood analyzed which needs to be verified by examining a properly stained peripheral blood smear. In a study conducted in Pune, it was found that Correlation between the WBC histograms and their peripheral smears was statistically significant.[6] Another study conducted by Depoorter et al, compared 5 different analysers to assess their flagging performance. They found out that flagging performance are inconsistent among different analysers of different manufacturers[7]. In our study also we observed diagnostic utility of instrument had 57.5% sensitivity.

Monocytosis is maybe the first presentation of a hematological neoplasm in an elderly patient however, monocytosis can also occur in chronic inflammatory disorders[8]. Blood smear examination is the gold standard to diagnoses neoplastic disorders when absolute monocyte count is more than 1.0 103 /µL and 10% of white blood cell (WBC) count[9]. In our study no significant correlation was seen with AML and monocyte percentages in diagnosis acute or chronic malignancy. Even the monocytosis flag did not demonstrate any significant correlation.

In a study conducted in Norway, observed that Blast flags did not significantly reduce the number of peripheral smears that were being made in their laboratory and only 41 cases with blast cells were identified among the 328 samples which all were flagged for blast cells. The study overall demonstrated very little clinical significance of blast flags in Sysmex analyzers.[10].

Conclusion

We conclude that although the Sysmex XN-9000 analyzer undoubtedly help us analyze the samples with precision with its 7-part differential count and can be successfully handle tremendous workload in high profile commercial lab but our study limits its diagnostic abilities as far as diagnosing acute and chronic leukemias are concerned. Relying solely on the flags generated by Sysmex XN 9000 does not warranty accurate diagnosis. Further, examining of the peripheral blood smear should be mandatory for the detection of suspicious cells whenever an abnormal flag is generated.

Limitations: This study was conducted on a limited number of samples during a short period of time. We recommend a much large-scale study and inclusion of research-based parameters to assess the overall performance of Sysmex XN 9000.

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