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SCATTERPLOT PATTERNS IN AUTOMATED HEMATOLOGY ANALYSERS AS RAPID SCREENING TOOL IN WHITE BLOOD CELL DISORDERS – A DESCRIPTIVE COMPARATIVE STUDY

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ABSTRACT

Automated hematology analyzers generate white blood cell (WBC) scatterplots that provide valuable information about leukocyte distribution and abnormalities. This study aimed to evaluate scatterplot patterns as a rapid screening tool for WBC disorders in comparison with peripheral blood smear (PBS) examination. This descriptive comparative study was conducted over one year and included 895 EDTA blood samples. Samples were analyzed using Sysmex XN-350 and Yumizen H500 hematology analyzers. The study was performed in three phases: identification of normal scatterplot patterns, evaluation of abnormal scatterplots, and comparison of scatterplot findings with PBS examination. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated. A prototype normal scatterplot was developed by analyzing 385 normal samples. Abnormal patterns were identified in cases of neutrophilia, lymphocytosis, eosinophilia, leukopenia, and leukemia. Characteristic changes included increased cellular clustering, altered distribution, overlapping cell populations, and greying of scatterplot regions. Scatterplot analysis demonstrated sensitivity ranging from 76.47% to 100%, specificity from 97.96% to 100%, and accuracy from 98.06% to 100%. Lymphocytosis showed the highest accuracy (100%), whereas leukemia showed comparatively lower sensitivity (76.47%). WBC scatterplot analysis is a rapid and reliable screening tool for identifying various WBC abnormalities. It demonstrates good diagnostic agreement with PBS findings and can assist in identifying samples requiring further evaluation. However, peripheral blood smear examination remains essential for morphological confirmation and accurate diagnosis, particularly in suspected leukemia.

INTRODUCTION

Hematology Analysers (also spelled Hematology analyzers in British English) are used to count and identify blood cells at high speed with accuracy.1 During the 1950s, laboratory technicians counted each individual blood cell underneath a microscope. Tedious and inconsistent, this was replaced with the first, very basic hematology Analyser, engineered by Wallace H. Coulter.[1] Hematology Analysers are used to conduct a complete blood count (CBC), which is usually the first test requested by physicians to determine a patients general health status. A complete blood count includes red blood cell (RBC), white blood cell (WBC), hemoglobin, and platelet counts, as well as hematocrit levels.[1] The diagnosis of diverse hematological disorders relies on blood counts obtained through automated cell counters and peripheral blood film examinations (PBFs).[1] The implementation of automated hematology Analysers has significantly decreased the necessity for manual counts, particularly when processing a large number of samples. However, when a comprehensive morphological diagnosis is essential, the examination of peripheral blood smears (PBS) remains valuable, albeit time-consuming and dependent on expertise.[2] Recent advancements in automated blood cell counting have facilitated the analysis of leukocyte differentials through the utilization of fluorescence RNA/DNA staining in conjunction with scattered light intensity.[3] This technique employs a combination of side scatter, forward scatter and nucleic acid fluorescence to generate a concise and accurate depiction of each individual white blood cell. The WBC-DIFF (White Blood Cell Differential) produced by the majority of cell counters exhibits a strong correlation with the manual reference method, demonstrating high sensitivity and excellent linearity in determining WBC differentials. This approach proves to be a reliable and effective means of characterizing white blood cells.[4,5] Anomalies observed in the WBC-DIFF and scatterplots generated by the system offer valuable insights into different leucocyte disorders and have demonstrated their utility in the diagnosis of malaria and dengue, enabling differentiation from other causes of febrile illnesses.[6,7] To achieve this objective, the current study was conducted with the aim of identifying scatterplot patterns associated with normal WBC counts, as well as those seen in various WBC disorders such as neutrophilia, eosinophilia, leukemia, reactive lymphocytosis, and leukopenia. Furthermore, the study aimed to evaluate the effectiveness of WBC scatterplot patterns in comparison to peripheral blood smear (PBS) analysis for diagnosing WBC disorders.

METHODOLOGY

A three-phased descriptive study was performed for a period of 1 year on a total of 895 EDTA blood samples and studied within three hours of collection. Sample size was calculated by n = [DEFF*Np(1-p)]/ [(d2/Z2 1- /2*(N-1)+p*(1-p)], where Population size (for finite population correction factor or fpc) (N): 4000000, Hypothesized % frequency of outcome factor in the population (p): 50% ± 5, Confidence limits as % of 100(absolute ± %)(d): 5%, Design effect (for cluster surveys-DEFF): 1 Calculated sample size was 385 at 95% confidence level.

Patients aged more than 18 years with abnormal white blood cell count and differential leucocyte count, scatterplot patterns as obtained by automated cell counter and PBS were enrolled in this study. Samples were analysed using the Sysmex XN-350 hematology analyser (manufactured by Sysmex, Sysmex Sverige, MariosGata 13, S-434 37, Kungsbacka, Sweden) and Yumizen H500 (manufactured by Horiba,India). The parameters included total and differential leukocyte counts, scatterplot patterns and peripheral smears. In phase 3, the scatterplots of abnormal flagged samples were analysed and provisional diagnosis made in each case which was then compared with the diagnosis on the PBS. Sensitivity, Specificity, Positive and Negative predictive values (PPV & NPV) were calculated.

Phase 1 Normal samples - Calculated to be 385 samples. Samples with normal total and differential counts as detected by the peripheral smear and the Sysmex Hematology Analyser were selected. The scatterplots of normal samples were studied and patterns among these were recognized.
Phase 2 Sample with WBC abnormalities - Specified pattern in at least 60% of cases depicts abnormal cell counts and patterns. Using this percentage with 15% relative precision at 95% confidence limits the sample size required for this objective. Hence for this phase sample size calculated to be 114. Although the study was done on 400 samples.
Phase 3: Taking the peripheral smear findings as the gold standard, scatterplots are compared with the peripheral

smear findings. About 110 samples were studied considering the feasibility. Consecutive sampling was done.

All the data was noted down in a pre-designed study proforma. Qualitative data were represented in the form of frequency and percentage. Association between qualitative variables was assessed by Chi-Square test. Quantitative data were represented using Mean ± SD. Analysis of Quantitative data was done using appropriate statistical test. A p-value < 0.05 was taken as level of significance. Results were graphically represented where deemed necessary. Microsoft Excel 2010 for graphical representation. Sensitivity, Specificity, Positive and Negative predictive values (PPV & NPV) were also calculated.

RESULTS

The scatterplots of all the 385 cases with normal values as determined by the cell counter and PBS were analysed to find specific patterns within them. After analysing the scatterplots of all the normal cases, 30 normal scatterplots were chosen which encompassed almost all the patterns found in the 385 scatterplots. Grids were superimposed on each scatterplot to get a better idea of the location of each cell region in the scatterplot. Further based on analysing the patterns of these 30 scatterplots, a prototype normal scatterplot was made manually. Neutrophils (Cyan blue): Thumbprint-shaped cluster with a dense centre. Immature granulocytes may appear above it. Lymphocytes (Magenta): Spindle/tear-drop-shaped cluster with a dense lower-right region. Monocytes (Green): Quadrilateral-shaped, unevenly distributed cluster without a dense centre. Eosinophils (Red): Irregular-shaped cluster with scattered cells. RBC debris (Dark blue): Seen in the bottom-right region, called the *Ghost region*. Basophils: Identified separately in the WBC-BASO scattergram. (Figure 1).

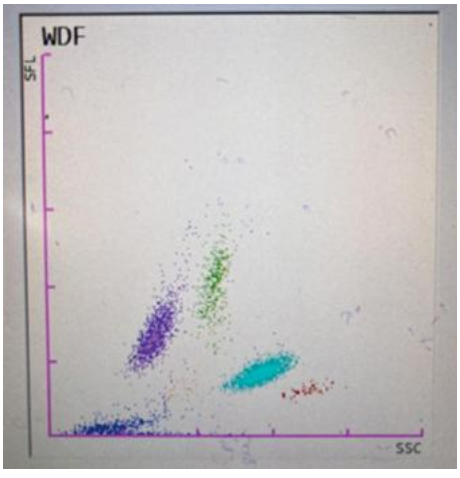


Fig 1: Normal Scatterplot of Phase

In second phase, a total of 400 samples in which the following abnormalities were detected by the PBS and flagged by the cell counter were selected. Neutrophilia – 190 (47.5%), Eosinophilia – 82 (20.5%), Lymphocytosis – 27 (6.75%), Leucopenia – 74 (47.5%), Leukemia – 27 (47.5%), Acute Myeloid Leukemia – 10, Acute Lymphoblastic Leukemia – 7, Chronic Myeloid Leukemia – 6 and Chronic Lymphocytic Leukemia – 4

Abnormal WBC Scattergram Patterns

- Neutrophilia: Increased neutrophil intensity, size and boomerang appearance. (Fig 2)
- Eosinophilia: Increased size, intensity and clustering of eosinophils. (Fig 3)
- Lymphocytosis: Increased lymphocyte clustering and merging with monocyte region. (Fig 4)
- Leucopenia: Reduced intensity and size of WBC regions with increased dispersion. (Fig 5)
- Acute Leukaemia: Grey, intense, meteor-shaped cell distribution. (Fig 6)
- Chronic Leukaemia: Grey scattergram with two peaks (twin mountain appearance). (Fig 7,8)

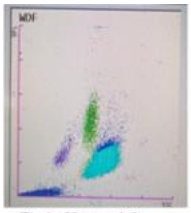


Fig 2 : Neutrophilia

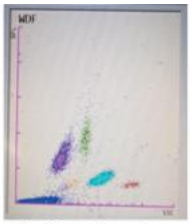


Fig 4: Lymphocytosis

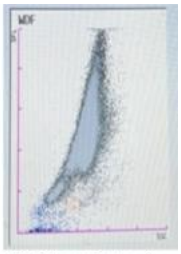


Fig 6: Acute Leukemia

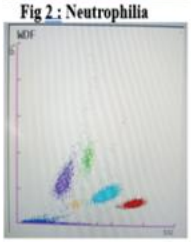


Fig 3: Eosinophilia



Fig 5: Leukopenia with neutrophilic preponderance



Fig 7: CML

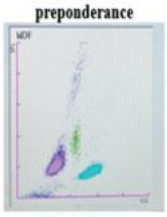


Fig 8: CLL

Table 1: Observed Values of True and False Positive Results and True and False Negative Results, Sensitivity and Specificity, Positive and Negative Predictive Values of all the Abnormalities

Scatter plot evaluation		Neutrophilia	Lymphocytosis	Eosinophilia	Leukopenia	Leukemia	Monocytosis
Scatter Plot status	Normal	73(66.36%)	89(80.9%)	94(85.45%)	92(8.64%)	97(88.18%)	105(95.45%)
	Abnormal	37(33.64%)	21(19.09%)	16(14.55%)	18(16.36%)	1(11.82%)	5(4.55%)
PBS	Normal	69(62.73%)	89(80.9%)	96(87.27%)	9(84.55%)	93(84.55%)	106(96.36%)
	Abnormal	41(37.27%)	21(19.09%)	14(12.73%)	17(15.45%)	17(15.45%)	4(3.64%)
True Positive		37	21	14	17	13	4
True Negative		70	89	94	92	93	105
False Positive		0	0	2	1	0	1
False Negative		3	0	0	0	4	0
Sensitivity		92.50%	100.00%	100%	100%	76.47%	100%
Specificity		100%	100	97.96%	98.92%	100%	99.06%
Positive Likelihood Ratio				49	93		106
Negative Likelihood Ratio		0.07	0	0	0	0.24	0
Disease prevalence		5%	5%	5%	55%	5%	5%
Positive predictive value		100%	100%	72.06%	83.04%	100%	84.80%
Negative predictive value		99.61%	100%	100%	100%	98.78%	100%
Accuracy		99.62%	100%	98.06%	98.98%	98.82%	99.10%

The scattergram evaluation showed high diagnostic performance in detecting WBC abnormalities. Sensitivity ranged from 76.47% to 100%, specificity from 97.96% to 100%, and accuracy from 98.06% to 100%. Lymphocytosis showed the highest accuracy (100%), while leukaemia showed the lowest sensitivity (76.47%). Overall, scattergram analysis demonstrated good agreement with peripheral blood smear findings. (Table 1)

DISCUSSION

Accurate measurement of hematological analytes is essential for correct interpretation of hematological disorders in both clinical and research fields.[8] A total of 895 samples were subjected to scrutiny during the study, that compares scatterplot patterns with peripheral smear findings in the existing literature.

This study successfully generated a grid-based standard scatter plot, serving as a reference, by overlaying 30 scatterplots obtained from normal samples. This approach allowed us to encapsulate the diverse patterns observed across all 385 normal scatterplots. Remarkably, this descriptive finding represents a novel contribution and has not been previously documented in the available literature up to the present time. The majority of cells within a given lineage exhibit distinctive characteristics, occupying well-defined regions in the scatterplot. These characteristics encompass characteristic intensity levels and clustering patterns, with each region forming a specific angle with respect to the x-axis, contingent upon factors such as cellular density, volume, and nuclear complexity. In light of the results obtained, any deviation observed from the aforementioned parameters typically indicates the presence of particular abnormalities warranting further investigation.

Throughout the scatterplot analysis conducted in the second phase, instances of neutrophilia, lymphocytosis, and eosinophilia revealed a notable overall trend characterized by an elevation in cell intensity, clustering, angles formed with the x-axis, and area occupied by specific regions, directly attributed to increased cell counts. In cases of neutrophilia, a higher number of cells were observed above the neutrophilic region, displaying enlarged size and heightened nuclear complexity, which were identified as immature granulocytes. Additionally, these cells appeared in proximity to the ghost region, representing older neutrophils undergoing 48 degeneration, resulting in a distinct boomerang-like appearance. It is noteworthy that such a morphological manifestation has not been documented in the available scientific literature. Lymphocytosis exhibited a distinctive pattern of convergence, wherein lymphocytes merged with the monocytic region, signifying the presence of activated or reactive lymphocytes with enhanced cell size and complex nuclear structures. This particular observation consistently manifested across all the dengue cases under investigation, suggesting that this morphological finding could serve as a valuable indicator aiding in the diagnosis of dengue infection.[9] Scatterplots generated from leukemia cases exhibited distinctive aberrations characterized by greying, stemming from significantly elevated counts of blasts and immature cells, along with noticeable clustering patterns. Furthermore, a discernible shift was observed, directing the scatterplots towards either the neutrophil or lymphocyte region, contingent upon the specific classification of leukemia as either lymphoid or myeloid type. The identification of such distinct abnormal scatterplot characteristics served as a critical indicator, prompting subsequent peripheral blood smear (PBS) examination and other diagnostic tests to facilitate the accurate diagnosis of leukemia.[10,11] Pillai KR et al (2020) in their study White Blood Cell (WBC) scattergrams were analysed and the cases with abnormalities were evaluated for presence of malarial parasites by peripheral smear examination. While analysing WBC scattergrams of Sysmex XN-1000, WDF channel, different abnormalities were noted in comparison with normal scattergrams. Many cases showed more than one abnormality. The abnormalities detected were : a. Greying of neutrophil and eosinophil populations b. Two eosinophil populations c. Overlapping of eosinophil and neutrophil populations d. Two neutrophil populations e. Greying of lymphocyte and monocyte groups f. Two lymphocyte populations g. Right shift of RBC ghost area. [12]

The detection of neutrophilia, lymphocytosis, eosinophilia, and leukopenia exhibited a notably high level of accuracy in third phase. Remarkably, a strong and positive correlation was established among the white blood cell (WBC) parameters concerning peripheral blood smear (PBS) findings, indicating a reliable concordance between the automated cell counter analysers utilized in the investigation. The sensitivity of Scatterplot in evaluation of Neutrophilia was 92.50%, for Lymphocytosis, Eosinophilia, Leukopenia and for Monocytosis it was 100% for each, for Leukemia it was 76.47%. The specificity of in evaluation of Neutrophilia, Lymphocytosis, Eosinophilia Leukopenia, Leukemia and for Monocytosis it was found to be 100.00%, 100%, 97.96%, 98.92%, 100% and 99.06% respectively. The accuracy of Scatterplot in evaluation of Neutrophilia was 99.62%, for Lymphocytosis was found 100%, for Eosinophilia was 98.06%, for Leukopenia 98.98 for Leukemia it was 98.82% and for Monocytosis it was 99.10%. The positive and negative predictive values were 100% and 99.61%, 100% and 100%, 72% and 100%, 83.04% and 100%, 100% and 98.78 and 84.80% and 100% for Neutrophilia, Lymphocytosis, Eosinophilia Leukopenia, Leukemia and for Monocytosis evaluation respectively. Scatter plot has a much better negative predictive value than positive predictive value in all WBC abnormalities. In present study, the sensitivity and specificity of Scatterplot in detecting Neutrophilia, Lymphocytosis, Eosinophilia Leukopenia, Leukemia and for Monocytosis was high and ranged from 92.50 to 100%. However, the sensitivity of Scatterplot in detecting Leukemia was low at 76.47% with a specificity of 100%. Few earlier studies conducted by Tavish Gupta T, and Basu D (2020), also showed low sensitivity of Scatterplot in detecting Leukemia. This excellent correlation underscored the consistency and precision of the WBC data obtained through various analysers, enhancing confidence in their clinical applicability and diagnostic utility.[5, 13] There was a discordance in a few cases . Increase cellularity due to neutrophilic toxic change was incorrectly determined as leukemia three cases. Six out of seven scatterplots showing prominent blue areas turned out to be CML with basophilia or toxic changes in neutrophils. Numerous haematology analysers (HA) employed in the enumeration of white blood cells (WBC) produce a WBC differential scattergram, serving as the foundational element for automated WBC differentials. These WBC scattergrams play a crucial role in the generation of flags or alarms, which serve the purpose of detecting potential inaccuracies in the WBC count (as well as the WBC differential) and providing pertinent insights into the potential origins of such anomalies. The automated hematology analyser readings are as reliable as the standard manual method even though the latter method gives additional diagnostic information through the blood pictures. Hence, manual microscopic blood examination should always be used to validate the automated methods.[8] Scatterplot patterns obtained from a complete blood count can give a clue in the diagnosis of the aetiologies of acute febrile illnesses.[14]

CONCLUSION

From this study we conclude that, WBC scatterplots can be used as a screening tool as it correlates well with various WBC disorders in the PBS. PBS methods produced comparable results with automated method for estimation of total WBC, lymphocytes and neutrophils. PBS methods estimation of differential cell counts showed higher sensitivity, specificity

and accuracy of the scatterplot findings keeping the PBS findings as gold standard.

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