International Journal of Research in Medical Sciences
Tailor T et al. Int J Res Med Sci. 2024 Mar;12(3):820-824
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20240524

Original Research Article

Study of automated hematology analyzer's scatterplot patterns in white blood cell disorders

Tanvi Tailor, Toral Jivani*, Ami Jagirdar, Shilpa Patel

Department of Pathology, Surat Municipal Institute of Medical Education and Research, Surat, Gujarat, India

Received: 02 January 2024
Revised: 02 February 2024
Accepted: 06 February 2024

***Correspondence:**
Dr. Toral Jivani,
E-mail: drtoraljivani@gmail.com

ABSTRACT

Background: Automated hematology analyzers produce scattergrams that can be used as screening tool for various hematological conditions and efficiently shorten turnaround times. Aim was to study scattergram patterns of various while blood cell disorders and assess their efficacy compared to a peripheral blood smear for diagnosis of various disorders.
Methods: Scattergram findings generated by UniCel® DxH 800 automated hematology analyzer, a 5-part differential analyzer. The graphic displays have been compiled over a period of 3 months from blood samples received for CBC. Samples that the counter flagged as abnormal for white blood cell were chosen. Based on the scatterplots, a preliminary diagnosis was formed. It was compared with the peripheral blood smear (PBS) findings which were taken as the gold standard.
Results: The scatterplots showed unique patterns for various disorders on the basis of location, shape, size, density of the cells and their clustering. The scattergram analyser showed 90% sensitivity and 88% specificity for diagnosing hematological disorders. A 97-100% accuracy rate was reported showing excellent correlation between PBS result and WBC parameter result in cell counter analyzers.
Conclusions: Not all cases of haematological malignancy exhibit cytopenias or cytosis at initial presentation. Therefore, these scatter plots offer helpful information that prompts a hematopathologist to suspiciously screen the peripheral smear in cases with normal counts. Scattergram analysis suspects a diagnosis earlier than peripheral smear examination. Given their strong correlation with a variety of WBC disorders and confirmed by PBS, WBC scatterplots can be used as a screening tool.

Keywords: Hematology analyzer, Leukemia, Scatterplots, White blood cell disorders

INTRODUCTION

Automated hematology analyzers are instruments designed to perform a complete blood count (CBC). It employs various methods such as coulter principle and volume, conductivity, and scatter (VCS) technology to count and differentiate blood cells.[1] Most instruments generate 2 types of data: numerical data and graphic displays with or without flags for internal laboratory review. Graphic displays take 2 basic forms: histograms in which relative numbers of red cells, white cells, and platelets are plotted against cell size and scatter plots in which white cell subpopulations and platelets are displayed.[2] Diagnoses of various hematological disorders depend on blood counts, derived from automated cell counter and peripheral blood film examination.[3] The ability to differentiate between normal and abnormal scatterplot patterns is beneficial in diagnosing conditions such as leukocytosis, leukopenia and leukemia. Automated hematology analyzers efficiently decrease turnaround time and produce scatter grams that offer valuable insights for considering diagnoses of various hematological conditions, especially during periods of high workload.[4] The established patterns can therefore be

utilized as a tool for the suspicion of hematological disorders when screening in resource-constrained laboratories and those with a high sample load. Additionally, lab technicians and staff members can be taught to sound the alert when they notice these patterns.[4] This makes these patterns a useful tool for pre-microscopic screening of hematological disorders. Aim was to study scattergram patterns of various while blood cell disorders and assess their efficacy compared to a peripheral blood smear for diagnosis of various disorders. Rapid, reliable access to information about a variety of hematologic disorders is provided; in some cases, review of peripheral smear along with clinical data may be sufficient enough to establish a diagnosis.[3,5]

METHODS

A descriptive study was performed with data collection conducted in the section of hematology, department of pathology in Surat Municipal Institute of Medicine Education and Research, Surat. Scattergram findings generated by Unicel® DXH 800 automated hematology analyzer, a 5-part differential analyzer. This instrument uses coulter method and VCS technology with volume analysis.[1]

The graphic displays of scatter plots compiled over a period of 3 months (January 2023-March 2023) from EDTA blood samples received for CBC analysis of indoor patients at our hospital. Scatter plot or scatter graph is a type of mathematical diagram using cartesian coordinates to display values for two variables for a set of data.[6]

Sample size was calculated using OpenEpi V3.01 considering proportion of sample flagged by counter as abnormal for WBC parameter for indoor patients which was 82.32% with level of confidence at 5% and allowable error at 5%.

Approximately 20,000 scattergrams were analyzed during the study period. A total of 272 samples were included in the study which was flagged by the counter for abnormal white blood cell parameters. 'Flags' are signals that occur when an abnormal result is detected by the analyzer. Flags are displayed to reduce false-positive and false-negative results by mandating a review of blood smear examination.[7] The exclusion criteria include inadequate or clotted samples, CBCs from non-admitted patients, and of indoor admitted patients whose samples were not flagged by the counter for abnormal white blood cell parameters.

A preliminary diagnosis was established upon reviewing the scatterplots of these selected samples. Then findings were confirmed by peripheral blood smear examination by keeping it as gold standard for diagnosis.[8] For cases of leukemias, they were confirmed by bone marrow aspiration and biopsy followed by immunophenotypic diagnosis.

RESULTS

To assess the efficacy of WBC scatterplot patterns, 272 flagged samples were taken. The cases were identified based on the flags generated on the hematology analyzer and there after peripheral smear examination was done for confirmation and for leukemia cases confirmation was done by bone marrow aspiration and biopsy and then after confirmed by immunophenotypic diagnosis.

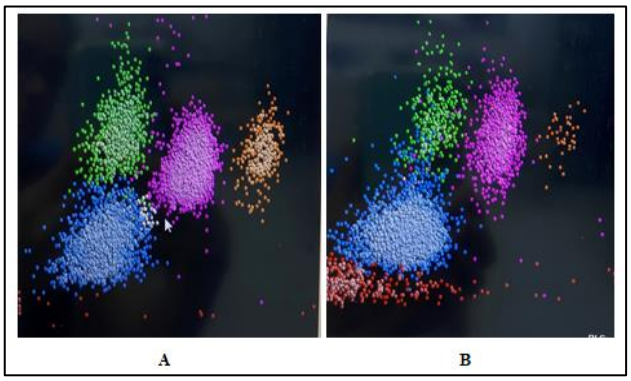

Figure 1: A) Normal scatterplot; B) leukopenia.

The normal scatterplots were analysed for location, shape, size, density of the cells and their clustering (Figure 1A).

Neutrophils are represented by colour pink and consists of centrally placed, singe, tear drop shaped, dense with mild dispersion toward top. Upper dispersion most likely represents the immature forms of cells like band forms, metamyelocytes and myelocyetes.

Lymphocytes are represented by colour blue and consists of single, thumbprint shaped, dense region in bottom left with mild dispersion in all directions due to wide variation in size of lymphocytes. Sometimes these cells merge with the monocytic region.

Monocytes are represented by colour green and consists of single, circular, which is dispersed unequally with a mildly dense area seen in the top left area.

Eosinophils are represented by colour orange and consists of single, irregular shaped with which is dispersed unequally with a non-dense area seen in the top right area.

Basophils are represented by colour white and consists of small, centrally placed, irregular shaped, non-dense cluster in left lower part of neutrophilic region.

A total of 272 cases that were flagged by the hematology analyzer showed following abnormalities that were confirmed by the PBS.

In neutrophilia, neutrophilic region shows increased intensity, increase in size and dispersion of cells and increase in number of cells in the upper part of the region (Figure 2A).

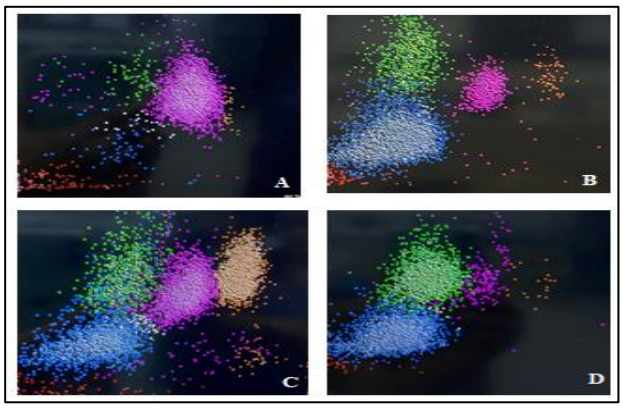

Figure 2: A) Neutrophilia; B) lymphocytosis; C) eosinophilia; D) monocytosis.

In lymphocytosis, lymphocytic region shows increase in the intensity, elongation of the region, reduced dispersion, increased clustering and cells above the region were seen (Figure 2B).

In eosinophilia, eosinophilic region shows increased intensity, increased size, elongation of shape, central clustering (Figure 2C).

In monocytosis, monocytic region shows increased intensity, increase in size and dispersion of cells (Figure 2D)

In leukopenia, decrease in the intensity and size of all WBC regions, reduced clustering, increased dispersion. As shown in Figure 1B.

In CLL, a compact cluster in the lymphocyte region which is touching the y axis and extending upwards but not as high as seen in acute leukemia scatter plot. However, the scatter plots of ALL and CLL may appear similar (Figure 3A).

In CML, dense neutrophilic cluster extending upwards which strongly indicate a shift to left represented by myelocyte, metamyelocytes, band cells and neutrophils. Clustering the in the upper part of the monocytic region probably represent early myeloid precursor. Prominent clustering is seen in the basophilic region (Figure 3B).

In CML-blast phase, Figure 3C depicts case which showed more than 20% basophil in the peripheral smear, with a prominent cluster in this "basophil region along with a prominent cluster in the monocytic region extending upwards which suggest blast population along with a large prominent cluster in the immature granulocyte region also seen.

In ALL, a large, compact cluster in the lymphocytic region which is encroaching monocytic region (Figure 3D).

In AML-M3 case showed small clusters, one in lymphocytic region and just above it a large neutrophilic cluster enroaching the monocytic region giving a "tear drop" appearance (Figure 3E).

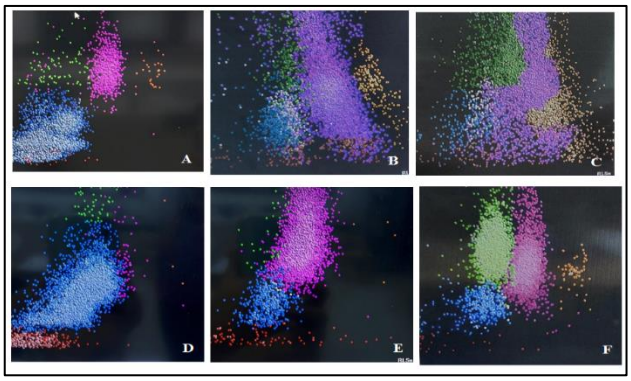

Figure 3: A) CLL; B) CML; C) CML-BP; D) ALL; E) AML-M3; F) AML-M4.

In AML-M4, two major clusters are seen, a dense neutrophilic cluster extending upwards in the immature granulocyte region representing myelocytes and metamyelocytes. A dense cluster in monocytic region is seen which is also extending upwards and touching the top margin representing myeloblast and monoblast (Figure 3F).

Using peripheral blood smear findings as the gold standard, the sensitivity, specificity, positive predicting value, negative predicting value and accuracy of the scatterplot patterns for various disorders was calculated (Table 2).

Table 1: Abnormalities detected on the scatterplots along with the peripheral findings and discordance among them.

Abnormalities	Scatterplot	Diagnosis confirmed on PBS	Discordant on PBS
Neutrophilia	97	96	1-Leukemia (AML)
Lymphocytosis	58	55	3-Leukemia (ALL)
Eosinophilia	34	34	0
Monocytosis	61	59	2- Leukemia (AML)
Leukemia	22	20	2- Neutrophilia (1) and Monocytosis (1)

Table 2: Sensitivity and specificity, positive, negative predictive values and accuracy of all the abnormalities detected on the scatterplots.

Abnormalities	Sensitivity (%)	Specificity (%)	Positive predictive values (%)	Negative predictive values (%)	Accuracy (%)
Neutrophilia	100	99.43	98.97	100	99.63
Lymphocytosis	98.21	88.61	94.83	99.5	98.53
Eosinophilia	100	100	100	100	100
Monocytosis	96.61	98.12	93.44	99.05	97.79
Leukemia	90	98.41	81.82	99.2	97.79

DISCUSSION

Automated analyzers have high sensitivity and hence reduce the need for manual examination. The purpose of automation is to provide faster reportable results and to reduce the technologist hands-on time, in addition to providing high quality and precision.

In our study, WBC disorders detected on the scatterplots along with the peripheral findings showed presence of discordance is shown in Table 1.

Diagnosis of neutrophilia was given in 97 cases on the scatterplots of which 96 were confirmed on PBS showing neutrophilia with left shift. But one was diagnosed as AML-M3. 58 cases of leucocytosis were diagnosed on scatterplots of which 55 were confirmed on PBS as leucocytosis and few showed reactive lymphocytosis as well, but 3 cases turned out to be ALL. Of the 34 cases with a presumptive diagnosis of eosinophilia on the scatterplots, all 34 showed eosinophilia on PBS. Among the 61 cases of monocytosis on scatterplot, 59 were confirmed under PBS whereas 2 cases were determined to be AML-M4. 22 cases were considered as leukemia on the scatterplot and 20 were confirmed. 2 cases turned out to be neutrophilia and 1 case of monocytosis. All the cases of CML which were detected on the scatterplot were confirmed to be CML under the PBS.

Our results were concurrent with Gupta et al and Gupta et al findings, which showed patterns of acute leukemias; which usually present with high total leucocyte count, anemia, and thrombocytopenia; however, in few cases when total leukocyte count is low or even normal, the scatter plots provide important clues to the diagnosis.[2,8]

In Gupta et al phase three of the study, 113 cases were taken which showed abnormalities in the scatterplots, out of which 13 cases showed discordance. In our study out of 272 cases, 8 discordances were found.[8] In Gupta et al study increased cellularity due to neutrophilic toxic change was incorrectly determined as leukemia in three cases.[8] In our study one case of was diagnosed as neutrophilia on scatterplot was diagnosed as AML on PBS.

In our study in neutrophilia, shows increased intensity, increase in size and dispersion of cells and increase in number of cells in the upper part of the region where as in Gupta et al study there was increased number of cells above the neutrophilic region having increased size and nuclear complexity (determined to be immature granulocytes) and near the ghost region (older neutrophils undergoing degeneration) giving a boomerang appearance.[8]

In Gupta et al study lymphocytosis showed merging of the lymphocytes with the monocytic region representing the reactive lymphocytes, with complex nuclear structure and increased cell size which was characteristically seen dengue cases and could provide a clue towards its diagnosis.[8] In our study we didn't have access to enough patient data to correlate such.

In contrast to our study, in Ningombam et al study, 38 cases CLL out of 53 showed "inverted comma" trailing where as in our study lymphocyte region was touching the y axis and extending upwards.[4] Our study result for CLL concurrent with Choccalingam et al study, in CLL the lymphoid cells are located lower than the normal lymphocytes area in the scattergram.[12]

Our CML results were concurrent with Ningombam et al that there was increased clustering and intensity in the basophilic region.[4]

Just like in Gupta et al study, Ningombam et al and in Mishra et al.[2,4,9] study, our study also showed two distinct clusters in AML-M3 cases. One smaller cluster in lymphocytic region and one large tear drop shaped cluster representing promyelocyte.

In our study, excellent correlation between WBC parameters and PBS results in cell counter analyzers has been documented with accuracy 97-100% (Table 2). There were 8 discordant on PBS where cases of leukocytosis were diagnosed as leukemia. Blast cells were incorrectly counted as monocytes and lymphocytes. Blasts were shown in the lymphocytic or monocytic region extending upwards. Increased counts of blasts and immature cells and clustering, with a shift towards the neutrophil or lymphocyte region depending upon whether the leukemia was lymphoid or myeloid type.

Scanning the scattergrams visually provides an initial sense of the size, shape, and other features of WBCs. Even before the peripheral blood smear review, these patterns from scattergram, along with numerical data and flags, provide a clue toward diagnosis. A large collection of data presented as a visual image can convey information far more effectively than numbers alone.

There are some limitations. While replacing the manual white cell differential count is the aim of any automated differential leukocyte counting system, this has not yet been entirely achieved in the clinical laboratory. The automated leukocyte differential count is superior to the manual differential count in many aspects, but in some cases it fails to provide important morphologic detail that only the microscopic review can provide.[11] Leukemia can be hard to diagnose when peripheral white blood cell levels are low or normal because a blast flag isn't.

CONCLUSION

Automated hematology analyzers provide useful information about common hematological conditions. Cytopenias and cytosis do not occur in all the cases of hematological malignancy. In cases where the counts are normal, these scatter plots provide a useful clue that alerts a hematopathologist to keep an eye while screening the peripheral smear. Typical pattern helps suspect a diagnosis of a disorder before peripheral smear examination. This can be useful in resource constrained laboratories and in centres where large sample size is processed every day. Automated cell counters are now an integral part of most hematology laboratories. Scatterplot patterns obtained from a complete blood count can aid in the diagnosis and it is necessary for a clinician and the laboratory personnel to be aware of differences from the usual scatterplot pattern. Identifying a normal scatterplot, on the other hand, may eliminate the need for PBS in each case, and hence reducing the amount of resources required, cost, and time. WBC scatterplots can be used as a screening tool as it correlates well with various WBC disorders in the peripheral blood smear.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Beckman coulter unicel® dxh 800 coulter® cellular analysis system Manual. Available from: https://www.beckmancoulter.com/products/hematology/dxh-800. Accessed on 20 August 2023.
2. Gupta M, Chauhan K, Singhvi T, Kumari M, Grover RK. Useful information provided by graphic displays of automated cell counter in hematological malignancies. J Clin Lab Anal. 2018;32:e22392.
3. Asad S, Ahmed I, Ali N. Utility of peripheral film findings and its correlation with automated analyzer- an audit from tertiary care hospital. J Lab Phys. 2017;9:1-4.
4. Ningombam A, Acharya S, Sarkar A, Kumar K, Chopra A. Scattergram patterns of hematological malignancies on sysmex XN-series analyzer. J Appl Hematol. 2021;12(2):83-9.
5. Adewoyin AS. Peripheral blood film-a review. Ann Ibadan Postgrad Med. 2014;12(2):71-9.
6. Lokwani DP. The ABC of CBC: Interpretation of complete blood count and histograms. 1st edn. JP Medical Ltd; 2013:127-130.
7. Kawthalkar SM. Essentials of clinical pathology. 1st edn. Jaypee Brothers Medical Ltd.; 2010:323-326
8. Gupta T, Basu D. Utility of scatterplot patterns of automated hematology analysers in white blood cell disorders- a comparative study with peripheral blood smears. Asian J Med Sci. 2020;11(5):29-34.
9. Mishra S, Kumar K, Panigrahi A, Das P, Padhi S, Chhabra G. The utility of leucocyte cell population data and scattergram in rapid identification of acute promyelocytic leukemia. Blood. 2020;136:19-20.
10. Juthani R, Basu D. Use of scatterplot patterns derived from automated haematological analysers in the diagnosis of acute febrile illnesses. J Clin Diagn Res. 2020;14(2).
11. Hoyer JD, Fisher CP, Soppa VM, Lantis KL, Hanson CA. Detection and classification of acute leukemia by the Coulter STKS Hematology Analyzer. Am J Clin Pathol. 1996;106(3):352-8.
12. Choccalingam C. Volume, conductance, and scatter parameters of neoplastic and nonneoplastic lymphocytes using Coulter LH780. J Lab Phys. 2018;10:85-8.

Cite this article as: Tailor T, Jivani T, Jagirdar A, Patel S. Study of automated hematology analyzer's scatterplot patterns in white blood cell disorders. Int J Res Med Sci 2024;12:820-4.