ORIGINAL RESEARCH PAPER
Volume - 15 | Issue - 06 | June - 2026 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

WBC SCATTERPLOTS IN AUTOMATED HEMATOLOGY ANALYZERS: AN EFFECTIVE SCREENING APPROACH FOR HEMATOLOGICAL DISORDERS

Hematology

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ABSTRACT

Introduction: Automated hematology analyzers are widely used with more and more sophisticated versions over the years. They provide valuable numerical as well as graphical data (histograms and scattergrams) which are helpful in predicting various diseases including RBC and WBC disorders, and are screening tool for various hematological conditions. They help reduce turnaround time.**Aim and Objectives:** To analyze scatterplot patterns of white blood cell (WBC) disorders. To evaluate their diagnostic effectiveness in comparison with peripheral blood smear in the diagnosis of WBC disorders. **Materials and Methods:** Scattergram findings were generated by the Yumizen H500 Hematology Analyzer, a 5-part differential analyzer. These graphic displays were collected over a period of three months from blood samples received for complete blood count (CBC) analysis. Samples that were flagged as abnormal for white blood cells by the analyzer were selected for the study. A preliminary diagnosis was made based on the scatterplot patterns and was subsequently compared with the findings of the peripheral blood smear (PBS), which is considered the gold standard.**Results:** Scatterplots showed distinct patterns for various disorders based on cell location, shape, size, density, and clustering. Of the 1432 samples analyzed, 572 were normal and 860 showed abnormal scatterplots with flagging; among these, 586 samples were flagged for WBC parameter abnormalities. A sensitivity of 98–100%, specificity of 99-100% and accuracy 99.6% was observed, demonstrating excellent correlation between WBC scatterplot findings and peripheral blood smear (PBS) results. **Conclusion:** Scattergram analysis allows for the preliminary detection of WBC abnormalities earlier than peripheral blood smear examination. Given their strong correlation with a variety of WBC disorders and confirmation by PBS findings, WBC scatterplots serve as an efficient screening tool, helping to reduce turnaround time, lower costs, and decrease laboratory workload.

KEYWORDS

Blood Counts, Cell Density, Hematology, Leukocytosis, Rna, Scatter Plots.

INTRODUCTION

Scatter plots are widely employed in exploratory data analysis across diverse scientific fields. Blood counts, which are derived from automated cell counter and peripheral blood film examination, are the most important factor in the diagnosis of various hematological disorders.[1] Most instruments generate 2 types of data: numerical data and graphic displays with or without flags. By using fluorescence RNA/DNA staining and scattered light intensity, we can analyze leukocyte differentials with the help of recent advances.[1] Manual counts have been reduced by the automated hematology analyzer, particularly when many samples are being analyzed.[2] Differentiating between normal and abnormal scatterplot patterns can help diagnose conditions such as leukocytosis, leukopenia, and leukemia. Automated hematology analyzers have the ability to efficiently shorten turnaround time and generate scatter grams that provide valuable insights for assessing various hematological conditions, particularly during times of heavy workload.[2]

Additionally, laboratory technicians and staff can be trained to recognize these patterns and raise an alert when they are observed. This makes scatterplot patterns a valuable tool for pre-microscopic screening of hematological disorders.[3]

With this objective, the present study was conducted to identify scatterplot patterns in normal WBC counts and in various WBC disorders, including neutrophilia, eosinophilia, leukemia, reactive lymphocytosis, and leukopenia, and to evaluate the efficacy of these patterns in comparison with peripheral blood smear (PBS) for the diagnosis of WBC disorders.[3]

Aim and Objectives

1. To analyze scatterplot patterns of white blood cell (WBC) disorders.
2. To evaluate their diagnostic effectiveness in comparison with peripheral blood smear in the diagnosis of WBC disorders

Materials and Methods

This cross-sectional descriptive study was conducted over a period of three months at the Central Laboratory of a tertiary care centre. A total of 1432 EDTA blood samples received for analysis were included in the study. All samples were processed and analyzed using the Yumizen H500 Hematology Analyzer and comparison with PBS was done for the diagnosis of WBC disorders.

RESULTS:

A total of WBC scatterplot patterns 1432 (100%) samples were taken out of which 572 (39.94%) samples were normal, 860 (60.06%) shows flagging/abnormal scatterplots. Out of 860 (100%) and 586 (68.14%) samples were flagged abnormal for WBC parameters. Flags are signals that occur when an abnormal result is detected by the analyzer. After analyzing the flags generated by the hematology analyzer, the cases were identified and confirmed through peripheral blood smear examination. The normal scatter plots were compared to others based on their location, shape, size, cell density, and clustering characteristics.

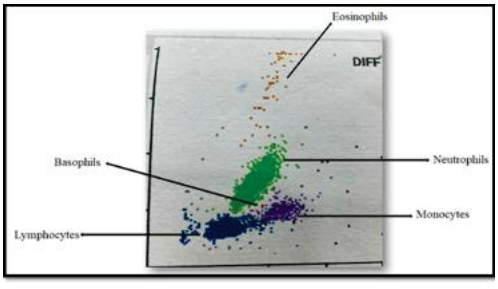


Figure 1: Normal scatterplot

Normal scatterplots were analyzed based on the location, shape, size, density, and clustering of cells (Figure 1).

Neutrophils (green) appear as a dense central cluster; show higher granularity and moderate fluorescence, which may represent immature forms such as band forms, metamyelocytes, and myelocytes.

Lymphocytes (blue) form a dense, thumbprint-shaped cluster in the bottom left region with mild dispersion in all directions due to variation in lymphocyte size, and may occasionally overlap with the monocytic region.

Monocytes (purple) are seen slightly above and to the right of lymphocytes; larger cells with moderate complexity.

Eosinophils (orange) are seen as an irregularly shaped cluster with uneven dispersion in the top right region and relatively lower density.

Basophils (red) appear as a small, non-dense cluster located in the lower part of the neutrophil region.

\Out of 860 abnormal samples, 586 showed abnormalities on peripheral blood smear (PBS). Neutrophilia was the most common finding, observed in 218 cases; among these, 215 were confirmed as neutrophilia on PBS, while 3 were subsequently diagnosed as leukemia (chronic myeloid leukemia – 2 cases; acute myeloid leukemia – 1 case). This was followed by lymphocytosis in 141 cases, of which 131 were confirmed as lymphocytosis and 3 were later identified as leukemia (chronic lymphocytic leukemia – 2 cases; acute lymphoblastic leukemia – 1 case) on PBS as tabulated in Table-1. Eosinophilia, monocytosis, and leukopenia were noted in 75, 74, and 70 cases, respectively.

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

Abnormalities	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Neutrophilia	100%	99.7%	99.5%	100%	99.8%
Lymphocytosis	100%	99.6%	98.6%	100%	99.7%
Eosinophilia	100%	100%	100%	100%	100%
Monocytosis	100%	99.8%	98.6%	100%	99.8%
Leukopenia	100%	100%	100%	100%	100%
Leukemia	100%	99.8%	87.5%	100%	99.8%

Overall, leukemic cases were relatively infrequent, with a total of 8 cases, including acute myeloid leukemia (2 cases), acute lymphoblastic leukemia (2 cases), chronic myeloid leukemia (2 cases), and chronic lymphocytic leukemia (2 cases). Notably, one leukemic case initially presented with neutrophilia on PBS.

The scatterplot patterns observed in the following cases are discussed below:

Neutrophilia:

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)

Lymphocytosis

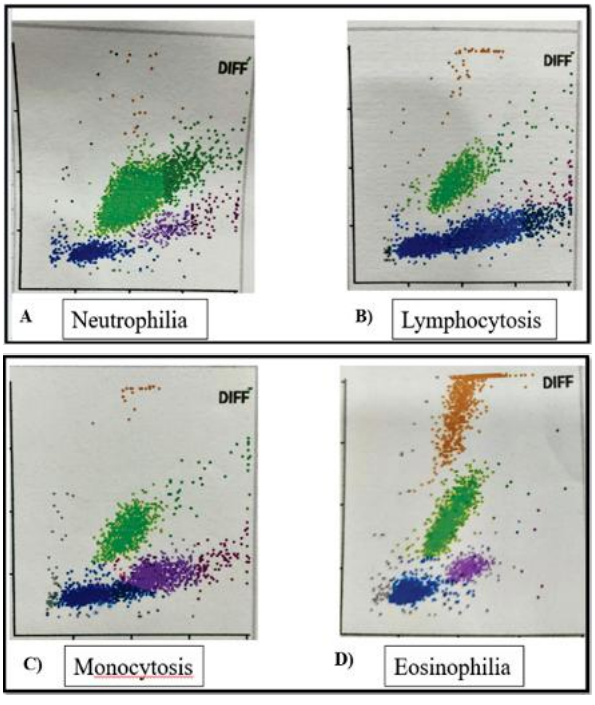
In lymphocytosis, lymphocytic region shows increase in the intensity of cell clustering, widening or elongation of the region, reduced dispersion, increased clustering and merging with the monocytic region. (Figure 2B)

Monocytosis

In monocytosis, the monocytic region shows increased intensity along with an increase in the size and dispersion of cells (Figure 2C).

Eosinophilia

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



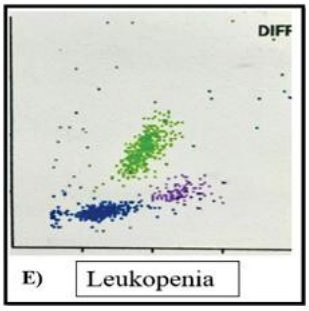


Figure 2: A) Neutrophilia; B) Lymphocytosis; C) Monocytosis; D) Eosinophilia; E)Leukopenia

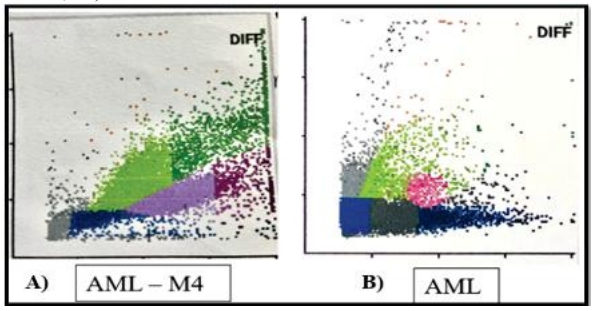
Leukopenia

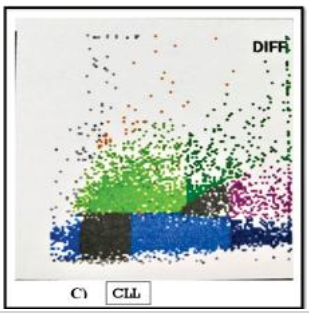
In leukopenia, decreased in the intensity and size of the WBC region, reduced clustering, increased dispersion. There is either an increased number of neutrophils or lymphocytes relative to the normal percentage. (Figure 2E)

Leukemia

Acute Leukemia – Most of the scatterplots had cell greying with an increased intensity and cell clustering, with some exhibiting a distinct neutrophilic region and others displaying a fusion of lymphocytic and monocytic regions.

- Leukemia scatterplots were distinctly abnormal with greying because of the significantly higher counts of blasts and immature cells, as well as clustering. The leukemia's lymphoid or myeloid type dictated a shift towards the neutrophil or lymphocyte region.
- The detection of the basic abnormal scatterplot is a clue for PBS examination and other tests to diagnose leukemia.
- In AML-M4, a dense neutrophilic cluster that extends upwards is observed, which is a representation of myelocytes and metamyelocytes. A dense cluster can be observed in the monocytic region, which is also spreading upwards and intersecting the top margin, and is a representation of myeloblast and monoblast. (Figure 3A, 3B)
- Chronic Lymphocytic Leukemia (CLL) shows marked expansion of the lymphocyte population in the lower region, appearing as a dense and broad horizontal cluster, indicative of lymphocytosis characteristic of CLL. (Figure 3C)
- Chronic Myeloid Leukemia (CML) shows that there was increased clustering and intensity in the basophilic region, and more than 20% basophil in the peripheral smear was noted. (Figure 3D, 3E)





PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

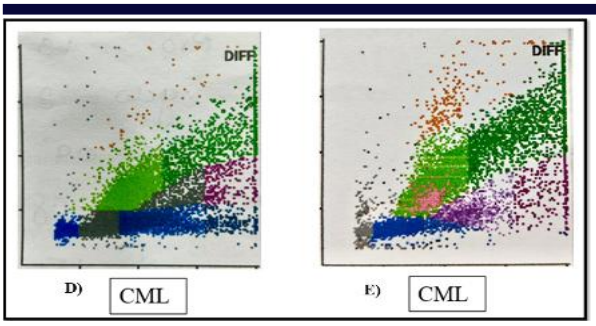


Table 2: The assessment of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the scatterplot patterns.

Abnormalities	Scatterplot	Diagnosis confirmed on PBS	Discordant on PBS
Neutrophilia	218 (37.2%)	215 (36.7%)	Leukemia AML – 1 (0.46%) , CML-2 (0.92%)
Lymphocytosis	141 (24.1%)	138 (23.5%)	Leukemia (ALL) -1 (0.71%) CLL -2 (1.42%)
Eosinophilia	75 (12.8%)	75 (12.8%)	0
Monocytosis	74 (12.6%)	73 (12.5%)	Leukemia (AML)–1 (1.35%)
Leukopenia	70 (11.9%)	70 (11.9%)	0
Leukemia	8 (1.4%)	7 (1.2%)	Neutrophilia – 1 (12.5%)

The table 2 demonstrates diagnostic performance, maintaining 100% Sensitivity and NPV across all categories to effectively rule out abnormalities. The slightly lower Positive Predictive Value (87.5%) for Leukemia reinforces that while the screening is highly accurate, manual confirmation remains necessary for specific positive results.

DISCUSSION

Automated analyzers demonstrate high sensitivity, thereby minimizing the need for manual examination. The primary aim of automation is to deliver faster and reliable results while reducing the manual workload of laboratory technologists, along with ensuring greater accuracy, consistency, and precision in testing.

In study done by *Jivani et al,* 272 cases were taken which showed abnormalities in the scatterplots, out of which 8 cases showed discordance. A study done by Gupta et al 113 abnormal flagged samples were analyzed in the scatterplots. 13 cases showed discordance. In our study out of 586 cases , 8 discordances were found. In *Jivani et al*[1], one case initially interpreted as neutrophilia on scatterplot was later diagnosed as Acute Myeloid Leukemia on peripheral blood smear (PBS), while Gupta et al[3] reported three discordant cases subsequently confirmed as leukemia on PBS; similarly, in our study, three cases were diagnosed as leukemia on PBS, which can be attributed to increased intensity, larger cell size, greater dispersion, and an upward shift in the neutrophilic region on scatterplot analysis.

In the study by *Gupta et a*[3]*l*, 13 cases identified as lymphocytosis on scatterplot correlated with peripheral blood smear (PBS) findings of reactive lymphocytosis, whereas *Jivani et al* could not establish such correlation due to insufficient data. In contrast, in our study, two cases were diagnosed as Chronic Lymphocytic Leukemia and one as Acute Lymphoblastic Leukemia, characterized on scatterplot by increased intensity of cell clustering, widening or elongation of the lymphocytic region, reduced dispersion, dense clustering, and partial merging with the monocytic region.

In a study done by *Jivani et al*[1] in monocytosis one turned out to be AML M2. In *Gupta et al*[3] study 2 cases showed discordance of leukemia showing AML M3 and AML M4. In our study 1 case was determined to be acute myelomonocytic leukemia because leukemic blasts and abnormal promonocytes in subtypes of AML can resemble monocytes in size and internal complexity on automated scatterplots, resulting in misclassification as monocytosis.

Strong concordance is seen in the cases of Eosinophilia and Leukopenia in comparison with the studies done by Jivani et.al[1] and Gupta et.al[3]

Both in *Jivani et al*[1] and *Gupta et al*[3] *study*, scatterplots of leukemia cases showed two distinct clusters in AML. Our results were consistent with *Jivani et al*[1] findings, and discordance of 1 case turned out to be neutrophilia. The discordant case occurred because immature granulocytic cells and reactive neutrophils can produce similar scatterplot clustering patterns to blasts in AML , leading to a presumptive leukemia pattern on automated analysis that was later identified as neutrophilia on PBS.

Blast cells are mistakenly identified as monocytes and lymphocytes. It was observed that lymphocytic or monocytic region had blasts that were extending upwards. clustering and increase blast cells count along shift towards the neutrophils or lymphocyte region, depend on whether the leukemia is lymphoid or myeloid type.

In our study we found that there is a strong correlation between scatterplots of WBC parameters and PBS results in cell counter analyzers with accuracy of 99% to 100%.

CONCLUSION :

The study demonstrates that WBC scatterplot patterns generated by automated hematology analyzers are useful for the preliminary detection of hematological abnormalities. Characteristic scatterplot changes were observed in disorders such as neutrophilia, lymphocytosis, eosinophilia, monocytosis, leukopenia, and leukemia. These findings showed high sensitivity, specificity, and diagnostic accuracy when compared with the peripheral blood smear (PBS), the gold standard.

Thus, scatterplot analysis serves as an effective pre-microscopic screening tool, helping laboratory personnel identify abnormal samples for further confirmation by PBS and enabling early detection and faster laboratory workflow.

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12. which showed patterns of leukemia, which typically has high leukocyte count, anemia and thrombocytopenia. However, in certain cases the scatter plots can provide important clues to the diagnosis despite the low or normal total leukocyte count. In our study the diagnosis of leukemia was given in 8 cases on scattered plot examination of which 7 were confirmed on PBS showing leukemia and