INTRODUCTION

A fully automated hematology analyzer, the Sysmex XN-1000, is a 7-part analyzer can run a number of hematological tests. A scattergram, which is a graphic representation of the distribution of the cell population, is one of the tests it can run. Scattergrams are produced in Sysmex XN-1000 using impedance and laser light scattering technologies. The analyzer distinguishes between various cell types and subpopulations using various angles of laser light scattering and impedance measurements.\(^1\)

Scattergram is a graphical representation of the distribution of cells based on their physical characteristics such as size and complexity. The principle of a scattergram in Sysmex is based on the Coulter principle, which uses impedance measurement to count and size cells as they pass through an aperture. In a Sysmex scattergram, cells are first classified based on their size, with smaller cells appearing on the left side of the graph and larger cells on the right. The complexity of the cells is then assessed, with less complex cells appearing on the bottom of the graph and more complex cells on the top. This creates four distinct quadrants on the scattergram that corresponds to different types of cells, including Figure 1 lymphocytes, monocytes, granulocytes, and abnormal cells.\(^2\)

The Sysmex XN-1000 analyzer is capable of producing a variety of scattergrams, including white blood cell (WBC). Scattergram which displays the different types of WBCs
based on their size and complexity and can differentiate between neutrophils, lymphocytes, monocytes, eosinophils, and basophils, and red blood cell (RBC). Scatter gram which displays the different types of RBCs based on their size and hemoglobin content and also differentiate between normal RBCs, microcytic RBCs, hypochromic RBCs, and macrocytic RBCs. Platelet Scatter gram displays the different types of platelets based on their size and granularity. This can differentiate between normal platelets, giant platelets, and clumped platelets. These scatter grams can provide valuable information about the different cell populations in a blood sample and can help in the diagnosis of various hematological disorders.

In the present study, we intended to analyze the specific scattergram patterns of Sysmex XN series in acute myeloid leukemia (AML), French American British Classification AML-M3 also known as acute promyelocytic leukemia (APL) which shows numerous promyelocytes having abundant cytoplasm with numerous azurophilic granules and characteristic rod-shaped Auer rods which is hallmark of APL. The overall survival rate has been increased from <50% in the pre-all transretinoic acid (ATRA) era to over 80–90% with the use of ATRA and chemotherapy, hence it should be diagnosed with the help of scattergrams which supports peripheral smears and also chronic myeloid leukemia (CML) and chronic lymphoproliferative disorder-chronic lymphocytic leukemia (CLL) so that scattergram can be used as a preliminary diagnostic tool, in identifying potential cases of acute and chronic leukemias.

Aims and objectives
Aim of the present study is to study the different Scattergram patterns of haematology analyser and to provide unique patterns shown by different haematological malignancies.

MATERIALS AND METHODS
This was a retrospective study done on 58 diagnosed cases of hematological malignancies during the time period of March 2021—February 2023 in Government Medical College and Hospital Nizamabad. Ethical Committee clearance has been taken. Peripheral smear and bone marrow morphology based diagnosis was done. The pre-treatment complete blood count (CBC) w/diff data for these patients were retrieved from the Sysmex Xn-1000 analyzer.

Internal quality checks were performed on all levels (low, normal, and high), calibration was performed once a year, and manufacturer-based standard operating procedures were followed. The blast count in the peripheral smear cut off 20% was considered in all of the acute leukemia cases WBC differential (WDF) (SSCSFL) side scatter (SSC) and side fluorescence (SFL), WDF (SSCFSC) SSC and forward scatter (FSC), WDF (FSCSFL) forward scatter (FSC) and SFL, and white cell nucleated (WNR) (SFLFSC), WNR (SSCFSC), and WNR (SFLSSC) scattergrams were analyzed. All samples were evaluated using peripheral blood smears and bone marrow smears stained with Leishman stain.

RESULTS
Among 58 cases, 20 cases were AML, 32 cases were CML, and 5 cases were CLL.

AML M3 (n=20) – of the 20 cases of AML, 6 cases were of French-American-British (FAB) type AML M3 were identified by peripheral smear, bone marrow, and flow cytometry. There were two distinct clusters to be seen. As seen in Figures 2 and 3, smaller cluster representing the normal lymphocyte region, while an abnormal promyelocytes are represented by a larger cluster resembling the shape of a “tear drop” with broad base.

A compact cluster that begins in the lymphocyte region and barely rises to the areas of higher SFL was visible in all five cases of chronic lymphoproliferative disorders (CLPD) of the CLL type. Out of the five cases, a small cluster was observed in four of them, as shown in Figure 4. It was located above the area of debris and directly beneath the compact cluster. Due to the observation of smudge cells in these four cases, we hypothesized that the cluster most likely represents smudge cells. We discovered a cluster with events trailing in the direction of higher FSC in the WNR (SFL FSC) plot, as shown in Figure 4. Three out of the five...
cases exhibited this cluster. As we observed prolymphocyte in the peripheral smear in each of these cases, we made the assumption that these prolymphocytic populations were those that displayed higher SFL.

Among the 32 cases, 30 involved chronic phase CML (CP). In the WDF (SSC SFL) plot, a significant cluster was observed above the baseline neutrophil population. Myelocytes, metamyelocytes, and band neutrophils are represented by these occurrences. Excessive events were also observed in the monocyte region, which most likely represented an early myeloid precursor. To the left of the neutrophil, toward the region of low SSC was visible another population that was typical of CML cases. This cluster most likely represents the basophil population. Figures 5 and 6 depict a representative scattergram of CML CP in WDF (SSC SFL) and WNR, respectively (SFL FSC).

**DISCUSSION**

AML can be diagnosed and classified using the information contained in the scattergrams generated by the Sysmex XN-1000 analyzer (AML). The WDF scattergram generated by the analyzer can display an increased number of cells in the blast region in the case of AML, which denotes a higher number of immature cells that are distinctive to AML. The high FSC and low SSC values of the blast cells are another way to recognize them.

The Sysmex XN-1000 analyzer can also be used to detect APL, which is a subtype of AML characterized by the presence of abnormal promyelocytes. Promyelocytes in APL have a distinctive morphology and are frequently referred to as “hypogranular” or “biloculated,” with an abnormal nuclear shape. These abnormal promyelocytes can be found in populations of cells with high SSC and high fluorescence intensity in the WDF scattergram generated by the Sysmex XN-1000 analyzer. In addition, the Sysmex XN-1000 analyzer’s CBC results for APL patients may reveal a decreased platelet count due to bone marrow suppression and an increased WBC count due to the presence of abnormal promyelocytes.

Acute lymphoblastic leukemia (ALL) and CLPD can be distinguished using compacted clusters and inverted comma trailing toward high SFL in the WNR (SFL-FSC) (ALL). Events with a higher SFL signal in the WDF (SSC-SFL) are more common in ALL, whereas these events are less common in CLPD unless there is a sizable prolymphocytic population present in the peripheral blood smear of CLL. Theoretically, these events with high SFL...
signal may be more frequent in non-Hodgkin lymphoma, such as diffuse large B-cell lymphomas, marginal zone lymphomas, and hairy cell leukemia variant, where there is a spill-over effect. In the case of CLPDs, such as CLL, the presence of "compacted clusters" and "inverted comma" trailing toward high SFL in the WNR (SFL-FSC) is a distinguishing feature. All the five CLPD cases that were included in the study's analysis of CLPDs showed the "inverted comma" trailing toward high SFL. It is crucial to remember that, in addition to the analysis of scatter gram patterns, the diagnosis of ALL and CLPDs frequently necessitates additional testing, such as molecular research and analysis of flow cytometry data.\textsuperscript{10,11}

CLPDs, which are frequently referred to as the “smudge cell region,” are more frequently associated with the presence of a smaller cell cluster immediately below a larger cell cluster in the lymphocyte region than ALL. Four out of five CLPD cases in the study mentioned displayed this cluster, suggesting that it may be a helpful diagnostic feature in the initial screening of these disorders. It is significant to remember that this characteristic is not unique to CLPDs and can also be observed in other illnesses such as viral infections, autoimmune diseases, and drug reactions.\textsuperscript{12} As a result, even though the existence of the smudge cell region can be a useful indicator in the diagnosis of CLPDs, it should be interpreted in conjunction with other clinical and laboratory findings as well as confirmatory tests such as flow cytometry and molecular studies.

Krause evaluated the use of Technicon H-1 (Technicon Instruments Corporation, Tarrytown, NY, USA) for the characterization of acute leukemias. Based on myeloperoxidase activity and nuclear characteristics of the cells, they were able to separate out AML and ALL. Among the AMLs, they suggested that FAB type AML-M3, M4, and M5 had characteristic cytograms. CML also was considered to have a distinctive pattern.\textsuperscript{13} Similarly, Kline et al. studied the usefulness of Technicon H-1 (Technicon Instruments Corporation, Tarrytown, NY, USA) to detect blast cells.\textsuperscript{14} The WDF scattergram combines fluorescence, side-angle light scatter, and forward-angle light scatter to identify and separate different cell types based on their physical and chemical properties. One can distinguish CML-CP patterns from leukemic reaction by analyzing the basophil region of the WDF scattergram and the excessive events in the monocyte region, which represent an abundance of early precursors.\textsuperscript{13,16} This suggests that, compared to someone who is having a leukemoid reaction, a person with CML-SCP will show distinctive patterns in their WDF scattergram. The possibility that CML-CP has changed into the accelerated phase (AP) and blast phase can be inferred from analysis of the events in the respective blast regions and the basophil region of the WDF scattergram (BP). Therefore, it appears possible to track the progression of the illness and predict when a person with CML-CP is likely to enter the AP or BP phases using the WDF scattergram.\textsuperscript{17}

Scattergram analysis can be a useful screening aid in differentiating various reactive and neoplastic conditions. In cases of already diagnosed leukemias, analysis of scattergram patterns confirms that each case has an individualized plot pattern. This can aid in proper triaging of cases for further molecular and cytogenetic evaluation. Furthermore, these patterns, in conjunction with definitive morphological assessment of peripheral blood and bone marrow examination, can also be used to monitor cases on therapy, whether they are in remission or relapse. However, it is important to note that scattergram patterns alone should not be used to make treatment decisions but rather as an adjunct to other diagnostic and monitoring tools. It is also important to consider the potential effect of treatment on scattergram patterns.

Limitations of the study

We were unable to provide ALL cases due to the fact that, during this time frame, ALL cases were not recorded in our hospital and also lack of flow cytometry prevented us from subclassifying AML. It is unknown whether treatment might affect these patterns since the study did not include post-treatment patients. Further research is required weather scattergram patterns can be used as a marker of treatment response or resistance.

CONCLUSION

The pattern analyses of scattergrams can be particularly useful in resource-constrained laboratories and in centers where a large sample size is processed every day. Scattergram analysis plays an important role as a preliminary diagnostic tool and has the potential to improve the efficiency and accuracy of leukemia diagnosis which helps in prioritizing the evaluation of cases that require further testing, such as molecular and cytogenetic evaluations particularly in settings with limited resources or high sample volumes. However, it is important to note that scattergram analysis should not be used as the sole diagnostic tool, and other clinical and laboratory evaluations are necessary to confirm the diagnosis and guide treatment decisions.

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REFERENCES


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