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Original Research Article

Value of scattergrams in screening of haematological malignancies on Sysmex XN analyser

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Abstract

Background: Automated haematology analysers are widely used with more & more sophisticated versions over the years. They provide valuable numerical as well as graphical data (histograms & scattergrams) which are helpful in predicting various diseases including RBC as well as WBC disorders. We aimed to analyse the pattern of white blood cell differential (WDF) and white cell nucleated region (WNR) scattergrams in cases of haematological malignancies.
Materials and Methods: This was an observational descriptive type cross-sectional study of a total 25 newly diagnosed cases of haematological malignancies over a period of 6 months confirmed by peripheral blood smear (PBS) examination and cytochemistry. All haematological malignancies were categorised according to French American British (FAB) classification and WHO classification. All cases were analysed using Sysmex XN 3100 haematology analyser and analysed for WDF & WNR scattergrams.
Results: Out of 25 cases 9 cases were of Chronic Myeloid Leukaemia (CML) chronic phase, 9 cases of Acute Myeloid Leukaemia (AML) including 6 cases of AML M2, single case of AML M3 and 2 cases of AML M5-2. There were 4 cases of Acute Lymphoblastic Leukaemia (ALL) and 3 cases of Chronic Lymphocytic Leukaemia (CLL). Our analysis of WDF channel scattergram in cases of CML and ALL showed unique patterns correlating with previous studies. Also, The WNR channel showed unique findings of scatter beyond the fourth decade in acute leukaemia, which also correlated with past studies.
Conclusion: Scattergrams can be used as screening aids in differentiating and screening haematological neoplastic conditions using pattern analysis.

Keywords: Automated cell counter, Haematological malignancies, Scattergram.

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1. Introduction

Complete blood count (CBC) is a simple, cheap and basic important routine haematological investigation used to identify infections, inflammatory conditions as well as haematological neoplastic conditions. CBC is carried out with the help of routine haematology analysers. In the past, haematology analysers were simple utilising semi-automated methods to measure single parameters. Over the years, more and more complex fully automated analysers are being developed giving multiple numerical as well as graphical parameters.[1]

Routine laboratories use haematology analysers to classify types of leukocytes for quick results. These analysers utilise the principle of flowcytometry for the same using specific reagents.

Sysmex Corporation (Sysmex, Kobe, Japan) launched the XN series of automated haematology analysers in 2011 where WDF channel is used for leukocyte differentiation. The WDF channel utilises a surfactant in specific reagents. This surfactant causes red blood cell (RBC) lyses and dissolution of red blood cells and platelets. Linear sheath flow and direct current impedance are used for counting RBCS and Platelets. Also, it disrupts the cell membrane of white blood cells. Then, the fluorescent dye in the specific reagent enters the cells and stains the nucleic acid. The principle of flowcytometry (633 nm laser beam) is utilised to measure the intensity of side fluorescent light (SFL) and side

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scattered light (SSC). Scattergrams are plotted in two-dimensional formats. XN series also gives the forward-scattered light (FSC) data giving SSC-FSC scattergram. Thus, leukocyte differential, nucleated RBCs, reticulocyte count and fluorescent platelet count can be obtained.[2-4]

WDF count is done by flowcytometry, after analyzing their cell-specific information such as size, internal complexity, and nucleic acid content. A polymethine fluorescent dye is used to stain the deoxyribonucleic acid and ribonucleic acid content of the cell. A semiconductor diode laser scatters the light beam of 633 nm wavelength at 90° giving Side Scattered Light (SSC) assigning information on the internal complexity of the cell or smoothness of the cell membrane, 0° giving forward scattered light (FSC) providing information on cell size and the side fluorescent light (SFL) reflecting information on total nucleic acid content of the cell. WDF channel distinguishes neutrophils, lymphocytes, eosinophils, and immature granulocytes except basophils and the WNR channel distinguishes nucleated RBCs and basophils from the other nucleated cells. The WDF scattergrams have the combinations of SSC-SFL, SSC-FSC, and FSC-SFL, and the WNR scattergrams have SFL-FSC, SSC-FSC, and SFL-SSC. **Figure 1A** and **Figure 2A** show normal scattergram patterns in WDF (SSC-SFL) and WNR (SFL-FSC) respectively.[3,5]

Various studies have shown use of the graphical or pictorial data to screen various infections or neoplastic conditions on different types of analysers.

Jayram et al.,[6] 2021 showed a specific figure of 8 like pattern or tilted hour glass like pattern on WDF scattergrams using XN 9000 analysers. Osman et al.,[7] 2020 described and evaluated the 'Sand glass-like pattern on WDF scattergram in the screening of patients of Covid 19 with Sensitivity, specificity and positive predictive values of 85.9%, 83.5% and 94.3% respectively. Dumas et al.,[8] in 2018 also described a specific pattern on WDF scattergram for the detection of plasmodium species using Sysmex 9000 or 3000 analysers. Antonio La Gioia et al.,[9] using Mindray BC 6000 analyser described specific pattern of 3D Diff scattergram for detection of infectious mononucleosis. Jain et al.,[10] described abnormal WNR scattergram in the case of mucopolysaccharidosis showing Alder Reily abnormality. Ningombam et al.,[11] also found usefulness scattergrams for rapid detection of malaria using Sysmex XN analysers.

In a similar way, various studies are conducted previously to differentiate leukaemia from reactive conditions using graphical data. Ahn A et al.,[12] suggested the use of leukaemia-specific scatterplots and blast detection using Sysmex XN 1000 analysers. Mishra et al.[13] in 2022 also found the usefulness of scattergrams using XN 1000 analysers in the detection of acute leukaemia also differentiating acute promyelocytic leukaemia, acute promyelocytic leukaemia and acute lymphoblastic leukaemia distinctively from reactive controls. In 2016 Schuff-Werner P et al.,[14] studied white pathological cell (WPC) channel flags in XN 2000 analyser flagging to differentiate reactive versus neoplastic leukocytic proliferations. Bigorra et al.,[15] using DxH 800 analysers suggested an abnormal characteristic round bottom flask-shaped volume-based scattergram to raise suspicion about persistent polyclonal B-cell lymphocytosis. In 1988, Krause JR et al.[16] using Technicon H-1 analysers tried to characterise acute leukaemia with further differentiation into acute myeloid and acute lymphoblastic leukaemia. Hoyer et al.,[17] using Coulter STKS haematology analyser studied the detection and characterization of acute leukaemia. They concluded that scattergrams are not specific to classify the acute leukaemia however specific flags can be used as screening tools for microscopic review to identify acute leukaemia.

In the context of previous studies, we aimed to analyse patterns of white blood cell differential (WDF) and white cell nucleated region (WNR) scattergrams in cases of haematological malignancies for specific patterns.

2. Materials and Methods

This was an observational descriptive type cross-sectional study which included Total 25 newly diagnosed cases of various subsets of haematological malignancies. Ethical approval was obtained from the institutional ethical committee. The cases were selected over a period of 6 months (January 2023 to June 2023). All cases were analysed using the Sysmex XN 3100 system and were confirmed by peripheral blood smear evaluation. Sysmex XN 3100 system uses two XN-10 automated haematology analysers along with SP 50 for automated peripheral smear preparation and staining. Already diagnosed cases and patients receiving chemotherapy for haematological malignancies were excluded. Two-millilitre blood samples in tubes containing K3 ethylene diamine tetra-acetic acid (K3EDTA) anticoagulant were used for analysis. XN-10 analyser was used for CBC analysis and peripheral smears were prepared using an SP50 automated slide maker and stainer (Wright-Giemsa stain). Diagnoses were established by evaluating peripheral blood smears and cytochemistry stains like Myeloperoxidase (MPO) and Periodic acid Schiff (PAS) stains. Acute leukaemia was classified using French American British (FAB) classification while chronic leukaemia was categorised according to and WHO classification. WDF and WNR scattergrams were analysed for each type of malignancy. As a control and comparison, scattergrams of leukemoid reactions were evaluated. We use daily all three level controls (low, normal and high) for internal quality control checks. Also, once a year instrument calibration is carried out as per the standard.

3. Results

Total 25 cases of new diagnosed cases of haematological malignancies were included out of which 9 cases were of chronic myeloid leukaemia (chronic phase), 9 cases of Acute

myeloid leukaemia including 6 cases of AML M2, 1 case of AML M3 and 2 cases of AML M5. There were 4 cases of Acute Lymphoblastic Leukaemia and 3 cases of Chronic Lymphocytic Leukaemia. The cases of Acute Leukaemia were categorized according to French American British (FAB) classification and chronic leukaemia, according to WHO classification. The analysis of scattergrams for each type is described below.

Chronic myeloid leukaemia. All 9 cases of CML were in chronic phase and showed similar pattern on WDF scattergram in the form of expanded region of neutrophils with bulge in monocytic region on WDF (SSC vs SFL) scattergram. The monocytic bulge was due to immature granulocytes seen due left shift as band forms, metamyelocytes, myelocytes, promyelocytes and blasts. Also, there was a tapering area of basophils are seen on left side of neutrophils region with low SFL events (**Figure 1B**). Thus, creating a hockey stick like pattern as previously described.

Acute lymphoblastic leukaemia. In all four Cases of Acute lymphoblastic leukaemia we found compact clusters of cells in the lymphocyte-monocyte region with high SFL events giving a comet tail-like appearance. (**Figure 1C**)

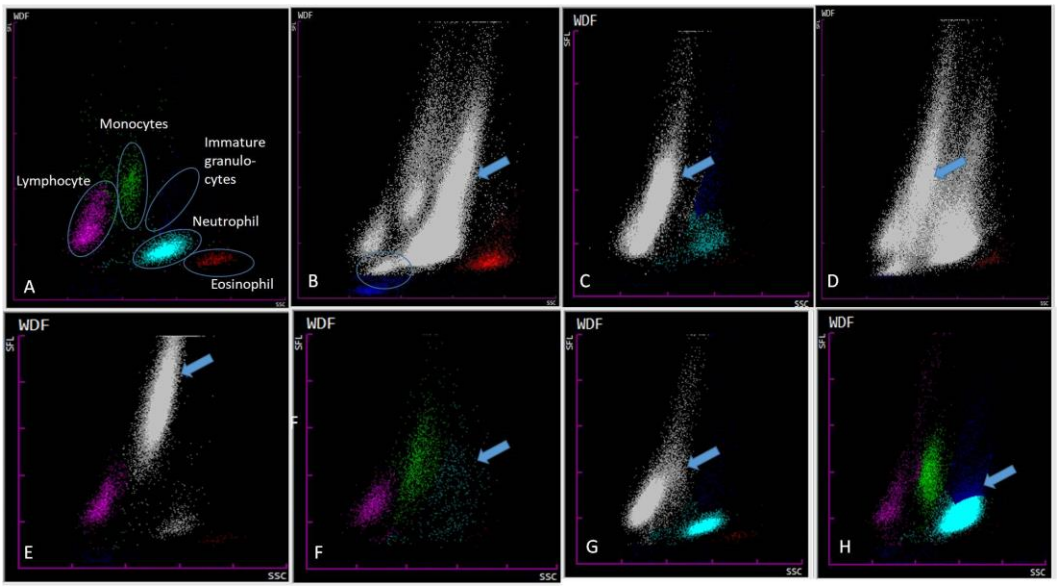

Figure 1: Representative images of WDF (SFL vs SSC) scattergram **A**): Normal position of leukocytes, **B**): Chronic myeloid leukaemia chronic phase showing Hockey stick-like pattern in the form of expanded immature granulocyte region (arrow) with a compact region on left base (circle) suggestive of Basophilia, **C**): Acute lymphoblastic leukaemia showing a compact cluster of lymphoblasts with high SFL events (arrow) **D**): Acute myeloid leukaemia AML M2 showing a compact cluster of myeloblasts (arrow) with a separate cluster of neutrophils, E) Acute myelomonocytic leukaemia, AML M5 showing a compact cluster of monoblast with high SFL events (arrow), **F**): Acute promyelocytic leukaemia, AML M3 showing loose scattered immature granulocyte region (arrow), **G**): Chronic lymphocytic leukaemia showing a compact cluster of lymphocytes with low SFL events (arrow), **H**): Leukemoid reaction showing the expanded cluster of immature granulocyte and neutrophils (arrow)

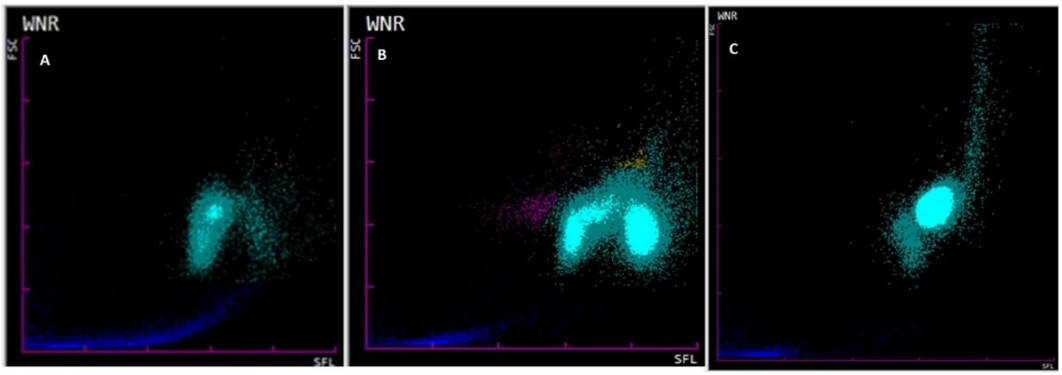

Figure 2: Representative image of WNR (FSC vs SFL) scattergram **A**): Normal, **B**): Acute leukaemia showing compact cluster with high SFL events (arrow) beyond fourth scale on x-axis, **C**): Chronic lymphocytic leukaemia showing inverted comma-like appearance (arrow)

Acute myeloid leukaemia. In acute myeloid leukaemia we found the following patterns. Six cases of AML-M2 showed more or less similar findings in the form of large separate expanded clusters of neutrophils & lymphocytes along with abnormally large clusters in the monocytic region showing high SFL events (**Figure 1D**). Two cases of AML-M5 showed a large compact cluster in a monocytic region with high SFL events suggesting monoblasts (**Figure 1E**). Lymphocytic and neutrophil regions were normal.

We had a single case of Acute Promyelocytic Leukaemia with leukopenia. This case and showed an overlap in the region of monocytes & immature granulocytes suggestive of increased promyelocytes (**Figure 1F**). Also, there was a large loose cluster with high SFL noted beyond the fourth scale of the X-axis on the WNR scattergram.

Chronic Lymphoproliferative Disorders- In the category of chronic lymphoproliferative disorders, total of 3 cases were of chronic lymphocytic leukaemia. Out of three, two of them showed large compact clusters in the lymphocyte-monocyte region with low SFL events (**Figure 1G**) as compared to acute lymphoblastic leukaemia where there were high SFL events due to blasts. However, a single case showed an ALL-like high SFL event. Two of the cases also showed WNR scattergram showed an inverted comma-like appearance. (**Figure 2C**)

Leukemoid reaction. For comparison, we also analysed a total of 25 cases of leukemoid reactions. These cases also showed similar scattergram findings in the form of separate zones of lymphocytes & neutrophils along with expanded zones of immature granulocytes. (**Figure 1H**) There was the absence of basophils or eosinophils.

WNR scattergram channel showed a unique finding of increased scatter beyond the fourth scale on X axis of scattergram (**Figure 2B**) was noted in cases of Acute Leukaemia irrespective of myeloid or lymphoid series. The findings were not seen in chronic leukaemia cases.

4. Discussion

Ningombam et al,[4] in 2021 studied a total of 291 newly diagnosed cases of haematological malignancies using a similar Sysmex XN 10 analyser. They diagnosed and classified cases on the basis of flowcytometry, bone marrow findings, immunohistochemistry and molecular studies. Among all the cases they had a total of 105 cases of acute myeloid leukaemia, 82 cases of chronic myeloid leukaemia, 51 cases of acute lymphoblastic leukaemia and 53 cases of chronic lymphoproliferative disorders mainly chronic lymphocytic leukaemia. Among AML cases M5 was the commonest while others were M4, M3, M2 and M1 in descending order having 42, 23, 20, 14 and 6 cases respectively. Our findings correlated well with their findings in cases of patterns in WDF scattergrams of ALL, CML CP, CLL as well as AML M2. However, we could not get a teardrop-shaped pattern on the scattergram in a single case of APML (M3) as described by them, maybe because of the low WBC count.

Similarly, Gupta et al.,[18] in 2017 studied a total of 271 cases of acute leukaemia, 164 cases of AML, 106 cases of ALL and a single case of undifferentiated acute leukaemia newly diagnosed cases of haematological malignancies using similar Sysmex XE 2100 analyser. In total 135 cases of AML characteristic patterns were noted on immature myeloid information channel patterns while 29 cases showed ALL-like patterns on a scattergram, giving sensitivity and specificity of approximately 82% & 99%. Five cases of acute promyelocytic leukaemia (APML) showed a characteristic pattern with increased lateral fluorescence and high side scatter in the monocytic and neutrophil regions. For ALL cases, overall sensitivity and specificity with respect to WBC-Diff channel was 100% and 90%. The sensitivity and specificity of the abnormalities detected in the WBC-Diff channel in CML was 95% and 100%, respectively. The sensitivity and specificity of the abnormalities detected in the WBC-Diff channel in CLL was 85% and 100%, respectively.

XE 2100 analysers differ from XN series analysers as they generate six different scattergrams including DIFF scattergram, WBC/BASO scattergram, IMI scattergram, NRBC scattergram, RET scattergram, and PLT-O scattergram.[2,18]

XN series analysers have WDF channel Like the DIFF channel of the XE-Series. The scattergrams of these 2 channels have different patterns due to the differences in the reagents used as well as differences in the hardware and software.

Our findings matched with the findings of Ningombam et al.,[4] and Gupta et al.,[18] in most of the cases.

In acute leukaemia, we observed the compact abnormal cluster beyond the 4th scale on the X-axis of the WNR scattergram, similar to the findings of Ningombam et al.[4] In AML cases, we observed an abnormal overlapping cluster of blasts and lymphocytes with high SFL events, similar to that of Ningombam et al.[4] However, in AML M3 we did not get isolated lymphocytes cluster with large cluster encompassing monocyte & immature granulocyte region giving “Teardrop appearance”. There was only a single case of APML in our study. In AML- M5, we found similar findings as that of Ningombam et al.[4] and Gupta et al.[18] in the form of the abnormal cluster with high SFL events separate from lymphocyte region suggestive of monoblasts. In ALL too our findings matched with the findings of continuous large clusters extending from lymphocyte to monocyte region with high SFL events.

In CML CP cases our study showed characteristic hockey stick patterns on WDF scattergram as described by Ningombam et al.[4] and Gupta et al.[18]

In CLL cases, we also found a single cluster in the lymphocyte region without increased SFL events as seen in Ningombam et al.[4] and Gupta et al.[18] However, in contrast to Ningombam et al.,[18] we found an inverted comma-like pattern on the WNR scattergram in a single case only.

Apart from these other studies have also highlighted the importance of WBC scattergram using Sysmex XN analysers.

Buoro et al.[19] in 2017 using XN 9000 analysers suggested that high immature platelet fraction combined with abnormalities like abnormal cell clusters next to the debris area in WDF scattergram and WBC differential clusters, abnormal cluster spreading over leukocyte region and extending into the PLT-clumps area on WNR scattergram as well as an abnormal cluster at the left bottom in WPC scattergram, were suggestive of altered megakaryopoiesis.

Seghezzi et al.[20] in 2017 using XN 9000 analysers suggested that the presence of an abnormal monocyte cluster in WDF scattergram, an abnormal lymphocyte double cluster in WNR scattergram and a complete lack of the monocyte cluster in WNR & WPC scattergrams, were suggestive of Hairy cell leukaemia. Moioli et al.[21] in 2019 using XN 20 analysers suggested that specific patterns in WDF, WNR and WPC scattergrams were suggestive of proerythroblasts.

Thus, all these studies suggest that WBC scattergrams can be used to screen the cases of various haematological at the level of analysers and further confirmed with standard modalities.

5. Conclusion

To conclude the scattergrams are graphical presentations that can be utilised for differentiating and suspecting haematological malignancies based on their specific patterns. Thus, these patterns can be used as simple effective screening tools for haematological malignancies before making a diagnosis on peripheral blood smears and flowcytometry.

6. Source of Funding

None.

7. Conflict of Interests

None.

8. Ethical Committee Approval

This study conducted after institutional ethical committee approval. (Approval No- DR/RMC/UG-PG/2023/178)

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References

1. Kawauchi S, Takagi Y, Kono M, Wada A, Morikawa T. Comparison of the leukocyte differentiation scattergrams between the XN-Series and the XE-Series of haematology analysers. *Sysmex J Int*. 2014;24(1):1–8.
2. Rowan RM, Linssen J. A Picture is Worth a Thousand Words. *Sysmex J Int*. 2005;15(1):27–38.
3. Briggs C, Longair I, Kumar P, Singh D, Machin SJ. Performance evaluation of the Sysmex haematology XN modular system. *J Clin Pathol.* 2012;65(11):1024–30.
4. Ningombam A, Acharya S, Sarkar A, Kumar K, Chopra A. Scattergram patterns of haematological malignancies on Sysmex XN-series analyser. *J Appl Hematol*. 2021;12(2):83–9.
5. Inaba T, Shimomoto B, Yuki Y, Okumura K, Nishimura H, Koshino K, et al. New alert message settings for the XN-series automated haematology analyser are useful for avoiding falsely high WBC counts and to detect specimens with giant platelets. *Ann Clin Lab Sci*. 2017;47(5):581–5.
6. Jayaram A, Mathews NS, Nair SC, Geevar T, Rose W, Verghese VP, et al. High fluorescent lymphocyte cell count and scattergram patterns on the Sysmex XN series cell counters-Novel parameters in early and reliable diagnosis of dengue on a background of acute febrile illness. *Int J Lab Hematol.* 2021;43(4):156–60.
7. Osman J, Lambert J, Templé M, Devaux F, Favre R, Flaujac C, et al. Rapid screening of COVID-19 patients using white blood cell scattergrams, a study on 381 patients. *Br J Haematol*. 2020;190(5):718–22.
8. Dumas C, Bienvenu AL, Girard S, Picot S, Debize G, Durand B. Automated Plasmodium detection by the Sysmex XN haematology analyser. *J Clin Pathol*. 2018;71(7):594–9.
9. La Gioia A, Fumi M, Fiorini F, Bombara M, La Gioia N, Pancione Y, et al. Mindray BC-6800 haematological analyser: 3D-DIFF scattergram usefulness in infectious mononucleosis diagnosis. *Int J Lab Hematol*. 2021;43(4):581–7.
10. Jain R, Khurana U, Bhan BD, Goel G, Kapoor N. Mucopolysaccharidosis: A case report highlighting haematological aspects of the disease. *J Lab Physicians*. 2019;11(1):97–9.
11. Ningombam A, Sarkar A, Acharya S, Chopra A, Kumar K, Subramanian A. Application of Sysmex XN-series automated haematology analyser in the rapid detection of malaria. *Indian J Hematol Blood Transfus*. 2020;36(3):512–8.
12. Ahn A, Han K, Kim Y. Leukaemia-specific scatterplots and blast detection using the XN-1000 haematology analyser. *Clin Lab*. 2022;68(7).
13. Mishra S, Chhabra G, Padhi S, Mohapatra S, Panigrahi A, Sable MN, et al. Usefulness of leucocyte cell population data by Sysmex XN1000 haematology analyser in rapid identification of acute leukaemia. *Indian J Hematol Blood Transfus*. 2022;38(3):499–507.
14. Schuff-Werner P, Kohlschein P, Maroz A, Linssen J, Dreißiger K, Burstein C. Performance of the XN-2000 WPC channel-flagging to differentiate reactive and neoplastic leukocytosis. *Clin Chem Lab Med.* 2016;54(9):1503–10.
15. Bigorra L, Larriba I, Gutiérrez-Gallego R. Abnormal characteristic “round bottom flask” shape volume-based scattergram as a trigger to suspect persistent polyclonal B-cell lymphocytosis. *Clin Chim Acta*. 2020;511:181–8.
16. Krause JR, Costello RT, Krause J, Penchansky L. Use of the Technicon H-1 in the characterization of leukaemias. *Arch Pathol Lab Med*. 1988;112(9):889–94.
17. Hoyer JD, Fisher CP, Soppa VM, Lantis KL, Hanson CA. Detection and classification of acute leukaemia by the Coulter STKS haematology analyser. *Am J Clin Pathol*. 1996;106(3):352–8.
18. Gupta M, Chauhan K, Singhvi T, Kumari M, Grover RK. Useful information provided by graphic displays of automated cell counters in haematological malignancies. *J Clin Lab Anal*. 2018;32(5):e22392.

19. Buoro S, Manenti B, Seghezzi M, Dominoni P, Vavassori M, Trezzi R, et al. Abnormal leukocyte scattergrams and immature platelet fraction on Sysmex XN-9000 analyser: a new diagnostic tool for altered megakaryopoiesis? *Scand J Clin Lab Invest*. 2017;77(1):73–5.
20. Seghezzi M, Manenti B, Previtali G, Gianatti A, Dominoni P, Buoro S. A specific abnormal scattergram of peripheral blood leukocytes that may suggest hairy cell leukaemia. *Clin Chem Lab Med.* 2018;56(5):e108–11.
21. Moioli V, Buoro S, Seghezzi M, Previtali G, Dominoni P, Ottomano C, et al. A specific abnormal scattergram of peripheral blood leukocytes suggestive for the presence of proerythroblast. *Scand J Clin Lab Invest*. 2020;80(1):55–8.

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