RESEARCH ARTICLE

Useful information provided by graphic displays of automated cell counter in hematological malignancies

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Background: Automated cell counters have become more and more sophisticated with passing years. The numerical and graphic data both provide useful clues for suspecting a diagnosis especially when the workload is very high.

Aim: We present our experience of useful information provided by graphic displays of an automated cell counter in hematological malignancies in a cancer hospital where a large number of complete blood count (CBC) requests are received either before or during chemotherapy. This study was conducted to assess the usefulness of hematology cell counter, viz. WBC-Diff (WBC differential), WBC/BASO (WBC basophil) and IMI (immature myeloid information) channel scatter plots, and the flaggings generated in various hematological malignancies.

Material & methods: The graphic displays have been compiled over a period of 1 year (October 2015–September 2016) from blood samples of various solid and hematological malignancies (approximately 400 per day) received for routine CBC in the laboratory. Approximately 50 000 scattergrams have been analyzed during the study period. The findings were confirmed by peripheral blood smear examination.

Results: The scattergram analysis on XE-2100 is very sensitive as well as specific for diagnosing acute leukemia, viz. acute myeloid leukemia, acute lymphoblastic leukemia; chronic myeloproliferative disorders, viz. chronic myeloid leukemia; and chronic lymphoproliferative disorder especially chronic lymphocytic leukemia.

Conclusion: It is suggested that the laboratories using the hematology analyzers be aware of graphic display patterns in addition to flaggings generated which provide additional information and give clue toward the diagnosis even before peripheral smear examination.

KEYWORDS

automated cell counter, hematological malignancies, immature myeloid information scattergram, WBC-Diff scattergram

1 | INTRODUCTION

The complete blood count (CBC) is the routinely performed laboratory investigation which demonstrates clinical usefulness. With the advent of electronic cell counters, which can enumerate all of these

test results in short time and with more precision and accuracy, labor requirements have been substantially reduced.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

[Correction added on 13 February 2018, after first online publication: The fourth author's name was corrected from 'Manisha Singh' to 'Manisha Kumari'.]

The aim of the study is to present graphic patterns commonly noted on flow cytometry-based cell counter XE-2100 (Sysmex, Kobe, Japan) in hematological malignancies, which invoke suspicion and give clue for establishing a diagnosis when the CBC indicates or does not indicate the same. The WBC differential scattergram, WBC/BASO scattergram, and the immature myeloid information (IMI) scattergram information generated have been incorporated.

2 | MATERIALS AND METHODS

This study presents a prospective evaluation and retrospective compilation of scattergram findings generated by XE-2100, a 5-part differential analyzer used in our laboratory, which provided a clue to the diagnosis. The graphic displays have been compiled over a period of 1 year (October 2015–September 2016) from blood samples of various solid and hematological malignancies (approximately 400 per day) received for routine CBC in the laboratory. The cases were identified based on the flags generated and subsequent peripheral smear examination followed by bone marrow aspiration and biopsy and confirmed on flow cytometric immunophenotyping.

The thresholds of flag alerts were optimized in the laboratory for the blasts, atypical lymphocytes, immature granulocytes, and platelet clumps by changing the cutoff from factory default setting after verifying on peripheral blood smear examination.

Approximately 50 000 scattergrams have been analyzed during the study period. Peripheral blood samples were collected into K₂EDTA tubes (Becton Dickinson) and were analyzed on XE-2100 hematology analyzer. All samples were analyzed within 2 hours of collection. The analyzer had been calibrated with the Sysmex

SCS-1000 calibrator once a year. The controls using 3 levels of e-check were run daily, and the instrument was maintained as per the manufacturer's instructions. The various abnormalities noted on the automated cell counter were analyzed for WBC-Diff, WBC/BASO, and IMI scattergrams.

This instrument uses combined impedance and radiofrequency conductance detection, semiconductor diode laser light 90° side scatter (SSC) and 0° forward scatter (FSC) detection, and polymethine side fluorescence (SFL) detection. It generates six different scattergrams including DIFF scattergram, WBC/BASO scattergram, IMI scattergram, NRBC scattergram, RET scattergram, and PLT-O scattergram. In addition, there are RBC and PLT-I histograms.³

In the WBC-Diff channel, WBCs are permeabilized to enable staining of their DNA and RNA with a fluorescence dye. Cells are then categorized according to their side-scattered light and fluorescence intensity characteristics. A 4-part WBC-diff is created from the WBC populations: lymphocytes, monocytes, eosinophils, and neutrophils plus basophils (Figure 1A). An adaptive cluster analysis system is used for identification of the different cell populations. All or parts of the differential count are not released (gray) when the instrument is not able to identify separate cell populations.⁴

In the WBC/BASO channel, measurement of WBCs is performed by flow cytometry using a semiconductor laser to detect forward- and side-scattered light information. Red cell lysis is performed by a reagent that selectively suppresses the degranulation of basophils, resulting in their separation from other forms of WBCs (Figure 1B).

In the IMI channel, a special reagent acts on the lipid pattern of the cell membrane to selectively protect immature WBC against disruption, whereas mature leukocytes are disrupted (Figure 1C). After this reaction, cells are categorized by radiofrequency (RF)/direct

FIGURE 1 (A) Normal DIFF scattergram on XE-2100. (B) Normal BASO scattergram on XE-2100. (C) Normal IMI scattergram on XE-2100. DIFF, differential; IMI, immature myeloid information; BASO, basophil

current (DC) signals, based on which an IMI scattergram is obtained. The IMI channel selectively detects cells of the immature granulocytic series not normally present in healthy human peripheral blood (Figure 1C).⁴

The presence of abnormal WBCs is indicated by the suspect messages: blasts, immature gran, left shift, atypical lympho, and abn

lympho/blasts. The suspect messages are generated by combining pattern abnormalities in the 4-DIFF and IMI scattergrams (Figure 2).

3 | RESULTS

The various scattergrams generated were studied, and peripheral smears as well as the bone marrow examination/flow cytometric immunophenotyping confirmed the diagnosis and following results were drawn. All the scattergram findings are summarized in Table 1.

3.1 | Scattergram displays

3.1.1 | Acute leukemia

The acute leukemias usually present with high TLC, anemia, and thrombocytopenia; however, in few cases, TLC could be low or even normal. Apart from the numerical data which provide a clue, the WBC-Diff scattergram shows a plot extending from the lymphocyte and monocyte areas upward toward the area of increasing lateral fluorescence, suggesting the appearance of blast cells (Figure 3). Even when the total

FIGURE 2 Suspect messages generated on XE-2100

TABLE 1 Scattergram abnormalities in various hematological malignancies

S. no	Hematological malignancy	Abnormality in the WBC-Diff scatter plot	Abnormality in WBC/BASO scatter plot	Abnormality in IMI channel
1.	Acute myeloid leukemia	Shows a plot extending from the lymphocyte and monocyte areas upward toward the area of increasing lateral fluorescence, suggesting the appearance of blast cells	–	Many red dots are found in the area in which RF is lowest, suggesting the presence of myeloid blasts.
2.	Acute promyelocytic leukemia	Characteristic pattern with increased lateral fluorescence and high side scatter in the monocytic and neutrophil regions	–	Two clusters of red dots were seen
3.	Acute lymphoblastic leukemia	Shows a plot with less lateral fluorescence as compared to acute myeloid leukemia	–	Red dots are absent in lymphoid blasts
4.	Chronic myeloid leukemia	In CML-CP, neutrophil cluster extends upward in the area where lateral fluorescence is strong indicates a shift to left. Also, there is extension of lymphocyte or monocyte cluster upward indicating the presence of blasts. Just beneath the lymphocyte cluster is the cluster in the nRBC area. In CML-BP, a prominent cluster from the lymphocyte or monocyte area extends upward in the direction of strong side fluorescence which suggests that there is increase in blast population. The neutrophils are also seen along with shift to left, however, appear reduced as compared to CML-CP. The findings in CML-AP appear intermediate of the CML-CP and CML-BP.	Prominent cluster of basophils is seen.	Many red dots are distributed all over the graph, suggesting the appearance of many immature granulocytes along with blasts appearing in the blast region.
5.	Chronic lymphoproliferative disorders	The border between the lymphocyte and monocyte areas is indistinct. The lymphocyte plot extends from the lymphocyte area upward toward the area in which lateral fluorescence is strong but not as upward as observed in acute leukemia scatter plot. In hairy cell leukemia, the DIFF scattergram shows a cluster in the region of lymphocyte area moving toward the monocyte area, but the extension is less than that seen in acute leukemias suggesting the presence of atypical cells.	–	Findings are insignificant.

RF, radiofrequency; DIFF, differential; IMI, immature myeloid information; CP, chronic phase; AP, accelerated phase; BP, blast phase.

WBC count is low, the presence of blasts is appreciable (Figure 3B, C). The monocytic leukemias show cluster in the monocytic region extending upward (Figure 3F). On the IMI scattergram, many red dots are found in the area in which RF is lowest, suggesting the presence of myeloid blasts. The acute lymphoblastic leukemia shows less lateral fluorescence as compared to acute myeloid leukemia (Figure 3D, E). The red dots are absent in lymphoid blasts (Figure 4A, B). The controls were the 250 consecutive samples analyzed on 5 days ($n = 250$). Two samples of the controls showed WBC-Diff scattergram abnormalities similar to seen in acute leukemia but was due to G-CSF therapy. Rest of the samples showed well-separated clusters of neutrophils, lymphocytes, eosinophils, and monocytes with unflagged TLC and DLC. Twenty-five control scatter plots of the 250 showed red dots in IMI region and it corresponded to left shift on the peripheral smear.

Two hundred and seventy-one cases of acute leukemia were diagnosed on the basis of peripheral smear/bone marrow smears and confirmed by flow cytometric immunophenotyping. These cases were analyzed for the abnormalities in the WBC-Diff and IMI scattergrams. Of these 22 cases (8%) presented with low TLC ($<4000/\text{mm}^3$), 7 cases (2.6%) with normal TLC ($4000\text{--}11\,000/\text{mm}^3$), and the rest presented with a high TLC ($>15\,000$). Of 271 acute leukemia cases, 164 were acute

myeloid leukemia (60%), 106 were acute lymphoblastic leukemia (39%), and a single case of acute undifferentiated leukemia (0.3%). All the cases (100%) showed abnormalities in WBC-Diff scattergrams as interpreted by a hematopathologist. Of the 164 AML cases, in 135 cases, characteristic abnormalities of AML in WBC-Diff scattergram were seen. In 29 cases, the scattergram appeared as in ALL. Therefore, sensitivity and specificity of the abnormalities detected in the WBC-Diff channel in AML was 82% and 99%, respectively. All cases of AML showed the characteristic IMI pattern (sensitivity-100%, specificity-100%).

Five cases of acute promyelocytic leukemia (APML) showed a characteristic pattern with increased lateral fluorescence and high side scatter in the monocytic and neutrophil regions. On IMI scattergram, 2 clusters of red dots were seen (Figure 3A). The IMI scattergram of AML-M2 closely mimics that of AML-M3 (Figure 5).

On scatter plot examination, 3 cases were thought to be AML-M2 type and rest of the 2 cases were thought to be AML-M3.

All the 117 cases suspected to be ALL on WBC-Diff channel, only 106 cases were ALL and rest 11 cases turned out to be CLL on morphology and subsequent immunophenotyping. Therefore, the sensitivity and specificity with respect to WBC-Diff channel was 100% and 90%. Of the 117 cases of ALL, 14 cases showed no red dots in IMI

FIGURE 3 DIFF scattergram generated by XE-2100 in acute leukemia: (A) acute promyelocytic leukemia; (B) (C) acute leukemia with a low TLC; (D) acute lymphoblastic leukemia; (E) acute myeloid leukemia; (F) acute monoblastic leukemia. DIFF, differential

FIGURE 4 DIFF & IMI scattergram in AML (A) & ALL (B). DIFF, differential; IMI, immature myeloid information

FIGURE 5 DIFF and IMI scattergram in acute promyelocytic leukemia. DIFF, differential; IMI, immature myeloid information

channel. The sensitivity and specificity with respect to IMI was 87.7% and 100%. Ten ALL cases where red dots were seen in IMI channel showed immature granulocytes on peripheral smear and immature granulocyte (IG) flag.

3.1.2 | Chronic myeloid leukemia

Chronic myeloid leukemia presents with a high TLC, with or without anemia and with or without thrombocytosis. In CML-chronic phase (CP), a characteristic pattern in WBC-Diff scattergram with neutrophil cluster extending upward in the area where lateral fluorescence is strong indicates a shift to left. Also, there is extension of lymphocyte or monocyte cluster upward indicating the presence of blasts. Just beneath the lymphocyte cluster is the cluster in the nRBC area. In CML-blast phase (BP), a prominent cluster from the lymphocyte or monocyte area extends upward in the direction of strong side fluorescence which suggests that there is increase in blast population. The neutrophils are also seen along with shift to left, however, appear reduced as compared to CML-CP. The findings in CML-accelerated phase (AP) appear intermediate of the CML-CP and CML-BP. On the IMI scattergram, many red dots are distributed all over the graph, suggesting the appearance of many immature granulocytes along with blasts appearing in the blast region (Figure 6A, B, C). The WBC/BASO scattergram shows prominent cluster of basophils (Figure 6D).

Sixty-seven cases were analyzed for the WBC-Diff and IMI scattergram abnormalities. Three phases of CML, namely CML-CP, CML-AP, and CML-BP have different appearance on the WBC-Diff scattergram. Of the 67 cases with CML-like scattergram abnormalities, 65 showed Philadelphia or BCR-ABL positivity. Fifty-one cases were CML-CP (78%), 5 cases were CML-AP (7.7%), and 6 cases were CML-BP (9.2%). The 3 cases where CML-like pattern was observed were on G-CSF therapy. The sensitivity and specificity of the abnormalities detected in the WBC-Diff channel in CML was 95% and 100%, respectively. The IMI channel abnormalities were seen in all the 67 cases.

3.1.3 | Chronic lymphoproliferative disorders

Chronic lymphoproliferative disorders especially CLL which commonly involves the blood usually present after 5th decade, a high TLC with or without anemia and usually a normal platelet count. On

the WBC-Diff scattergram, the border between the lymphocyte and monocytes areas is indistinct. The lymphocyte plot extends from the lymphocyte area upward toward the area in which lateral fluorescence is strong but not as upward as observed in acute leukemia scatter plot (Figure 7A, B, C). However, the scatter plots of ALL and CLL can appear similar. In hairy cell leukemia, the DIFF scattergram shows a cluster in the region of lymphocyte area moving toward the monocytes area but the extension is less than that seen in acute leukemias suggesting the presence of atypical cells (Figure 7D). A blast flag is usually given by the analyzer. The IMI scattergram findings are insignificant.

Of the 68 cases of chronic lymphoproliferative disorders, 58 cases were chronic lymphocytic leukemia, 4 cases were mantle cell lymphoma, 5 cases were hairy cell leukemia, and 1 case was CLL-PLL. Ten cases of the 68 gave a blast flag. The sensitivity and specificity of the abnormalities detected in the WBC-Diff channel in CLL was 85% and 100%, respectively.

3.1.4 | Carcinocythemia

Carcinocythemia or the circulating malignant cells or any infiltration of bone marrow can present with a leukoerythroblastic picture with or without atypical cells. In a known case of solid malignancy, such findings indicate metastasis to bone marrow by the primary tumor or involvement by acute leukemia. Leukoerythroblastic picture with few atypical cells presents in the WBC-Diff scatter plot as neutrophil cluster extending upward toward the area of increased lateral fluorescence along with blue dots in the nRBC region and lymphocyte cluster moving upward indicating the presence of atypical cells (Figure 8).

Over a period of 1 year, 10 cases with such features were seen out of which 2 cases were of solid malignancies with peripheral blood and bone marrow involvement could be picked up by the characteristic WBC-Diff scattergram. This included a case of rhabdomyosarcoma and breast carcinoma. The rest 8 cases were due to G-CSF therapy (6 cases) and underlying infection with fever (2 cases).

4 | DISCUSSION

Most of the laboratories use automated hematology instruments to generate cell counts, red cell indices, and white cell differential counts.

FIGURE 6 DIFF and IMI scattergram in (A) CML-CP (B) CML-AP (C) CML-BP. (D) WBC/BASO scattergram in CML-CP. DIFF, differential; IMI, immature myeloid information; CP, chronic phase; AP, accelerated phase; BP, blast phase

FIGURE 7 DIFF scattergram in CLL (A, B, C) & HCL (D). DIFF, differential

The instruments use flags to notify the user that a specific abnormality is present.² However, relying alone on the flags increases the false positivity by triggering morphologic reviews. For instance, in a case

of neutrophilic leukocytosis, a blast flag in most of the cases carries a minimal value. In such cases, analyzing the scatter plots, for example, the WBC-Diff, helps to suspect/rule out the presence of atypical cells.

FIGURE 8 DIFF, IMI, and BASO scattergram in a case of Carcinocythemia. DIFF, differential; IMI, immature myeloid information; BASO, basophil

The patterns generated on analyzers if studied for large number of cases help in not only suspecting atypical cells but also giving a clue to the diagnosis.

Technicon H¹ is an automated hematology analyzer and the data generated by it provides a host of useful information to clinician and laboratories pertaining to anemias, typing of leukemias, blast detection for diagnosis and monitoring response to treatment, acute inflammation and parasitic infestation.⁵

Hoyer et al⁶ used a combination of appropriate suspect and definitive flags to trigger checking criteria and microscopic review and concluded that the Coulter STKS Hematology Analyzer will be successful in detecting virtually all cases of acute leukemia involving the peripheral blood. Here, we describe few instances where the graphic display provided clues of suspicion while reporting peripheral smears, especially when the workload is high.

The acute leukemias usually present with high TLC, anemia, and thrombocytopenia; however, in few cases when TLC is low or even normal, the scatter plots provide important clues to the diagnosis. In all the cases, blast flag was generated. The DIFF scattergram was displayed in gray color, pink color, or a mixture of pink and green colors. The blast population lies in the lymphoid or monocytic region extending upward. The IMI channel shows red dots in the blast region in most cases of acute myeloid leukemia and no dots in the blast region in acute lymphoid leukemia cases; however, confirmation by flow cytometric immunophenotyping is needed.

Chronic myeloid leukemia shows a characteristic pattern in the WBC-Diff channel; however, the IMI channel pattern is not very different for the 3 phases, unless only a blast population is seen without neutrophils and shift to left as in some cases of CML-BP. In such cases, a differential of acute myeloid leukemia is also considered. CML-CP-like picture can also be seen with G-CSF therapy or with any underlying infection.

Leukoerythroblastic reaction refers to a condition accompanied by teardrop-shaped cells, prematurely released nucleated red cells and

immature myeloid cells. The presence of a leukoerythroblastic peripheral blood picture serves as a valuable clue to the presence of some underlying disease stressing hematopoiesis or as a signal to investigate further for the presence of malignancy.⁷ This usually correlates with the degree of bone marrow fibrosis and tumor cells “crowding out” of marrow elements due to their interaction with hematopoietic cytokines rather than with the extent of malignant infiltration. Various studies have reported the frequency of leukoerythroblastic changes ranging from 30% to 35%.^{8,9,10} The automated hematology analyzers also give a clue to the presence of leukoerythroblastic reaction and therefore should not be ignored while releasing CBC reports of patients with nonhematological malignancies (Figure 4). However, a picture similar to leukoerythroblastic reaction is seen in patients receiving G-CSF therapy with a shift to left.

Chronic lymphoproliferative disorders show same pattern as in acute leukemias but the lateral fluorescence as seen in WBC-Diff scattergram is lesser than acute leukemias. Some cases of CLPDs may show increased lateral fluorescence as in acute leukemias when there are larger cells on peripheral smear. In such cases, flow cytometric immunophenotyping using immaturity markers is helpful in making a definitive diagnosis. A low WBC count along with lymphocytosis and cluster extending from lymphocyte area to the monocyte area is quite characteristic of hairy cell leukemia.

Visual scanning of the scattergrams gives a good initial sense of the size, shape, and other salient features of the WBCs. These new positional parameters along with the numerical data and flags provide clue toward diagnosis even before the peripheral blood smear review. A large collection of data, displayed as a visual image, can convey information with far more impact than the numbers alone.

The speed and reliability of the modern analyzers allow technologists time to evaluate abnormal blood films, consider diagnostic clues, and correlate clinical findings to histograms and other hematological parameters with greater confidence and efficiency, all of which produce high returns in terms of patient health care.

5 | CONCLUSION

The automated hematology analyzers provide valuable information regarding common hematological conditions. Every case of hematological malignancy does not present with cytopenias or cytosis, and hence in cases with normal counts, these scatter plots provide useful clue that alerts a hematopathologist to screen the peripheral smear with a suspicion. Also, the typical pattern helps suspect a diagnosis before peripheral smear examination. It is important that operators and end users have an understanding of the parameters while interpreting through numerical and graphic data. This can enhance the detectability rate even when there is no peripheral smear request.

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