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SHORT COMMUNICATION

Leukemia-Specific Scatterplots and Blast Detection Using the XN-1000 Hematology Analyzer

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SUMMARY

Background: **The complete blood cell count with differential is particularly important in the diagnosis and monitoring of hematologic malignancies. The visual examination of blood films is a labor-intensive process and the results are affected by the experience of the hematopathologist. Automated hematology analyzers are used to count and identify blood cells in most laboratories.**
Methods: **EDTA-treated blood samples were collected from 318 patients diagnosed with acute and chronic leukemia and 150 normal controls. Twelve XN-1000 white blood cell scatterplots of each case were displayed and characteristic differences from the normal scatterplot were evaluated. We describe the specific scatterplots of normal controls and patients in each disease.**
Results: **Each type of leukemia shows characteristic scatterplots, some of which report blast counts. The WPC plot revealed an oblique downward trend in the low-FSC blast population typically, whereas the leukemic cells in acute promyelocytic leukemia showed the characteristic signal in the oblique upward direction on the SSC-FSC plot. The high:low FSC lymphocyte ratio was decreased in chronic lymphocytic leukemia (CLL), whereas B-cell prolymphocytic leukemia (B-PLL) showed increased high:low FSC lymphocyte ratio. Both B-PLL and T-PLL showed the same scatterplot.**
Conclusions: **XN scatterplots represent morphology of the blood cells. Each type of leukemia shows a characteristic scatterplot including WPC plot. XN reveals blast counts and leukemia type.**
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KEY WORDS

scatterplot, blast, Sysmex XN, WDF, WPC, WNR

INTRODUCTION

The complete blood cell count (CBC) with differential is one of the most commonly used laboratory tests for the diagnosis of a broad variety of clinical conditions [1]. It is particularly important in the diagnosis, treatment and monitoring of hematologic malignancies [2]. The visual examination of blood films to diagnose malignancy is a labor-intensive and subjective process dependent on the experience of hematopathologist [3]. In addition, many patients visit hospitals without the hematopathologist and the diagnostic delay leads to adverse patient outcomes [4].

Automated hematology analyzers are used to count and identify blood cells with high levels of accuracy, efficiency, throughput, and short turnaround times in most laboratories [5]. However, the automated differential count is not adequate and limited flags are provided to users. Few studies have evaluated the blast flagging capabilities of Sysmex XN series, specifically in patients with hematologic malignancies [5,6]. Scatterplots representing the blood cell morphology can be used to identify differences and flag patients with hematologic diseases compared with those without. In this study, the XN scatterplots of samples obtained from patients with various hematologic malignancies were analyzed to identify the unique disease characteristics and detect the blasts in the respective diseases.

MATERIALS AND METHODS

The study enrolled a total of 468 patients who underwent a CBC with differential at Seoul St. Mary's Hospital. EDTA-anticoagulated blood samples were obtained from patients. The study involved 318 patients with hematologic malignancies (79 acute myeloid leukemia (AML), 10 acute promyelocytic leukemia (APL), 85 acute lymphoblastic leukemia (ALL), 51 chronic myeloid leukemia (CML), 90 chronic lymphocytic leukemia (CLL), 2 B-cell prolymphocytic leukemia (B-PLL), and 1 T-cell prolymphocytic leukemia (T-PLL)) who were diagnosed with or treated for the disease. Normal controls included 150 subjects undergoing worker's health examination. The study was approved by our institutional review board (KIRB-00626_5-002).

Sysmex XN-1000 hematology analyzers (Sysmex, Kobe, Japan) were used to display white blood cell (WBC) scatterplots of each case. For each case, image files related to the WBC differential (WDF), white precursor and pathological cell (WPC), and WBC and nucleated red blood cell (WNR) channels were generated with the respective parameters: forward scatter (FSC), side fluorescence light (SFL), and side scatter (SSC). The value of FSC and SSC are proportional to the size and internal complexity of the cell, respectively. In addition, the intensity of SFL reflects the cell's DNA/RNA content [7]. The biparametric analyses were performed as regards the populations of WBC. According to the characteristic differences of scatterplot, each case was classified and each formula for blast counting was described by two independent, blinded hematopathologists.

RESULTS

In normal scatterplots, the WDF channels showed excellent separation of neutrophils, lymphocytes, monocytes, and eosinophils especially on an FSC-SFL plot. Each WBC class was displayed in unique color: neutrophils in pale blue, lymphocytes in pink, monocytes in green, eosinophils in red, and debris in blue. The WPC channels were distinct from WDF or WNR channels. The lymphocytes were clearly divided into low- and high-FSC populations, based on SSC-FSC plots. All WBCs showed relatively similar SFL. The monocytes showed a higher SFL population than lymphocytes but lower than neutrophils on FSC-SFL plots. The WNR channels separated basophils and nucleated red blood cells from other WBCs. The basophils showed higher FSC and SFL than other WBCs, and were displayed as yellow dots.

The leukemic blast population including both myeloblasts and lymphoblasts showed low FSC and SSC and diffuse SFL distribution, and were usually counted as monocytes on WDF channels. In AML, the myeloblast population was separated from other WBCs, especially lymphocytes, using WDF and WPC channels. In WPC channels, the myeloblasts and lymphoblasts showed low SSC and low-to-medium FSC. The low-FSC population showed an oblique downward trend on the FSC-SFL plot. The true monocytes with medium FSC and high SFL were separated from other WBCs on the FSC-SFL plot. Thus, the number of myeloblasts can be calculated using the monocyte count on the WDF and WPC channels.

Myeloblast count = monocyte count on the WDF - monocyte count on the WPC

Lymphocytes could not be separated on any channel in ALL. Thus, the number of lymphoblasts is limited by the monocyte count on the WDF and WPC channels.

Lymphoblasts + lymphocytes = monocytes count on the WDF - monocytes count on the WPC

The leukemic cells of APL showed low FSC and SSC and diffuse SFL distribution, and were usually counted as monocytes on WDF channels. In WPC channels, the leukemic cells showed medium SSC and low to high FSC, and the characteristic signal with an oblique upward direction on the SSC-FSC plot. The monocytes could not be separated on any channel. Thus, the number of leukemic cells is limited by the monocyte count on the WDF channels (Figure 1).

APL leukemic cells + monocytes = monocyte count on the WDF

CML typically presents as severe leukocytosis, immature granulocytosis, neutrophilia, eosinophilia, and basophilia. The number of basophils and blasts are increased depending on disease progress. A huge population of the immature granulocytes and neutrophils with low FSC and medium-to-high SFL can be separated from other WBCs on WDF channels. In WPC channels, the basophils and/or blasts with medium SSC and FSC were separated from other WBCs, and showed lower SSC and higher FSC than monocytes. In case of increased blasts, the low-FSC population showed an oblique downward trend on the FSC-SFL plot. The basophils showed medium SSC and high FSC, but were under-counted on WNR channels.

CLL and PLL typically manifest severe lymphocytosis, and leukemic cells are separated from other WBCs, except lymphocytes. They show low SSC and FSC, and

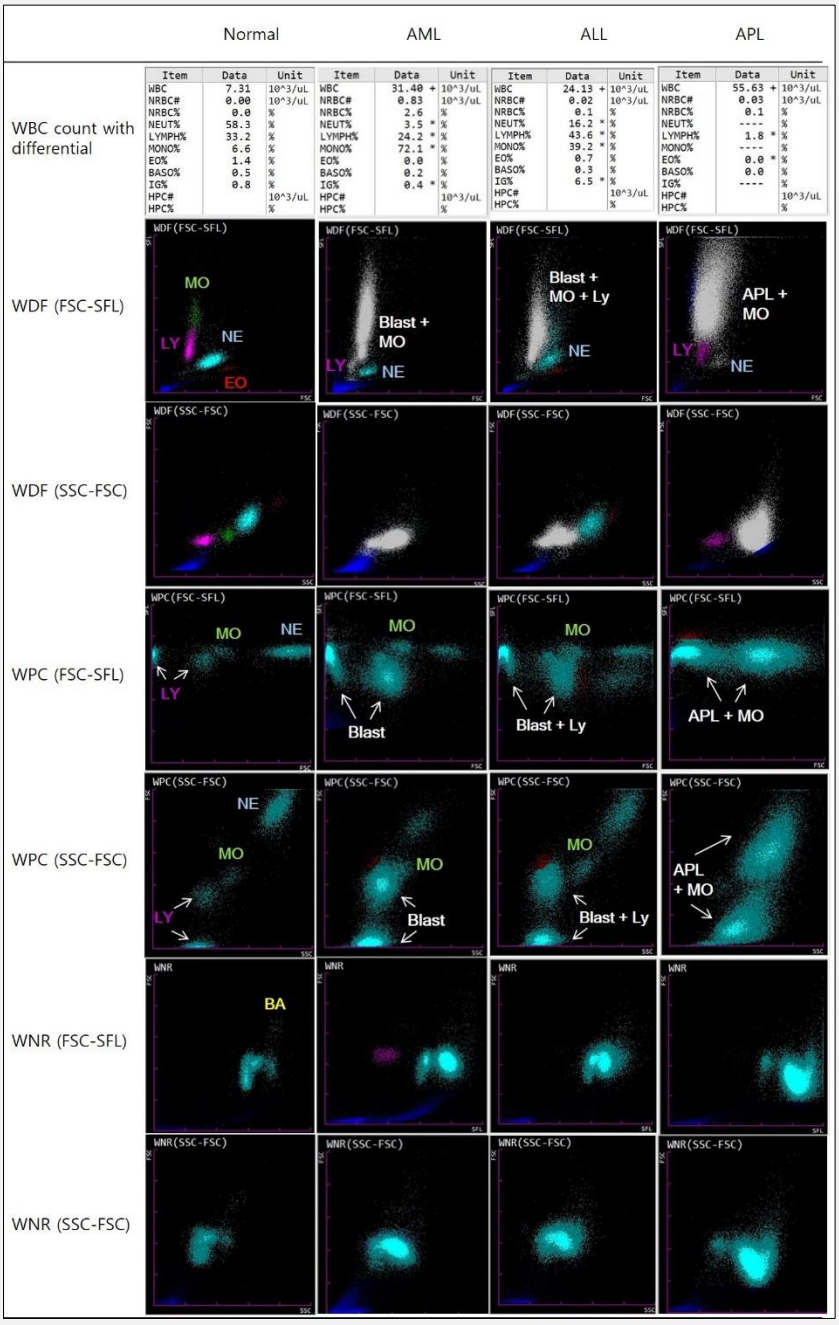

Figure 1. Sysmex XN scatterplots of normal controls and patients with acute leukemia.

In normal controls, each white blood cell (WBC) class is displayed in a unique color, neutrophils (NE) in pale blue, lymphocytes (LY) in pink, monocytes (MO) in green, eosinophils (EO) in red and debris in blue on WDF channels. The basophils (BA) showed higher SFL and FSC than the other WBCs and are displayed in yellow on WNR channels. In acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), the blast population with low FSC and diffuse SFL distribution were counted as monocytes on WDF channels. The blasts showed low SSC and low or medium FSC on WPC channels, and the low-FSC population showed an oblique downward direction on the FSC-SFL plot. The true monocytes with medium FSC and high SFL were separated from other white blood cells on the FSC-SFL plot, but lymphoblasts and lymphocytes could not be separated on any channel. In acute promyelocytic leukemia (APL), the leukemic populations with low FSC and SSC and diffuse SFL distribution were usually counted as monocytes on WDF channels. The leukemic cells on WPC channels showed medium SSC and low-to-high FSC, and the characteristic signal with oblique upward direction on the SSC-FSC plot. The monocytes could not be separated on any channel.

Abbreviations: WDF - white blood cell differential, SFL - side fluorescence light, FSC - forward scatter, WNR - white blood cell and nucleated red blood cell, SSC - side scatter, WPC - white precursor and pathological cell.

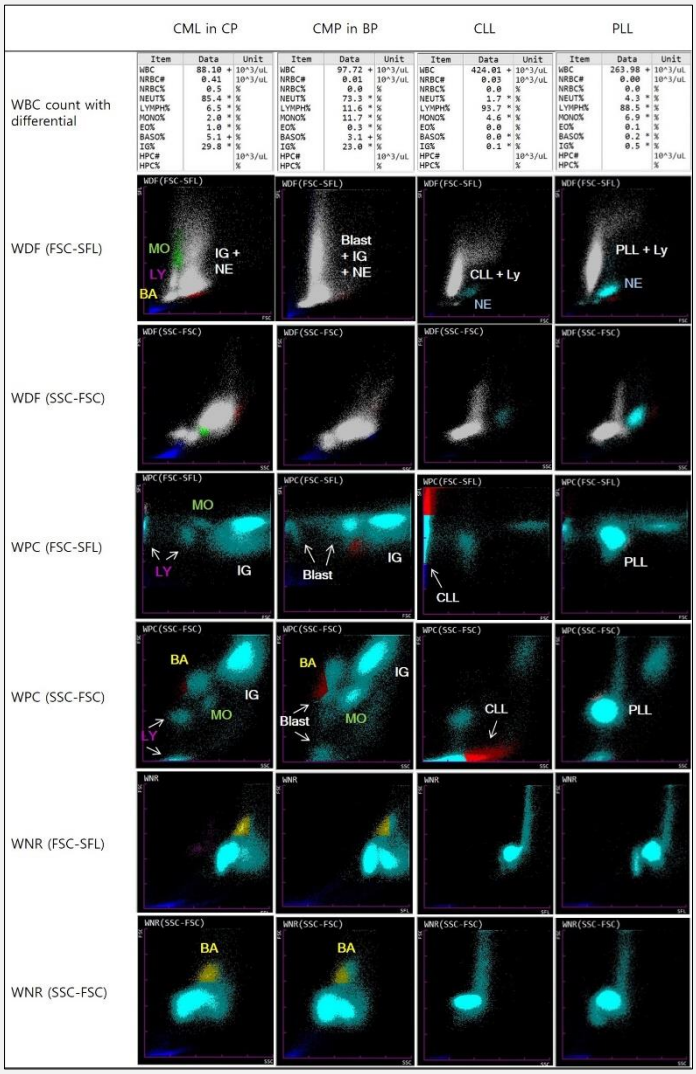

Figure 2. Sysmex XN scatterplots of patients with chronic leukemia.

In chronic myeloid leukemia in chronic phase (CML-CP), a huge population of the immature granulocytes (IG) and neutrophils (NE) showed low FSC and medium-to-high SFL and were separated from other white blood cells (WBCs) on WDF channels. The basophils (BA) and/or blasts with medium SSC and FSC were clearly separated from other WBCs on WPC channels and showed lower SSC and higher FSC than monocytes (MO). In blast phase (CML-BP), the low-FSC population showed an oblique downward direction on the FSC-SFL plot. The basophils showed medium SSC and high FSC on WPC and WNR channels. In chronic lymphocytic leukemia (CLL) and T-cell prolymphocytic leukemia (PLL), the leukemic cells were separated from other WBCs, except lymphocytes. They showed low SSC and FSC, and were usually counted as lymphocytes on WDF channels. On WPC channels, CLL cells showed low FSC and diffuse SSC and SFL distribution. Thus, the high:low FSC lymphocyte ratio was decreased. However, PLL cells showed medium FSC, SSC, and SFL on WPC channels. Thus, the high:low FSC lymphocyte ratio was increased.
Abbreviations: FSC - forward scatter, SFL - side fluorescence light, WDF - white blood cell differential, WPC - white precursor and pathological cell, WNR - white blood cell and nucleated red blood cell, LY - lymphocytes.

are usually counted as lymphocytes on WDF channels. In WPC channels, CLL cells showed low FSC and diffuse SSC and SFL distribution. Thus, the high:low FSC lymphocyte ratio was decreased and most CLL cases showed the characteristic scatterplots even under a low lymphocyte count during treatment. However, PLL cells showed medium FSC, SSC, and SFL on WPC channels. Thus, the high:low FSC lymphocyte ratio was increased, and both B-PLL and T-PLL showed the same scatterplot (data not shown) (Figure 2).

DISCUSSION

In this study, we identified malignant cells using XN-1000 WBC scatter plots based on CBC tests of patients with several hematologic malignancies. The WDF and WPC channels were used to identify leukemia blasts. The increased blasts in samples are misclassified as normal mature WBCs, especially monocytes on WDF channels. The blasts in WPC channels showed low SSC and low-to-medium FSC, and the low-FSC population typically showed an oblique downward trend on the FSC-SFL plot in both AML and ALL.

We described the blast counting formulas in the respective diseases. In AML, since the monocytes were separated from other WBCs on WPC channels, the myeloblasts have been clearly described. However, lymphoblasts and lymphocytes could not be separated on any channel, and so were reported as a mixture of blasts and lymphocytes. In APL, the monocytes could not be separated on any channel, and the number of leukemic cells is limited to leukemic cells plus monocytes. APL is an AML with predominantly abnormal promyelocytes [8]. It is divided into hypergranular (so-called typical) or microgranular (hypogranular) types [9]. Prompt diagnosis of APL is essential for accurate treatment to prevent the development of lethal hemorrhagic complications [10]. APL, unlike most other leukemias, is frequently associated with leukopenia [9]. In this case the XN scatter plots are not helpful and require further analysis.

Chronic leukemia is usually detected during health examination and early diagnosis is difficult because of the absence of specific signs and symptoms [11]. Therefore, detection of malignant cells in the CBC of asymptomatic chronic leukemia patients is very meaningful. In addition, CML, CLL and PLL show characteristic XN scatterplots. The increase in blasts and basophils in CML is also reflected in the CML-accelerated phase diagnostic criteria, indicating disease progression [9]. The basophils need to be measured on both WNR and non-WNR channels.

In XN scatterplots, B- and T-PLL showed similar scatterplots, whereas CLL and PLL differed. In CLL, the high:low FSC lymphocyte ratio was decreased and most CLL cases showed the characteristic scatterplots even at a low lymphocyte count during treatment. In contrast, PLL showed increased high:low FSC lymphocyte ratio, and both B-PLL and T-PLL showed the same scatterplot. Interestingly, the leukemic cells of CLL and T-PLL show morphologically overlapping features [12], whereas XN showed their characteristic scatterplots.

In the future, the use of artificial intelligence in the analysis of XN scatterplots could increase the accuracy of leukemia diagnosis. XN can be used to report accurate WBC differential counts and flags in leukemic samples, heralding the next generation of CBC.

Declaration of Interest:
The authors have no competing interests.

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