Deviation in White Blood Cell Count Measurement between WNR and WDF Channels on an Automated Hematology Analyzer XN-3000: A Case Report

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Abstract

BACKGROUND: At present, complete blood count (CBC) testing is one of the most common clinical tests. The XN-3000 used in our hospital utilizes the principles of impedance and semiconductor laser flow cytometry, which contains two types of leukocyte fractionation methods: White cell nucleated (WNR) and white cell differential (WDF) channels.

CASE PRESENTATION: Herein, we present the case of a 63-year-old male patient who was admitted to our hospital for the treatment of non-small cell lung cancer (T4N3M1 [LYM, HEP, and OSS]). Biochemical data showed abnormal liver function and anemia. WNR results showed poor leukocyte and debris fractions compared with WDF results, and leukocyte counts were different between the two channels. However, the poor fractionation of the leukocyte and debris fractions in the WNR was resolved at the twofold dilution stage. To investigate the cause of the problem, precipitates affecting the WNR measurement were visually checked. Consequently, insoluble precipitates were observed in the WNR mixture of the specimen of the patient. In our hospital, we measure 1200 CBCs/day and very rarely encounter abnormal specimens such as in the presented case. Normally, leukocyte counts are reported in priority to those measured by the WNR channel; however, in this case, the switching function was effective in giving priority to WDF count results because of the appearance of abnormal WNR plots. However, a system that can promptly confirm events when results are reported is necessary.

CONCLUSION: We very rarely encounter abnormal specimens such as in the presented case. Normally, leukocyte counts are reported in priority to those measured by the WNR channel; however, in this case, the switching function was effective in giving priority to WDF count results because of the appearance of abnormal WNR plots. However, a system that can promptly confirm events when results are reported is necessary.

Introduction

At present, complete blood count (CBC) testing is one of the most common laboratory tests. Routine CBC testing in outpatients and inpatients, combined with patient history and clinical symptoms, helps confirm the clinical diagnosis and therapeutic efficacy of many diseases, particularly those related to the blood system and may suggest further testing [1]. Nowadays, most laboratories use automated analyzers for CBC testing. It provides information on all blood cell production, can assess the presence of anemia and the quality of red blood cell (RBC) by evaluating the RBC index; hemoglobin (Hb); and hematocrit, and provide insight into the immune system by evaluating white blood cell (WBC) count. These CBC tests are useful not only for evaluating anemia but also for acute hemorrhagic states, infections, certain blood disorders such as hematologic malignancy, allergies, and immunodeficiency and for monitoring the side effects of certain drugs that cause hematological disorders [2], [3], [4], [5], [6].

Automated hematology analyzers combine impedance and flow cytometry to ensure the accuracy and reproducibility of the clinical specimen results, and these analyzers can detect abnormalities in RBCs, WBCs, and platelets (PLTs), triggering a peripheral blood smear test [7], [8]. However, the complexity and marked variability of the blood components still pose several challenges for automated analyzers.

In our hospital, we use a XN-3000 multiparameter automated hematology analyzer (Sysmex Corporation, Hyogo, Japan) for blood cell testing. XN-3000 is based on the principles of impedance and semiconductor laser flow cytometry as previously described for blood cell counting methods. Specifically, leukocyte counts are based on two different leukocyte fractionation methods, namely white cell nucleated (WNR) and white cell differential (WDF) channels, using flow cytometry with a...
Case Report

The patient was a 63-year-old man who was admitted to our hospital for the treatment of non-small-cell lung cancer (T4N3M1 [LYM, HEP, and OSS]) and was in the terminal stage of his disease. The patient had been treated with docetaxel as an anticancer drug 1 month before the finding of the deviation of white blood cell counts presented here, but no further anticancer drugs had been administered.

The blood specimen was collected directly from the veins using a drawing needle, tube holder, and a K2-EDTA-containing vacutainer. It was processed within 1 h after collection and analyzed at our hospital.

This study was approved by the ethics board of the Fujita Health University (HM23-219), and written informed consent from the patient was not required because the present case did not require additional blood sampling and data were obtained with routine CBC.

Laboratory data are shown in Table 1. The white blood cell count (WBC) was 5200/µL, which was within the normal range, whereas Hb was 8.6 g/L, which was low. The aspartate aminotransferase: 203 U/L, alanine aminotransferase: 48 U/L, and γ-glutamyl transpeptidase: 258 U/L were high, indicating abnormal liver function, and C-reactive protein: 15.67 mg/dL, which was high and confirmed an inflammatory response.

During a leukocyte count on the XN-3000, a WBC Abn scattergram and lymphopenia were displayed on the device as a WBC flag. During visual leukocyte classification by the medical technologist, the previous WNR results of the device showed poor fractionation of the leukocytes and debris and discrepancies in the leukocyte count between the two channels. For this reason, the sample did not require additional blood sampling and data were obtained with routine CBC.

In this study, the control specimen and the patient’s remaining specimen were mixed in vitro with the hemolytic agents Lysercell WNR and WDF, Fluorocell WNR and WDF, and blood samples, each used in a different channel, to visually check for sediments. The mixing ratio of each reagent and sample was manually mixed according to the mixing ratio used in the actual measurement on the XN-3000. The mixing ratios are as follows:

i. Lysercell WNR or WDF: 1 mL
ii. Fluorocell WNR or WDF: 20 µL
iii. Blood sample: 17 µL

In this case, insoluble precipitates were observed in the mixed ratio of the WNR (Figure 4). However, the insoluble precipitates identified in Figure 4 have not been analyzed, and the causative agent has not been elucidated. Therefore, as a basic study, hyaluronic acid sodium salt (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was dissolved

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<th>Table 1: Laboratory data</th>
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<td><strong>Complete blood count</strong></td>
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in normal saline to achieve a final concentration of 0–4000 ng/mL, 1 mL of Lysercell WNR and 20 µL of Fluorocell WNR mixture were added, and the WNR was measured. In the results, no debris was observed even when 4000 ng/mL hyaluronic acid was added (data not shown). However, when hyaluronic acid was added at a very high concentration (40 μg/mL), some debris was observed; however, no obvious expansion of the plot was noted, as in this case (Figure 5).

**Discussion**

In this report, we described a case of deviations in the WNR and WDF leukocyte counts on an automated hematology analyzer XN-3000.

Manual microscopy is a well-established reference method for blood cell counts; however, given
its low accuracy and validity, it is not suitable for clinical practice [9] and most facilities currently use automated hematology analyzers such as the XN-3000. The XN-3000 is based on the principles of impedance and flow cytometry, as previously described for blood cell counting methods. Specifically, leukocyte counting can be performed by two different leukocyte fractionation methods, WNR and WDF, using three parameters, FSC, side fluorescence light, and side scattered light, by flow cytometry with a red scatter laser light having a wavelength of 633 nm (front and side) [10], [11].

The WNR counts leukocytes and classifies and counts basophils and nucleated erythrocytes. The surfactant in the Lysercell WNR causes erythrocytes to hemolyze and permeate the cell membrane of leukocytes. Each leukocyte has an external shape and internal structure depending on its cellular characteristics. This morphological difference is captured by FSC to distinguish and count basophils from other leukocytes. Fluorocell WNR fluorescently stains nucleic acids and organelles in the leukocytes and nucleated erythrocytes. Leukocytes tend to retain more staining sites in Lysercell WNR than nucleated erythrocytes, and their fluorescence intensity is stronger than that of nucleated erythrocytes. This difference in fluorescence intensity is used to distinguish between nucleated RBCs and other leukocytes for counting.

By contrast, WDF classifies and counts neutrophils, lymphocytes, monocytes, and eosinophils and detects abnormal cells such as juvenile leukocytes and atypical lymphocytes. Surfactants in the Lysercell WDF hemolyze and lyse RBCs and PLTs and permeate the cell membrane of leukocytes. Each leukocyte changes its cell morphology according to its characteristics, and the differences are distinguished by lateral light scattering. Fluorescent dyes in the Fluorocell WDF then enter the cell and stain nucleic acids and organelles. The type and abundance of nucleic acids and organelles cause differences in the fluorescence intensity of each leukocyte. By clustering differences in light scatter and fluorescence of each leukocyte with a proprietary algorithm, various cell types can be counted, classified, and flagged for abnormal cells.

In the presented case, the light-blue leukocyte area in the WNR (FSC-FSCW) shows a widening of the plot, and the presence of interfering substances should be considered. FSCW is usually a signal of leukocyte size, and the widening of the plot may be due to (1) the presence of leukocyte aggregation or (2) the detection of abnormal particles because of reactions between acid hemolytic...
agents in the reagents. The appearance of such interfering substances in the WNR can affect the measurement as a discrepancy in the WBC count between channels [12], [13].

For the detection of abnormal particles due to reactions between acid hemolytic agents in the reagents, we considered the possibility of hyaluronic acid influence; at the time, the present case occurred. The reason for this is that a deviation in white blood cell count between the WNR and WDF of the multiparameter automated hematology analyzer XE-2100 (Sysmex Corporation, Hyogo, Japan) has been reported in past reports, and the quaternary ammonium salts (QAS) used as a red blood cell lysis reagent has been suggested as a possible cause on the reagent side [13]. In the WNR measuring reagent (Lysercell WNR) of the XN-3000, 0.2% QAS was also used. QAS is widely used in industry and medicine; however, during its use, caution should be exercised because of the risk of precipitation of hyaluronic acid in the solution when in contact with hyaluronic acid [14], [15].

The presence of large amounts of hyaluronic acid in specimens from patients with cancer [16], [17] or liver disease [18], [19] has also been reported; however, since no Alcian blue staining was performed on the precipitates, determining whether hyaluronic acid itself would affect the leukocyte assay was not possible. However, the possibility that unexplained plots (ghosts), such as the one in this case, may result from the reaction of hyaluronic acid with acidic substances in the hemolytic agent should be fully considered. The patient was found to have very high concentrations (765 ng/mL) of hyaluronic acid not only in the pericardial fluid but also in the blood by additional testing (reference range, <50 ng/mL).

However, in the basic experiment shown in Figure 5, where hyaluronic acid was added to the WNR reagent, a wide range of debris was not observed, and only a few plots were observed when a very high concentration of hyaluronic acid (40 µg/mL) was added. Therefore, we suspect that not only hyaluronic acid but also other interfering substances present in the patient’s blood may be responsible for the falsely high WNR channel in the WBC assay.

In Sysmex XN, the reagent for the WNR channel is more acidic and less osmotic than that for the WDF channel [20]. The use of the WNR reagent may enhance leukocyte destruction compared to the WDF reagent due to the osmotic effect, and when comparing WBC counts between WNR and WDF, WNR may result in falsely low values due to enhanced destruction. However, the results of this study suggest that insoluble precipitates from interfering substances in the patient’s specimen caused the falsely high WBC counts in WNR. In the future, we plan to analyze the precipitates and investigate in detail the interfering substances in the WBC measurement when we observe a deviation in WBC counts, as in the present case.

**Conclusion**

Although we perform CBCs of approximately 1200/day at our hospital, we very rarely encounter abnormal specimens such as in the presented case. Normally, leukocyte counts are reported in priority to those measured by the WNR channel; however, in this case, the switching function was effective in giving priority to WDF count results because of the appearance of abnormal WNR plots. However, a system that can promptly confirm events when results are reported is necessary. When no clinical request for WDF measurement was made, the WNR scattergram must be confirmed, dilution measurement, if necessary, must be performed, or the WDF must be measured and reported to the clinician as useful criteria for diagnosis.
References

PMid:27565129

PMid:12710004

PMid:24038228


PMid:35413229

PMid:22646275

PMid:28903099


PMid:34840629

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