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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. 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.

Introduction

At present, complete blood cell 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

semiconductor laser, and WNR results are usually used and reported.

Herein, we describe a case of deviations in the WNR and WDF leukocyte counts and discuss the causes and remedies.

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 study, a sample that showed similar Hb concentration and leukocyte counts to the sample that showed a discrepancy in leukocyte counts was selected as the contrast control, and a scattergram is shown in Figure 1.

We hypothesized that interferents on the patient's plasma were involved in the appearance of the debris fraction in the WBC assay. Therefore, to reduce the influence of interfering substances on the patient's plasma, the remaining patient blood sample was used and diluted twofold and threefold with the diluent used in the XN-3000, and the leukocyte count was measured again using the WNR and WDF channels. The results showed that the poor fractionation of leukocyte and debris fractions in the WNR channel was resolved at the twofold dilution stage, and the leukocyte counts were like those measured in the WDF channel at the first measurement. In the WDF channel, leukocytes were well fractionated from the original measurements, and dilution had little effect on the counts (Figure 2).

In addition, we have identified a scattergram with forward scattered light signal width (FSCW) on the X-axis and forward scatter (FSC) on the Y-axis. In the contrast control, the FSCW on the X-axis did not show any spread on the X-axis in the leukocyte fraction, which is shown in light blue; however, in the sample with a divergent leukocyte count, a clear spread in the plot can be seen (Figure 3). FSCW is a signal of leukocyte size, and some sediments possibly formed in the reaction of the blood sample with the reagents used for WBC measurement in the divergent patient specimens.

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

Table 1: Laboratory data

Complete blood count		Biochemistry			
WBC	5,200/μL	TP	6.3g/dL	Na	134mmol/L
Stab	3.3%	Alb	2.5g/dL	K	4.6mmol/L
Seg	80.6%	T-Bil	0.2mg/dL	Cl	99mmol/L
Eo	2.7%	D-Bil	0.1mg/dL	Ca	8.2mg/dL
Lym	4.7%	AST	203U/L	Glucose	69mg/dL
Mono	8.7%	ALT	48U/L	NH_3	33μg/dL
RBC	273×10^4/μL	γ-GT	258U/L	Hyaluronic Acid	765ng/mL
Hb	8.6g/dL	ALP	261U/L	Pericardial effusion	
Hct	27.0%	AMY	106U/L	TP	6.5g/dL
Plt	15.8×10^4/μL	LD	923U/L	Alb	3.4g/dL
MCV	99fL	CRP	15.67mg/dL	CYFRA	918.0ng/mL
MCH	31.5pg	BUN	23.0mg/dL	CEA	10169.8ng/mL
MCHC	31.9%	Cre	0.97mg/dL	Hyaluronic Acid	20,700ng/mL

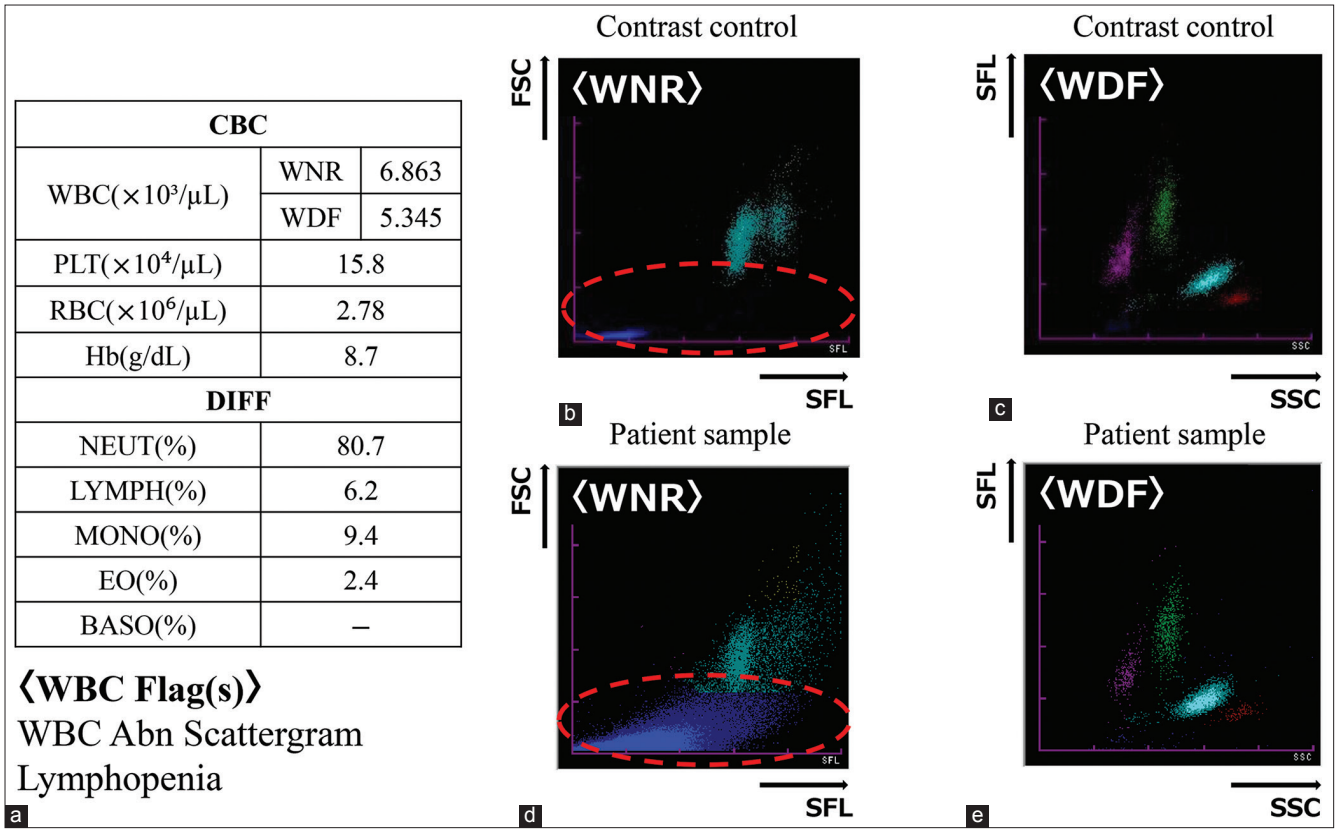

Figure 1: Leukocyte counts in WNR and WDF channels and scattergrams of each channel during WBC measurement. (a) White blood cell counts in WNR and WDF channels as indicated by XN-3000 and measurement error flags output during WBC measurement, (b) scattergram in the WNR channel during leukocyte measurement of the contrast control, (c) scattergram in the WDF channel during leukocyte measurement of the contrast control, (d) scattergram in the WNR channel during leukocyte measurement of the patient sample, (e) scattergram in the WDF channel during leukocyte measurement of the patient sample

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

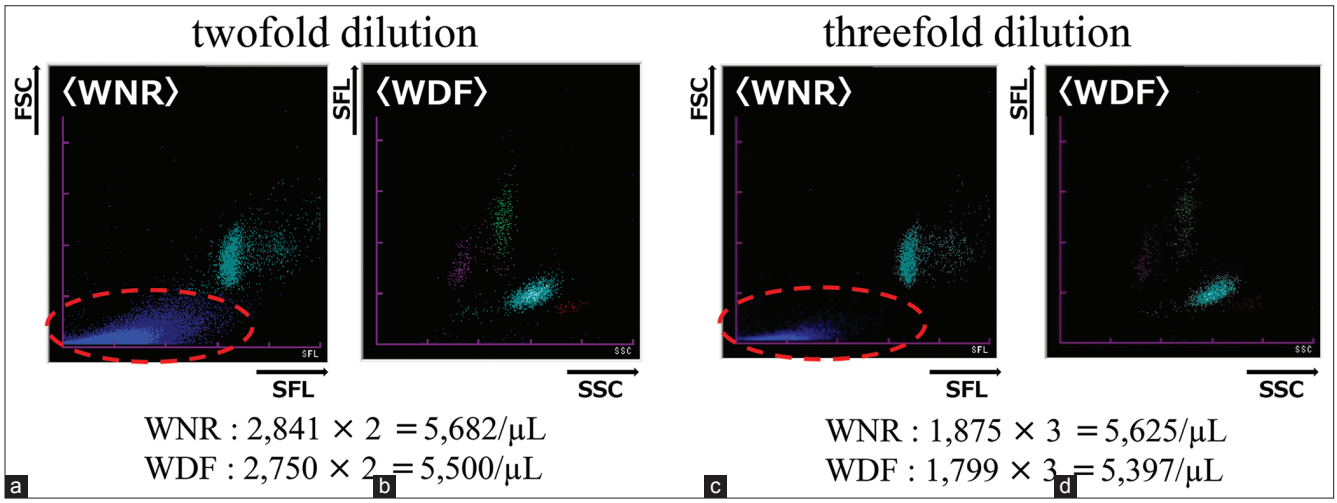

Figure 2: Scattergrams of WNR and WDF after dilution at various multiples. (a) Scattergram in the WNR channel after twofold dilution, (b) scattergram in the WDF channel after twofold dilution, (c) scattergram in the WNR channel after threefold dilution, (d) scattergram in the WDF channel after threefold dilution

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].

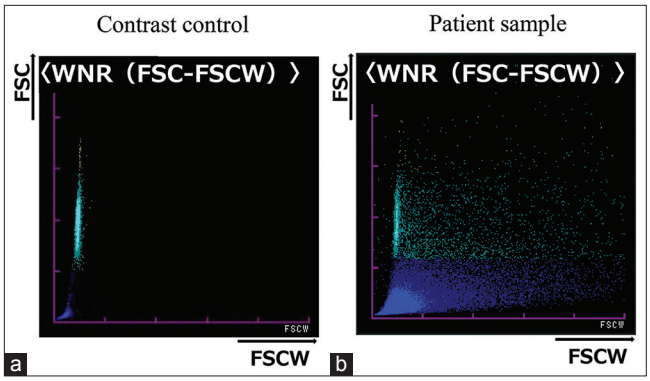

Figure 3: Scattergram of WNR forward scattered-forward scattered light signal width (FSC-FSCW) channel. (a) Scattergram in the WNR (FSC-FSCW) channel of the contrast control, (b) scattergram in the WNR (FSC-FSCW) channel of the patient sample

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

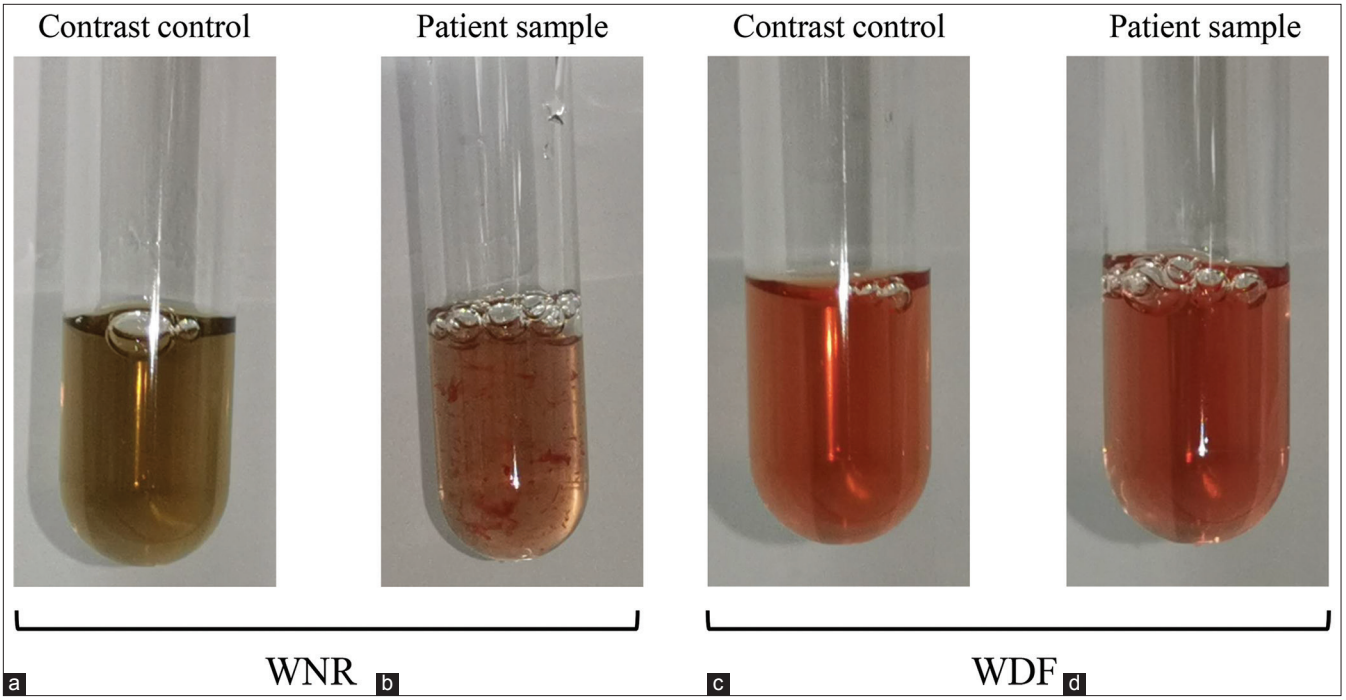

Figure 4: Confirmation of precipitates in WNR and WDF channels with control and patient samples. (a) Confirmation of the precipitates in the WNR channel in the contrast control, (b) confirmation of the precipitates in the WNR channel in the patient sample, (c) confirmation of the precipitates in the WDF channel in the contrast control, (d) confirmation of the precipitates in the WDF channel in the patient sample

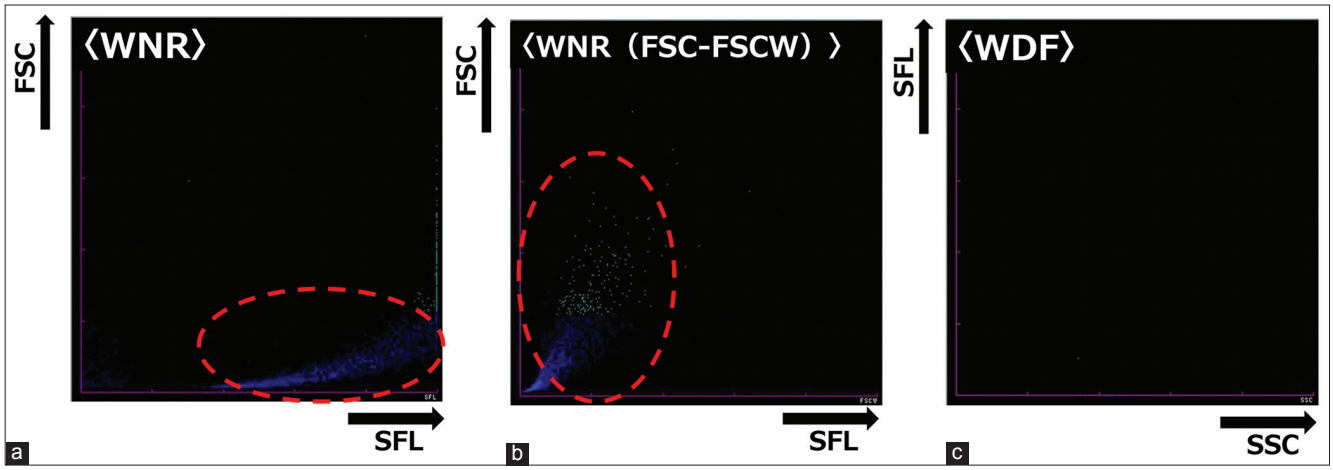

Figure 5: Scattergrams of WNR, WNR forward scattered-forward scattered light signal width (FSC-FSCW), and WDF channels after the addition of 40 μg/mL hyaluronic acid sodium salt. (a) Scattergram in the WNR channel after the addition of 40 μg/mL hyaluronic acid, (b) scattergram in the WNR (FSC-FSCW) channel after the addition of 40 μg/mL hyaluronic acid, (c) scattergram in the WDF channel after the addition of 40 μg/mL hyaluronic acid

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 the cause on the reagent side used as an erythrocyte lysing 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.

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