International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology

ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Comparison of white blood cell counts by WNR, WDF, and WPC channels in Sysmex XN hematology analyzer

H. KIM*, M. HUR*, S.-G. CHOI*, K.-M. OH†, H.-W. MOON*, Y.-M. YUN*

*Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea
†Department of Nursing, Konkuk University Medical Center, Seoul, Korea

Correspondence:
Mina Hur, Department of Laboratory Medicine, Konkuk University School of Medicine, Konkuk University Hospital, 120-1, Neungdong-ro, Hwayang-dong, Gwangjin-gu, Seoul 143-729, Korea.
Tel.: +82 2 2030 5581;
Fax: +82 2 2636 6764;
E-mail: dearmina@hanmail.net

doi:10.1111/ijlh.12421

Received 14 April 2015;
accepted for publication 31 July 2015

Keywords
Sysmex XN, white blood cells, comparison, WNR, WDF, WPC, channel

SUMMARY

Introduction: The Sysmex XN modular system (Sysmex, Kobe, Japan) uses a novel technology for white blood cell (WBC) count and differential, using separate channels: white cell nucleated (WNR), WBC differential (WDF), and white progenitor cell (WPC) channels. We questioned how concordant WBC counts would be between them.

Methods: In a total of 6327 consecutive specimens, WBC counts were compared between WNR and WDF channels. They were also compared in three groups of WBC counts and two groups of chemotherapy status. In 508 specimens from the 4361 specimens that were run on the XN-20 module, the WPC channel was used for reflex test. Data were compared using Pearson's correlation, absolute difference, and percent difference (%D).

Results: WBC counts between WNR and WDF channels showed very high correlations in total specimens ($r = 0.9976$) and in the groups of WBC counts and chemotherapy. As WBC count increased, absolute difference increased, while %D decreased ($P < 0.0001$, both). Percent difference was 1.55% in total specimens and showed the highest value in the severe leukopenia group ($<1.0 \times 10^9$/L, 6.18%).

Conclusions: This is the first large-scale study on novel channel technology for WBC counts in the Sysmex XN. WBC counts by WNR, WDF, and WPC channels are highly correlated, and they are overall interchangeable and reliable.

INTRODUCTION

Complete blood count (CBC) gives information on patients' blood cells and is one of the most frequently requested tests in clinical laboratories. Since the first automated hematology analyzer in the 1950s, based on the Coulter principle, there have been remarkable technical evolutions in automated hematology analyzers; current state-of-the-art hematology analyzers are characterized by their own technologies and characteristics to provide comprehensive reports on blood cells with good levels of precision and accuracy [1–3]. Regarding the white blood cell (WBC) count and differential cell count,

various technologies have been applied, such as light scattering, absorption spectrometry, electric impedance, radiofrequency conductivity, and/or flow cytometry [4]. In spite of reliable reporting of WBC count and differential cell count in most cases, manual smear review, although its clinical value is still under debate, is usually triggered according to specialized rules when automated hematology analyzers show a flag [3, 5–7].

The Sysmex XN modular system (Sysmex, Kobe, Japan) is a new-generation analyzer using principles, channels, and reagents different from those of its previous version, Sysmex XE-2100, and its performance has been evaluated in recent studies [7–11]. The Sysmex XN has been developed to measure nucleated red blood cells (NRBC) automatically along with the basic CBC. For this function, WBC counts and differentials are analyzed by two separate channels, the white cell nucleated (WNR) and WBC differential (WDF) channels, respectively. Recently, there was an interesting report on spurious WBC counts in Sysmex XN; WBC counts by WNR and WDF channels were remarkably different, such as 0.11×10^9/L (WNR) *vs.* 6.93×10^9/L (WDF) [12]. In that case, the estimated WBC counts on blood smear were compatible with WBC counts by the WDF channel, and there was a flag message 'Difference between WNR and WDF. Check the results' on the screen of analyzer. However, it was not transmitted to the laboratory information system, so the laboratory staff did not recognize this problem. In addition, four accidental cases with discrepant WBC counts between the WNR and WDF channels were observed in patients suffering from adenocarcinomas [13]. The discrepancy between the WNR and WDF channels was thought to be due to different reagents between the channels. The reagent in the WNR channel was more acidic, and this might have caused WBC to be more fragile in the WNR channel.

An accurate WBC count is fundamental for exact diagnosis and prompt treatment. In this study, we aimed to evaluate the performance of Sysmex XN in terms of WBC counts. We wondered whether WBC counts could be obtained reliably from both WNR and WDF channels and whether there would be discrepant WBC counts between the two channels in consecutive, large-scale clinical specimens.

MATERIALS AND METHODS

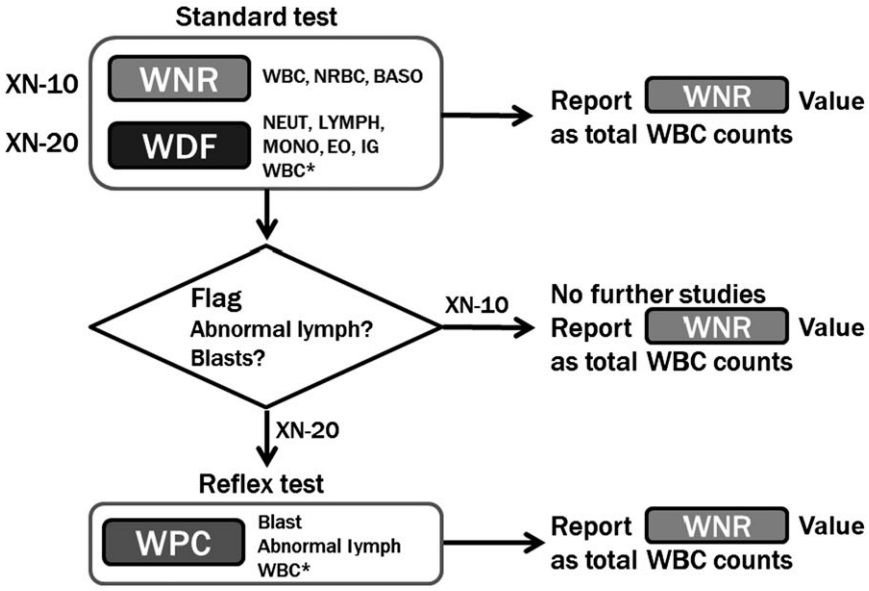
Specimens

For 5 days in April 2014, a total of 6327 specimens were consecutively enrolled from 4088 patients (including inpatients and outpatients). They were sent to the laboratory for routine CBC analysis from all departments in Konkuk University Hospital (KUH), Seoul, Korea. The specimens were collected directly from veins using drawing needle, tube holder, and K_3-EDTA-containing vacutainer (Greiner Bio-One GmbH, Frickenhausen, Germany) and were run within 4 h after collection. Hemolytic or clotted specimens were excluded to avoid poor data and false results. The study protocol was designed following the criteria of the Declaration of Helsinki and was approved by the Institutional Review Board of KUH. Written informed consent from the enrolled patients was exempted, because the data were obtained during routine CBC without additional blood sampling.

Study design

All 6327 specimens were analyzed using the Sysmex XN. Sysmex XN has two basic models: XN-10 and XN-20. Each model has different optional parameters with various needs. Both XN-10 and XN-20 report CBC, WBC differential, and NRBC counts with the WNR and WDF channels. Additionally, XN-20 has the white progenitor cell (WPC) channel, which discriminates blasts and abnormal lymphocytes when 'abnormal lymph?' or 'blasts?' flags are indicated from the WNR or WDF channel. The WNR channel is used for WBC, NRBC, and basophil counts, whereas the WDF channel is used for counts of neutrophils, lymphocytes, monocytes, eosinophils, and immature granulocytes. Both channels use flow cytometry with semiconductor laser. In both Sysmex XN-10 and XN-20, the value from the WNR channel is reported as total WBC count, regardless of reflex test in Sysmex XN-20. Total WBC counts from the WDF and WPC channels are used as research-use-only (RUO) parameters. Figure 1 schematically shows the reporting of WBC counts in the Sysmex XN [9, 12].

Among the reagents used (LYSERCELL and FLUOROCELL), LYSERCELL is different in each channel; LYSERCELL in the WNR channel is more acidic



WBC*: measured but not reported value
XN-10 module: varied as B1, B2, B3 and B4 modules according to PLT-F and RET channels availabilities
XN-20 module: A1 and A2 according to PLT-F channel availability
Abbreviations: EO, eosinophils; IG, immature granulocytes; LYMPH, lymphocytes; MONO, monocytes; NEUT, neutrophils; NRBC, nucleated red blood cells; PLT-F, platelets-fluorescent; RET, reticulocytes; WBC, white blood cells; WDF, WBC differential; WNR, white cell nucleated; WPC, white progenitor cells;

Figure 1. Brief scheme for reporting total white blood cell (WBC) counts in Sysmex XN modular system.

(pH 2.95–3.05, 26–32 mOsm/kg H_2O) than that in the WDF channel (pH 5.95–6.05, 98–108 mOsm/kg H_2O). If this channel detects blasts or abnormal lymphocytes, a reflex test is performed in the WPC channel, where LYSERCELL is more neutral (pH 7.25–7.35, 32–42 mOsm/kg H_2O).

The WBC count is calibrated at least every 6 months using internal standard material (XN CAL, Sysmex); it is calibrated only in the WNR channel. This calibration does not apply to the other WBC channels, where the WBC count is generated as a RUO parameter. Internal quality control is checked at least every 8 h using three-level quality control materials (XN CHECK, Sysmex). All 6327 specimens were randomly analyzed between XN-10 and XN 20 modules; 1966 specimens were analyzed with the XN-10 module and 4361 specimens with the XN-20 module. In XN-20, the reflex test by the WPC channel was conducted in 508 of 4361 specimens (11.6%).

Patients' medical records were reviewed for the clinical and laboratory data. The median age of enrolled patients was 57 years (range, 0–96 years), and females were 50.6% (3202/6327). The median value of WBC counts from all specimens was 6.74×10^9/L (range, 0.03–294.58 $\times 10^9$/L). All specimens were divided into three groups based on WBC counts: leukopenia ($<4.0 \times 10^9$/L, $n = 716$); normal WBC count (4.0–10.0 $\times 10^9$/L, $n = 4419$); and leukocytosis ($>10.0 \times 10^9$/L, $n = 1192$). The leukopenia group was further divided into severe leukopenia ($<1.0 \times 10^9$/L, $n = 82$) and mild-to-moderate leukopenia (1.0 to $<4.0 \times 10^9$/L, $n = 634$) groups. They were also compared in two groups of patients according to chemotherapy status at the time of blood sampling: chemotherapy ($n = 1304$) *vs*. no chemotherapy ($n = 5023$).

We compared correlation, absolute difference, and percent difference (%D) of WBC counts between WNR and WDF channels. Percent difference of WBC counts was calculated by dividing the difference in WBC counts between WNR and WDF channels by the WBC count in the WNR channel; the WBC count in WNR channel was regarded as a baseline value. For WBC counts, the manufacturer-claimed maximum within-run coefficient of variance (CV) was 3% in normal samples (WBC $\geq 4.0 \times 10^9$/L); this value was used for method comparison.

Statistical analysis

Data were expressed as medians and ranges. Pearson's correlation coefficient (with 95% confidence interval [CI]) was used to compare WBC counts. The *r* coefficients ≤0.35 were considered as representing low correlations, 0.36 to 0.67 as moderate correlations, and 0.68 to 1.0 as high correlations, with *r* coefficients ≥0.90 as very high correlations [14]. The Bland–Altman plot was used to identify mean difference and 95% limits of agreement of WBC counts between WNR and WDF channels. The Kruskal–Wallis test with *post hoc* test was used to compare absolute difference and %D in the groups of WBC counts. The Mann–Whitney *U*-test was used to compare absolute difference and %D between the two groups of chemotherapy status. The Wilcoxon paired test was used to compare absolute difference and %D of WDF *vs*. WPC and WDF *vs*. WNR channels.

For the statistical analysis, Analyse-it Software (version 3.76.4 Analyse-it Software, Ltd., Leeds, UK) and MedCalc Software (version 13.1.2, MedCalc Software, Mariakerke, Belgium) were used. *P* values equal to or less than 0.05 were considered statistically significant.

RESULTS

Pearson's correlation coefficient (*r*), absolute difference, and %D between WNR and WDF channels are presented in Table 1. In all 6327 specimens, WBC counts showed a very high correlation between the two channels ($r = 0.9976$) without significant deviation from linearity. Such a very high correlation was observed across all subgroups of WBC counts and chemotherapy status. %D was 1.55% in total specimens and less than 3% in each subgroup, except in the severe leukopenia group. %D in the severe leukopenia group ($n = 82$) was significantly higher than that in mild-to-moderate leukopenia group (6.18 *vs*. 2.36%, $P < 0.0001$). As WBC count increased, absolute difference in WBC counts increased while %D decreased ($P < 0.0001$, both). The absolute difference in WBC counts was significantly lower in 1304 chemotherapy patients than in nontreated patients (0.08×10^9/L *vs*. 0.11×10^9/L, $P < 0.0001$). However, %D was significantly higher in chemotherapy patients than in nontreated patients (1.56 *vs*. 1.55%, $P = 0.0008$).

In 508 specimens for which the reflex test was conducted, WBC counts showed very high correlations in each pair of channels: WNR *vs*. WDF; WNR *vs*. WPC; and WDF *vs*. WPC channels (Table 2). Such a very high correlation was consistently observed across all subgroups of WBC counts and chemotherapy status. Similar to total specimens, as WBC count increased, absolute difference in WBC counts increased, while %D of WBC count decreased. Of note, in the severe leukopenia group, %D between WNR and WPC channels was 0.10%, showing a remarkable difference compared to %D of other channel comparisons (6.66% between WNR and WDF channels; 7.69% between WDF and WPC channels).

DISCUSSION

In spite of the new advanced technologies in automated hematology analyzers, spurious or erroneous cell counts may be encountered. WBC count with its differential is one of the most important CBC parameters. The present study was conceived based on a previous case that showed remarkably different WBC counts between WNR and WDF channels (0.11×10^9/L *vs*. 7.4×10^9/L) and revealed the WBC counts by WDF channel as an accurate value [12]. In that case, a flag message that appeared on the screen of the analyzer, 'Difference between WNR and WDF. Check the results', was not transmitted to the laboratory information system, and the laboratory staff could not recognize it. This case underscores the importance of checking every single flag message from the instrument. In our 6327 consecutive specimens reflecting various clinical settings and typical patient populations in clinical laboratories, the same flag message was not detected. If we had included investigations of the few occurrences of this flag, this study would have been substantially strengthened. Although we could not get further information on which occasion such a flag message would appear, either from our study or from the manufacturer, this flag seems to appear very rarely. Therefore, users should pay attention to spurious, severely leukopenic specimens and should confirm the results with further tools such as blood smears.

In this study, WBC counts by WNR, WDF, and WPC channels showed very high correlations regardless of WBC counts and chemotherapy status (Tables 1 and 2). Although we used %D for channel comparison, we had no information on the bias of any of the three WBC channels, as a reference

Table 1. Pearson's correlation coefficient, absolute difference, and percent difference between white cell nucleated (WNR) and WBC differential (WDF) channels in Sysmex XN

	N	Median from WNR (range, x 10^9/L)	Pearson's correlation coefficient (95% CI)	Absolute difference (range, x 10^9/L)	Percent difference (range, %)
Total	6327	6.74 (0.03–294.58)	0.9976 (0.9973–0.9978)	0.18 (0.00–4.77)	1.55 (0.00–33.33)
Groups according to WBC counts					
Leukopenia	716	3.12 (<4.00)	0.9962 (0.9956–0.9967)	0.06 (0.00–0.30)	2.61 (0.00–33.33)
Severe	82	0.62 (<1.00)	0.9900 (0.9845–0.9936)	0.03 (0.01–0.16)	6.18 (0.00–33.33)
Mild to moderate	634	3.23 (1.00–<4.00)	0.9918 (0.9904–0.9930)	0.07 (0.00–0.37)	2.36 (0.00–10.45)
No chemotherapy	362	3.37 (0.11–<4.00)	0.9912 (0.9892–0.9928)	0.07 (0.00–0.37)	2.34 (0.00–14.29)
Chemotherapy	354	2.58 (0.04–<4.00)	0.9973 (0.9966–0.9978)	0.05 (0.00–0.30)	2.90 (0.00–33.33)
Normal WBC counts	4419	6.49 (4.0–10.0)	0.9956 (0.9953–0.9959)	0.10 (0.00–0.84)	1.53 (0.00–9.24)
Leukocytosis	1192	12.60 (>10.00)	0.9995 (0.9995–0.9996)	0.17 (0.00–4.77)	1.29 (0.00–8.21)
Groups according to chemotherapy status					
No chemotherapy	5023	6.93 (0.11–68.03)	0.9992 (0.9992–0.9992)	0.11 (0.00–2.85)	1.55 (0.00–14.29)
Chemotherapy	1304	5.59 (0.04–299.35)	0.9997 (0.9997–0.9998)	0.08 (0.00–4.77)	1.56 (0.00–33.33)

Abbreviations: WNR, white cell nucleated; WDF, WBC differential; CI, confidence interval.

method of WBC count was not used in this study. Given that there is no suggested cutoff that is applicable to compare these kinds of novel channels for WBC counting, we used the manufacturer-claimed maximal within-run CV of 3% [15, 16]. Although this 3% cutoff was suggested for normal specimens (WBC $\geq 4.0 \times 10^9$/L), the %D between channels was less than 3% even in leukopenic specimens, except in severely leukopenic specimens. However, it is doubtful whether the statistical significance of %D can be regarded as a clinically significant difference.

It was noteworthy that %D was very low (0.10%) between WNR and WPC channels, even with specimens with severe leukopenia, in contrast to the high % D between WDF and WPC channels and between WNR and WDF channels (6.66% between WNR and WDF channels; 7.69% between WDF and WPC channels) (Table 2). In XN-20, which uses the WPC channel for the reflex test, the WBC data generated from the WNR and WPC channels seemed to be more related to each other than that from WDF channel. The osmolarity of LYSERCELL in the WDF channel (98–108 mOsm/kg H_2O) was higher than that in WNR and WPC channels (26–32 and 32–42 mOsm/kg H_2O, respectively). This might be one of the explanations for such differences between channels. The analytical imprecision for neutrophil counts increases quickly in severely leukopenic specimens [15]; this finding is supported by the present study. In severely leukopenic specimens, such aspects should be considered, and blood smear review would be necessary to confirm the WBC counts.

In Sysmex XN, the reagent for the WNR channel is more acidic and has lower osmolarity than that for the WDF channel. We assumed that chemotherapy would influence WBC membrane fragility and cause different WBC counts between channels, but the present study demonstrated that current chemotherapy status did not produce a clinically significant difference in WBC counts between channels. This finding is in line with a previous report that there was no significant difference in WBC counts between WNR and WDF channels with different pH, incubation times, and temperatures [13].

According to the Clinical Laboratory Improvement Amendment (CLIA) regulations, each laboratory must demonstrate that it can obtain performance specifications comparable to those established by the manufacturer when it introduces a new hematology analyzer [16]. However, the judgment of acceptability depends on which amount of analytical error is allowable without affecting or limiting the use and interpretation of individual test results, when validating method comparison between two instruments. Hence, each laboratory should develop its own evaluation criteria for acceptable results. New judgment criteria or comparison guidelines would be necessary for this novel channel technology. The criteria should not depend on technology but rather on the potential medical consequences of incorrect laboratory results.

Table 2. Absolute difference and percent difference in each pair of channels in 508 specimens with reflex test

			WNR *vs*. WDF		WNR *vs*. WPC		WDF *vs*. WPC	
	N	Median from WPC (range, x 10^9/L)	Absolute difference (range, x 10^9/L)	Percent difference (range, %)	Absolute difference (range, x 10^9/L)	Percent difference (range, %)	Absolute difference (range, x 10^9/L)	Percent difference (range, %)
Total	508	6.53 (0.14–60.41)	0.10 (0.00–2.52)	1.46 (0.00–18.18)	0.11 (0.00–1.83)	1.76 (0.00–11.25)	0.11 (0.00–3.92)	0.02 (0.00–0.21)
Groups according to WBC counts								
Leukopenia	76	2.90 (<4.00)	0.07 (0.00–0.33)	3.20 (0.00–18.18)	0.06 (0.00–0.32)	2.61 (0.00–11.25)	0.07 (0.00–0.33)	0.03 (0.00–0.21)
Severe	12	0.80 (<1.00)	0.03 (0.00–0.16)	6.66 (0.00–18.18)	0.04 (0.01–0.10)	7.69 (2.50–11.25)	0.04 (0.01–0.11)	0.10 (0.01–0.21)
Mild to moderate	64	3.15 (1.00–<4.00)	0.09 (0.00–0.33)	2.86 (0.00–10.28)	0.06 (0.00–0.32)	2.09 (0.00–10.46)	0.08 (0.00–0.33)	0.03 (0.00–0.09)
Normal WBC counts	348	6.58 (4.0–10.0)	0.10 (0.00–0.84)	1.38 (0.00–9.12)	0.11 (0.00–0.57)	1.63 (0.00–7.81)	0.11 (0.00–0.77)	0.02 (0.00–0.09)
Leukocytosis	84	12.94 (>10.00)	0.18 (0.00–2.52)	1.29 (0.00–5.74)	0.23 (0.00–1.83)	1.61 (0.00–6.13)	0.22 (0.01–3.92)	0.01 (0.00–0.10)
Groups according to chemotherapy status								
No chemotherapy	394	6.76 (0.32–60.41)	0.10 (0.00–2.52)	1.42 (0.00–14.29)	0.12 (0.00–1.83)	1.70 (0.00–10.34)	0.12 (0.00 – 3.92)	0.02 (0.00–0.16)
Chemotherapy	114	5.51 (0.14–20.66)	0.08 (0.00—0.72)	1.78 (0.00–18.18)	0.08 (0.01–0.88)	1.92 (0.17–11.25)	0.08 (0.00–1.23)	0.02 (0.00–0.21)

Abbreviations: WDF, WBC differential; WNR, white cell nucleated; WPC, white progenitor cell.

This study is limited in that detailed information on chemotherapy was not available. We can speculate that different types of chemotherapy (e.g., alkylating agents, topoisomerase II inhibitors) may affect WBC membrane differently. Additionally, any changes in %D during the course of chemotherapy schedule could not be evaluated. Serial investigation of %D may provide more information on which channel would be useful for WBC counts in relation to chemotherapy. Further studies would be necessary to prove these speculations. Lastly, because all specimens were tested within 4 h after collection, the effect of sample transport and/or processing delays were not evaluated in this study.

In conclusion, this is the first study that explored a novel WBC counting method using different channels in the Sysmex XN modular system. In our large-scale study including 6327 clinical specimens, WBC counts with the WNR, WDF, and WPC channels were highly correlated, and the results were overall interchangeable and reliable. Our single institutional study could not detect any cases with significant discrepancy of WBC counts between channels, leaving room for further investigation on Sysmex XN and its WBC channels in various clinical situations.

ACKNOWLEDGEMENT

This work was supported by Konkuk University in 2015.

CONFLICT OF INTEREST

The authors report no conflict of interests. The authors alone are responsible for the content and writing of the article.

AUTHOR CONTRIBUTIONS

Kim H analyzed the data and wrote the draft. Hur M designed the study and finalized the draft. Choi SG and Oh KM participated in data collection. Moon HW and Yun YM participated in data analysis and reviewed the draft.

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