RESEARCH ARTICLE

Extent of agreement between the body fluid model of Sysmex XN-20 and the manual microscopy method

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Background: Although the correlations concerning cellular component analysis between the Sysmex XN-20 body fluid (BF) model and manual microscopy have been investigated by several studies, the extent of agreement between these two methods has not been investigated.

Methods: A total of 90 BF samples were prospectively collected and analyzed using the Sysmex XN-20 BF model and microscopy. The extent of agreement between these two methods was evaluated using the Bland-Altman approach. Receiver operating characteristic (ROC) curve analysis was employed to evaluate the diagnostic accuracy of high-fluorescence (HF) BF cells for malignant diseases.

Results: The agreements of white blood cell (WBC), red blood cell (RBC), and percentages of neutrophils, lymphocytes, and monocytes between the Sysmex XN-20 BF model and manual microscopy were imperfect. The areas under the ROC curves for absolute and relative HF cells were 0.67 (95% confidence interval [CI]: 0.56-0.78) and 0.60 (95% CI: 0.48-0.72), respectively.

Conclusion: Due to the Sysmex XN-20 BF model's imperfect agreement with manual microscopy and its weak diagnostic accuracy for malignant diseases, the current evidence does not support replacing manual microscopy with this model in clinical practice.

KEYWORDS

agreement, Bland-Altman approach, cellular component analysis, high-fluorescence cell, SysmexXN-20

1 | INTRODUCTION

Cellular analysis of body fluids (BFs) is clinically useful because it can assist clinicians in diagnosing many diseases.^{1,2} Traditionally, manual microscopy has been regarded as the gold standard for qualifying and classifying the cellular components of BF. However, this method has limitations, including being labor intensive and time consuming, as well as having high observer variability. Recently, hematology analyzers for assessing BF has gained much attention because they can improve laboratory efficiency and cost-effectiveness.³ Many hematology

analyzers have been developed to analyze BF automatically, such as Sysmex XE-5000⁴ and coulter LH 750.⁵

Recently, Sysmex launched a new hematology analyzer named XN-20, which has a BF model that can automatically analyze cellular components of BF. The cells in BF are differentiated into white blood cells (WBC) and high-fluorescent (HF) cells, and WBCs are further categorized as polymorphonuclear (PMN) and mononuclear (MN) cells. According to the manufacturer's instructions, the PMN cells comprise neutrophils, eosinophils, and basophils, and the MN cells comprise lymphocytes and monocytes. Because basophils and eosinophils are uncommon in BF, they are usually not reported. HF cells have large amounts of nucleic acids and are a useful index to identify suspected malignant samples.

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To date, many studies have evaluated the analytical performance of the Sysmex XN-20 BF model.^{6–9} However, these studies only evaluated the correlations between the XN-20 and the manual microscopy method using Spearman or Pearson approaches. The extent of agreement between the two methods has not been assessed using the Bland-Altman approach. Indeed, good correlation does not mean good agreement.¹⁰ In this study, we investigated the extent of agreement between the Sysmex XN-20 hematology analyzer and manual microscopy using the Bland-Altman approach.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethical review committee at Changhai Hospital of the Second Military Medical University (Shanghai, China).

2.2 | Experimental procedure

A total of 90 specimens sent to the clinical laboratory of Changhai Hospital for routine analysis were prospectively collected. These specimens included 41 pleural effusions, 48 ascites, and one joint effusion. All specimens were mixed by inversion and then divided into two samples: one was analyzed using microscopy and the other was analyzed using Sysmex XN-20.

Manual cellular analysis was performed using a Neubauer counting chamber and a standard microscope. White blood cell (WBC) differentiation was performed using cytocentrifugation followed by Wright staining and microscopy.

In manual analysis, three technicians independently performed the analyses, strictly following the standard operation procedure (SOP) of our laboratory. Their mean values were regarded as the final

results. Specimens with higher cell counts were diluted if necessary. Laboratory technicians were blind to the clinical details of all samples. All specimens were analyzed within 2 hours after they were received.

2.3 | Statistical analysis

Statistical analyses were performed using Graphpad Prism 6.0 software (GraphPad Software Inc., San Diego, CA, USA). The Bland-Altman approach was used to estimate the extent of agreement between the manual and Sysmex XN-20 methods. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic accuracy of HF and relative HF for malignant diseases. Optimal thresholds were adopted at the highest Youden index. A *P* value of <.05 was regarded as statistically significant.

3 | RESULTS

3.1 | Agreement between microscopy and XN-20

Figure 1 is a Bland-Altman plot analyzing the agreement between microscopy and XN-20. The biases and 95% limits of agreement are listed in Table 1. Generally, agreement between microscopy and XN-20 was imperfect. The monocyte percentage obtained from microscopy was higher than that obtained from XN-20, while RBC and WBC counts as well as neutrophil and lymphocyte percentages obtained from microscopy were lower than those obtained from XN-20. The 95% limits of agreement for cellular analysis were computed.

3.2 | Diagnostic accuracy of HF parameters for malignant diseases

Forty-three of the 90 specimens were diagnosed as malignant diseases, and tumor cells were found in 20 cases. Therefore, the

FIGURE 1 Bland-Altman plots for microscopy and XN-20

TABLE 1 Biases and 95% limits of agreement between microscopy and XN-20

	Bias ((XN20 - microscopy)/average × 100%)	95% limit of agreement (%)	
		Lower	Upper
White blood cell count (mL ⁻¹)	-1.246	-30.88	28.39
Red blood cell count (mL ⁻¹)	14.43	-72.00	100.90
Neutrophils (%)	27.09	-101.90	156.10
Lymphocytes (%)	10.62	-95.98	117.20
Monocytes (%)	-18.19	-148.30	111.90

FIGURE 2 Receiver operating characteristic (ROC) curves for absolute high-fluorescent (HF) cell count and relative HF (HF%) cell count

diagnostic sensitivity of microscopy for malignant disease was 0.47 (20/43).

Figure 2 is an ROC plot depicting the diagnostic accuracy of absolute and relative HF cell counts for the diagnosis of malignant diseases. The areas under the curve (AUCs) for absolute (HF) and relative HF (HF%) cell counts were 0.67 (95% confidence interval [CI]: 0.56-0.78) and 0.60 (95% CI: 0.48-0.72), respectively. In addition, among patients with malignant diseases, the HF and HF% in patients with tumor cells in BF and those without were similar, without a statistically significant difference between them. Table 2 lists optimal thresholds and their corresponding sensitivity, specificity, positive and negative predictive values.

4 | DISCUSSION

Although some studies have investigated the correlations of cellular analysis results between Sysmex XN-20 and manual microscopy,⁶⁻⁹ the

TABLE 2 Diagnostic accuracy of absolute and relative high-fluorescent cell counts for malignant disease

	Absolute HF cell count	Relative HF cell count
Area under curve (95% CI)	0.67 (0.56-0.78)	0.60 (0.48-0.72)
Optimal threshold	39	6.5
Sensitivity (95% CI)	0.74 (0.59-0.86)	0.65 (0.49-0.79)
Specificity (95% CI)	0.62 (0.46-0.75)	0.62 (0.46-0.75)
Positive predictive value (95% CI)	0.64 (0.49-0.77)	0.61 (0.45-0.75)
Negative predictive value (95% CI)	0.73 (0.56-0.85)	0.66 (0.50-0.80)

extent of agreement between these two methods has not previously been evaluated. Correlation analysis only measures the strength of the linear association between two methods, but not the strength of agreement. Even perfect correlation does not necessarily mean good agreement. For example, if WBC counts detected by microscopy were only half of those detected by Sysmex XN-20, the two methods would show perfect correlation, yet would not show high agreement. To the best of our knowledge, this is the first study investigating the agreement between Sysmex XN-20 and microscopy. We found that the agreement between microscopy and the BF model of Sysmex XN-20 was imperfect because the limit intervals of all indices in the Bland-Altman plots were wide. For example, the 95% limit of agreement for neutrophil percentage was -101.9% and 156.1%. This means that approximately 95% of specimens would have a difference (%) in neutrophil percentage, as measured using these two methods, between -101.9% and 156.1%. It seems that this large difference is not clinically acceptable.

In addition, we found that the diagnostic accuracy of both the absolute and relative counts of HF cells for malignant diseases is fair because the area under each ROC curve was small. At the optimal threshold, the diagnostic sensitivity for HF and HF% was 0.74 and 0.65, respectively. This means that about one-third of patients with malignant diseases would be missed if HF or HF% was used as the sole diagnostic tool. Although these sensitivities seem to be higher than that of microscopy (0.47), their corresponding specificities were only 0.62, each. This means that only two-thirds of nonmalignant patients could be safely ruled out when HF or HF% was used alone. Taken together, these results indicate that HF and HF% are not reliable indices for diagnosing malignant diseases.

This study has some limitations. First, this is a single center study and the sample size is small; therefore, bias in patient selection cannot be excluded. Second, only pleural effusion and ascites were included in this study; cerebrospinal fluid (CSF) should be analyzed in future studies.

5 | CONCLUSION

This study indicates that the agreement between the Sysmex XN-20 BF model and manual microscopy is imperfect, and the diagnostic

values of HF and HF% are not high. Therefore, current evidence does not support replacing manual microscopy with the BF model of XN-20.

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