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Evaluation of Sysmex XN-1000 hematology analyzer for cell count and screening of malignant cells of serous cavity effusion

Weiyi Xu, BS[a,b], Qian Yu, BS[a,b], Lixia Xie, BS[a,b], Baode Chen, MS[a,b,*], Ling Zhang, BS[c]

Abstract

Over the years, with the advancement in hematology analyzer technology, the use of fluid analysis method has seen a drastic increase in clinical examinations. Cell counting and classification in independent body fluid analysis method are conducted by semiconductor laser flow cytometry and nucleic acid fluorescence staining techniques. This study is to evaluate the efficacy of Sysmex XN-1000 hematology analyzer in cell counting and to screen malignant cells with serous cavity effusion. Specimens (N = 206) with serous cavity effusion from our hospital were included in this study. Manual and instrumental methods for cell counting, nucleated cell classification, and high-fluorescent cells (HFC) were used in this study. The correlation between RBC, nucleated cell count (NUC), the percentages of polymorphonuclear cell (PMN%), and mononuclear cells (MN%) was statistically analyzed using manual and instrumental methods. The regression equations of RBC, NUC, PMN%, and MN% in the manual and instrumental methods were RBC $y = 0.88x + 426.4$; NUC $y = 0.85x + 33.4$; PMN% $y = 0.91x + 4.2$; and MN% $y = 0.91x + 5.1$. Correlation coefficient R^2 was 0.99, 0.98, 0.90, and 0.90 ($P < .001$). ROC curve analysis showed that when the cut-off value of HFC% was 4.4% and HFC# was 24.5/μL, area under curve (AUC), sensitivity, specificity, and 95% confidence interval were 0.707, 0.792, 0.558, 0.637–0.777; 0.708, 0.753, 0.550, 0.635–0.780, respectively. XN-1000 hematology analyzer body fluid method can accurately and rapidly count cell and nucleated cell classification with serous cavity effusion. HFC can indicate the possible existence of malignant cells; however, further investigations are required to validate its efficacy.

Abbreviations: HE = hematoxylin–eosin, HFC = high-fluorescent cells, MN = mononuclear cells, NUC = nucleated cell count, PMN = polymorphonuclear cell.

Keywords: HFC, malignant cells, serous cavity effusion, XN-1000 hematology analyzer

1. Introduction

Cell count, nucleated cell classification, and exfoliative cytology of serous cavity effusion are critical in clinical judgment of the fluid nature.[1] These methods are not only convenient and rapid, but also provide cytological evidence for the clinical examinations. Until now, manual *Neubauer* hemocytometer and nucleated cell classification after slide-making and staining are still the "gold standard."[2] However, these methods are time-consuming, laborious, and demand stringent specifications for technical personnel, with poor reproducibility.[3,4] Over the years, with the advancement in hematology analyzer technology, the use of fluid analysis method has been found to be effective for clinical examinations. Cell counting and classification in independent body fluid analysis method in Sysmex XE-5000 and XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan) are carried using semiconductor laser flow cytometry and nucleic acid fluorescence staining techniques. There have been a few reports on the performance evaluation of body fluid mode and malignant cell screening,[4–7] the evidence to validate the efficacy of automatic nucleated cell counting, nucleated classification, and malignant cell screening of serous cavity effusion is still very rare.

2. Specimen sources

Two hundred six specimens with serous cavity effusion were collected from inpatients in the First Affiliated Hospital of Zhejiang University from October 2015 to May 2017. Among them, 146 cases were male, with an average age of 59 years old, and 60 cases were female, with an average age of 55 years old. Ninety-five cases were associated with pleural effusion, while 111 cases were associated with ascites. Based on the existence of tumor cells in the effusion cytology, these cases were divided into malignant effusion group of 77 cases and nonmalignant effusion group of 129 cases. Specimens with more than 10% of denatured cells or viscous specimens were excluded from the test. This

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This project was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University Medical College. Informed consents have obtained.

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[a] Department of Laboratory Medicine, The First Affiliated Hospital, College of Medicine, Zhejiang University, [b] Key Laboratory of Clinical In Vitro Diagnostic Techniques of Zhejiang Province, Hangzhou, [c] Renji College, Wenzhou Medical University, Wenzhou, China.

[*] Correspondence: Baode Chen, Department of Laboratory Medicine, The First Affiliated Hospital, College of Medicine, Zhejiang University, Key Laboratory of Clinical In Vitro Diagnostic Techniques of Zhejiang Province, Hangzhou 310003, China (e-mail: cbd39@163.com).

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3. Specimen detection

Specimens were collected, stored, transported, and detected according to the requirements of CLSI H56-A document.[2] Specimens with EDTA-K2 anticoagulation were collected and transported immediately after collection. Cell detection was performed by instrumental method and manual cell counting, centrifuged for 5 min at 400g, and sediments were kept on slides for nucleated cell classification using Wright–Giemsa staining and pathologic examination was performed using hematoxylin–eosin (HE) stained, followed by immunocytochemistry if applicable. Manual cell count and nucleated cell classification were completed using 2 experienced microscope operators independently, and the count results required CV <10%. Nucleated cells in each specimen were classified by identifying 200 nucleated cells, and identifications from cytology experts were taken for dissents. The neutrophils, eosinophils, and basophils were classified as PMN cells, and lymphocytes, plasma cells, mesothelial cells, macrophages, and malignant cells were classified as MN cells. Cell counting and classification of the samples were manually detected by XN-1000 hematology analyzer in the body fluid mode through the instrument electrical impedance, flow cytometry, and nucleic acid fluorescence staining and other techniques. Main detecting parameters were RBC, WBC, PMN#, PMN%, MN#, MN%, HFC#, HFC%, and WBC and HFC# were classified as NUC. Cell counting, slides making and staining, and instrumental analysis were completed within 2 h after receiving the samples.

4. Instruments and reagents

Cells were stained by Wright–Giemsa stain (BASO) and manually counted by *Neubauer* hemocytometer. Exfoliative cells were detected by HE stain. RBC, NUC, and NUC differential counts were measured in duplicate on the Sysmex XN-1000 in body fluid open mode. Two levels (low and high) of body fluid XN-check were measured before sample analysis.

5. Statistical method

SPSS17.0 statistical software and linear regression analysis were used to compare the results of the 2 methods, whereas Mann–Whitney *U* test was used for comparison between the groups. ROC curve was used to analyze the cut-off value, AUC, sensitivity, and specificity of HFC% and HFC# in malignant effusion screening, and the difference of $P < .05$ was considered statistically significant.

6. Results

The linear regression analysis of the cell count and nucleated cell classification by manual method and instrumental method were red blood cell count $y = 0.88x + 426.4$, $R^2 = 0.99$, $P < .001$; nucleated cell count $y = 0.85x + 33.4$, $R^2 = 0.98$, $P < .001$; nucleated cell classification PMN% $y = 0.91x + 4.2$, $R^2 = 0.90$, $P < .001$; and MN% $y = 0.91x + 5.1$, $R^2 = 0.90$, $P < .001$, respectively. The correlation scatter plots of each parameter in 2 methods are shown in Fig. 1.

ROC curve was used to analyze the ability of HFC% and HFC# in malignant cell screening. When the cut-off values of HFC% and HFC# were 4.4% and 24.5/μL, AUC, sensitivity, specificity, and 95% confidence interval were 0.707, 0.792,

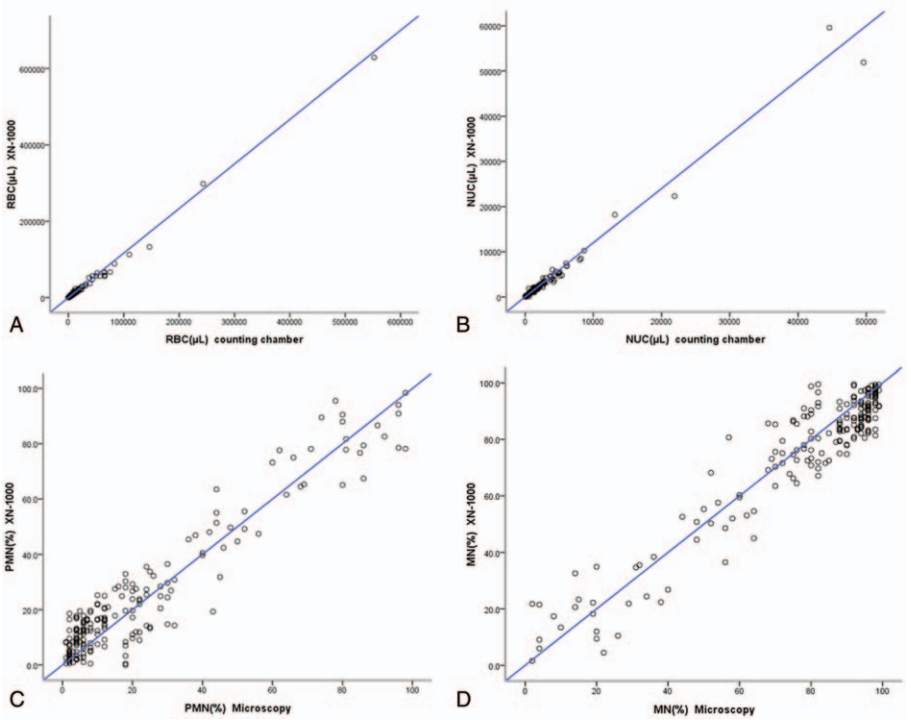

Figure 1. Correlation of cell counts from body fluids between automated and manual counting methods. (A) RBC count; (B) NUC count; (C) PMN cell count; (D) MN cell count. MN = mononuclear cells, NUC = nucleated cell count, PMN = polymorphonuclear cell, RBC = red blood cell.

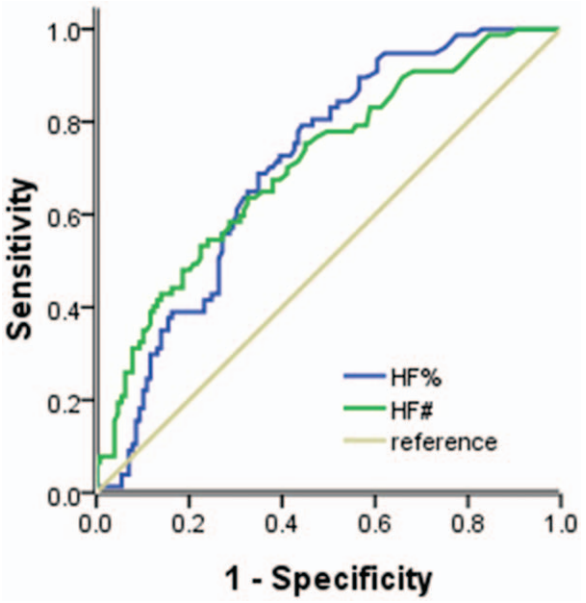

Figure 2. HFC% and HFC# receiver operating characteristic curves for predicting fluids containing malignant cells. HFC = high-fluorescent cells.

0.558, 0.637–0.777 and 0.708, 0.753, 0.550, 0.635–0.780, respectively (Fig. 2).

Cell count, nucleated cell classification, and high fluorescent cell count in malignant effusion group and nonmalignant effusion group were compared using XN-1000 hematology analyzer body fluid mode (Table 1). There were no significant differences in RBC, NUC, PMN%, or MN% between the 2 groups. However, HFC# and HFC% were significantly different between the 2 groups ($P < .001$; Fig. 3).

7. Discussion

It is well known that cell count and nucleated cell classification of serous cavity effusion are effective in identifying the damage or infection to the organs and provide useful laboratory evidence for the diagnosis and treatment of diseases.[8,9] The traditional method of manual cell counting by *Neubauer* hemocytometer is time-consuming and is usually associated with poor reproducibility. In order to develop automated testing, blood mode of hematology analyzer is used in the analysis of body fluid cells. However, blood mode detected body fluid and blood components with the same stroma, which could not overcome the matrix effect caused by the different components of the blood and body fluid. Also, the interference from mesothelial cells, macrophages, and tumor cells may contribute to the error of cell classification results.[10–12] Reports on the cell count of body fluid by urine flow cytometry analyzer are also available.[13] This method has the advantage of rapid and accurate cell count, but it cannot classify nucleated cells. In addition, Walker et al[14] detected body fluid cells using iQ200 urine sediment analyzer. The study showed good correlation with manual cell counts, but could not compare the 2 methods for efficacy in nucleated cell classification. In addition, the requirement for sample volume of this method is relatively high.

These findings showed that XN-1000 hematology analyzer body fluid mode could be used for the cell count of serous cavity effusion. Also, the method exhibits a good correlation with manual method. The correlation coefficients of RBC and NUC were 0.99 and 0.98, respectively, which were consistent with the reported literature.[5,7] XN-1000 hematology analyzer body fluid

Table 1

Comparison of cell counts and classification results by XN-1000 hematology analyzer between the 2 groups.

Project P^{50} (P^{25}–P^{75})	Malignant effusion group (n = 77)	Nonmalignant effusion group (n = 129)	*P*
NUC, μL	763.0 (274.0–1790.5)	504.0 (233.0–2123.0)	.786
RBC, μL	3900.0 (976.3–15,500.0)	5000.0 (807.5–13,850.0)	.764
HFC, %	13.0 (5.1–27.1)	3.3 (0.7–15.0)	.000
HFC, μL	63.0 (24.0–188.0)	21 (7.0–54.5)	.000
MN, %	84.1 (74.6–90.4)	84.3 (62.0–92.4)	.796
PMN, %	15.9 (9.6–25.4)	15.5 (7.5–36.5)	.850

HFC = high-fluorescent cell count, MN = mononuclear cell, NUC = nucleated cell count, P^{25}–P^{75} = 25th and 75th percentiles, P^{50} = median, PMN = polymorphonuclear cell, RBC = red blood cell.

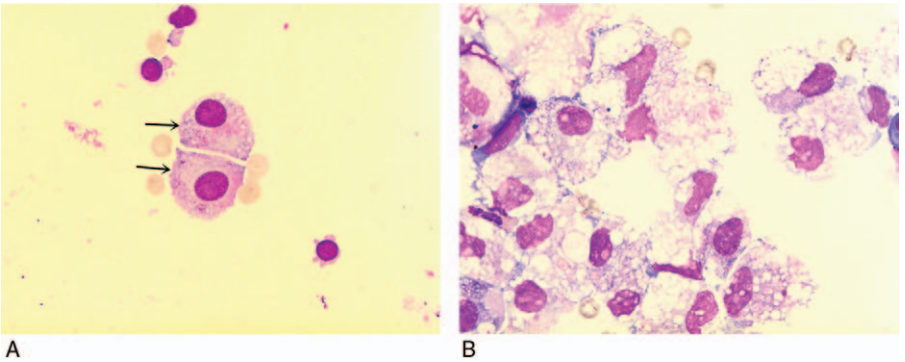

Figure 3. High proportion of macrophages and/or mesothelial cells results in the high values of HFC% and HFC#, causing false positive. (A) The arrow signified is mesothelial cells; (B) a lot of macrophages. Magnification A and B (×1000). HFC = high-fluorescent cells.

mode is capable of classifying and counting nucleated cells, including neutrophils, lymphocytes, monocytes, eosinophils, and HFC. The neutrophils, eosinophils, and basophils were classified as polymorphocytes (PMN), and lymphocytes, macrophages, mesothelial cells, and malignant cells were classified as monocytes (MN). HFC was able to bind more nucleic acid fluorescent dyes, thereby resulting in a high-fluorescent reading of the cells, including tumor cells, mesothelial cells, and macrophages.[15] The cell classification by instrumental method and manual staining microscopy method had a high correlation, PMN% was similar to MN%, both were 0.90, respectively. This finding was consistent with the report of Cho et al,[7] but slightly weaker than what was reported by Fleming et al. The main reason for this was that the testing specimens had a high proportion of peritoneal dialysis fluid, and the test was not focused on malignant effusions, which resulted in a great improvement in the correctness of the instrument. The cut-off values of HFC% and HFC# were 4.4% and 24.5/μL, respectively, whereas the values for AUC and sensitivity and specificity were 0.707, 0.792, 0.558 and 0.708, 0.753, 0.550, respectively, which were slightly lower than what was reported in the literature.[6,7] During chronic inflammation, the number of mesothelial cells and macrophages were significantly increased in the effusion, and their nuclei contained more nucleic acids, which can be combined with more nucleic acid fluorescent dyes, and thus can be classified as high-fluorescent nucleated cells. In this experiment, we observed that liver cirrhosis ascites specimens contained many high-fluorescence nucleated cells, which were mainly classified as mesothelial cells and/or macrophages by microscopic examination Fig. 3. In addition, false negative results may appear in the specimens, with little tumor cells.

In conclusion, XN-1000 hematology analyzer fluid mode is capable of rapid cell count and nucleated cell classification rapidly and accurately and can be used as a rapid screening tool for laboratory analysis of humoral cells. However, the composition of body fluid samples is complex. Therefore, when the scatter plot is abnormal, HFC exceeds the threshold value, or there are clinical suspicions, the nucleated cell classification of specimens should be confirmed to improve the quality of the analysis. The clinical operations should develop internal quality control for automated body fluid analysis and establish the corresponding standard operating procedures, quality control procedures, and inspection procedures to guide daily works.[16,17]

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