Clin. Lab. 2026;72:XXX-XXX
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ORIGINAL ARTICLE

Real-World Evaluation of the Sysmex XN-350 Body Fluid Mode for Automated Cell Analysis in Body Fluids

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ABSTRACT

Background: **Automated hematology analyzers with dedicated body fluid (BF) modes are increasingly used in routine BF analysis, but large real-world assessments across diverse specimen types remain limited. Therefore, this retrospective study of 1,777 consecutively processed BF specimens aims to investigate the agreement between leukocyte differentials generated by the Sysmex XN-350 BF mode and cytospin-based manual counts. Additionally, the study examined differences in high-fluorescent body fluid cell counts per 100 white blood cells (HF-BF/100 WBC) and body fluid carcinoembryonic antigen (CEA) concentrations between malignant and non-malignant specimens.**

Methods: **This retrospective analysis included 1,777 routine BF specimens (pleural, synovial, cerebrospinal, ascitic, and pericardial) submitted for automated BF cell counts and leukocyte differentials between January 2023 and December 2024. For each specimen, white blood cell (WBC) counts, two-part leukocyte differentials comprising polymorphonuclear (PMN) and mononuclear (MN) cells, and HF-BF/100 WBC values were obtained using the Sysmex XN-350 BF mode. Cytospin-based manual leukocyte differentials, cytopathology reports, and BF CEA concentrations measured on the Cobas e801 analyzer were also collected for comparison.**

Results: **Among the 1,777 BF specimens analyzed, PMN counts generated by the Sysmex XN-350 demonstrated excellent concordance with cytospin-based manual differentials ($R^2 = 0.991$), while MN counts showed moderate correlation ($R^2 = 0.437$). After excluding 107 specimens with unreliable manual differentials owing to cellular degeneration, correlations improved for both PMN ($R^2 = 0.997$) and MN ($R^2 = 0.829$) across all fluid types. HF-BF/100 WBC values were significantly higher in malignant than in non-malignant specimens. In the 602 specimens with both HF-BF/100 WBC and BF CEA results available, CEA exhibited stronger discriminative ability for malignancy than HF-BF/100 WBC (AUC 0.850 vs. 0.753), although both markers showed significant diagnostic value.**

Conclusions: **Automated BF analysis revealed strong agreement with cytospin-based manual leukocyte differentials, with discrepancies largely restricted to specimens with marked cellular degeneration, where manual differential counting was limited or not feasible. The HF-BF/100 WBC parameter provided supplementary screening value for malignancy when interpreted alongside BF CEA and cytology.**

(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.251217)

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Manuscript accepted December 19, 2025

KEYWORDS

body fluids, Sysmex XN, HF-BF, automated cell count, CEA

INTRODUCTION

Cellular analysis of body fluids (BF) such as pleural, pericardial, peritoneal, cerebrospinal, and synovial spec-

imens is routinely performed to support the differential diagnosis of infectious, inflammatory, and malignant conditions [1,2]. Accurate and timely assessment of these cellular components is crucial for effective clinical management [3]. Traditionally, manual microscopic techniques using counting chambers and cytospin preparations remain the reference standard for leukocyte differentials and abnormal cell detection. However, these procedures are labor-intensive, time-consuming, and prone to imprecision, high inter-observer variability, and limited reproducibility, particularly in high-throughput laboratory settings [4,5].

Automated hematology analyzers with dedicated BF modes are increasingly used in clinical laboratories to streamline serous effusion analysis [4]. The Sysmex XN series provides total cell counts, white blood cell (WBC) counts, and a two-part differential, and additionally reports high-fluorescence body fluid (HF-BF) cells, representing large nucleated cells with high nucleic acid content such as mesothelial cells, macrophages, and tumor cells [6]. To date, studies show strong concordance between automated BF analyzers and conventional manual methods for total cell counts and leukocyte differentials, with HF-BF consistently serving as a reliable screening parameter for detecting malignant cells [7-11].

Tumor markers in BFs, particularly carcinoembryonic antigen (CEA), have been explored as adjunctive indicators for distinguishing malignant from benign effusions [12]. More recently, a study proposed a diagnostic approach combining high-fluorescence cells quantified by an automated hematology analyzer with pleural fluid and serum tumor markers (CEA, CA19-9, and CA15-3) to enhance the identification of malignant pleural effusion, highlighting the complementary value of HF parameters and tumor markers [13]. However, studies were limited by short study periods or selected pleural effusion cohorts, resulting in scarce real-world data on the performance of automated BF modes in routine clinical practice.

In this study, 1,777 consecutive BF specimens processed on the Sysmex XN-350 analyzer over several years at a single tertiary-care center were retrospectively analyzed, using data generated during routine clinical testing. This study aims to investigate, under real-world conditions, the agreement between leukocyte differentials obtained using the XN-350 BF mode and cytospin-based manual counts. Additionally, the study examined differences in HF-BF per 100 WBC (HF-BF/100 WBC) and BF CEA concentrations between malignant and non-malignant specimens as supplementary indicators of malignancy.

MATERIALS AND METHODS

Between January 2023 and December 2024, routine test data were retrospectively collected for 1,777 BF specimens for which automated cell counts and differential counts were requested. For each specimen, BF WBC counts and differential data generated by the Sysmex XN-350 (Sysmex Corporation, Kobe, Japan) were retrieved along with the corresponding cytospin microscopic differential findings, cytopathology reports, and, where available, BF CEA measurements. The specimens included 664 pleural, 487 synovial, 364 cerebrospinal, 220 ascitic, and 42 pericardial fluid samples (Table 1).

Automated analysis using the Sysmex XN-350

The XN series analyzers determine total WBC counts and leukocyte differentials by integrating measurements of cell size (forward-scattered light), internal complexity (side-scattered light), and nucleic acid content (fluorescence). In the BF mode of the Sysmex XN-350, the instrument reports total cell counts (TC-BF), HF-BF, WBC counts (WBC-BF), a two-part differential - polymorphonuclear cells (PMN-BF) and mononuclear cells (MN-BF), and red blood cell counts (RBC-BF) [14].

Cytospin-based manual analysis

For manual WBC differentials, cytospin preparations of each specimen were stained with Wright-Giemsa. A minimum of 100 leukocytes was counted under 400 x magnification. Leukocytes were classified as neutrophils, lymphocytes, monocytes/macrophages, eosinophils, or basophils. Lining cells (e.g., mesothelial cells) and malignant cells were excluded from the 100-cell leukocyte differential count and recorded separately when present. To calculate PMN and MN counts for comparison with the automated analyzer, a separate manual total WBC count was not performed; instead, we used the WBC count from the automated analyzer in conjunction with the manual differential percentages. In the manual differential, neutrophils, eosinophils, and basophils were grouped as PMNs, while lymphocytes and monocytes/macrophages were grouped as MNs.

BF specimens were classified as malignant if malignant cells were identified on cytospin preparations and/or if the corresponding cytopathology report confirmed malignancy. Specimens lacking cytologic evidence of malignancy were classified as non-malignant.

Carcinoembryonic antigen analysis

CEA concentrations in BFs were measured using an electrochemiluminescence immunoassay on the Cobas e801 analyzer (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

The agreement between PMN and MN counts obtained by the Sysmex XN-350 analyzer and cytospin-based manual counts was evaluated using Pearson's correlation and simple linear regression analysis. Scatter plots with fitted regression lines and coefficients of determination (R^2) were generated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Differences in HF-BF/100 WBC between malignant and non-malig-

nant specimens were assessed using an unpaired *t*-test with Welch's correction owing to unequal group variances. To evaluate the discriminatory performance of BF CEA and HF-BF/100 WBC for malignancy, receiver operating characteristic (ROC) curves were constructed with malignancy status as the binary outcome. Analysis was restricted to BF specimens in which both markers were measured. The area under the ROC curve (AUC) and 95% confidence intervals (CIs) were calculated non-parametrically, testing the null hypothesis of AUC = 0.5. Group comparisons and ROC analyses were performed using GraphPad Prism version 10.6.1 (Graph Pad Software, San Diego, CA, USA). Two-sided p-values < 0.05 were considered statistically significant.

RESULTS

Characteristics of the studied specimens

Overall, 1,777 BF specimens were analyzed, including 664 pleural, 487 synovial, 364 cerebrospinal, 220 ascitic, and 42 pericardial fluid samples (Table 1). The overall mean WBC count was 12,245 ± 56,856 cells/µL (range, 0 - 839,221 cells/µL). Synovial fluid exhibited the highest mean WBC and PMN counts (27,129 ± 70,168 and 22,500 ± 54,266 cells/µL, respectively), while cerebrospinal fluid had the lowest WBC counts.

Correlation between automated and manual differentials

Figure 1 presents the correlation between PMN and MN counts obtained by the Sysmex XN-350 and manual cytospin differential count. Across all 1,777 specimens, PMN counts demonstrated a strong linear relationship between both methods ($y = 1.0214 x - 152.3$, $R^2 = 0.9914$), whereas MN counts exhibited a weaker correlation ($y = 0.7065 x + 377.98$, $R^2 = 0.4372$). After excluding 107 specimens with unreliable manual differentials owing to cellular degeneration, regression analysis of the remaining 1,670 specimens yielded $y = 0.9926 x + 25.138$ ($R^2 = 0.9971$) for PMNs and $y = 0.9161 x + 121.05$ ($R^2 = 0.8293$) for MNs (Figure 1B).

Table 2 shows the correlation analyses by BF type. Among specimens without cellular degeneration (n = 1,670), PMN counts showed correlation coefficients (r) ranging from 0.9797 to 0.9994 with R^2 values from 0.9598 to 0.9989. MN counts exhibited r values of 0.8469 - 0.9907 and R^2 values of 0.7173 - 0.9814, with regression slopes approximating 1.0 across all BF types (Table 2).

Distribution of high-fluorescence body fluid per 100 white blood cells in malignant and non-malignant groups

HF-BF/100 WBC was analyzed in 1,777 BF specimens, including 1,610 non-malignant and 167 malignant samples (Table 3). The mean HF-BF/100 WBC was 2.82 ± 16.31 (range, 0 - 583.3) in the non-malignant group and 24.29 ± 139.64 (range, 0 - 1,735.5) in the malignant group. In pleural fluid, the mean HF-BF/100 WBC was 4.94 ± 25.96 (range, 0 - 583.3) for 546 non-malignant specimens and 29.06 ± 164.94 (range, 0 - 1,735.5) for 118 malignant specimens. In ascitic fluid, corresponding values were 3.46 ± 7.87 (range, 0 - 54.3) for 191 non-malignant specimens and 12.3 ± 23.92 (range, 0.5 - 112.4) for 29 malignant specimens. HF-BF/100 WBC values were generally low in cerebrospinal and pericardial fluids for both malignant and non-malignant samples, and no malignant synovial fluid specimens were identified in this cohort.

Overall, mean HF-BF/100 WBC was significantly higher in malignant than in non-malignant specimens (Welch's *t*-test, $p < 0.05$; Figure 2).

Receiver operating characteristic analysis of carcinoembryonic antigen and high-fluorescence body fluid per 100 white blood cells

ROC analysis was performed on 602 BF specimens with both CEA and HF-BF/100 WBC measurements, including 564 pleural, 34 pericardial, and 4 ascitic fluid samples. Of these, 120 were malignant and 482 non-malignant. Fluid CEA showed strong discriminative ability for distinguishing malignant from non-malignant effusions, with an AUC of 0.850 (95% CI: 0.806 - 0.893; $p < 0.001$). HF-BF/100 WBC also demonstrated significant discriminatory ability, with an AUC of 0.753 (95% CI: 0.712 - 0.795; $p < 0.001$).

DISCUSSION

With the increasing adoption of automated hematology analyzers for BF analysis, many laboratories have implemented these systems to streamline routine testing [15-17]. Instruments such as the Sysmex XN series provide total nucleated cell counts and a two-part differential (PMNs vs. MNs), and studies on pleural effusions report excellent concordance between automated and manual methods for total nucleated cells and leukocyte differentials [16]. Most published studies focus on exudative pleural effusions with a limited range of etiologies, and data on the performance and limitations of automated BF differentials across diverse specimen types and broader clinical settings remain scarce [6,10,15-17]. This study represents one of the largest real-world evaluations of an automated BF mode across multiple BF types, including specimens with advanced cellular degeneration often excluded from analytical studies.

The dataset reflected real-world clinical conditions and included cases with cellular degeneration that precluded reliable differential counting. In 6% of specimens (107/1,777), leukocyte differentials remained uninterpretable even after cytospin preparation and staining, indicating that laboratories relying solely on wet preparations must accept some imprecision. Where stained slide review is impractical or significantly delayed, automated analyzers provide a clear practical advantage. Correlation coefficients for MN counts were consistently lower than

Table 1. WBC, PMN, and MN cell counts in BF specimens.

BF type	Number of specimens (n)	WBC count (cells/μL)		PMN count (cells/μL)	MN count (cells/μL)
		Mean ± SD	Range	Mean ± SD	Mean ± SD
Pleural fluid	664	9,764 ± 66,497	4 - 796,164	7,213 ± 53,508	2,550 ± 16,966
Synovial fluid	487	27,129 ± 70,168	10 - 839,221	22,500 ± 54,266	4,585 ± 22,559
Cerebrospinal fluid	364	360 ± 5,702	0 - 108,568	258 ± 4,303	102 ± 1,407
Ascitic fluid	220	8,374 ± 32,670	1 - 355,276	7,107 ± 28,060	1,267 ± 4,961
Pericardial fluid	42	2,173 ± 2,355	18 - 12,613	861 ± 1,114	1,312 ± 2,014
Total	1,777	12,245 ± 56,856	0 - 839,221	9,824 ± 45,208	2,420 ± 15,901

WBC: white blood cells, PMN: polymorphonuclear cells, MN: mononuclear cells, BF: body fluid.

Table 2. Correlation between Sysmex XN and cytospin-based manual differentials for PMN and MN counts by BF type.

BF type	* Number of specimens (n)	PMN			MN		
		r	R^2	Regression slope	r	R^2	Regression slope
Pleural fluid	631	0.9976	0.9953	1.0974	0.9122	0.8321	0.6996
Synovial fluid	448	0.9981	0.9962	0.9857	0.8469	0.7173	0.9355
Cerebrospinal fluid	361	0.9984	0.9968	1.0145	0.9907	0.9814	0.9857
Ascitic fluid	188	0.9994	0.9989	1.0045	0.9850	0.9703	0.9275
Pericardial fluid	42	0.9797	0.9598	0.9579	0.9804	0.9611	1.0317
Total	1,670	0.9985	0.9971	0.9926	0.9107	0.8293	0.9161

*** Only specimens without cellular degeneration were included.**
PMN: polymorphonuclear cells, MN: mononuclear cells, r: correlation coefficient, R^2: coefficients of determination.

Table 3. Distribution of HF-BF/100 WBC in malignant and non-malignant specimens.

BF type	Non-malignant			Malignant		
	n	Mean ± SD	Range	n	Mean ± SD	Range
Pleural fluid	546	4.94 ± 25.96	0 - 583.3	118	29.06 ± 164.94	0 - 1,735.5
Synovial fluid	487	1.46 ± 3.54	0 - 34.5	0	NA	NA
Cerebrospinal fluid	358	0.46 ± 2.87	0 - 33.3	6	0.72 ± 1.76	0 - 4.3
Ascitic fluid	191	3.46 ± 7.87	0 - 54.3	29	12.3 ± 23.92	0.5 - 112.4
Pericardial fluid	28	10.93 ± 34.82	0 - 178.7	14	18.96 ± 45.59	0.4 - 175.6
Total	1,610	2.82 ± 16.31	0 - 583.3	167	24.29 ± 139.64	0 - 1735.5

HF-BF: high-fluorescent body fluid cells, HF-BF/100 WBC: high-fluorescent body fluid per 100 white blood cells, NA: not available, BF: body fluid.

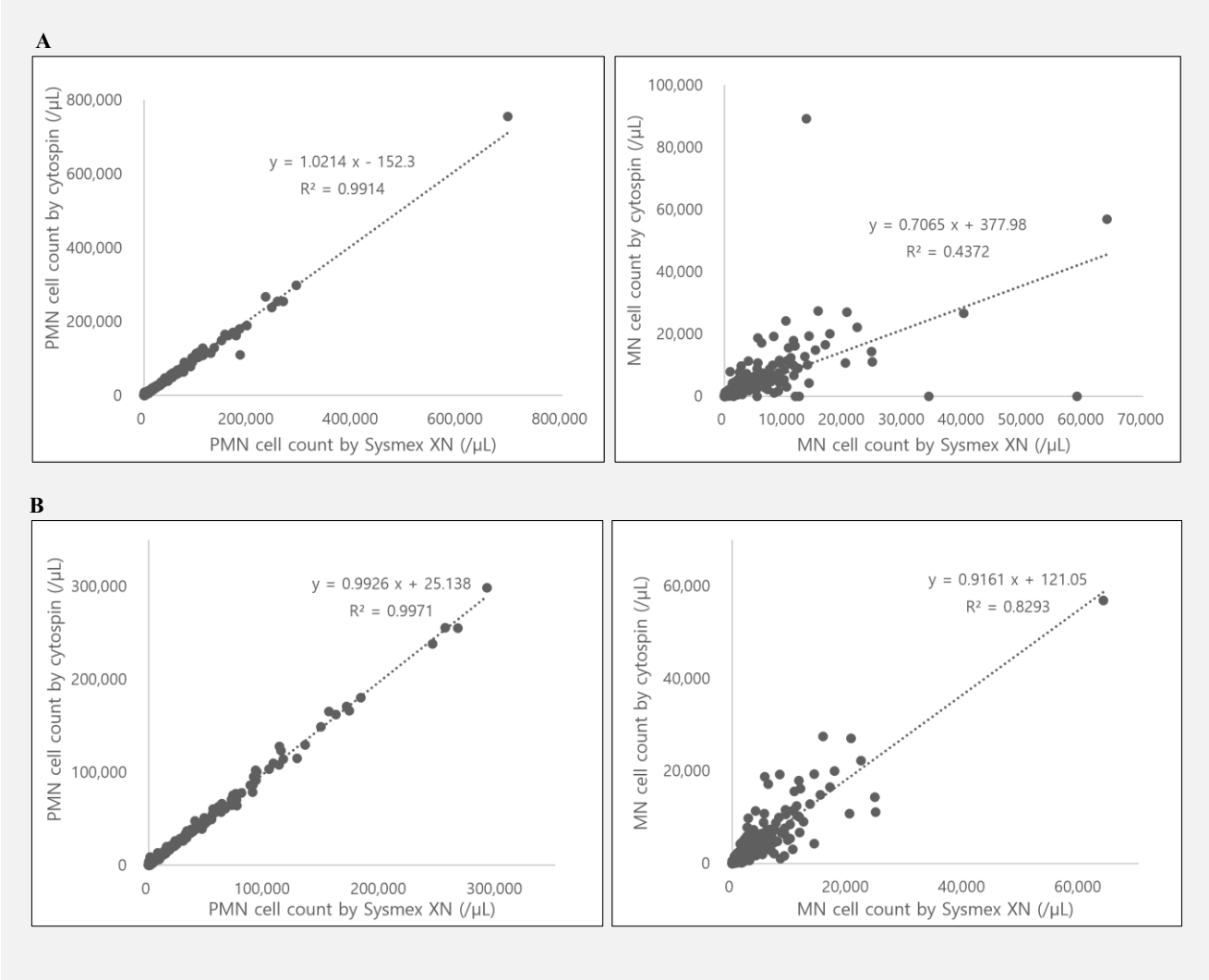


Figure 1. Correlation between Sysmex XN and cytospin-based manual differential counts for PMN and MN cells.

A: Scatter plots illustrating the correlation between PMN and MN cell counts obtained using the Sysmex XN and cytospin-based manual differentials across 1,777 BF specimens. Dotted lines denote linear regression fits with corresponding regression equations and R^2.
B: Correlation analysis after excluding 107 specimens with unreliable manual differentials owing to cellular degeneration (n = 1,670).
PMN: polymorphonuclear cell, MN: mononuclear cell, BF: body fluid, R^2: coefficients of determination.

those for PMN counts (R^2 = 0.829 vs. 0.997, Figure 1). Table 2 presents correlation analyses by BF type. Among specimens without overt cellular degeneration (n = 1,670), PMN counts demonstrated very strong correlations across all fluid types ($r \geq 0.98$, $R^2 \geq 0.96$), while MN correlations were slightly lower but remained high (r 0.85 - 0.99, R^2 0.72 - 0.98). This difference potentially reflects the intrinsic characteristics of BFs, where transitional forms between monocytes and macrophages are common. In cytospin-based manual differentials, monocytes and macrophages are typically combined into a single mono-macrophage category. In contrast, the XN-350 excludes macrophages from the WBC differential, classifying them as HF-BF, which possibly contributes to the weaker correlation observed for MN counts compared to PMN counts.

Beyond the leukocyte differential, the HF-BF parameter warrants consideration as a potential marker for malignant effusions. HF-BF represents large, highly fluorescent nucleated cells, including mesothelial cells, macrophages, and neoplastic cells. Studies in pleural and peritoneal effusions report that increased HF-BF counts or percentages are associated with malignancy and may serve as a useful screening tool [5,7-9,11]. Concurrently, tumor markers such as CEA in BFs have long been used alongside cytology for the diagnosis and monitoring of malignant effusions [12].

In this cohort, HF-BF/100 WBC values were significantly higher in malignant than in non-malignant specimens (Figure 2), supporting a relationship between in-

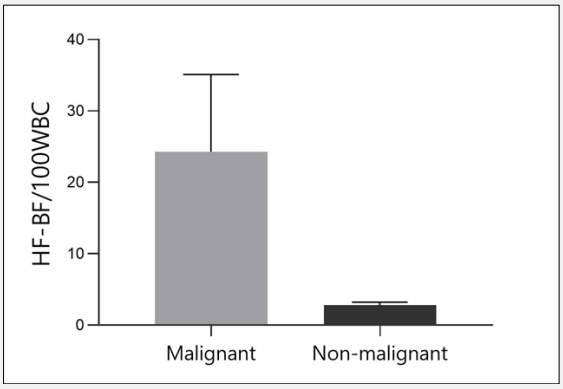


Figure 2. Comparison of HF-BF/100 WBC between malignant and non-malignant groups.

Bars indicate mean ± standard error of the mean (malignant, n = 167; non-malignant, n = 1,610). HF-BF/100 WBC values were significantly higher in the malignant specimens than in non-malignant specimens (Welch's *t*-test, p < 0.05).
HF-BF: high-fluorescent body fluid cells, HF-BF/100 WBC: high-fluorescent body fluid per 100 white blood cells.

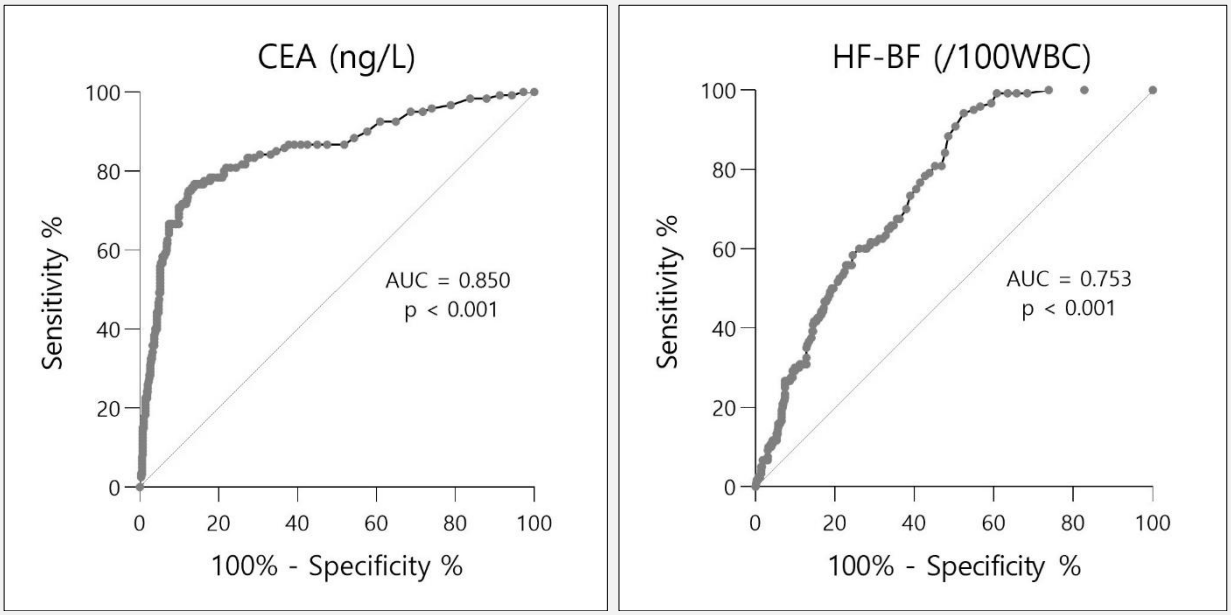


Figure 3. ROC curves of fluid CEA and HF-BF/100 WBC for distinguishing malignant from non-malignant specimens.

Analyses included only BFs specimens with both markers measured (n = 602). The AUC was 0.850 (95% CI: 0.806 - 0.893; p < 0.001) for CEA and 0.753 (95% CI: 0.712 - 0.795; p < 0.001) for HF-BF/100 WBC. The dashed line represents the line of no discrimination (AUC = 0.5).
CEA: carcinoembryonic antigen, HF-BF: high-fluorescent body fluid cells, WBC: white blood cells, ROC: receiver operating characteristic, BF: body fluid, AUC: area under the curve, HF-BF/100 WBC: high-fluorescent body fluid per 100 white blood cells.

creased HF-BF and the presence of malignant cells. ROC analysis revealed that HF-BF/100 WBC had lower discriminative performance than BF CEA (AUC 0.753 vs. 0.850), indicating that HF-BF/100 WBC alone is less accurate than CEA but may serve as a complementary screening marker in routine automated BF analysis.
This study has some limitations. First, manual total WBC counts using a hemocytometer were not performed alongside automated measurements. Consequently, this prevented direct comparison of automated and manual total nucleated cell and WBC counts; the analysis was therefore limited to leukocyte differentials. However, hemocytometer-based manual counting has largely been phased out in our institution and is no longer routine in many high-throughput laboratories, so these data potentially remain representative of contemporary practice in similar real-world settings where automated WBC counts are used without systematic manual confirmation. Second, differences in macrophage classification between the XN-350 automated differential and the cytospin-based manual differential potentially influenced the accuracy of correlation analyses for leukocyte subsets. Since the automated analyzer serves as the routine method in this laboratory, standardizing macrophage categorization and reporting across automated and manual differentials would help prevent confusion in clinical interpretation. Third, this single-center study was conducted in a tertiary-care hospital, and CEA measurements were available for only a subset of specimens, which may limit the generalizability of findings on HF-BF/100 WBC and CEA. Finally, HF-BF/100 WBC and BF CEA were evaluated separately, without constructing multivariable models combining HF-BF parameters with tumor markers or clinical data. A recent 2025 study shows the combined use of high-fluorescence cells and tumor markers (CEA, CA19-9, and CA15-3) in pleural fluid and serum for diagnosing malignant pleural effusion, reporting excellent performance for multivariable models, including HFc (AUC 0.91) [13]. These findings suggest that future research should develop integrated algorithms combining HF-BF, tumor markers, and clinical variables in larger, more heterogeneous BF cohorts.
In conclusion, automated BF analysis revealed strong agreement with cytospin-based manual differentials across diverse specimen types and was particularly advantageous for specimens with advanced cellular degeneration, where reliable manual counting was often not feasible. These findings also indicate that the HF-BF parameter can serve as a complementary screening marker for malignancy when interpreted alongside other diagnostic tests, such as BF tumor markers and cytology, in the routine evaluation of BF specimens.

Declaration of Generative AI in Scientific Writing:
During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to improve language clarity and readability. All suggested edits were carefully reviewed and revised by the authors, who take full responsibility for the final content.

Declaration of Interest:
The authors declare no conflict of interest.

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