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ORIGINAL ARTICLE

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Two-site evaluation of a new workflow for the detection of malignant cells on the Sysmex XN-1000 body fluid analyzer

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Abstract

Introduction: The presence of high fluorescent cells high fluorescent cells (on the body fluid analyzer) (HF-BF) on the Sysmex XN-1000 hematology analyzers has gained interest regarding the prediction of malignant cells in body fluids, but lacks sensitivity. We aimed to increase this sensitivity by combining HF-BF value, automated results, and clinical information.

Methods: We evaluated a new workflow for the management of body fluids in the hematology laboratory, including the HF-BF criterion and clinical information. In two laboratories, 1623 serous fluids were retrospectively analyzed on the XN-1000 BF mode. All samples were morphologically screened for malignant cells. Optimal HF-BF cutoffs were determined to predict their presence. Thereafter, the added value of clinical information was evaluated. Other reflex testing rules (eosinophilic count >5% and presence of the WBC Abnormal Scattergram flag) were also used to refine our workflow.

Results: Optimal HF-BF cutoffs in the two hematology centers were 108 and 45 cells/μL, yielding a sensitivity/specificity of 66.7/93.6% and 86.8/66.6% for malignant cell detection. When adding clinical information, sensitivity/specificity evolved to 100.0/68.9% and 100.0%/not determined. Of 104 samples containing malignant cells, 97 had positive clinical information; the remainder had a HF-BF > cutoff.

Conclusion: Combining clinical information and HF-BF reached 100% sensitivity for malignant cell detection in body fluid analysis. Lack of robustness of the optimal HF-BF cutoff deserves the use of local cutoffs. Rapid automated results reporting from the XN-1000 BF mode are also feasible in clinical practice. Prospective evaluation of the workflow is needed before its implementation in clinical practice.

KEYWORDS

body fluids, cell count, malignant cell detection, reflex testing rules, Sysmex XN-1000

1 | INTRODUCTION AND OBJECTIVES

Body fluid (BF) cytology analysis following paracentesis or thoracentesis is recommended for the detection of malignant cells (MC) in ascitic fluids (AF) or pleural fluids (PF).[1,2] Whereas the reported sensitivity of the technique reaches up to 96.7% in AF,[3] it is approximately 60% in PF,[2] in some cases requiring a second sample [2,4] or even tissue samples.

The BF module on the XN-1000 (Sysmex Corporation) represents a convenient platform for accurate and fast quantification of total nucleated cells by flow cytometry in multiple types of BF.[5-8] The advantages of BF analyzers are no sample preparation, reduced turnaround time (TAT), decreased biohazard exposure, low sample volume, low limits of detection (LOD) and quantification (LOQ), flagging system, and a differential module.[9-11] Multiple studies have already shown good performance of the XN-1000 for cell counting and the differential nucleated count.[5-8,12-14] However, it is currently still recommended to use manual methods in case of doubtful results or suspicion of MC.[9]

In the same way as it has been performed for peripheral blood film review,[15,16] multiple authors have shown that several parameters on BF analyzers could be used to determine which BF needs microscopic examination (ME) and which does not (ie, white blood cells (WBC) Abn. Scattergram or eosinophil count [EO]-BF > 5.0%).[12,17,18] This is of importance, mainly because it would improve the TAT for BF not needing ME. One of the parameters that can be used on XN analyzers for this purpose is the presence of high fluorescent cells (HF)-BF, which aims to be a predictor of the presence of MC. This parameter has gained interest in the literature for this purpose, but still lacks sensitivity (ranging from 73.0% to 88.0%) and specificity (ranging from 55.0% to 87.0%).[13,19,20]

In this study, we first aimed to evaluate the performances of locally determined high fluorescent cells (on the body fluid analyzer) (HF-BF) cutoffs for the detection of MC. Secondly, we aimed to assess a new workflow for the management of BF in the hematology laboratory. While our current approach consists of reviewing all BF microscopically before reporting results, the strategy we evaluated relies on the integration of clinical information (history and/or high clinical suspicion of a neoplastic disorder) obtained prior to BF collection, HF-BF value, and automated results. Such a combined approach has already been encouraging,[9,19-21] but no experimental data have yet been published.

2 | MATERIALS AND METHODS

2.1 | Patient samples

This was a retrospective two-site study. In the first center (Yvoir, Belgium), 141 consecutive BF (84 AF and 57 PF) had ME for the detection of MC. All BF were collected in K_2-ethylenediaminetetraacetic acid (EDTA) tubes (Vacuette, Greiner Bio-One, Courtaboeuf). BF obtained during opening hours (8:30 AM-5:00 PM) between January 2018 and June 2018 were included. In the second center (Brussels, Belgium), 1482 consecutive BF (649 AF and 833 PF) had ME for the detection of MC. BF obtained between January 2017 and January 2018 were included. All BF were collected in K_3-EDTA tubes (S-Monovette; Sarstedt). The collection and analysis of all BF were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines H56-A.[21] No samples were excluded for technical reasons. The study protocol was in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the CHU UCL Namur, Université catholique de Louvain (Yvoir, Belgium, approval number 122/2018) and by the Medical Ethics Committee of CUSL Saint-Luc (Brussels, Belgium, approval number 2019/06FEV/056).

2.2 | Sample flow

In both centers, all samples were first analyzed on the XN-1000 BF mode analyzer (Sysmex Corporation). The XN-BF mode provides total cell (TC-BF), WBC (WBC-BF), polymorphonuclear cells, PMN (PMN-BF), and monocellular cells, MN (MN-BF) counts. Research parameters are also provided and include neutrophil (NE-BF), eosinophil count (on the body fluid analyzer) (EO-BF), lymphocyte (LY-BF), monocyte (MO-BF), and HF-BF classification. These latter cells have a high nucleo-cytoplasmic ratio and higher nucleic acid content and have been proposed as a useful tool for the identification of MC.[19] All the analyses were performed according to the manufacturer's recommendations.

After the XN-1000 BF analysis, all samples had ME in the hematology laboratories. For ME, two cytospin slides per BF were prepared by cytocentrifugation (Cytospin 2; Shandon, Thermo Electron Corporation, Waltham, MA, USA, in center 1 and Cellspin I; Tharmac GmbH, Wiesbaden, Germany in center 2) followed by May-Grunwald-Giemsa staining (Merck) in both centers. All slides were screened (at least 100 cells) for the presence of MC with ×10 and ×50 oil immersion objective lenses by at least two skilled laboratory professionals who were blinded to the clinical information. Every sample was examined for the presence of MC. Cytological features of nonhematopoietic MC were defined as large size, multinuclearity, high nuclear to cytoplasmic ratio, variable nuclear shape and size, irregular and smooth nuclear contour, prominent nucleoli, nuclear molding, cannibalism, or presence of tridimensional clusters.[19,21,22] Samples were considered positive if at least one MC was observed. A manual differential count was thereafter performed before reporting the results. This flow is shown in Figure 1A.

Regarding the clinical aspects of this study, patients with a history or suspicion of malignancy found in clinical records (ie, no history of malignancy but suspicious as claimed by prescribing clinicians in clinical records mainly based on imaging results) prior to BF collection were considered as clinically suspicious for malignancy, while all the other patients (ie, cirrhosis, infection, cardiac or renal disorders or other etiologies) were considered as clinically negative. This categorization was established based on

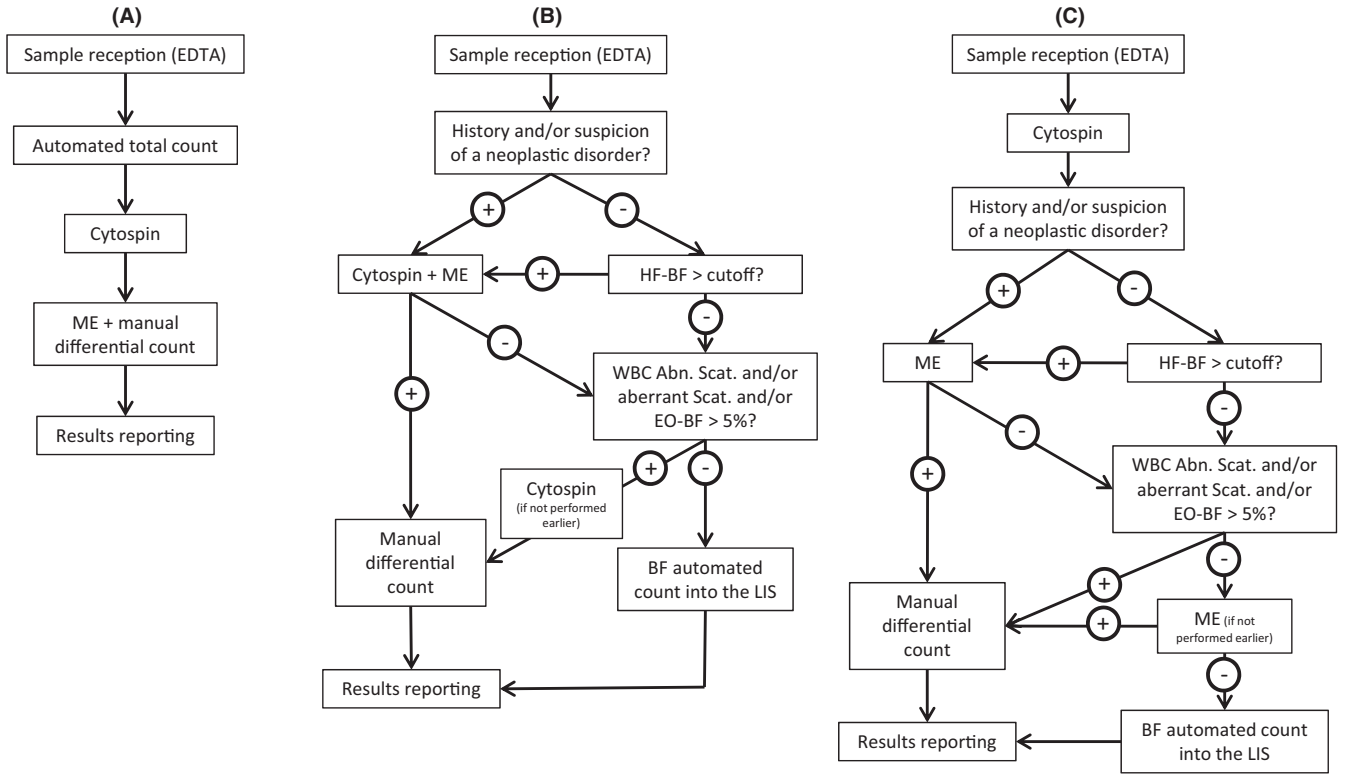

FIGURE 1 Setup of our proposed workflow for the detection of malignant cells in hematology laboratories. A, Represents the existing workflow. B, Represents the tested workflow. C, Represents the workflow that we propose to use in the absence of prospective validation of the workflow (B)

clinical record review by laboratory scientists. The clinical information obtained was always available prior to BF collection. This review was performed for all patients from the first center. For the second center, clinical record review was only performed for patients with a positive ME.

2.3 | End-points of the study

For all end-points, ME was used as the reference for the detection of MC in AF and PF combined. Separate data on AF and PF are presented in Appendix.

- The first end-point was to evaluate the HF-BF cell count as a predictor of the presence of MC.

For this evaluation, the optimal HF-BF cutoff (in absolute values or in %) value was determined for each center. Thereafter, a contingency table was established for sensitivity/specificity calculation: The number of samples containing MC with HF-BF > cutoff (true-positive samples based on HF-BF cutoff) and the number of samples containing MC with HF-BF < cutoff (false negatives based on HF-BF cutoff) were determined for each center, as were the number of samples without MC, while having HF-BF > cutoff (false positives), and the number of samples without MC and having HF-BF < cutoff (true negatives).

The logistic regression analysis between HF-BF count and MC detection is presented in Appendix S1.

- The second end-point was to evaluate the clinical information as a predictor of the presence of MC.

As proposed in several papers,[9,19-21] every BF obtained from patients with a known history of neoplastic disorder or in a patient with high clinical suspicion of a neoplastic disorder (ie, suspicious imaging studies) should always be checked by ME. Analogically to end-point one, a contingency table was established for sensitivity/specificity calculation of the clinical information alone in the first center. In the second center, only the sensitivity was calculated.

- The third end-point was to assess a new workflow for the management of BF in the hematology laboratory that integrates clinical information, HF-BF cell count, and automated parameters (TC-BF, NE-BF, LY-BF, and MO-BF + HF-BF).

The new workflow evaluated in both centers is shown in Figure 1B. Based on this workflow, a ME takes place without considering the HF-BF count in the case of a positive history and/or clinical suspicion of a neoplastic disorder. In the case of a negative history and/or clinical suspicion of a neoplastic disorder, a ME for the detection of malignant cells takes place only if the HF-BF count is superior to the optimal locally validated HF-BF cutoff, or in the case

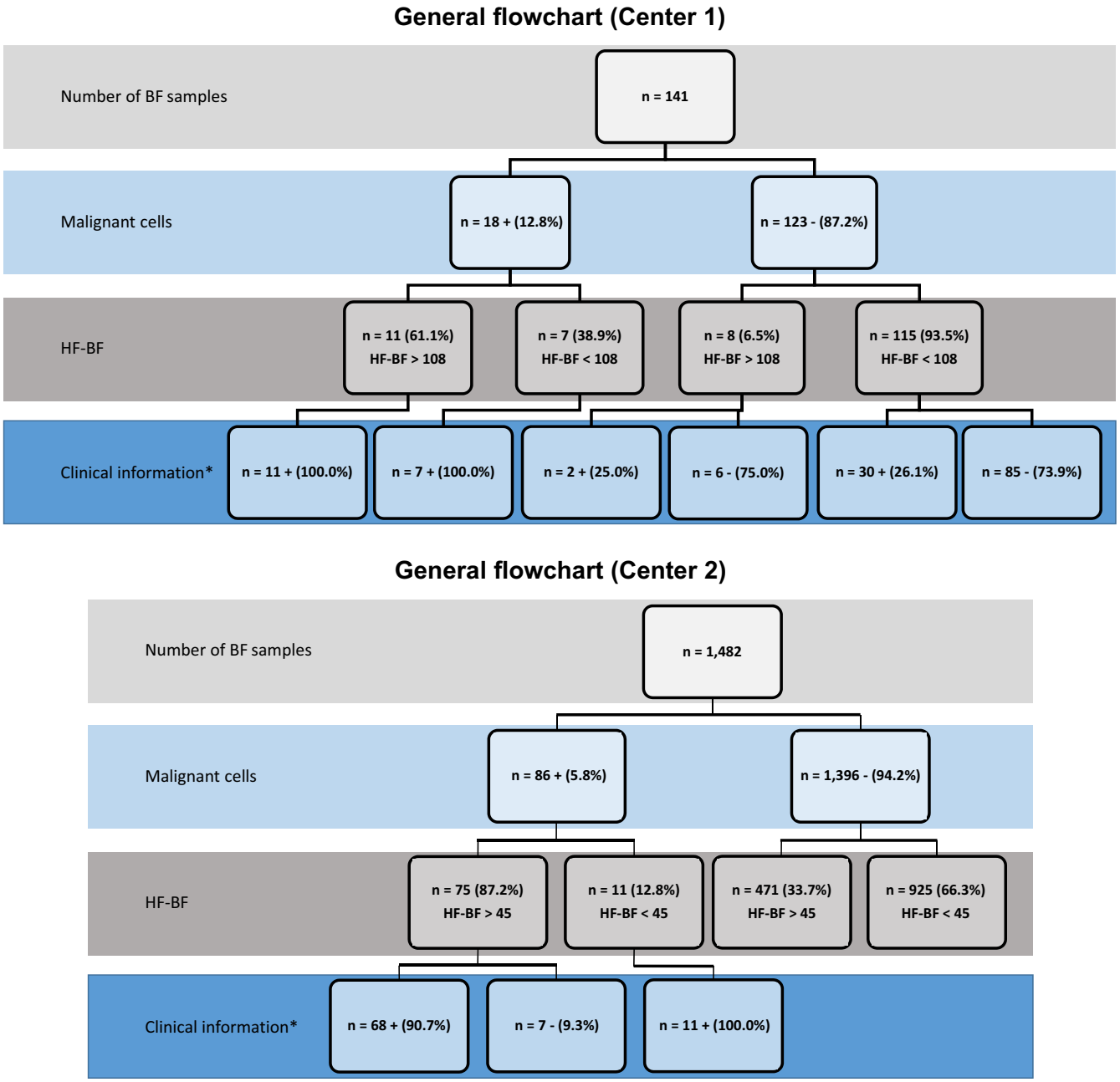

FIGURE 2 Flowchart of body fluids (BF) analyzed in both centers. *History and/or high suspicion of neoplastic disorder

of aberrant scattergrams, WBC Abn. Scattergram flag, and elevated EO-BF (ie, >5%).[12,23] The number of BF for which rapid automated results could be sent more rapidly to the clinician (ie, no history and/or high suspicion of a neoplastic disorder and HF-BF < cutoff) in the first hematology laboratory was also assessed based on this approach.

2.4 | Statistical analysis

Receiver operating characteristic (ROC) curves were calculated to evaluate the diagnostic performance (sensitivities and specificities) of HF-BF cells as predictors of the presence of MC by ME. Optimal HF-BF cutoffs were arbitrarily defined as cutoff values with the highest sum of sensitivity and specificity (Youden's index). Furthermore, contingency tables were used to evaluate sensitivities and specificities of (a) the clinical information (history and/or high suspicion of neoplastic disorder as predictor of MC and (b) of combined clinical information with the previously defined HF-BF cutoffs in the first end-point. The HF-BF count was compared in AF and PF samples containing MC or not with a Mann-Whitney test. Data analysis was performed using MEDCALC software (version 14.8.1, Ostend, Belgium). Logistic regression analysis and likelihood-ratio test were determined using GraphPad Prism 8.3.0 (California, United States). A P value <.05 was considered as statistically significant.

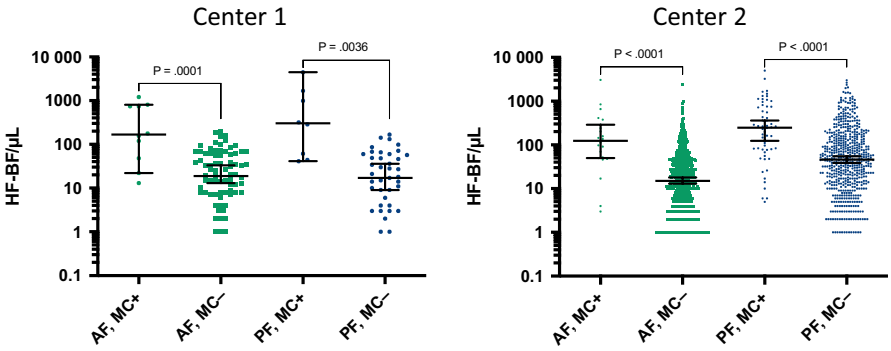

FIGURE 3 Distribution of high fluorescent cells (on the body fluid analyzer) (HF-BF) count (mean and 95% confidence interval) in ascitic fluids (AF) and pleural fluids (PF) obtained from both centers in the absence/presence of malignant cells (MC). AF and PF are represented in green and blue, respectively

3 | RESULTS

Among the 141 BF analyzed in the first center, 18 (10 AF and 8 PF; 12.8%) contained MC as observed in the hematology laboratory (mean MC count = 564.0/µL; 5%-95% percentiles: 1.0-4.876/µL) (Figure 2). While reviewing retrospectively the clinical records of every patient, we found that 41% had cirrhosis (mainly ethylic), 26.4% had history of neoplastic disorder (ie, mesothelioma, epidermoid carcinoma, metastases of the pancreas, lung adenocarcinoma, cholangiocarcinoma), 8.6% had suspicion of neoplastic disorder (ie, hypermetabolic infiltrate, suspicious nodules), 8.4% had infection (ie, bacterial peritonitis, sepsis), 8.4% had cardiac or renal disorders (ie, acute renal failure, cardiac failure, fibrillation), and 7.2% had other conditions (ie, hepatic encephalopathy, esophagitis, gastrostomy, gastrointestinal bleeding). Among the 1482 BF analyzed in the second center, 86 (27 AF and 59 PF; 5.8%) contained MC as observed in the hematology laboratory (mean MC count = 3500.0/µL; 5%-95% percentiles: 176.0-13 229.0/µL) (Figure 2).

- First end-point: HF-BF cell count as a predictor of the presence of MC.

In the first center, the mean HF-BF count in AF and PF was, respectively, 37.4 (5%-95% percentiles: 0.0-145.1) and 34.3 (5%-95% percentiles: 0.0-139.8) cells/µL in samples not containing MC, and 403.0 (5%-95% percentiles: 13.0-1211.0) and 980.6 (5%-95% percentiles: 41.0-4437.0) cells/µL in samples containing MC. In the second center, the mean HF-BF count in AF and PF was, respectively, 40.5 (5%-95% percentiles: 0.0-151.4) and 154.4 (5%-95% percentiles: 2.0-720.0) cells/µL in samples not containing MC, and 323.5 (5%-95% percentiles: 3.2-2729.0) and 536.4 (5%-95% percentiles: 6.0-1801.0) cells/µL in samples containing MC. A significant higher HF-BF count was observed in samples containing MC ($P < .005$) (Figure 3).

Because Youden's index was lower if using the HF-BF criteria in % (Appendix S2), we chose to use the HF-BF cutoff in absolute value. Our logistic regression analysis confirmed that HF-BF count is significantly related to the probability of MC detection ($P < .0001$ in both centers) and that an ideal cutoff does not exist (Appendix S1).

The optimal HF-BF cutoff found using ROC curve analysis was >108 cells/µL in the first center (Figure 4A). This cutoff yields sensitivity and specificity of 66.7% (95% confidence interval [CI]: 41.0-86.7%) and 93.6% (95% CI: 87.7-97.2%) for MC by ME (Table 1). In the second center, the optimal HF-BF cutoff found using ROC curve analysis was >45 cells/µL (Figure 4B). This cutoff yields a sensitivity and specificity of 86.8% (95% CI: 77.5%-93.2%) and 66.6% (95% CI: 64.1%-69.0%) for MC detection (Table 1).

The optimal HF-BF cutoff found using ROC curve analysis was >76 cells/µL if data from both centers are analyzed together (Figure 4C). This cutoff yields sensitivity and specificity of 72.4%

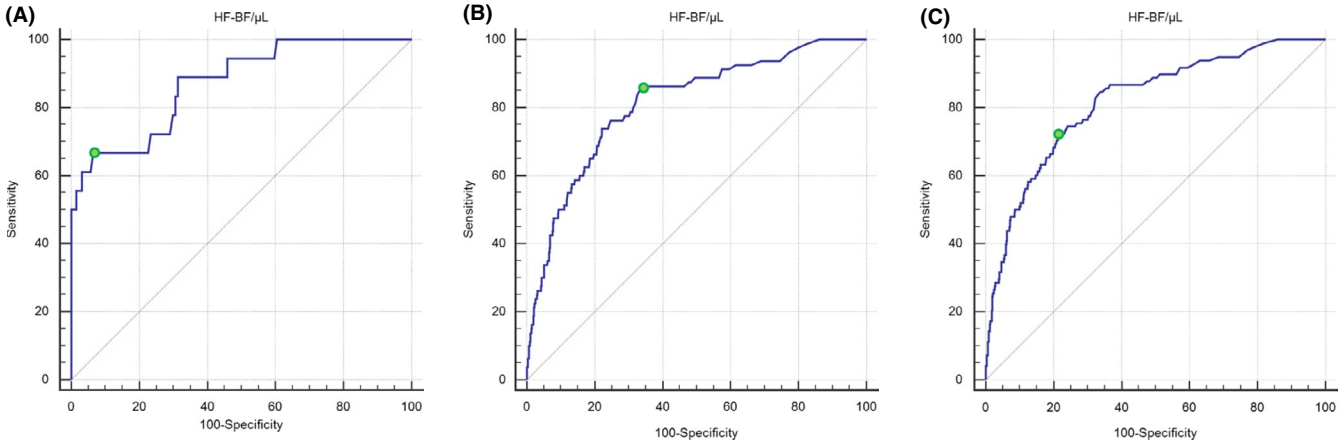

FIGURE 4 Receiver operating characteristic curve analysis of the optimal high fluorescent cells (on the body fluid analyzer) (HF-BF) cutoff to predict the presence of malignant cells (MC) in the first (A), second (B), and (C) both hematology centers

TABLE 1 Performance of the HF-BF cutoff, clinical information, and their combination for MC detection using ME as reference

	End-point 1 HF-BF cutoff only	End-point 2 Clinical information only	End-point 3 HF-BF + clinical information
Center 1 (n = 141) HF-BF > 108			
Sensitivity	66.7% (95% CI; 41.0%-86.7%)	100.0% (95% CI; 81.5%-100.0%)	100.0% (95% CI; 81.5%-100.0%)
Specificity	93.6% (95% CI; 87.7%-97.2%)	73.8% (95% CI; 65.0%-81.3%)	68.9% (95% CI; 59.8%-76.9%)
Center 2 (n = 1482) HF-BF > 45			
Sensitivity	86.8% (95% CI; 77.5%-93.2%)	91.9% (95% CI; 84.0%-96.7%)	100.0% (95% CI; 95.7%-100.0%)
Specificity	66.6% (95% CI; 64.1%-69.0%)	ND	ND

Abbreviations: CI, confidence interval; HF-BF, high fluorescent cells (on the body fluid analyzer); MC, malignant cells; ME, microscopic examination; ND, not determined.

(95% CI: 62.5%-81.0%) and 78.5% (95% CI: 76.4%-80.5%) for MC by ME.

- Second end-point: clinical information as predictor of MC.

In the first center, the use of clinical information alone yields sensitivity and specificity of 100.0% (95% CI: 81.5%-100.0%) and 73.8% (95% CI: 65.0%-81.3%) for MC by ME (Table 1). By using a strategy combining clinical information and reflex testing rules (EO-BF count >5% and WBC Abn. Scattergram flag), the results of 59.6% BF could have been reported more rapidly to the clinician, with a low probability of containing MC. Therefore, 40.4% still required a ME because of history and/or suspicion of a neoplastic disorder (n = 50), EO-BF count >5% (n = 4), and presence of the WBC Abn. Scattergram flag (n = 3).

In the second center, the clinical information alone yields a sensitivity of 91.9% (95% CI; 84.0%-96.7%) for MC detection (Table 1). This strategy would therefore have missed seven cases of samples containing MC.

- Third end-point: HF-BF cell count along with clinical information as a predictor of MC.

The HF-BF criteria, combined with a history and/or high suspicion of a neoplastic disorder, brought a sensitivity and a specificity of 100.0% (95% CI: 81.5%-100.0%) and 68.9% (95% CI: 59.8%-76.9%), respectively, for MC detection in the first center (Table 1). All samples containing MC with fewer than 108 HF-BF cells had a history of neoplastic disorder (ie, adenocarcinoma, breast cancer) (n = 7). In patients without suspicious clinical information, no MC were identified in patients with HF-BF > cutoff (Figure 2). Using the workflow presented in Figure 1B, 44.7% of BF still required ME because of a history and/or suspicion of a neoplastic disorder (n = 50), HF-BF > cutoff not associated with a history and/or suspicion of a neoplastic disorder (n = 6), EO-BF count > 5% (n = 4), and presence of the WBC Abn. Scattergram flag (n = 3). Therefore, the results of 55.3% of BF could have been reported more rapidly to the clinician, with a low probability of containing MC.

Similarly, in the second center, the HF-BF criteria combined with clinical information increased the sensitivity to 100.0% (95% CI: 95.7%-100.0%) (Table 1). All samples containing MC with fewer than 45 HF-BF cells had a history of neoplastic disorder (ie, adenocarcinoma, breast cancer) (n = 11) (Figure 3). In patients without clinical information, MC were retrieved in seven (9.3%) patients in accordance with the HF-BF criterion.

If considering AF and PF separately, the HF-BF criteria combined with clinical information also increased the sensitivity to 100.0% (Appendix S3).

4 | DISCUSSION

To the best of our knowledge, this is the first study that highlights the interest of adapting structurally BF management in the hematology laboratory by using clinical data. The combination of HF-BF cell count, together with clinical information as a predictor of MC, appeared to be the best compromise to achieve a high sensitivity in both centers. We also confirmed that HF-BF criteria alone present a suboptimal sensitivity to identify MC in AF and PF.

Good performance for TC-BF, PMN-BF, MN-BF, NE-BF, LY-BF, and MO-BF + HF-BF has been published on the XN-BF module.[5-9,12,14,17] However, the current analyzer cannot directly identify the presence of MC.[9] To overcome this drawback, the use of HF-BF cells has gained interest in the literature.[6,13,14,17,19,20] HF-BF cutoffs exist either in absolute or as percentages. In this study, we confirmed that the use of cutoffs with absolute values is a more effective predictor of MC (Appendix S2).[17,19,20] However, several studies have shown that the HF-BF criteria could not efficiently resolve this drawback. The sensitivity for MC detection of this parameter ranges from 73.0% to 88.0% in the literature, and we obtained similar results in this study, with a sensitivity ranging from 61.1% to 86.8%.[13,19,20]

Given this lack of performance, the HF-BF criteria are of low added value. Accurate staging of tumors is indeed of paramount importance to correctly assess the patient's prognosis and to choose the appropriate treatment.[24] Involvement of BF (ie, lung adenocarcinoma with malignant effusion, peritoneal carcinomatosis) is

particularly associated with poor overall survival in cancer patients,[25,26] representing a more advanced stage of disease. Therefore, the ME of every BF has been advised to avoid false-negative results.[18] However, the major drawback of ME of all BF is that it requires experienced cytologists and may lead to a prolonged TAT.

To propose a solution, we aimed to identify BF for which ME is of no added value. Because the HF-BF criteria alone did not seem sufficient for this purpose, we evaluated the added value of clinical data and established a new workflow (Figure 1B). The basis of this workflow lies in the fact that every BF obtained from patients with a known history of neoplastic disorder or in a patient with high clinical suspicion of a neoplastic disorder (ie, suspicious imaging studies) should always be checked by ME. In this context, it is however important to remember the CLSI guidelines claiming: "it is important to understand that BF differential counts are not an appropriate screening or diagnostic test for malignancy in previously undiagnosed patients".[21]

Using this workflow, we found a 100.0% sensitivity (95% CI: 81.5%-100.0% and 95% CI: 95.7%-100.0% in the first and second centers, respectively) for detecting MC based on our patient cohort (Table 1). The interest of HF-BF appeared to be useful in only seven samples (in the second hematology center, only for PF) of 104 containing MC (6.1%) as a positive history and/or high suspicion of a neoplastic disorder was observed in the other samples containing MC (n = 97; 93.9%) (Figures 2 and 3). The absence of such cases in the hematology center 1 is probably related to the low number of BF included. However, the use of clinical information alone as a trigger for ME achieved a lower sensitivity in the second hematology center (91.9%; 95% CI: 84.0%-96.7%) (Table 1). Based on the new workflow (Figure 1B), a correct automated differential leukocyte count would have been immediately available for 55.3% of the BF in the first center with a low probability of containing MC and could have led to potentially faster clinical decision-making.

Although we achieved a 100% sensitivity for MC detection with the proposed workflow, every laboratory should evaluate locally the benefits of rapid results over the potential risk of rare false-negative results and find their optimal workflow. This recommendation is due to the wide variability for the optimal HF-BF cutoff (108 and 45 HF-BF cells/µL in the first and second hematology centers, respectively) and HF-BF sensitivity (from 61.1% to 86.8%) we observed in this study, despite the use of the same instrumentation. These results are concerning, but consistent with the literature, showing various cutoffs (ie, 16, 17, 24.5, 67, or 68 HF-BF cells/µL).[12,13,19,20] Cutoffs are also different if considering AF and PF separately (Appendices S2 and S3). The inclusion of different BF numbers, with different prevalences of MC (12.8% and 5.8% in the first and second centers, respectively), with various amounts of normal mesothelial cells, reactive mesothelial cells, and MC in each BF, may, at least in part, explain such discrepancies between studies and between laboratories 1 and 2. This observation highlights the lack of robustness of the HF-BF criterion and the need to define center-specific cutoffs before implementing a HF-BF cutoff in routine clinical practice.[27]

With the exception of some situations already discussed in the literature [aberrant scattergrams, WBC Abn. Scattergram flag, and elevated EO-BF (ie, > 5%)]—where a manual differential remains advisable[9,17,18,23]—We propose to directly import the BF data in the laboratory information system (LIS) to decrease the overall TAT, namely TC-BF, NE-BF, LY-BF, and MO-BF + HF-BF, based on the recent method comparison we published.[8] However, since automated counts of BF samples containing MC are less correlated to optical microscopy,[8,14] they still need a manual differential count in case of positive ME for MC (Figure 1B,C).

Pending local prospective evaluation of our first workflow proposal (Figure 1B), we indeed recommend transmitting the automated BF data while mentioning that the BF will be subsequently revised microscopically, even if the probability of having MC in those samples would be extremely low (Figure 1C).

Our study has some limitations. Firstly, we were not able to provide the specificity of HF-BF cells combined with clinical information in the second center because the clinical files of all patients were not reviewed. Secondly, the clinical information for determining history and/or suspicion of malignancy was retrieved retrospectively based on patient record. Because the efficiency of our workflow mainly relies upon the correct transmission of patient information, well-informed and trained clinicians are of crucial importance for the implementation of such a workflow. Therefore, well-defined clinical criteria for identifying patients with a "suspicion of malignancy" will be clearly established for prospective evaluation.

In conclusion, we evaluated a workflow combining clinical information and HF-BF count that allows rapid automated results reporting from the XN-1000 BF mode for AF and PF. Prospective evaluation of this workflow is needed before doing without ME for these samples.

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CONFLICT OF INTEREST

F. Mullier reports institutional fees from Stago, Werfen, Nodia, Roche, Sysmex, and Bayer. He also reports speaker fees from Boehringer Ingelheim, Bayer Healthcare, Bristol-Myers Squibb-Pfizer, Sysmex, Stago, Werfen, and Aspen all outside the submitted work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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