Original Article

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Pathology Section

Utility of Automated Analyser-derived High Fluorescence Cell Counts for the Screening of Malignancy in Body Fluids: A Cross-sectional Study

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ABSTRACT

Introduction: Conventional microscopic Body Fluid (BF) analysis is time-consuming, requires a skilled Cytopathologist, and is prone to inter-observer variability, while automated analysers have a faster turnaround time and a higher throughput rate. The present study explores whether High Fluorescence (HF)- BF parameters can support more efficient preliminary screening.

Aim: The present study investigates the utility of automated analyser-derived HF-BF parameters in screening for the presence of malignancy.

Materials and Methods: A retrospective cross-sectional study was conducted at the Department of Pathology, Apollo Institute of Medical Sciences and Research, Hyderabad, Telangana, India, from July 2022 to July 2025. A total of 243 samples, including ascitic (AF), pleural (PF), cerebrospinal (CSF), and pericardial fluids, were collected and analysed using the Mindray BC-780(R) in the BF mode. Smear examination was done for all the samples to detect the presence of malignant cells. HF-BF relative (HF-BF%) and absolute counts (HF-BF#) were used to predict cytopathological malignancy by logistic regression. Receiver Operating Characteristic (ROC) curve analysis was used to determine the optimal cut-off values.

Results: Out of 243 samples, 14 were suspicious/positive for malignancy. Both HF-BF% (β =0.0595, p=0.006) and HF-BF# (β =1.92, p=0.001) were positive predictors for the outcome. ROC-derived AUC and cut-off values for HF-BF% are 0.698 and ≥1.95%; corresponding values for HF-BF# are 0.729 and ≥51 cells/µL. Overall accuracy for HF-BF% is 52.67%, and for HF-BF# is 88.89%. Both parameters have high Negative Predictive Values (NPV) and low Positive Predictive Values (PPV) {HF-BF%: NPV=99.14%, PPV=10.24%, sensitivity=92.86%, specificity=50.22%; HF-BF#: NPV=97.64%, PPV=29.03%, sensitivity=64.29%, specificity=90.39%}

Conclusion: HF-BF# showed numerically higher discriminative performance than HF-BF%; however, formal statistical comparison of the AUCs was not performed. Automated analysers enable rapid assessment; however, the moderate sensitivity and low PPV of HF-BF#, together with the low PPV of HF-BF% despite its high sensitivity, demonstrate that neither HF-BF parameter is suitable for use as a primary screening tool. Expert cytopathological examination continues to be the gold standard for diagnosing malignancy, with HF-BF counts serving mainly as supportive, exclusionary tools rather than primary diagnostic indicators.

Keywords: Automated cytometry, High fluorescence cell populations, Rapid screening of malignancy

INTRODUCTION

The BF analysis is a crucial tool in the diagnosis, management, and prognosis of various diseases [1]. Neutrophilic predominance in a BF sample suggests an acute inflammation, while lymphocytic excess indicates chronic inflammation [2]. Polymorphonuclear (PMN) leucocytes >250/µL in the ascitic fluid are pathognomonic of Spontaneous bacterial peritonitis [3]. The BF assessment includes cell count (Neubauer chamber), differential count based on smear examination, and cytopathological evaluation for the presence of malignant cells. The cytopathological examination of the BF is regarded as the reference standard for the diagnosis of malignancy [4].

Traditional microscopic analysis has the following limitations: a high degree of imprecision, delayed results, requirement of skilled personnel, and non availability of trained cytopathologists for 24 hours/day [5]. These issues have led to the introduction of analyser-based automated BF analysis methods for the evaluation of cell counts [6]. However, the HF cell fraction of the BFs includes macrophages, mesothelial cells, and malignant cells, and as such is not specific to malignancy [7]. Although automated analysis reduces the inter-observer variability, improves the turnaround time and precision of the results [8], diagnostic performance of the HF-BF fraction needs to be carefully evaluated against cytopathological examination for rapid screening of malignancy in the BFs [9]. Although automated BF analysis is increasingly adopted in diagnostic laboratories, published evidence evaluating HF-BF parameters on the Mindray BC-780 (R) remains limited. Furthermore, available reports typically examine single-fluid types, predominantly pleural or ascitic fluids, rather than mixed cohorts including cerebrospinal and pericardial fluids. This creates a clear gap in understanding the diagnostic utility and performance characteristics of HF-BF parameters [10] across diverse BF specimens, especially in real-world, high-volume laboratory settings. The present study aimed to evaluate the diagnostic performance of automated BF analyser derived HF-BF parameters (HF-BF%: relative cell counts and HF-BF#: absolute cell counts) for detecting cytopathologically suspicious/positive for malignancy using logistic regression, and to determine their optimal cut-off values through ROC curve analysis.

MATERIALS AND METHODS

A retrospective cross-sectional study was conducted at the Department of Pathology, Apollo Institute of Medical Sciences and Research, Hyderabad, Telangana, India, from July 2022 to July 2025, to evaluate the potential utility of HF-BF parameters. The study was approved by the Institutional Ethics Committee (IEC No: EC/NEW/INST/1527/2025/04/253, dated 30-04-2025) and conforms to the guidelines laid down under the Declaration of Helsinki.

Inclusion criteria: 1) All ascitic, pleural, CSF, and pericardial fluid samples submitted for routine diagnostic analysis to the laboratory between July 2022 and July 2025; 2) Samples obtained from unique individuals (only one BF sample per patient included); 3) Samples with sufficient volume (≥1 mL) to allow parallel processing for microscopy and analyser based measurements were included.

Exclusion criteria: 1) Duplicate or repeat samples from the same patient during the study period; 2) Mismatch between details (like patient identity and sample type) on requisition form and sample containers were included.

All consecutive samples that arrived in the laboratory and met the following selection criteria were included. The BF samples were received in plain tubes and processed within two hours of receipt in the laboratory. Each sample was aliquoted into two tubes, one for microscopy and the other for the analyser. A formal sample-size calculation was not performed because all eligible consecutive specimens received during the predefined study period were included. Given the retrospective, exploratory design, the study was intended to characterise the diagnostic performance of HF-BF parameters within the available cohort. Given the exploratory aim of characterising HF-BF parameters for screening use, analysing the full consecutive cohort was the most appropriate approach to maximise statistical power and characterise diagnostic performance within the available consecutive cohort.

Study Procedure

The BF samples were cytocentrifuged using Cyto-tek® 2500 (5 min at 500 rpm, ×30 g), sediments smeared, Giemsa stained, and examined microscopically; at least 100 cells were counted at 400× magnification, and the differential counts were determined (neutrophil% and lymphocytes%). Malignancy was assessed qualitatively as suspicious (Indian Academy of Cytopathologists-IAC [11] Category 4: mesothelial cells admixed with atypical aggregates or dispersed cells showing nuclear atypia insufficient for frank malignancy) or definitively positive (IAC Category 5: high cellularity with 3 D clusters and atypical cells showing high N/C ratio, irregular nuclear membranes, coarse chromatin, and prominent nucleoli).

The samples were run in the BF mode in the Mindray BC-780(R) automated haematology analyser. Highly fluorescent cells are identified as the HF-BF fraction in the research mode of the equipment. The HF-BF parameters are: 1) HF-BF% reported as the number of cells/100 WBCs; 2) HF- BF cell count (HF-BF#) measured as cells/μL. Additional parameters included Red Blood Cells (RBC) -BF, Total count-BF, White Blood Cell (WBC)-BF, neutrophil%, lymphocyte%, and monocyte%. The HF-BF% and HF-BF# values were compared with the microscopic findings obtained by cytopathological examination. The cytopathological examiner was blinded to the HF-BF analyser reports to avoid potential observer bias. Cytology interpretations were performed by consensus review, in which two cytopathologists jointly evaluated each case and resolved findings through agreement.

The Mindray BC-780(R) does not employ a dedicated BF calibration procedure. Instead, according to the manufacturer's recommendations, analytical performance in BF mode is ensured through a combination of automated instrument quality assurance functions and performance verification procedures. Whenever the analyser is switched from whole blood mode to BF mode, it automatically initiates a rinse program followed by a background check to eliminate residual blood cells and minimise sample carryover. In addition, an automated rinse cycle is performed after each BF sample analysis to prevent cross-contamination between consecutive blood and BF specimens. Before BF analysis, it is verified that background total WBC and RBC counts are within acceptable limits (WBC-BF ≤0.001 $\times 10^9$/L and RBC-BF ≤0.003 $\times 10^{12}$/L). In addition, routine analyser calibration was maintained using the manufacturer's whole-blood calibration procedure, as recommended in the Mindray BC-780(R) service manual, since no separate BF calibration is provided by the manufacturer.

STATISTICAL ANALYSIS

Statistical analysis was performed using Jamovi v2.7.4.0, with cytopathological status as the outcome and HF-BF% or HF-BF# as predictors. Baseline demographic and laboratory variables are summarised as median (Interquartile range-IQR) for continuous variables, and categorical variables are summarised as frequencies. For analysis, the "suspicious" and "definite" malignant categories were combined into a single class to reflect their shared screening implication (i.e., as both warrant cytopathologist review), improve sensitivity and statistical power, and stabilise statistical estimates in small subgroups. This binary grouping (suspicious/positive for malignancy vs. normal) also enables the use of interpretable binary logistic regression models. Mann-Whitney U test was used to compare baseline continuous variables between normal and suspicious/positive for malignancy groups. Binary logistic regression provided regression coefficients (β) and odds ratios (OR) to quantify associations, while ROC curve analysis assessed discriminative ability through the Area Under the Curve (AUC). Optimal cut-offs for HF-BF% and HF-BF# were determined using Youden's index, and diagnostic performance at these thresholds was evaluated by sensitivity, specificity, PPV, and NPV. For all the metrics, 95% confidence intervals were determined and reported.

RESULTS

The laboratory and demographic features of the participants from whom samples were collected are summarised in [Table/Fig-1]. Of the 243 samples, 14 (5.76%) were suspicious/positive for malignancy, while the remaining 229 were normal based on cytopathologic examination. The suspicious/positive group had a higher median age than the normal group. The categorical variables, including frequencies of gender, fluid type, and haemorrhagic status in normal and suspicious/positive for malignancy groups, are summarised in [Table/Fig-2].

Binary logistic regression and ROC analyses to assess association with suspicious/positive cytopathological status from analyser-derived HF-BF parameters in the BFs, including 95% confidence intervals, are shown in [Table/Fig-3], and the ROC curve demonstrating the discriminative performance of HF-BF% and HF-BF# for identifying samples suspicious/positive for malignancy [Table/Fig-4]. Briefly, the key findings are as follows:

S. No.	Variables	Normal median (IQR)	Suspicious/ Positive median (IQR)	p-value
1.	Age (years)	43 (26)	50 (5.5)	0.024
2	Volume (mL)	12 (31.4)	39 (23.8)	0.005
3	RBC-BF(analyser) ($\times 10^6$ cells/ μL)	0.0001 (0.005)	0.003 (0.008)	0.713
4	TC-BF(analyser) ($\times 10^3$ cells/ μL)	0.229 (0.848)	1.134 (2.87)	0.026
5	WBC-BF(analyser) ($\times 10^3$ cells/ μL)	0.221 (0.815)	0.882 (0.935)	0.069
6	HF-BF# ($\times 10^3$ cells/ μL) (analyser)	0.005 (0.013)	0.123 (0.312)	0.004
7	HF-BF % (analyser)	1.8 (5.72)	5.3 (9.65)	0.027
8	Neutrophil % (analyser)	16.9 (33.1)	24.9 (21.7)	0.571
9	Lymphocyte % (analyser)	42.2 (58.5)	51.2 (18.4)	0.648
10	Monocyte % (analyser)	13.9 (29.4)	24.3 (23.3)	0.446
11	Neutrophil % (smear-based)	20 (42)	25 (31)	0.737
12	Lymphocyte % (smear-based)	76 (52)	69 (22)	0.813
	Total sample size (N=243)	n=229	n=14 (5.76%)	

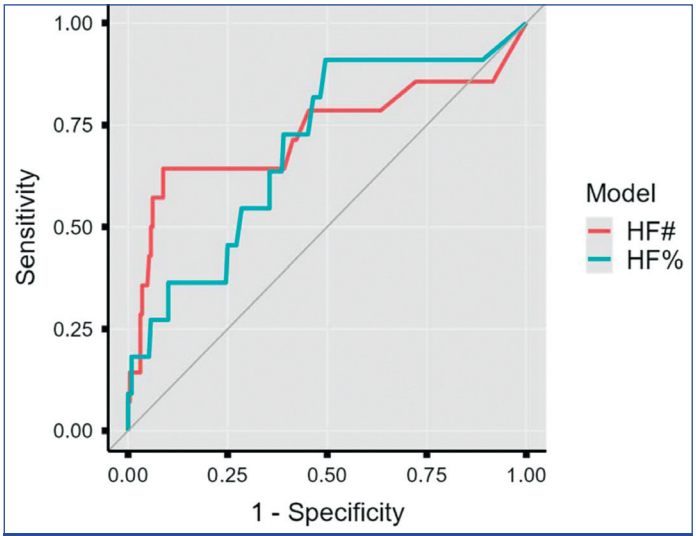
[Table/Fig-1]: Demographic and laboratory parameters-continuous variables. TC-BF: Total Count in Body Fluids (BF) (analyser); RBC-BF: Red blood cell count in BFs (analyser); WBC-BF: White blood cell count in BF (analyser); HF-BF%: Percentage of HF cells in BFs (analyser). HF-BF#: Absolute counts of High Fluorescence (HF) cells in BFs (analyser).

S. No.	Variables	Categories	Normal	Suspicious/Positive
1	Gender	Males	155	8
		Females	74	6
2	Fluid type	AF	96	10
		CSF	59	0
		PF	70	4
		Pericardial fluid	4	0
3	Fluid colour	Haemorrhagic	34	2
		Non haemorrhagic	195	12

[Table/Fig-2]: Demographic and laboratory characteristics - categorical variables.
AF: Ascitic fluid; CSF: Cerebrospinal fluid; PF: Pleural fluid. Frequencies (number of samples) of different categorical variables is shown as a function of cytopathological status.

Model estimates	HF-BF%	HF-BF#
β (regression coefficient)	0.0595	1.92
p-value	0.006	0.001
Odds ratio (95% CI)	1.0613 (1.02-1.11)	6.7963 (2.15-21.5)
ROC AUC	0.698 (0.531-0.865)	0.729 (0.542-0.916)
Optimal cut-off (Based on Youden's index)	≥1.95%	≥51 cells/µL
Sensitivity	92.86% (66.13-99.82%)	64.29% (35.14-87.24%)
Specificity	50.22% (43.56-56.87%)	90.39% (85.82-93.88%)
PPV	10.24% (8.58-12.17%)	29.03% (18.99-41.66%)
NPV	99.14% (94.54-99.87%)	97.64% (95.34-98.82%)
Accuracy	52.67% (46.19-59.09%)	88.89% (84.25-92.55%)

[Table/Fig-3]: Binary logistic regression and ROC analysis results for HF-BF% and HF-BF#.
ROC: Receiver operating characteristic; AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value. Model: Outcome (Probable malignancy Yes/No) ~HF-BF% or HF-BF#



[Table/Fig-4]: ROC analysis curve results.

- HF-BF% performance: HF-BF% showed a statistically significant association with malignancy (β=0.0595, p=0.006) with an odds ratio of 1.06. Its optimal cut off (≥ 1.95%) yielded high sensitivity (92.86%) and high NPV (99.14%), though specificity and PPV remained low.
- HF-BF# performance: HF-BF# demonstrated a stronger association with malignancy (β=1.92, p=0.001) and a markedly higher odds ratio (6.80). At its optimal cut off (≥ 51 cells/µL), it achieved high specificity (90.39%), high NPV (97.64%), and better overall accuracy (88.89%) compared with HF-BF%.

ROC characteristics: The AUC values for HF-BF% (0.698) and HF-BF# (0.729) indicate moderate discriminative ability, with HF-BF# showed numerically higher discriminative performance.

However, these results must be cautiously interpreted due to the low number (n=14/243) of suspicious/positive malignant cases.

DISCUSSION

The present study explored the utility of analyser-derived HF-BF% and HF-BF# to predict malignancy in the BFs. A positive association was found between HF-BF parameters and the odds of suspicious/positive for malignancy. Both HF-BF% and HF-BF# have a high NPV, while HF-BF# has a higher specificity. Values below the respective thresholds were associated with a lower probability of cytopathological suspicion/positivity in this cohort; however, neither threshold can independently exclude malignancy. Overall, HF-BF# is better than HF-BF% due to higher NPV and specificity, but the low event rate (i.e., 14/243) could potentially explain these results. The ROC AUC values indicate a moderate discriminative power with HF-BF parameters for the detection of malignancy in BFs.

Malignant cells show HF with abnormal forward and side scatter in the analyser assessment due to large amounts of nucleic acids in them [12]. HF-BF parameters have been previously studied as a surrogate marker for malignant effusions [13].

Ivady G et al., suggested that a lack of a distinct population in the HF-BF region with similar TC-BF and WBC-BF values indicated the absence of malignant cells in the BFs [14]. Low levels of HF-BF parameters shown in the present study reduce the likelihood of malignancy, consistent with findings reported previously. Huang WH et al., analysed routinely collected BFs to assess the diagnostic accuracy of HF-BF% and HF-BF# for the detection of malignancy [4]. This study reported an AUC of 0.6, an optimal cut-off of 6.5%, a sensitivity of 65%, a specificity of 62%, a PPV of 61%, and an NPV of 66% for HF-BF%. The corresponding values for HF-BF# are: AUC=0.67, cut-off=39cells/µL, sensitivity=74%, specificity=62%, PPV= 64% and NPV=73%. The present study obtained a similar but slightly greater AUC and cut-off of 0.698 and 1.95%, respectively, for HF-BF%, while HF-BF# had an AUC and cut-off of 0.729 and 51 cells/µL, respectively. The present work also found higher sensitivity and NPV than this study. The differences can be attributed to sample size (the proportion of malignant cases in the cited study is 40/90 compared to 14/243 in the present study).

Rastogi L et al., reported a prevalence of 6.39% for malignancy in 924 BF samples. They found higher HF-BF% in malignant samples with an ROC-derived optimal cut-off of 2.85%, yielding the following values: AUC-0.709; Sensitivity- 64.4%; Specificity- 61.4%; PPV- 10.2% and NPV- 96.1% [1]. The corresponding metrics for HF-BF# are optimal cut-off =12 cells/µL, AUC-0.76; Sensitivity- 71.2%; Specificity-71.2%; PPV- 14.7% and NPV- 97.3%. These values closely matched the results of the present study except for the HF-BF# cut-off.

Cho YU et al., and Labaere D et al., found higher levels of HF-BF parameters in BFs with malignant cells [9,10]. Cho YU et al., reported the following: HF-BF% (AUC=0.791, cut-off=6.9%). Labaere D et al., found the following: HF-BF% (AUC=0.69, cut-off=2.1%); HF-BF# (AUC=0.77, cut-off=17 cells/µL). Both studies found high sensitivity/NPV and low specificity/PPV when using HF-BF parameters for the assessment of malignancy. The discrepancy between the numerical values of AUC and cut-off in the above studies and the present study can be attributed to the lower sample size, differences in the analyser device, and fluid types analysed.

Although HF-BF metrics can be rapidly assessed, they suffer from both false negative and false positive results, and any interpretation of these metrics in a clinical context must account for these potential errors. False negatives could be observed in the following conditions: 1) Tumour burden: sparse cellularity in samples or highly diluted effusions may fail to reach the threshold event count required to register an elevated HF-BF parameter. Similarly formation of cell clusters with high tumour burden underestimates HF-BF counts; 2) Fluorescence characteristics: well-differentiated or hypodiploid malignant cells have low baseline RNA/DNA density or may have poor dye permeability leading to lower fluorescence, and non detection as a HF-BF cell; 3) Cell degeneration: Degenerating or apoptotic tumor cells undergo rapid nucleic acid degradation and membrane fragmentation, reducing dye

binding and causing abnormal cells to be detected as non fluorescent cells. False positive results are seen with reactive mesothelial cells, activated/transformed lymphocytes, or activated large macrophages.

Limitation(s)

The present study is limited by its role as a rapid assessment tool rather than a confirmatory test, which still requires cytopathologist review. Combining suspicious (Category IV) and definite malignant (Category V) cases introduces heterogeneity and potential misclassification bias. Pooling different BF types may affect diagnostic performance. The small sample size and low event rate reduce the precision and stability of the logistic regression estimates and diagnostic-performance measures restricting interpretation to exploratory univariate associations. The single centre design, risk of false positives from reactive mesothelial cells, and inability to detect low malignant cell counts limit generalisability and sensitivity, underscoring the need for larger validation studies.

CONCLUSION(S)

HF-BF% and HF-BF# demonstrated moderate discriminative ability for identifying body-fluid samples classified as suspicious/positive for malignancy. Their low PPV and imperfect sensitivity preclude their use as standalone screening or diagnostic tests. HF-BF# showed numerically higher specificity and AUC than HF-BF%; however, formal statistical comparison between the two parameters was not performed. Both parameters should therefore be considered adjunctive tools to cytopathological examination and require validation in larger, multicentre cohorts.

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