Original Article

High Fluorescent Cells on Automated Body Fluid Analysis as Discriminator for Malignant Cell Detection

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Abstract

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Keywords

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► body fluid
► high fluorescent cells
► malignant cells screening
► Sysmex XN-1000

The automated examination of body fluids (BF) serves as a valuable screening tool for the presence of malignant cells in such samples. Malignant cells are identified as high fluorescence cells (HFC) when analyzed using the Sysmex XN-1000 automated analyzer. This study aimed to assess the correlation between HFC cell counts generated by the automated analyzer and manual cytological examination for detecting malignant cells. Additionally, it sought to establish reliable cutoff values for malignant cells since there is a lack of literature on this subject. Conducted at the department of pathology hematology and cytology laboratory in a tertiary care hospital in India from January 2019 to May 2020, this hospital-based comparative study analyzed 120 BF samples, each subjected to cytological evaluation. The mean age of the study population was 52 years, with 70 male and 50 female patients (male-to-female ratio of 1.4:1). The samples consisted of 53 ascitic fluids (44.17%), 46 pleural fluids (38.33%), and 21 cerebrospinal fluids (CSF; 17.50%). Cytopathological examination revealed malignant cells in 50 (41.67%) of the BF samples, with 70 (58.33%) samples classified as nonmalignant. Specifically, among the ascitic fluids, 24 (48%) were malignant, while 29 (41.43%) were nonmalignant. For pleural fluids, 24 (48%) were malignant, and 22 (31.43%) were nonmalignant. In CSF, 2 (4%) samples were malignant, and 19 (27.14%) were nonmalignant. The total white blood cell counts provided by automated hematology analyzers were significantly higher in malignant samples, ranging from a minimum of 100 cells to a maximum of 60,000, with a median count of 800. Nonmalignant samples had white blood cell counts ranging from 2 to 12,000, with a median count of 100. Subgroup analysis for ascitic, pleural, and CSF samples revealed significantly higher median HFC counts in malignant samples. Receiver operating characteristic curve analysis indicated that the HF-BF parameter could effectively distinguish between benign and malignant fluids. For HF#, the area under the curve (AUC) was 0.844, with a sensitivity of 82% and specificity of 81%, while HF% had an AUC of 0.706, with sensitivity and specificity values of 72% and 72.9%, respectively. This study highlights that the HFC count in the BF mode of Sysmex XN-1000 can be a

valuable tool for predicting the presence of malignant cells in serous fluids and for selecting samples for further microscopic examination. Based on this study, cutoff values of 15.70/µL for absolute HFC count and 5.05% for relative HFC count can be applied to screen BF samples for malignancy, offering good sensitivity and specificity.

Introduction

The analysis of body fluids (BF)—pleural, ascitic, cerebrospinal fluid (CSF)—is an important diagnostic modality that gives evidence of malignancy[1,2] and therefore it has vital role in the management of cancer patients. The gold standard of BF examination is manual hemocytometer followed by cytomorphological examinations of BF with Papanicolaou[3] and other stain like hematoxylin and eosin. The manual methods have high turnaround time and large interobserver variability; therefore, automated BF analysis is useful as it enables laboratories to cut short the turnaround time affording rapid diagnostic information to clinicians.[4–6] The BF module on the XN-1000 (Sysmex Corporation) represents a convenient platform for accurate and fast quantification of total nucleated cells.[7] The BF mode (Sysmex-1000) differentiates cells as neutrophils and eosinophils, lymphocytes, and monocytes. This channel records high fluorescence body fluid (HF-BF) cells, which are not included in the white blood cell (WBC) count, but are included in the total nucleated cell count.[4] HFCs have higher fluorescence signal because of large amount of nucleic acids. The HF-BF cells are segregated by their differential fluorescence intensity, and they include malignant cell, mesothelioma cells, and macrophages.

The cutoff percentage of HF cells is not defined in literature, and it therefore is a limitation in the use of hematology (XN-1000) as a screening tool. This study was aimed to reliably estimate the cutoff for HF cells. More such studies are needed to validate the findings of this study.

Materials and Methods

A descriptive type of observational (validation) cross-sectional study was conducted over a period of 1 year, extending from January 2019 to May 2020, in the department of pathology in a tertiary care hospital in west India. This study group comprised of patients who submitted BFs sample such as CSF, pleural fluid, and ascitic fluid to the laboratory for examination for malignant cells. This study was approved by institutional ethics committee and subjects were included in study after written informed consent was obtained from the participants.

The following exclusion criteria were applied:

- Cases with inadequate quantity of sample for both manual count and automated analysis
- Samples were excluded if more than 10% degenerated cells were identified in the counting chamber
- If WBC count using the manual method was less than 5 cells/µL in non-CSF samples.

A total of 120 specimens of BFs, including 53 (44.17%) ascitic fluid, 46 (38.3%) pleural fluid, and 21 (17.50%) CSF, were analyzed on BF mode on Sysmex XN-1000 (Sysmex, Kobe, Japan). Sample collection, preservation, transport, and analysis were performed in accordance with the Clinical & Laboratory Standards Institute (CLSI) H56-A guideline.[7] The cell identification was done by both manual and automated methods. All fluid samples were collected in K2-ethylene-diaminetetraacetic acid anticoagulants tubes or sterile recipients without anticoagulant. Sample were received by laboratory immediately after collection and automated analysis and slide preparation was completed within 2 hours. Cytology section received separate sample and manual microscopy was done on slide stained by special stains.

The samples were first run in the BF mode in XN-1000 automated hematology analyzer. Manual microscopic counting was performed using a Neubauer hemocytometer using samples diluted 1:10 (50 µL CSF + 450 µL of solution) in Turk's solution (crystal violet and glacial acetic acid dissolved in distilled water). Cell counting was performed at 200× magnification using a standard light microscope. The average number was converted into cells/µL according to the following formula provided in the CLSI H56-A guidelines.[7] The differential WBC counts were performed after sample cytocentrifugation (7 minutes at 1250 rpm) and later smear preparation stained with Wright staining. At least 100 cells were counted at 400× magnification.

The results of automated XN 1000 analyzer were compared with manual method for BF examination. The statistical analysis was done using online free statistical calculator (Medicals).

Results

This study comprised of 160 samples of BF received in the department of pathology between January 2019 and May 2020. The majority of samples were from the age group 41 to 50 years (20.83%) followed by 51 to 60 years (20.0%). The mean age group for this study population was 52.48 years (►Fig. 1). Out of 120 patients, 70 patients were male and 50 were female with male female ratio of 1.4:1 (►Fig. 2).

There were three types of BF samples: 53 ascitic fluid (44.17%), 46 pleural fluid (38.33%), and 21 CSF (17.50%; ►Fig. 3). Fifty (41.67%) fluids had malignant cells on cytopathological examination (►Fig. 4).

Of the 53 ascitic fluids, 24 (45.3%) samples were reported as malignant, and 29 (54.7%) samples were reported as
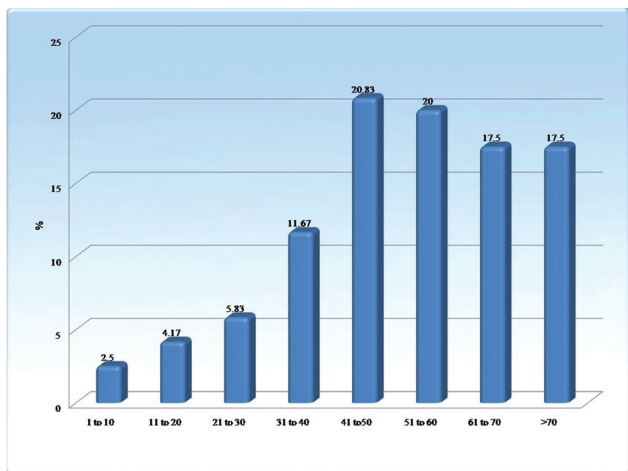
Fig. 1 Distribution of the cases according to age groups.

nonmalignant cells. Out of 46 pleural fluids, 24 (52.2%) samples were reported as malignant and 22 (47.8%) as nonmalignant. For 21 CSF only, 2 (9.5%) were reported as malignant and 19 (70.5%) CSF as nonmalignant (►Fig. 5). Mean value of total WBC count for malignant sample was higher than nonmalignant samples (statistically nonsignificant as p-value more than 0.05; ►Table 1).

In our study, the mean polymorphonuclear cells (PMN)% for malignant and nonmalignant samples were 34.80 and 36.03%, respectively, with p-value 0.78. There is no significant correlation found in between PMN% and malignant status of fluid (statistically in significant, p-value 0.96; ►Table 2).

In this study, mean mononuclear cells (MN)% for malignant and nonmalignant samples were 58.40 and 62.57%, respectively. MN% was slightly higher in nonmalignant sample in our study; however, the results (statistically in significant, p-value >.05; ►Table 3). ►Table 4 shows mean WBC count for malignant and nonmalignant BFs—ascetic, pleural, and CSF.

The mean WBC count for malignant fluid was 995.83 and for nonmalignant fluid it was 254.48. For pleural fluid, mean WBC count for malignant fluids was 3433.33 and for nonmalignant fluids, it was 802.27. Similarly, for CSF mean count was 645 and 21.16 for malignant and nonmalignant fluids, respectively (►Table 4).

The mean absolute HFC count for malignant fluids was 450 and 16.40/μL for nonmalignant fluids in all BFs. For ascitic fluid, mean HFC count for malignant/nonmalignant cell was 458.05 and 12.37/μL, for pleural fluid mean HFC count for malignant/nonmalignant cell was 467.58 and 29.49/μL, and for CSF it was 164.50 and 7.41/μL, respectively (►Table 5).

Mean absolute HF% for all malignant fluids was 21.1 and 9.9% for nonmalignant fluids. Mean HF% for ascitic fluid was 18.6 and 7.0% for malignant and nonmalignant fluids, respectively.

Mean HF% for pleural fluid was 20.7 and 16.9% for malignant and nonmalignant fluids, respectively. Mean HF% for CSF was 55.4 and 6.1% for malignant and nonmalignant sample, respectively (►Table 6).

The receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cutoff values of significant variables (Hf#) detected between the two groups
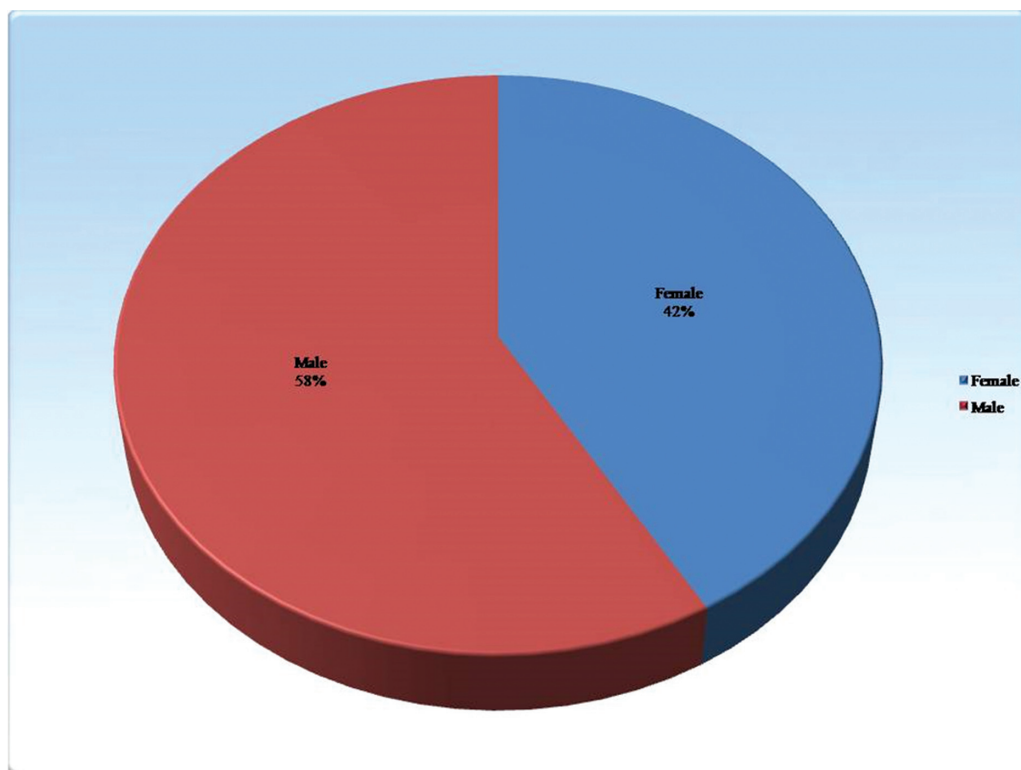

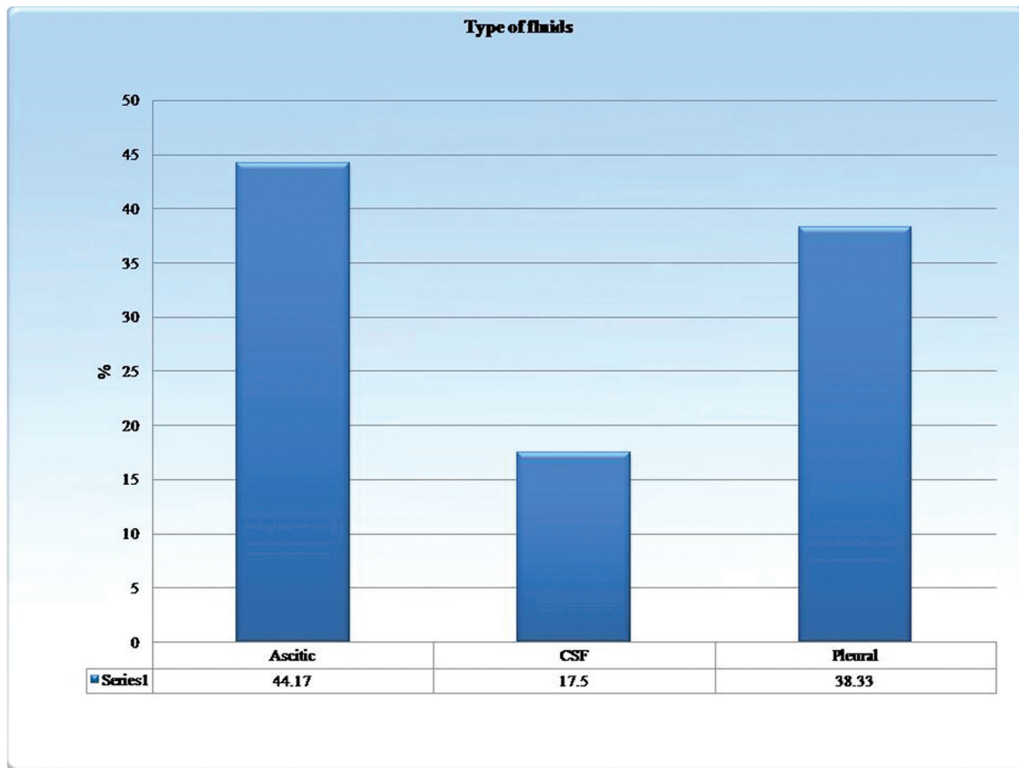**Fig. 2** Distribution of the cases according to sex.

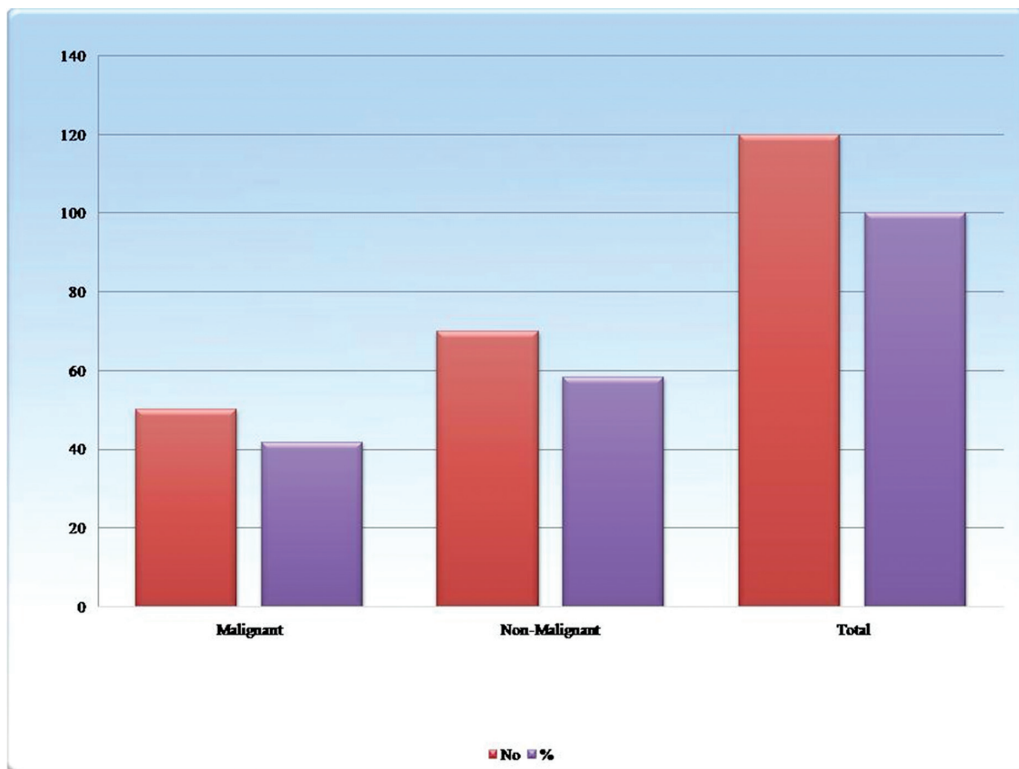**Fig. 3** Distribution of the cases according to type of fluids. CSF, cerebrospinal fluid.

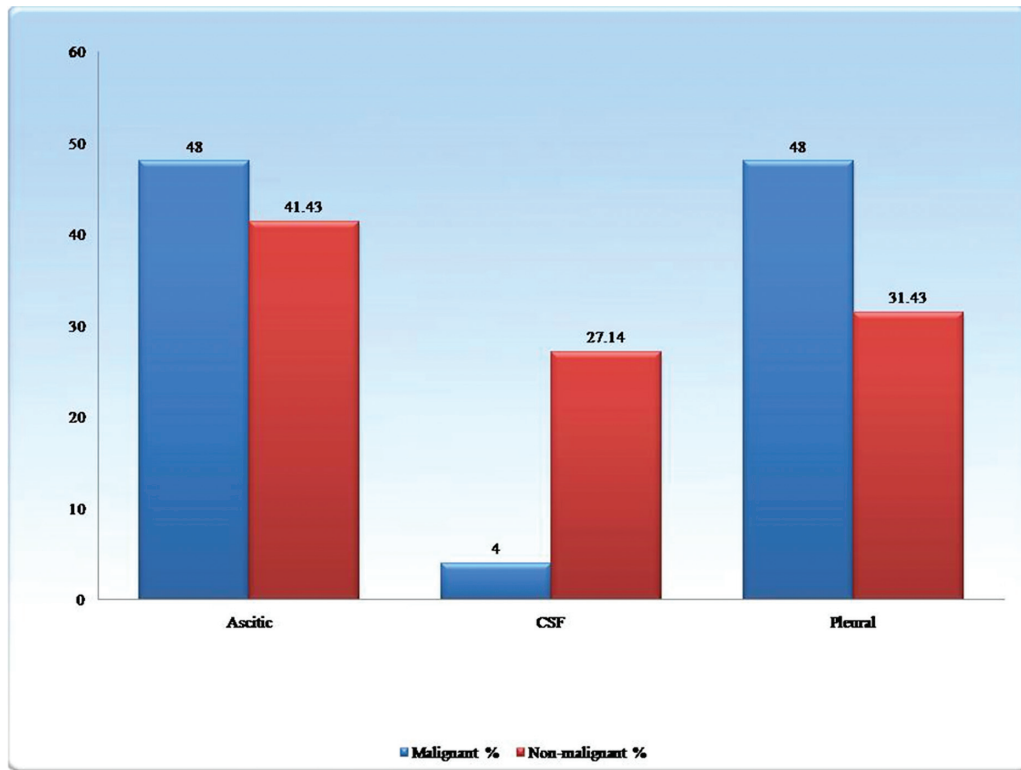**Fig. 4** Distribution of the cases according to malignant status.

Fig. 5 Distribution of the cases according to malignant status with type of fluid. CSF, cerebrospinal fluid.

Table 1 Total WBC count in analyzer with malignancy status (TLC/µL)

Cytology results	n	Mean	SDs	Minimum	Maximum	p-Value
Malignant	50	2151.8	8403.1	100.0	60000.0	0.083
Nonmalignant	70	363.3	1431.5	2.0	12000.0	
Total	120	1108.5	5572.0	2.0	60000.0	

Abbreviations: TLC, total leucocyte count; SD, standard deviation; WBC, white blood cell

Table 2 Correlation of PMN% with malignant status of fluid (by microscopic methods)

Cytology results	n	Mean	SD	Minimum	Maximum	p-Value
Malignant	50	34.8	19.4	10.0	80.0	0.96s
Nonmalignant	70	35.0	24.6	0.0	90.0	
Total	120	34.9	22.5	0.0	90.0	

Abbreviations: PMN, polymorphonuclear cells; SD, standard deviation.

Table 3 Correlation of MN% with malignant status of fluid (by microscopic method)

Cytology results	n	Mean	SD	Minimum	Maximum	p-Value
Malignant	50	58.40	20.011	15	85	
Nonmalignant	70	62.57	24.697	10	100	0.37
Total	120	60.83	22.865	10	100	

Abbreviations: MN, mononuclear cells; SD, standard deviation.

Table 4 Total WBC count (/μL) comparison between malignant and nonmalignant fluid

Total WBC count (/μL)		n	Mean	SD	Median
Ascitic	Malignant	24	995.83	797.81	850.00
	Nonmalignant	29	254.48	238.17	200.00
	Total	53	590.19	671.47	300.00
CSF	Malignant	2	645.00	502.05	645.00
	Nonmalignant	19	21.16	38.40	3.00
	Total	21	80.57	221.68	3.00
Pleural	Malignant	24	3433.33	12102.93	525.00
	Nonmalignant	22	802.27	2516.30	150.00
	Total	46	2175.00	8921.25	400.00
Total	Malignant	50	2151.80	8403.09	800.00
	Nonmalignant	70	363.31	1431.47	100.00
	Total	120	1108.52	5572.04	250.00

Abbreviations: CSF, cerebrospinal fluid; SD, standard deviation; WBC, white blood cell.

Table 5 Absolute HFC count comparison between malignant and nonmalignant fluid

Hf #(/μL) (absolute HFC count)		n	Mean	SD	Median
Ascitic	Malignant	24	458.05	1584.98	103.00
	Nonmalignant	29	12.37	20.557	7.50
	Total	53	214.19	1077.747	11.00
CSF	Malignant	2	164.50	232.638	164.50
	Nonmalignant	19	7.41	10.048	4.40
	Total	21	22.37	70.921	4.40
Pleural	Malignant	24	467.58	1402.768	91.50
	Nonmalignant	22	29.49	38.225	9.50
	Total	46	258.06	1027.317	38.00
Total	Malignant	50	450.89	1451.70	96.00
	Nonmalignant	70	16.40	26.948	7.15
	Total	120	197.44	959.272	13.60

Abbreviations: CSF, cerebrospinal fluid; HFC, high fluorescence cell; SD, standard deviation.

ROC for Hf# showing (1-specificty) on the X axis and sensitivity on Y Axis exercise different cut off value to provide the greatest sum of sensitivity and specificity (►Fig. 6).

The optimum cutoff value was obtained by points of test values that grants the highest Youden Index comes out to be 15.70% with a sensitivity of 82% and a specificity of 81% (►Table 7). Similarly, for HF%, the optimal cutoff was 5.05%. The sensitivity and specificity of 72% were determined with SE 0.039 (►Table 8; ►Fig. 7).

Discussion

The past two decades have seen rapid advancement in automation in the field of laboratory medicine, from high output automatic analyzers to the use of machine learning algorithms of artificial intelligence.[8–17] The scarcity of trained manpower, need for rapid turnaround time, and minimal subjective bias are some of the factors that have driven manufacturers to bring new equipment for automated analysis. This study included three types of BFs similar to studies by Rastogi et al[18] and Cho et al[19] for performance evaluation of automated BF analyzer using HF-BF parameters as discriminators, whereas others like Wu et al,[20] Wrong et al,[21] and Zimmerman et al[22] used single type of BF for analysis (►Figs. 8–10).

The total WBC count in this study was higher for malignant BFs than nonmalignant ones. The finding is similar to studies by Huang et al,[23] Xu et al,[24] Labaere et al,[25] and Sun et al.[26]

As per ROC analysis, the HF% cutoff of 5.5% equivalent to HF cell count of 15.70/μL should yield good sensitivity and

Table 6 Relative HFC count comparison between malignant and nonmalignant fluid

Hf % (relative HFC count)		n	Mean	SD	Median
Ascitic	Malignant	24	18.6	26.5	11.3
	Nonmalignant	29	7.0	8.7	3.7
	Total	53	12.2	19.6	4.4
CSF	Malignant	2	55.4	78.3	55.4
	Nonmalignant	19	6.1	7.7	1.8
	Total	21	10.8	24.1	1.8
Pleural	Malignant	24	20.7	27.9	8.7
	Nonmalignant	22	16.9	14.6	14.4
	Total	46	18.9	22.4	10.7
Total	Malignant	50	21.1	29.5	10.7
	Nonmalignant	70	9.9	11.6	3.9
	Total	120	14.5	21.6	5.2

Abbreviations: CSF, cerebrospinal fluid; HFC, high fluorescence cell; SD, standard deviation.
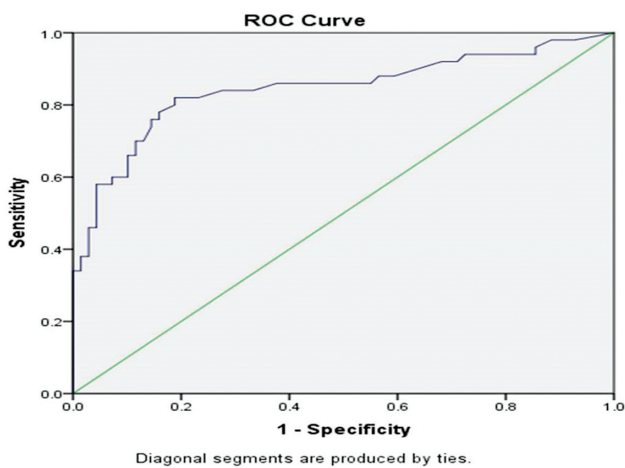

Fig. 6 Receiver operating characteristic (ROC) curve for the optimal cutoff value of absolute high fluorescence cells count.

specificity for malignant cell detection in this study that could be used as discriminator. The comparison with other literature studies is shown in ►Table 9. The findings of the study are in line with those by Rastogi et al,[18] Buoro et al,[27] Huang et al,[23] Xu et al,[24] and Larruzia et al[28] had higher cutoff value determination than this study. The use of automated analyzer is mainly as a screening tool, whereas the cytological examination remains the gold standard. The cutoff selected in this study is justified as the automated analysis being used as screening tool should not compromise on sensitivity. The results are comparable to studies by Labaere et al,[25] Cho et al,[19] and Zimmerman et al[22] who found false positive similar to this study —11 malignant cases out of 70 nonmalignant cases. They also reported interference by histiocytes, mesothelial cells, and plasma cells. In terms of false negatives, there were 7 out of 50 cases; majority of them were CSF samples that have low cell yield. There was no carry over of WBC in this study as reported

Table 7 ROC curve for the optimal cutoff value of absolute HFC count

Area under the curve				
Test result variable(s): HF#				
Area	SE[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
0.844	0.040	0.000	0.766	0.922
Positive if greater than or equal To[a]	Sensitivity	1-Specificity	Specificity	Y
15.700	0.820	0.188	0.812	0.632

Abbreviations: HFC, high fluorescence cell; ROC, receiver operating characteristic; SE, standard error.
The test result variable(s): HF# has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
[a]Under the nonparametric assumption.
[b]Null hypothesis: true area = 0.5.

Table 8 ROC curve for the optimal cutoff value of relative HFC count

Test result variable(s): HF%				
Area	SE[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
0.706	0.049	0.000	0.610	0.802
Positive if greater than or equal To[a]	Sensitivity	1-Specificity	Specificity	Y
5.050	0.720	0.271	0.729	0.449

Abbreviations: HFC, high fluorescence cell; ROC, receiver operating characteristic; SE, standard error.
The test result variable(s): HF% has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
[a]Under the nonparametric assumption.
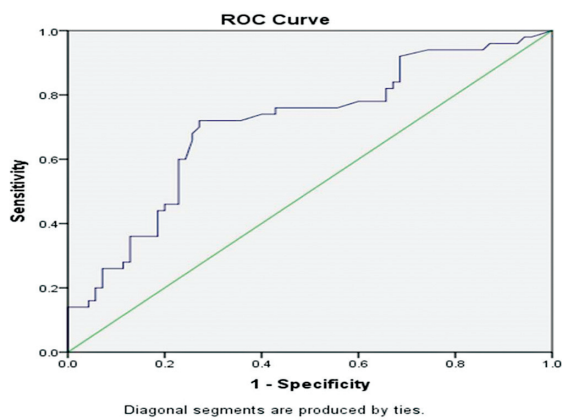[b]Null hypothesis: true area $= 0.5$.

Fig. 7 Receiver operating characteristic (ROC) curve for the optimal cutoff value of relative high fluorescence cells count.

by Caroline et al.[9] The advantage of automated BF analyzer could not be undermined as the analysis is quick and reliable as stressed by Caroline et al[29] and Aguadero et al.[30]

We acknowledge that the limited sample size and inability to capture time interval between sample collection and analysis were limitations of the study. It mandates that in the absence of clear-cut discriminatory value for HF cells to detect malignant cells, each laboratory should establish its own cutoff in BF analysis on automated analyzer.

Conclusion

Automated BF analysis using Sysmex XN-1000[31] could be a sensitive tool in predicting the nature of BF offering quick screening as adjuvant to routine cytological examination.
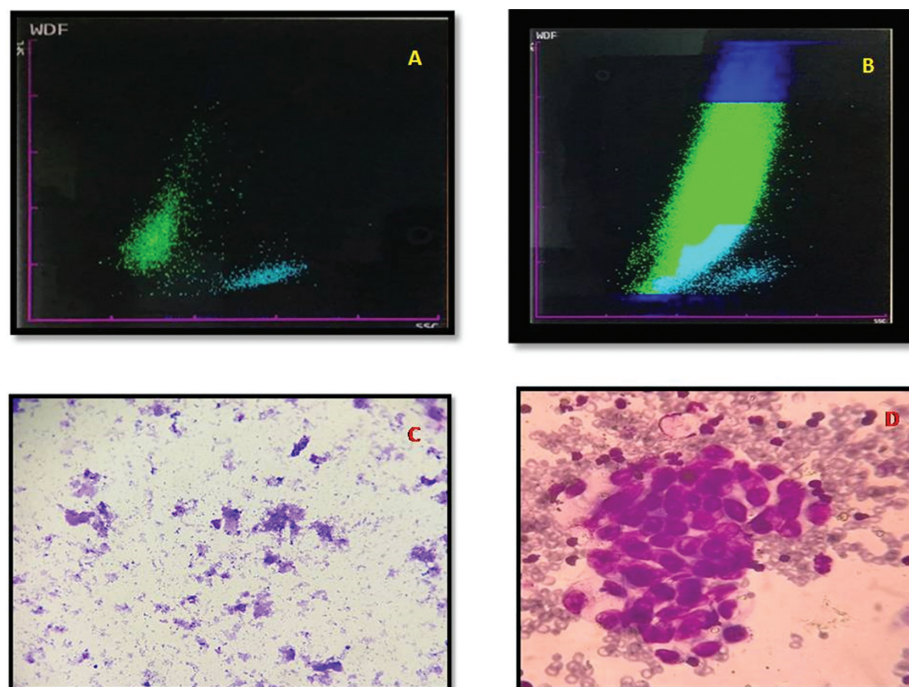

Fig. 8 Ascitic fluid—(**A**) Benign scatter gram, (**B**) malignant scatter gram, (**C**) benign microscopy, and (**D**) malignant microscopy.
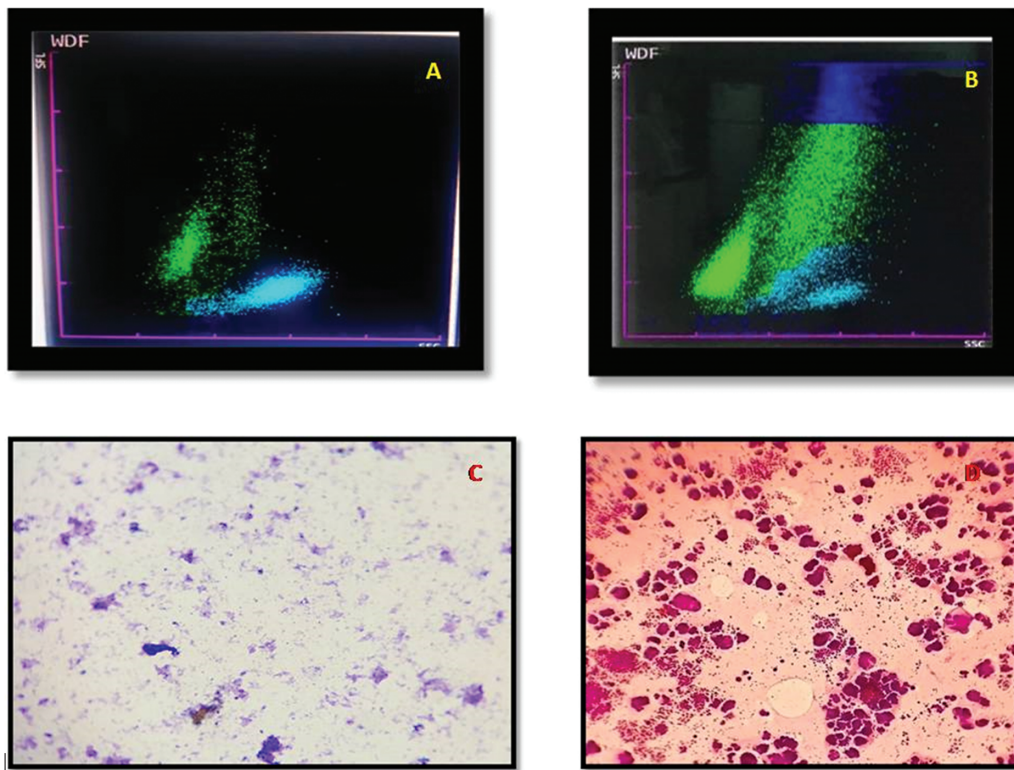
Fig. 9 Pleural fluid—(**A**) Benign scatter gram, (**B**) malignant scatter gram, (**C**) benign microscopy, and (**D**) malignant microscopy.
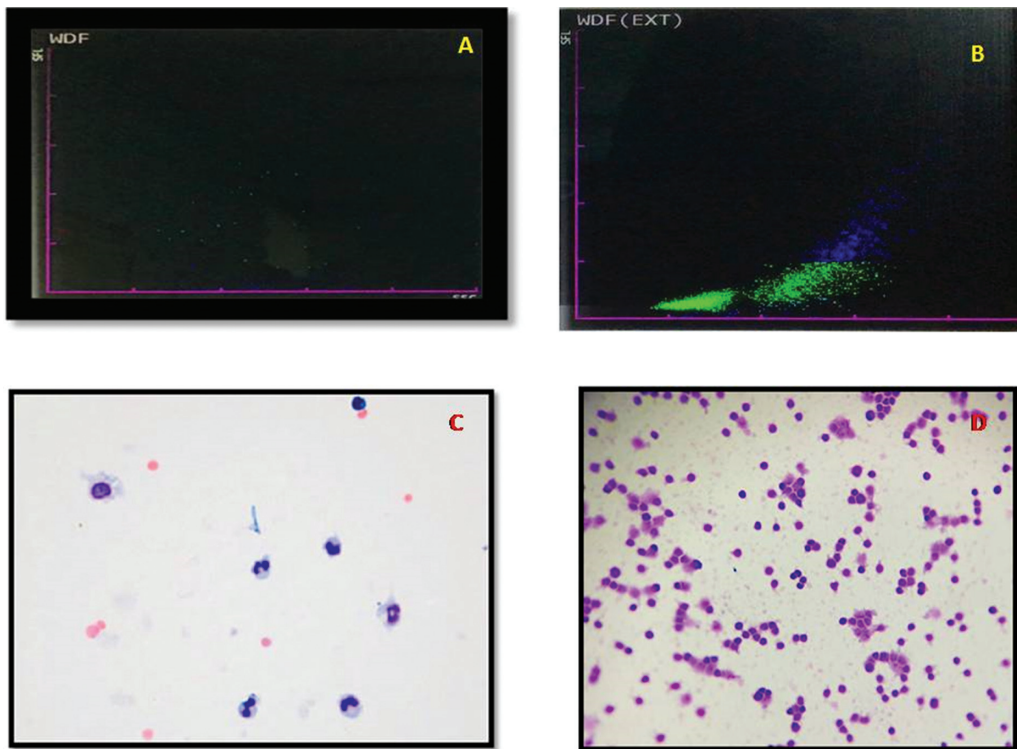

Fig. 10 Cerebrospinal fluid—(**A**) Benign scatter gram, (**B**) malignant scatter gram, (**C**) benign microscopy, and (**D**) malignant microscopy.

Table 9 Distribution of study group according to HF-BF# cutoff

Sl no.	Study name	HF-BF#	AUC	95% CI	Sensitivity	Specificities
1.	Rastogi et al[18]	>12	0.76	0.690–0.830	71.2	71.2
2.	Huang et al[23]	39	0.67	0.56–0.78	74	62
3.	Wu et al[20]	29.5	0.66	0.569–0.743	70.4	61.8
4	Xu et al[24]	24.5	0.708	0.635–0.780	75.3	55
5.	Labaere et al[23]	17	0.77	0.69–0.84	88	61
6.	Buoro et al[27]	50	0.75	0.61–0.79	49	90
7.	Present study	15.700	.84	0.766–0.922	82	81

Abbreviations: AUC, area under the curve; CI, confidence interval; HF-BF, high fluorescence body fluid.

Conflicts of Interest
None declared.

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