EVALUATION OF HIGH-FLUORESCENCE BODY FLUID (HF-BF) PARAMETER AS A DIAGNOSTIC TOOL FOR MALIGNANCY IN BODY FLUIDS USING AUTOMETATED HEMATOLOGY ANALYSER

BY

Dr.SULTANA.SHAHANAZ

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Dr.SUREKHA.B.H.

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IN

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LIST OF ABBREVIATIONS

ABBREVIATION	PARAMETER
HF-BF	High Flurescence Body fluid
WDF	WBC Differential Fluorescene
AF	Ascitic Fluid
PF	Pleural fluid
CSF	Cerebrospinal fluid
BF	Body Fluid
NFM	Negative for malignancy
AUS	Atypia of undetermined significance
SFM	Suspicious for malignancy
MAL	Malignant
SD	Standard Deviation
AUC	Area Under Curve
ROC	Receiver operated curve

ABSTRACT

INTRODUCTION

Body fluid (BF) analysis is an essential test for the diagnosis and management of various diseases. In suspected cases of malignancy, body fluids are evaluated in cytopathology laboratories for early diagnosis. The interest towards the development of automated BF analyzers has enormously increased due to the existing limitations of manual cell count techniques.

OBJECTIVES

To analyze the High Fluorescence-Body Fluid parameter(both HF-BF# and HF-BF%) given by Sysmex XN-1000 and to study its correlation with conventional cytological method to detect the presence of malignant cells in the BFs.

MATERIALS AND METHODS

This prospective hospital-based study of 56 Body Fluid samples in suspected cases of malignancy is conducted in Central Clinical Laboratory, of BLDE's Shri B.M.Patil Medical College, Vijayapura. All the body fluid samples were collected in EDTA tube and will be processed within 2 hours of receipt in the laboratories. The cell identification was made by both manual microscopic method and automated method using Sysmex XN-1000.

RESULTS

HF-BF%/100 WBCs for NFM, AUS, SFM, and MAL Body Fluid samples are (1.17± 1.69), (6.34± 1.7), (11.02 ± 6.74) and (26.65 ± 6.56) respectively. HF-BF# μL for NFM, AUS, SFM, and MAL Body Fluid samples are (5.99 ± 10.75), (13.32 ± 10.60), (70.18 ± 104.8) and (151.78 ± 134.9) respectively. The coefficient of variation between manually calculated cell count and TC-BF in our study is 1.00 showing a perfect positive correlation between both methods. P value is <0.0001 which is statistically significant.

CONCLUSION

Sysmex XN-1000 hematology analyzer BF method is capable of rapid and reliable differential count in the BFs. HF-BF parameters are higher in malignant BFs than benign fluids.

KEYWORDS - Hematology analyzer, Body fluid analysis, HF-BF mode.

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INTRODUCTION

Body fluid (BF) analysis is a key diagnostic and management tool in various diseases. In suspected cases of malignancy, body fluids are evaluated in cytopathology laboratories for early diagnosis[1]

Microscopic evaluations of body fluids are still considered standard techniques for the analysis of ascitic and pleural fluids.[2]

The Sysmex XN is an automated hematology analyzer that has the capability of discriminating non-hematopoietic cells and it can be an alternative to manual techniques[4]. This automated hematology analyzer has a specified BF mode (XN-BF) for Body Fluid analysis which helps to count total and differential cell counts. [1]

Development of automated BF analyzers has increased due to the existing limitations of manual techniques such as inter-observer variability, cellular degeneration and longer TAT.[3]

In XN-BF mode in automated analyser, to evaluate different cells in body fluids, the cell membranes are treated with Lysercell WDFTM and then cells are stained with Fluorocell WDFTM so that Fluorescence flow cytometers can detect specific side scattered signals generated by intracellular organelles and nucleic acids, according to their type and quantity.[4]

This study considers various parameters such as HF-BF%, HF-BF#, along with other parameters given by the analyser and all of which has been evaluated both by microscopy and automated technique.

Moreover, recent study by **Ai T et al** [4] observed few normal constituents of serous fluids such as macrophages and mesothelial cells have been reported to be counted falsely as High Fluorescent cells along with the atypical/ malignant cells and thus giving false-positive results. [4] Additionally a study conducted by **Rastogi L, et al.** [1] observed that HF-BF parameters gives rapid and reliable assistance in body fluid analysis to detect malignant cells.

So this study was undertaken to evaluate the efficacy of HF-BF mode of Sysmex XN 1000 automated hematology analyzer in detecting atypical/malignant cells in various Body Fluids.

AIM AND OBJECTIVES OF THE STUDY

- To analyze the High Fluorescence Body Fluid parameters (both “HF-BF%” and “HF-BF#”) by “ Sysmex XN 1000” automated hematology analyzer and to correlate these parameters with the conventional cytological method in the detection of malignant cells in the body fluids.

REVIEW OF LITERATURE

Automated hematology analyzers quantify and categorize the body fluids using various combinations of technology. These techniques include nuclear fluorescent staining intensity measurement using principle of flow cytometry, electrical impedance, cytochemical composition, cell lysis, and many other. Hematology analyzer with BF mode's performance in detecting malignant cells has been assessed in the majority of recent studies [5]

In addition to being tested for automated BF analysis, an automated analyzer that was first introduced for urine analysis uses digital imaging and neural network for quantification and classification of cells and other constituents.[16,17]

The combined data from these research shows that automated BF analysis is now a common practise in many clinical laboratories. In terms of cellular composition, stability and matrix, BFs are different from whole blood. Addition of BF mode to the hematology analyzers is a significant advancement in automated BF analysis.

The aim of BF mode is to maximize the automated analyser's technologies for the analysis of BF samples. Given that these elements will define the method's capabilities and constraints, it is crucial to comprehend the technical properties and in-built software algorithm of the XN-BF mode. 'BF mode' values are more precise than in 'CBC mode' because of a gating method that excludes normal mesothelial cells. However, compared to the ability of whole blood differential to distinguish between 5 or 6 cell types, the BF mode WBC differential count may only provide

two cell differential count (i.e PMN and MN). In addition BF mode provides HF-BF parameters as research parameters by its ability to discriminate non-hematopoietic cells.[4]

VALIDATION OF AUTOMATED BODY FLUID ANALYSIS

The types of BFs must be verified before processing on the automated analyzers and the normal diagnostic range for every specific fluid type must be stated in a statement of intended use that is supplied by the manufacturers.

The evaluation of at least 40 samples is often advised by method validation methods. The prerequisites for validation studies are thoroughly explained in publications by the International Council for Standardization in Hematology and Clinical Laboratory Standards Institute. [18,19]

ACCURACY OF AUTOMATED BODY FLUID ANALYSIS

Accuracy is obtained by comparing two methods, one existing reference method and another new method of interest. Low coefficient of correction can be obtained when co-efficient of variation is high in comparison studies. [20]

Sample degeneration after collection presents another difficulty when conducting correlation research to assess ability of BF mode to detect presence of malignant cells. To reduce discrepancies brought on by cellular degeneration, it is crucial to evaluate the split samples quickly after splitting. Accuracy can also be evaluated by incorporating regular assay of analyser with commercially available quality control samples.

Although an analyser might report a sample as abnormal/ atypical which is labelled as normal by reference method, thus relying only on automated analysis, the management and diagnosis of patients may be impacted.[10]

SPECIFICITY AND LIMIT OF QUANTIFICATION OF AUTOMATED ANALYZER

The minimum cell count that meets the laboratory's criteria is known as the LOQ. [19] Practically, LOQ is the minimum total count that has a Coefficient of Variation is less than 20%.
Automated systems can achieve higher accuracy (lower CVs) at reduced cell counts because they evaluate a larger amount of sample compared to hemocytometers. [7,8,11,13]

In the domain of automated BF analysis, the concept analytical specificity has distinct but related interpretations. The manufacturer should specify any known contaminants, that could affect the analysis.[9,21] Analytical specificity provides rate of false positive reports [12] The determination of the false positive rate is a helpful tool to illustrate the analytical variations.

BODY FLUID TYPES AND RELATED SPECIFIC ISSUES

The various Body Fluids present various difficulties for automated analysis in detection of malignnacy. In contrast to serous cavity effusions, which are pathologic diseases, CSF which is typically present in healthy individuals. Several obstacles have been encountered in detecting malignant cells in different body fluids.

CEREBROSPINAL FLUID (CSF)

The biggest obstacles to automated BF analysis is analysis of CSF samples. Numerous medical conditions are required to investigate a CSF sample, and the majority of patient samples come out normal. As a result, investigations on technique correlation are slanted toward very few cell counts. When cell counts are low, both automated and manual approaches to detect malignant cells are less precise.

There are a number of possible causes for the false positive reports by an automated analyser, such as “electronic noise” and other complex cellular debris [8,11]

As a result, certain samples with acceptable manual cell counts could appear aberrant or might show HF-BF cells when analyzed using automated methods. Due to this, some researchers[10] have advised against using automated analysis on samples that have low cell counts, while others have proposed that alternative reference limits for automated procedures may need to be established.[15]

SEROUS FLUIDS (PLEURAL FLUID, ASCITIC FLUID, PERITONEAL LAVAGE FLUIDS)

Detection of malignancy in serous BFs have been reported in a confusing and contradictory manner. The mesothelial cells, which are frequently found in these serous fluids in numerous numbers, frequently cause inconsistency in methods of automated examination and interferes with HF-BF values. Similar inconsistencies can be found in the case of macrophages. Gating techniques are used by automated analyzers with specific HF-BF mode to keep tissue cells out of the WBC count and thus reducing their interference in High Flurescence cells. Hence, automation provides the opportunity to standardize BF reporting.

AUTOMATED BODY FLUID ANALYSIS- EFFICIENCY AND COST EFFECTIVENESS

In comparison to labor-intensive manual processes, automated technologies are generally thought to enhance laboratory turnaround times (TATs). However, each laboratory's particular set of variables will determine if faster TATs can actually be attained. Automation will undoubtedly be advantageous to laboratories that screen a lot of BF for malignant cells.

AUTOMATED HEMATOLOGY ANALYZERS

SYSMEX ANALYZERS

Sysmex hematology analyzers contain a specialized BF mode ("HF-BF mode") which is having approval from FDA. This mode is capable of performing body fluids analysis. In 2011, and 2015, the XN and XN-L series were introduced, respectively. However, XT nad XE series are still on use for many studies. [27,28]

Sysmex XN 1000 has a specific in-built BF- mode for body fluid analysis. This analyzer gives HF-BF% and HF-BF# values for each body fluid under its research parameters. Whenever these values exceed the laboratory-specific cut-off [1], (Table 1) cytopathologists screen these samples by routine cytological techniques to rule out the presence of malignant cells in the samples. This also allows to reduce the turnaround time(TAT) and enables increased efficiency in a high sample load laboratory.

PRINCIPLE OF MALIGNANT CELLS DETECTION BY XN-BF MODE

This specialized mode called as XN-BF mode works on basic principle of flow cytometry. To calculate and differentiate non hematopoietic cells from hematopoietic cells, the cell membrane

of the cells are perforated with a reagent buffer called as Lysercell and then intracellular organelles and nucleic acids are stain with a fluoroscent reagent that is Fluorocell. Then, by the principle of flow cytometry, specific side scatter are generated on the basis of complexity of intracellular organelles and nucleic acids. Due to the treatment with the buffers, leucocytes do not aggregate and platelets are also lysed, thus they do not affect forward scatter signals.[4]

XN-BF mode utilizes WDF channel[4] which generates four simultaneous signals for each cell passing through the focused laser beam in detecting chamber i.e Flurocell-

1. Forward scatter signal indicating volume of the cell.
2. Side scatter signals indicating intracellular complexity and granularity.
3. Fluorescence intensity signal indicating the amount of intracellular nucleic acids.
4. Forward scatter width signals indicating "time of flight", that implies that large aggregates of cells passing through.

The combination of all four signals are analyzed and categorized into different parameters by using in-built software.[4]

BF- mode utilizes fluorescence signals to differentiate WBC from the non-haematopoietic HF-BF cells. HF-BF cells are not included in the WBC counts and the amounts of HF-BF cells are expressed as a ratio over the WBCs (HF-BF/100 WBCs, abbreviated as 'HF-BF%') or absolute cell counts (number of HF-BF/μL, abbreviated as 'HF-BF#'). Atypical/malignant cells as well as Reactive mesothelial cells and macrophages are also counted as HF-BF cells.[4]

Cut off values given by **Rastogi L, et al. [1] in t**heir study to evaluate HF-BF parameters in Sysmex XN 1000 automated analyser in detecting presence of malignant cells are taken as reference value for cut off in our study.

TYPE OF FLUID	Cut off(HF-BF%)	Cut off(HF-BF#)
ASCITIC FLUID	3.95	17
PLEURAL FLUID	4.05	17
CSF	0.75	1
ALL FLUIDS	2.85	12

TABLE 1- Cut off values- HF-BF% and HF-BF# - **Rastogi l, et al.** [1]

Xu W, et al [45] studied serous cavity effusions to evaluate XN-BF mode in Sysmex XN 1000 hematology analyzer in detecting presence of malignant cells. The found a cut off value of 4.4/100WBC for HF-BF% and a cut off value of 24.5/μL for HF-BF# to suspect presence of malignant cells and to subject the cases showing a HF value above this cut off to microscopic review.[45]

TYPE OF FLUID	Cut off (HF-BF%)	Cut off (HF-BF#)
ALL SEROUS FLUIDS	4.4	24.5

Table 2- Cut off value- HF-BF% and HF-BF# - **Xu W, et al** [45]

Labaere D et al [26] studied ability of BF mode in Sysmex XN 2000 in serous body fluids. When ROC analysis was done, they found that malignant fluids showed a higher mean of HF-

BF% which is 10.2/100WBC, whereas 2.6/100 WBC for benign fluids. Similarly, malignant fluids showed higher mean for HF-BF# which was 65/µL, whereas 10/ µL for benign fluids.[26]

CATEGORY	HF-BF%/100 WBCs (MEAN)	HF-BF#/Ml (MEAN)
BENIGN BODY FLUID	2.6	10
MALIGNANT BODY FLUID	10.2	65

Table 3- MEAN- HF-BF% and HF-BF# - **Labaere D et al [26]**

Labaere D et al [26] suggested a **cut off > 17#/ µL** to screen serous fluid samples for microscopic review. With this cut of HF-BF# showed sensitivity 88% and specificity 61%

SYSMEX XN-1000 ANALYSER-

Reportable parameters by channel

XN_CBC NRBC	WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT (PLT-I), NRBC%, NRBC#, RDW-SD, RDW-CV, MPVWBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT (PLT-I), NRBC%, NRBC#, RDW-SD, RDW-CV, MPV
XN_DIFF IG	NEUT%, LYMPH%, MONO%, EO%, BASO%, IG%, NEUT#, LYMPH#, MONO#, EO#, BASO#, IG#
WPC	Provides improved differentiation of blast cells and abnormal lymphocyte flagging; no reportable parameters.
XN_BF DIFF	WBC-BF, RBC-BF, TC-BF, MN%, MN#, PMN%, PMN#, HF-BF%, HF-BF# (Body Fluid)
PLT_F IPF	PLT (PLT-F), IPF (Platelets, P License)
RET RET-He	RET%, RET#, IRF, RET-He (Reticulocytes, R License)

Figure-1: Sysmex XN 1000 automated analyzer

These analyzers offer “HF-BF” under research parameters in addition to TC-BF, WBC-BF, and RBC-BF counts. The term "research parameters" refers to analyzer-provided parameters that are under research, such as high-fluorescence cells (HF-cells). These analyzers reduce turnaround time (TAT) and need a lesser volume of fluid than conventional method [24]

Many recent studies have conducted comparative studies to determine the utility of the XN series automated analyzers as a reliable and practical alternatives to manual examination for detecting malignant cells in body fluids.[30–32] Despite the fact that many studies have found a tendency of automated method to overdiagnose some cell populations [31,37], due to the strong concordance (95%) between the two techniques, these differences are usually of no clinical significance.[31] Few studies have found that when sample is hypocellular, the degree of concordance increases between automated and manual merhod. [27,31]

The presence of fungal elements such as yeasts may interfere with HF-BF, producing a distinct pattern on the scattergrams. ("blue surfboard pattern") [34] This is why routine cytological reviews are important.

According to FDA reports, these analyzers have adequate limit of detection (LOD) for processing serous fluids. Comparison studies in ascitic and pleural fluids [12,35] support this analyzer as an alternative to manual examination for detection of malignancy, but they are not yet validated for peritoneal fluids. [36]

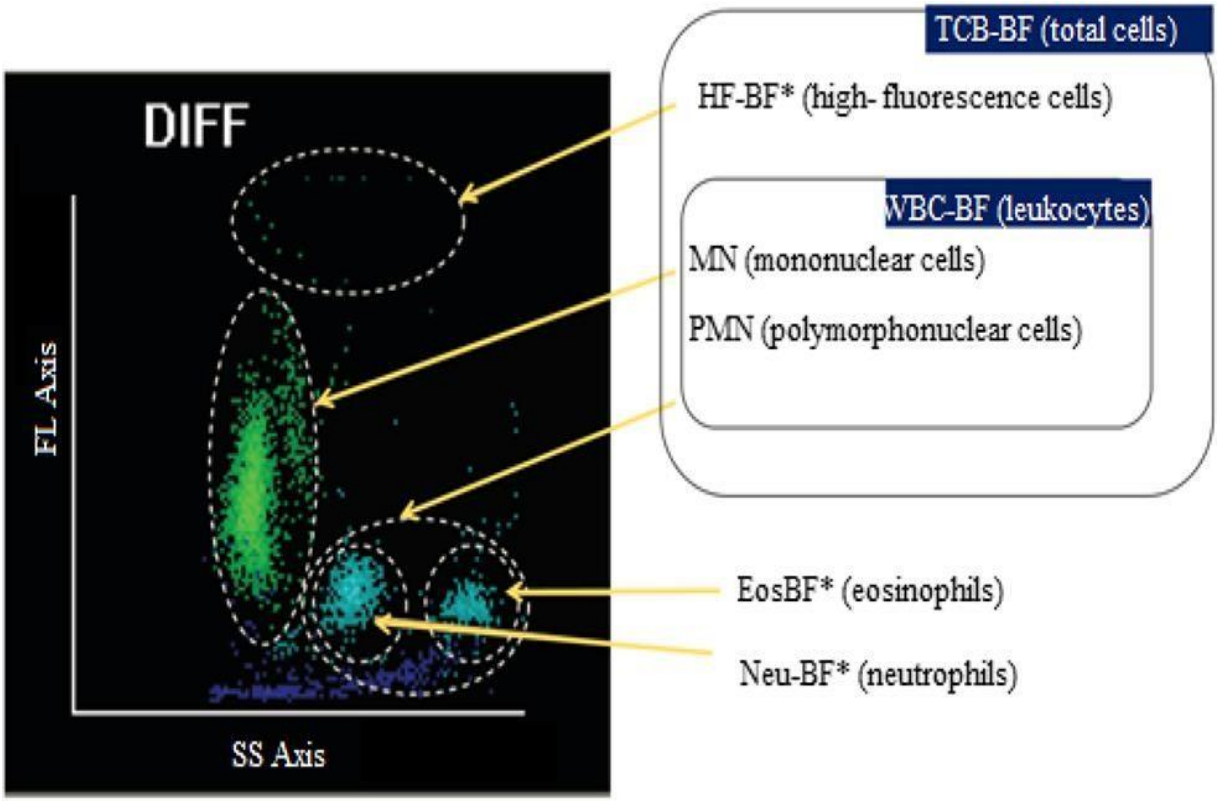

Figure 2: WBC scattergram for BF mode of automated body fluid analyzer.

The key benefits of automated analyzers are the availability of internal quality control, the samples donot need to be prediluted and it has a short turnaround time (3 min).

A close review of HF-BF parameters can act as an excellent aid in screening large number of body fluid for microscopic review.[38] In this regard, Buoro et al. [37,38] concluded in their study that microscopic inspection is required in the presence of WBC-BF ranging from 4.0 to 7.0/L in CSF sample and/or high HF-BF values, leading to misdiagnosis. Only a few studies have found

a difference between TC-BF count and WBC-BF counts, resulting in an increase in the value of HF-BF parameters. [37]

CYTOLPATHOGICAL EXAMINATION AND CATEGORIZATION OF BODY FLUIDS

Cytopathology has always been one of the reliable standard techniques in diagnosing malignancy in body fluids. The International System for reporting serous fluid cytopathology[48] provides an excellent format for better understanding of the serous fluid reports and thus reducing inter-laboratory variability in reporting serous fluids. Another goal of TIS is that it allows easier and efficient comparison between various research results and provides good correlation and follow up between cytopathology and clinical course.[48]

TIS targets to improve the diagnostic outcome of serous fluids via its diagnostic categories which also have well-defined risk of malignancy (ROM) in the form of percentage.[48]

In our study, cytopathological examination on routine cytology smears are done for all samples and were categorised according to protocols provided by TIS of reporting serous fluid cytopathology.

TIS provides five diagnostic categories for reporting serous fluids, which are depicted below-

DIAGNOSTIC CATEGORY	CRITERIA
1. NONDIAGNOSTIC (ND)	Samples with insufficient cellular elements for a cytologic interpretation
2. NEGATIVE FOR MALIGNANCY (NFM)	Samples with cellular changes which completely lacks evidence of mesothelial/ non- mesothelial malignancy
3. ATYPIA OF UNDETERMINED SIGNIFICANCE (AUS)	Samples showing limited cellular and/or architectural atypia (includes extreme reactive atypia, degenerated tumor cells)
4. SUSPICIOUS FOR MALIGNANCY (SFM)	Samples showing features suspicious but not definitively diagnostic for malignancy
5. MALIGNANT (MAL)	Samples with definitive findings/ supportive studies indicating mesothelial or non- mesothelial malignancy

Table-4: The International System of reporting serous fluid cytopathology

MATERIALS AND METHODS

Source of data: A prospective hospital-based study of Body Fluid samples in suspected cases of malignancy is carried out in the Central Clinical Laboratory, of " BLDE Deemed to be university Shri. B. M. Patil medical college, Hospital and Research Centre, Vijayapura"

Study period: January 2021 to July 2022.

Type of Study- Cross-sectional study.

Inclusion criteria

- All Body Fluids of clinically suspected cases of malignancy which can be run in "Sysmex XN 1000 automated hematology analyzer" were included in the study.

Exclusion Criteria

- There are no exclusion criteria to be mentioned.

Sample Size-

With anticipated sensitivity and specificity of the Automated method in correlation with Microscopy as 98% and 95%, with a 99% confidence level and precision of 0.08 the sample size (N) calculated was 112 using the following formula-

$$\mathbf{N}=\frac{Z^2P(1-p)}{\Delta^2}$$

N = (a+c) if we use sensitivity as p.

N= (a+c)/Prevalence

Methods of collection of data

The study includes all Body Fluid samples of suspected cases of malignancy.

All the body fluids are collected in EDTA vacutainer and processed as per laboratory "SOP" of body fluids within 2 hours. The body fluid analysis was done by both manual microscopic method and automated method using BF mode of Sysmex XN-1000.

Automated fluid processing [51]

(According to SYSMEX Automated Hematology Analyzer XN series Administrator's Guide)

- All the samples were run in BF- mode of XN 1000 automated hematology analyzer.
- The high fluorescence cells were identified above the MN cluster in scattergram and were given as HF-BF% and HF-BF#.
- The High fluroscence parameters were given as HF-BF%/100 WBCs and HF-BF# as /μL.
- Other additional parameters of body fluids were also obtained.

Cytological examination [49]

- A corresponding cytopathological examination was done for all samples.
- The body fluid samples were centrifuged, and cytospin smears were prepared from the sediment and stained with Papanicolaou stain.
- Smears directly prepared from the fluid sample were also stained with special stains like Giemsa, PAP, and H & E stain.
- All slides were evaluated manually using Microscope.
- Cytopathology reporting was done for all fluids according to The International System of reporting serous fluid cytopathology (Table 4)

Manual Technique [50]

- Manual cell counting was done for all fluids in Neubauer chamber as a part of routine laboratory protocol.

Statistical Analysis-

- All the data obtained were entered into a Microsoft Excel sheet, and statistical analysis was performed using a statistical package for the social sciences (Version 20).
- Results were presented as Mean±SD, counts and percentages, and pie/bar diagrams.
- For normally distributed continuous variables between two methods were compared using an Independent t-test. For not normally distributed variables, the Mann-Whitney U test was used. Categorical variables between the two methods was compared using the Chi-square test.
- ROC was used to find cutoff values and to find sensitivity and specificity.
- $p<0.05$ was taken as statistically significant. All statistical tests were performed in two-tailed.

RESULTS

Our study was done at the Department of Pathology, B.L.D.E (Deemed to be University), Vijayapura, Karnataka. In our study, we studied the body fluids of 112 patients who were suspected cases of malignancy. Here, we present an evaluation of the results of our study.

AGE DISTRIBUTION

In this study, the minimum age was 10 years and the maximum was 80 years and the mean age of presentation in this study was 48.6 years. Among all the patients (N = 112) in the study, the majority of patients were in the age group 40 to 69 years comprising 75 cases (66.9% of the study population). The detailed representation is shown below.

Age(Years)	No. of patients	Percentage
< 20	5	4.5
20 – 29	9	8.0
30 – 39	16	14.3
40 – 49	23	20.5
50 – 59	27	24.1
60 – 69	25	22.3
70+	7	6.3
Total	112	100

Table 5- Age of all the patients and the number of patients in each group with

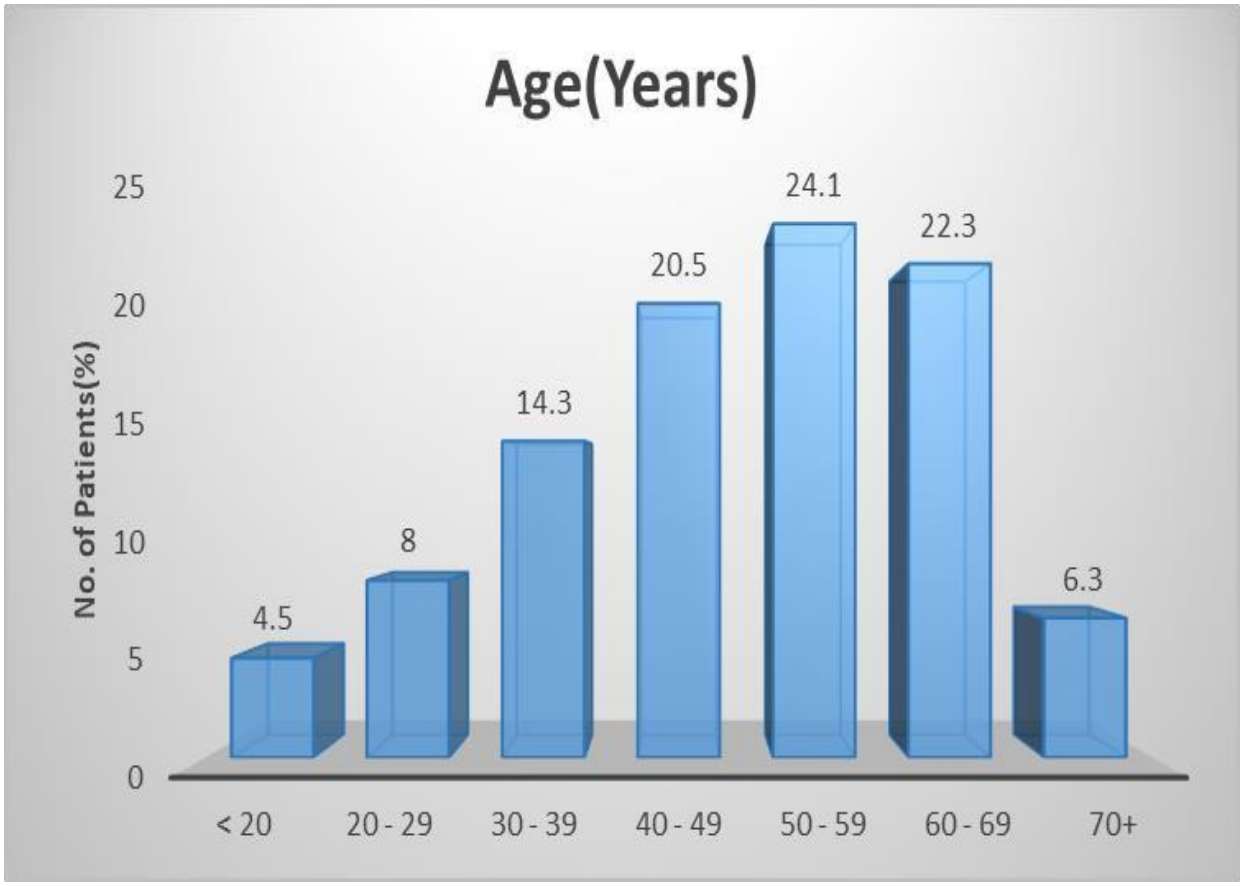

Fig. 3- Distribution of patients according to Age

GENDER DISTRIBUTION

Among all the patients included in this study, 66 were males and 46 were females comprising 58.9% and 41.1 % of total cases respectively.

Gender	No. of patients	Percentage
Female	46	41.1
Male	66	58.9
Total	112	100.0

Table 6- Gender distribution of all the patients and the number of patients in each group

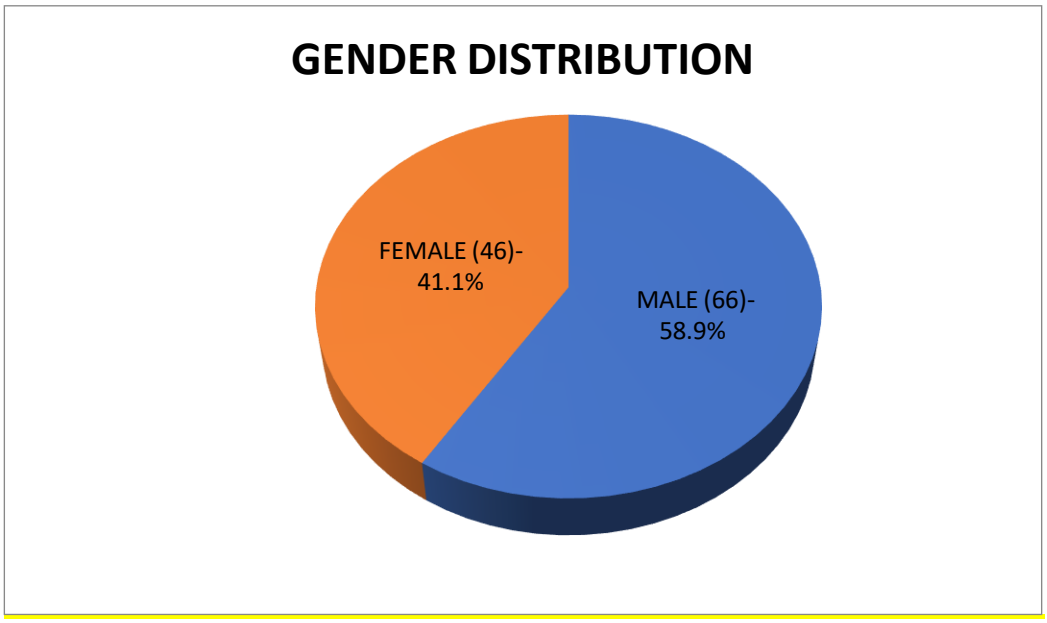

Fig. 4- Distribution of patients according to gender.

DISTRIBUTION OF PATIENTS ACCORDING TO TYPE OF BODY FLUID

Out of 112 body fluid samples included in this study, 47 were ascitic fluid, 46 were pleural fluid and 19 were cerebrospinal fluid, comprising 41.96%, 41.07 %, and 16.97% of total cases respectively.

TYPE OF FLUID	NUMBER	PERCENTAGE (%)
ASCITIC FLUID	47	41.96
PLEURAL FLUID	46	41.07
CEREBROSPINAL FLUID	19	16.97
TOTAL	**112**	**100**

Table 7- Type of body fluid with percentage.

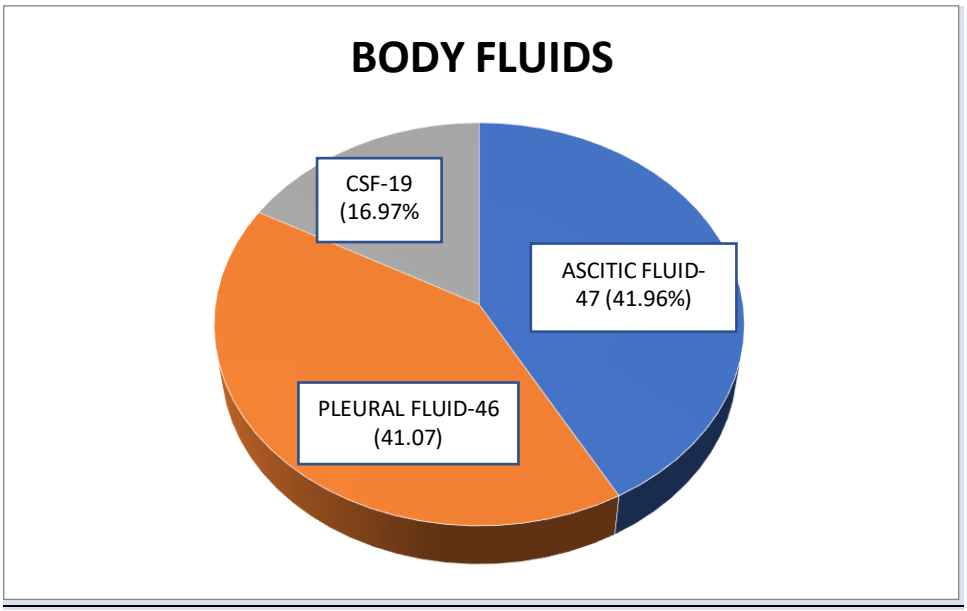

Fig 5- Type of body fluid with percentage.

CATEGORISATION OF BODY FLUID ACCORDING TO CYTOLOGICAL DIAGNOSIS

Out of 112 body fluid samples, none of the cases were categorised as Nondiagnostic, 75 were categorized as Negative for malignancy, 6 were Atypia of undetermined significance, 20 were Suspicious for malignancy and 11 were Malignant, comprising 0%, 66.97%, 5.36 %, 17.85% and 9.82% of total cases respectively.

CYTOLOGICAL CATEGORY	NUMBER	PERCENTAGE (%)
NONDIAGNOSTIC (ND)	00	00
NEGATIVE FOR MALIGNANCY (NFM)	75	66.97
ATYPIA OF UNDETERMINED SIGNIFICANCE (AUS)	6	5.36
SUSPICIOUS FOR MALIGNANCY (SFM)	20	17.85
MALIGNANT (MAL)	11	9.82
TOTAL	**112**	**100**

Table 8- Cytological category of body fluids with percentage.

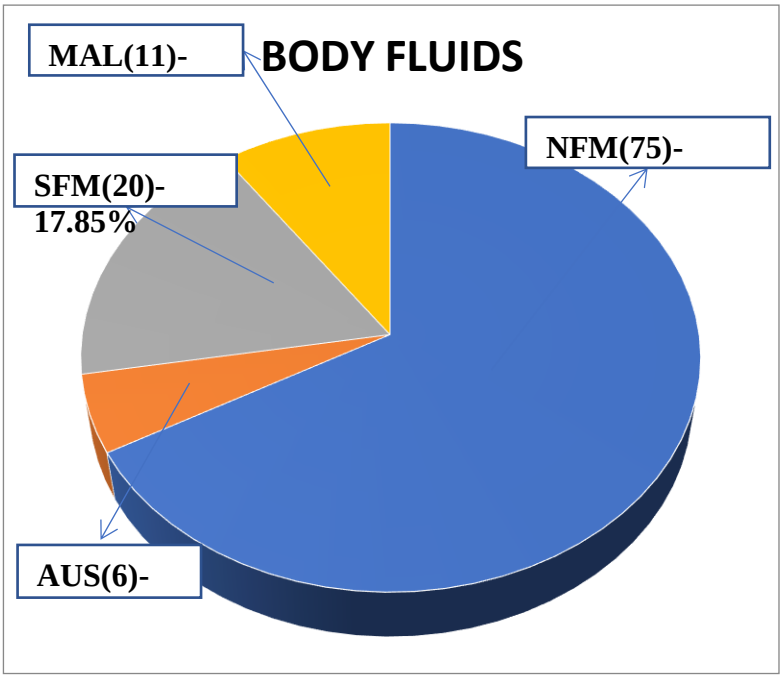

Fig 6- Cytological category of body fluids with percentage.

MEAN, MEDIAN, STANDARD DEVIATION, AND PERCENTILES OF HF-BF PARAMETERS

Mean, Median, Standard Deviation, and Percentiles (25, 50, and 100) have been calculated and all statistical values for HF-BF% and HF-BF# are depicted in the table 9 below-

STATISTICAL PARAMETERS	**HF-BF%/100WBC (All fluids)**	**HF-BF#/ µL (All fluids)**
Mean	5.71	32.15
Median	2.00	8.00
Std. Deviation	8.565	54.877
Percentiles-25	1.00	3.25
Percentiles -50	2.00	8.00
Percentiles-100	8.00	35.00

MEAN ±SD for HF-BF%/100WBC and HF-BF#/ µL for each cytological category of body fluids is depicted in the table 10 below-

CYTOLOGICAL DIAGNOSIS	MEAN ±SD (HF-BF%/100WBC)	MEAN ±SD (HF-BF#/ µL)
NEGATIVE FOR MALIGNANCY	1.17± 1.69	5.99 ± 10.75
ATYPIA OF UNDETERMINED SIGNIFICANCE	6.34± 1.7	13.32 ± 10.60
SUSPICIOUS FOR MALIGNANCY	11.02 ± 6.74	70.18 ± 104.8
MALIGNANT	26.65 ± 6.56	151.78 ± 134.9

MEAN OF HF-BF%/100WBC FOR EACH CYTOLOGICALCATEGORY OF BODY FLUIDS

Among 112 body fluid samples, the Mean of HF-BF%/100WBC for the Negative for malignancy category is 1.17, Atypia of undetermined significance category is 6.34, the Suspicious for malignancy category is 11.02 and the Malignant category is 26.65.

CYTOLOGICAL CATEGORY	MEAN OF HF-BF%/100WBC
NEGATIVE FOR MALIGNANCY	1.17
ATYPIA OF UNDETERMINED SIGNIFICANCE	6.34
SUSPICIOUS FOR MALIGNANCY	11.02
MALIGNANT	26.65

Table 11- MEAN of HF-BF%/100WBC for each category of body fluids

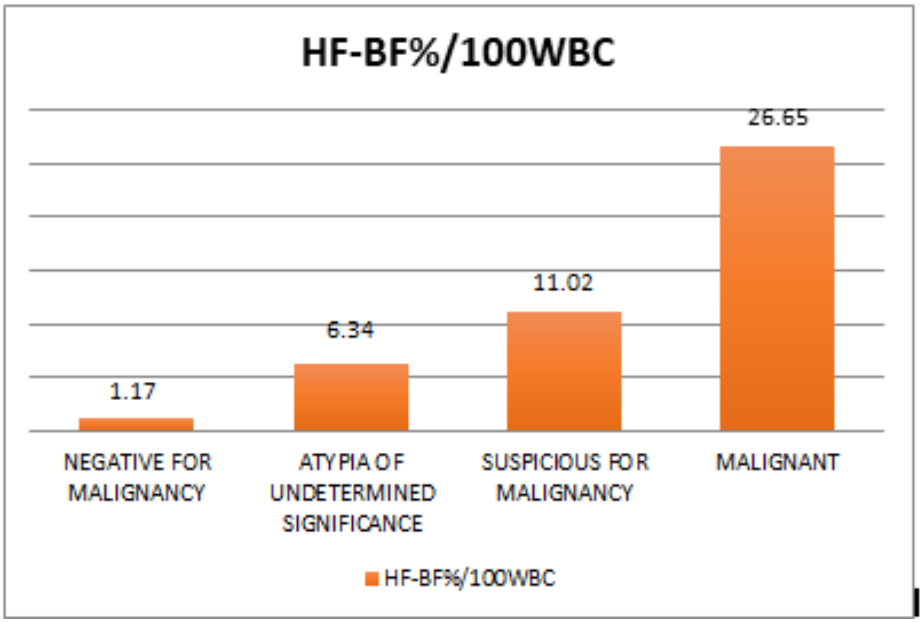

Fig 7- MEAN of HF-BF%/100WBC for each category of body fluids

MEAN OF HF-BF#/µL FOR EACH CYTOLOGICAL CATEGORY OF BODY FLUIDS

Among 112 body fluid samples, the Mean of HF-BF#/µL for the Negative for malignancy category is 5.99, Atypia of undetermined significance category is 13.32, Suspicious for the malignancy category is 70.18 and the Malignant category is 151.78.

CYTOLOGICAL CATEGORY	MEAN OF HF-BF#/µL
NEGATIVE FOR MALIGNANCY	5.99
ATYPIA OF UNDETERMINED SIGNIFICANCE	13.32
SUSPICIOUS FOR MALIGNANCY	70.18
MALIGNANT	151.78

Table 12- MEAN of HF-BF#/µL for each category of body

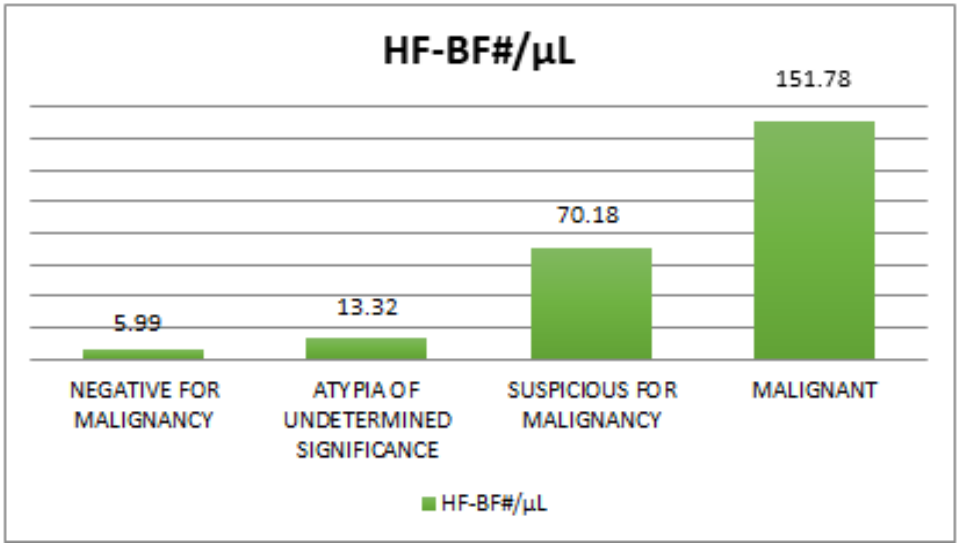

Fig 8- MEAN of HF-BF#/µL for each category of body fluids

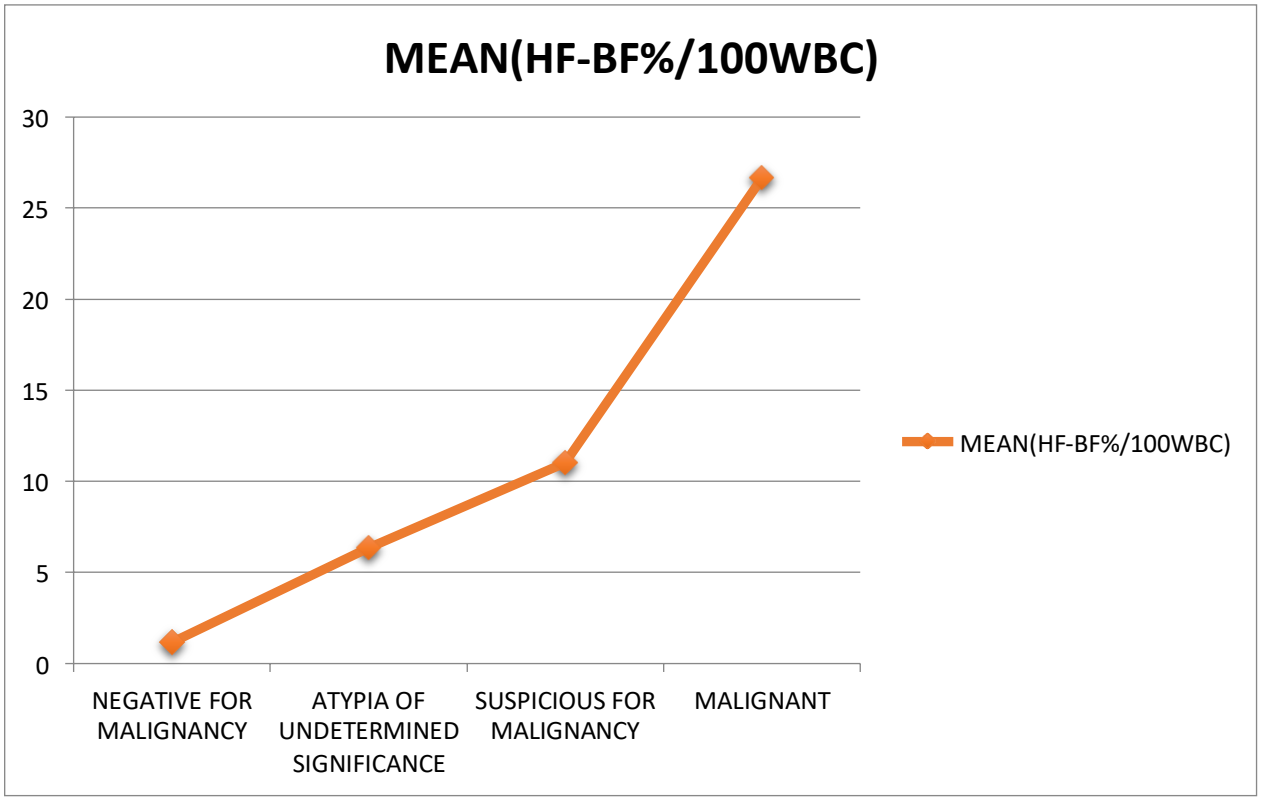

Fig 9- MEAN of HF-BF%/100WBC for each category of body

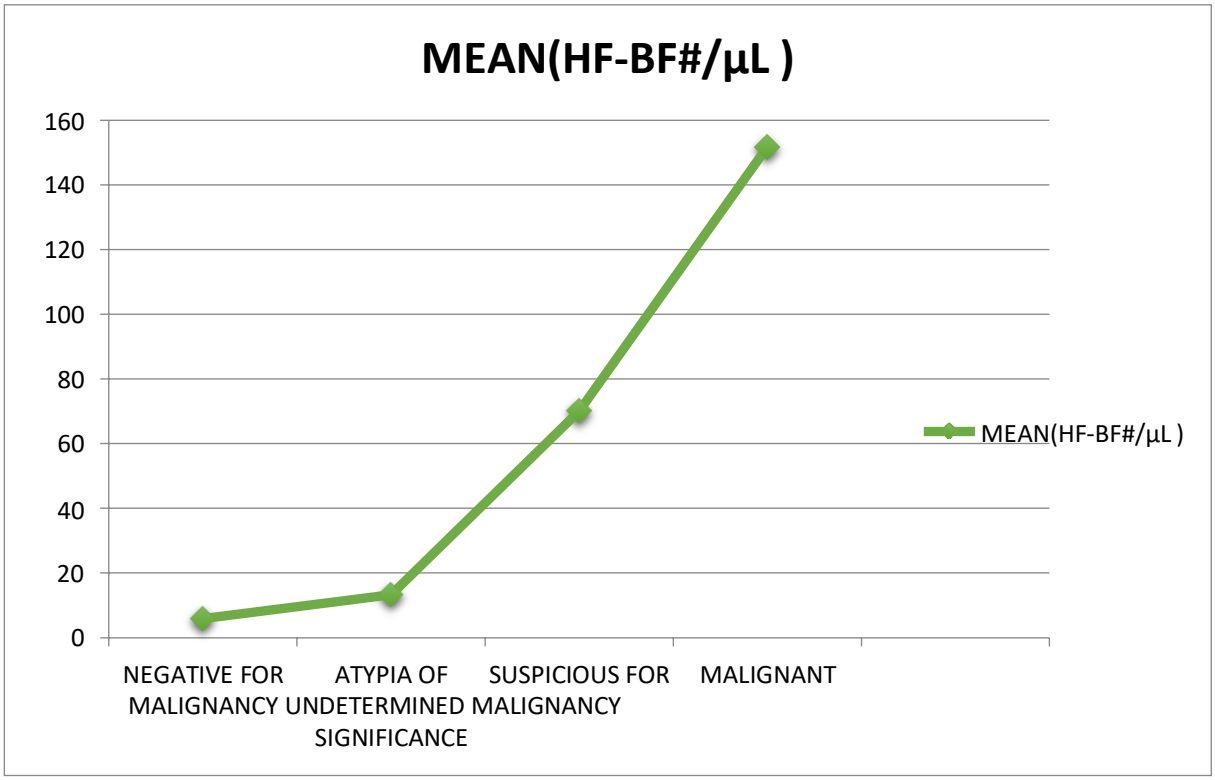

Fig 10- MEAN of HF-BF#/µL WBC for each category of body

ROC ANALYSIS-

ROC analysis was done to evaluate the ability of HF-BF parameters to differentiate benign fluids from malignant fluids. For ROC analysis, cases with cytology diagnosis as NFM and AUS were combined together as negative cases and are designated code “0”, similarly cases with cytology diagnosis SFM and MAL are combined together as positive cases and designated code “1”.

ROC ANALYSIS OF HF-BF%/100 WBC PARAMETER-

ROC analysis was done to evaluate the ability of HF-BF parameter to differentiate benign fluids from malignant fluids. The cutoff for HF- BF%/100WBCs, sensitivity, specificity and p- value obtained for all body fluids together as well as for individual body fluids. All data obtained are being presented below (Table 13 and Table 14).

The corresponding ROC for all categories are plotted subsequently.

TYPE OF FLUID	BENIGN	MALIGNANT	CUT OFF(%/100 WBCs)
ASCITIC FLUID	34	13	>4.3
PLEURAL FLUID	30	16	>6.32
CSF	17	2	>1.03
ALL FLUIDS	81	31	>4.56

Table 13- cutoff values for HF-BF% to differentiate benign and malignant fluids

TYPE OF FLUID	AUC	P value	95% CI	SENSITIVITY (%)	SPECIFICITY (%)
ASCITIC FLUID	0.95	<0.001	0.96 to 0.84	84.6	91.18
PLEURAL FLUID	0.92	<0.001	0.81 to 0.98	93.75	96.67
CSF	0.64	0.68	0.39 to 0.84	50.0	100.0
ALL FLUIDS	0.92	<0.001	0.85 to 0.96	87.1	93.8

Table 14- Statistical parameters obtained for HF-BF% in detecting malignant cells

ROC ANALYSIS OF ALL BFS FOR HF-BF% TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 15) (Fig 11)

TYPE	AUC and P value	CUT OFF (%/100 WBCs)	SENSITIVITY (%)	SPECIFICITY (%)	REMARK
ALL FLUIDS	AUC-0.92 P <0.001	>4.56	87.1	93.8	Statistically significant

Table 15- ROC analysis of all fluids for HF-BF%/100 WBCs

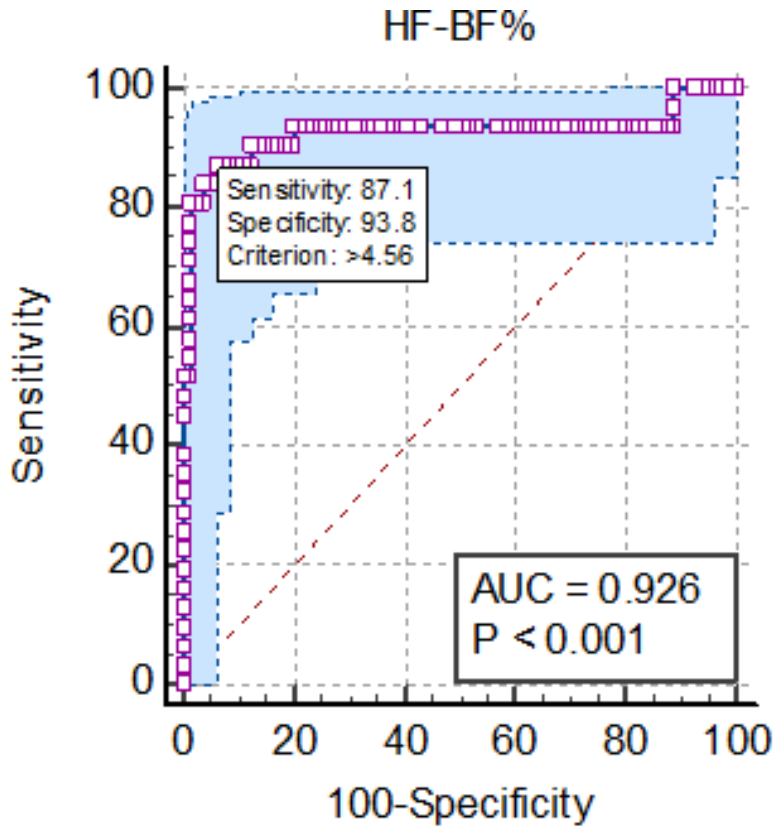

Fig 11- ROC of all fluids for HF-BF%/100 WBCs

ROC ANALYSIS OF ASCITIC FLUIDS FOR HF-BF% TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 16) (Fig 12)

TYPE	AUC and P value	CUT OFF (%/100 WBCs)	SENSITIVITY (%)	SPECIFICITY (%)	REMARK
ASCITIC FLUID	AUC-0.95 P <0.001	>4.3	84.6	91.18	Statistically significant

Table 16- ROC analysis of AFs for HF-BF%/100 WBCs

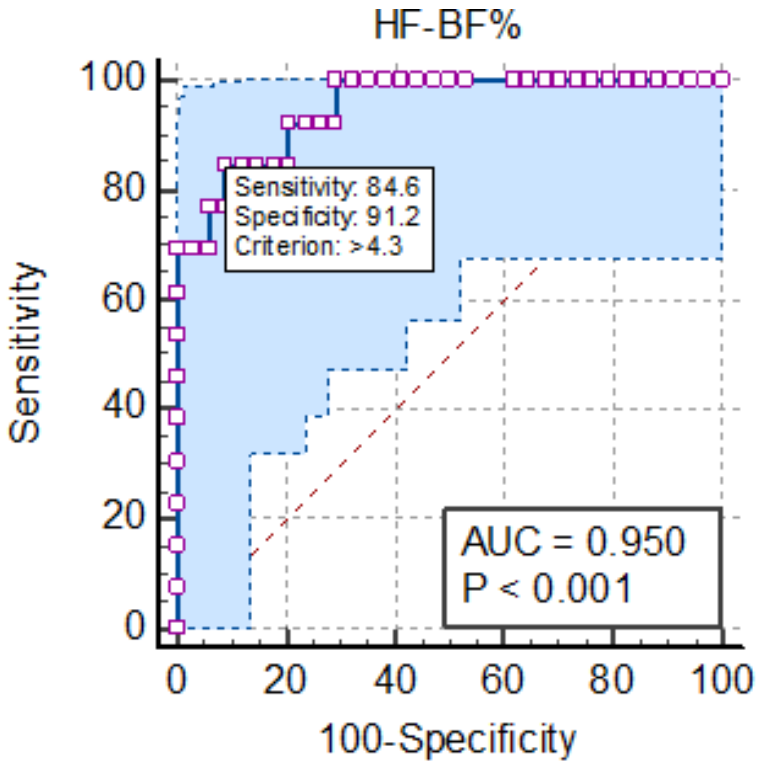

Fig 12- ROC of AFs for HF-BF%/100 WBCs

ROC ANALYSIS OF PLEURAL FLUIDS FOR HF-BF% TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 17) (Fig 13)

TYPE	AUC and P value	CUT OFF (%/100 WBCs)	SENSITIVITY (%)	SPECIFICITY (%)	REMARK
PLEURAL FLUID	AUC-0.92 P <0.001	>6.32	93.75	96.67	Statistically significant

Table 17- ROC analysis of pleural fluids for HF-BF%/100 WBCs

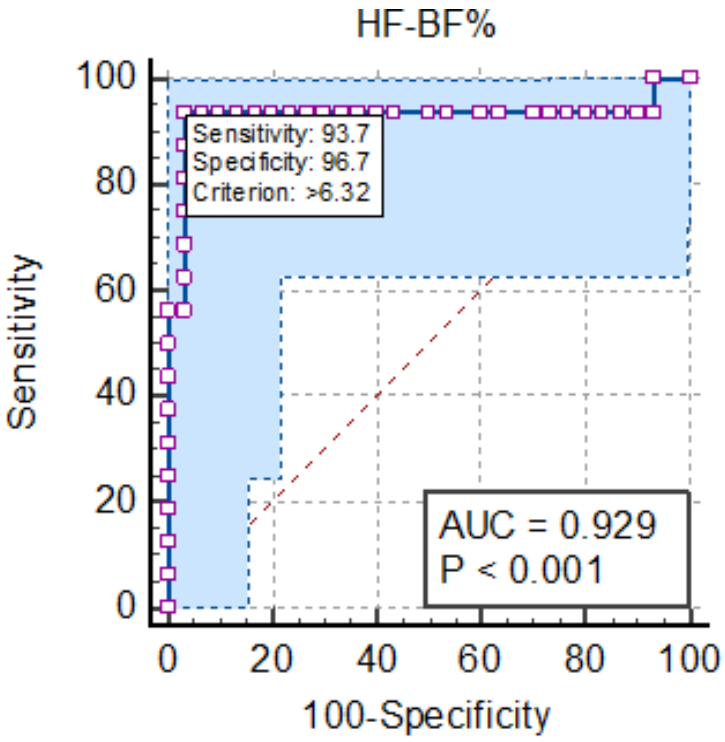

Fig 13- ROC of pleural fluids for HF-BF%/100 WBCs

ROC ANALYSIS OF CSF FOR HF-BF% TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 18) (Fig 14)

TYPE	AUC and P value	CUT OFF (%/100 WBCs)	SENSITIVITY (%)	SPECIFICITY (%)	REMARK
CSF	AUC-0.64 P <0.68	>1.03	50.0	100.0	Statistically significant

Table 18- ROC analysis of CSF for HF-BF%/100 WBCs

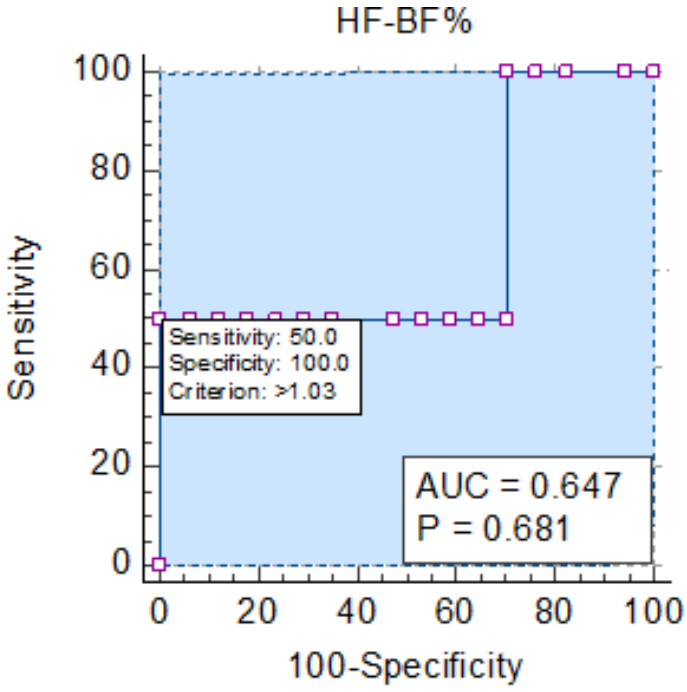

Fig 14- ROC of CSF for HF-BF%/100 WBCs

ROC ANALYSIS OF HF-BF# PARAMETER TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 19 and Table 20)

TYPE OF FLUID	BENIGN	MALIGNANT	CUT OFF(#/µL WBCs)
ASCITIC FLUID	34	13	>9.32
PLEURAL FLUID	30	16	>12.25
CSF	17	2	>1.7
ALL FLUIDS	81	31	>12.25

Table 19- cutoff values for HF-BF#/µL to differentiate benign and malignant fluids

TYPE OF FLUID	AUC	P value	95% CI	SENSITIVITY (%)	SPECIFICITY (%)
ASCITIC FLUID	0.76	<0.004	0.61 to 0.87	69.23	82.35
PLEURAL FLUID	0.91	<0.001	0.79 to 0.97	87.5	93.33
CSF	0.70	0.40	0.45 to 0.88	100.0	47.06
ALL FLUIDS	0.814	<0.0001	0.72 to 0.88	74.19	90.12

Table 20- Statistical parameters obtained for HF-BF#/µL in detecting malignant cells

ROC ANALYSIS OF ALL BFS FOR HF-BF# TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 21) (Fig 15)

TYPE	AUC and P value	CUT OFF (#/µL)	SENSITIVITY (%)	SPECIFICITY (%)	REMARK
ALL FLUIDS	AUC-0.814 P <0.0001	>12.25	74.19	90.12	Statistically significant

Table 21- ROC analysis of all fluids for HF-BF#/µL

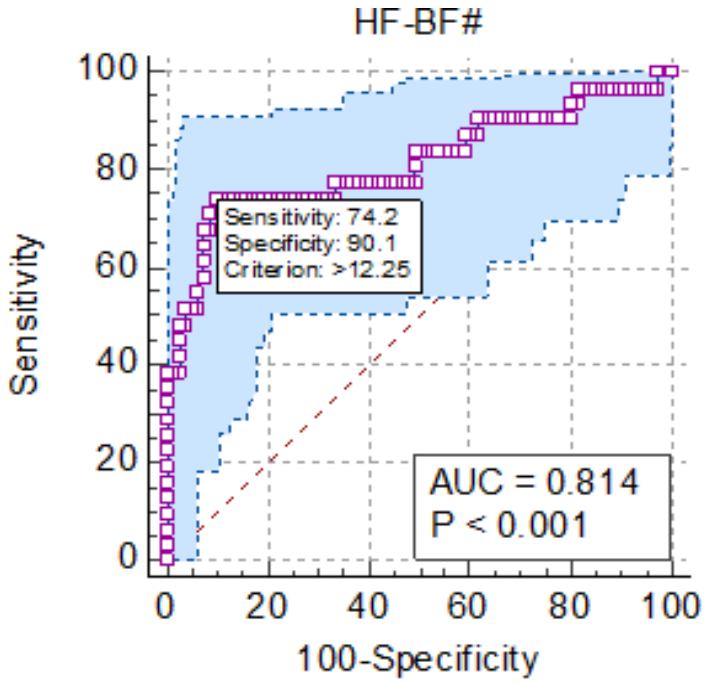

Fig 15- ROC of all fluids for HF-BF#/µL

ROC ANALYSIS OF ASCITIC FLUIDS FOR HF-BF# TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 22) (Fig 16)

TYPE	AUC and P value	CUT OFF (#/µL)	SENSITIVITY (%)	SPECIFICITY (%)	REMARK
ASCITIC FLUID	AUC-0.76 P <0.004	>9.32	69.23	82.35	Statistically significant

Table 22- ROC analysis of AFs for HF-BF#/µL

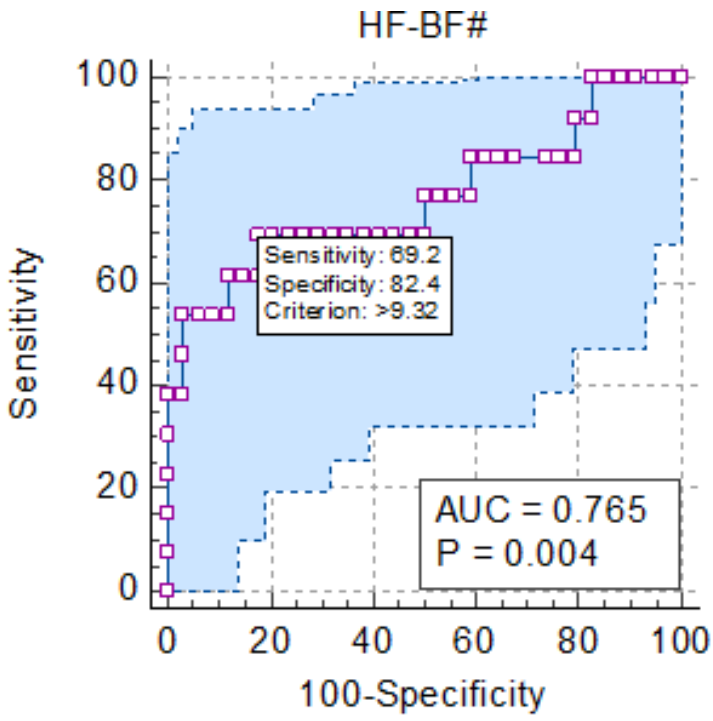

Fig 16- ROC of AFs for HF-BF#/µL

ROC ANALYSIS OF PLEURAL FLUIDS FOR HF-BF# TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 23) (Fig 17)

TYPE	AUC and P value	CUT OFF (#/μL)	SENSITIVITY (%)	SPECIFICITY (%)	REMARK
PLEURAL FLUID	AUC-0.91 P <0.001	>12.25	87.5	93.33	Statistically significant

Table 23- ROC analysis of pleural fluids for HF-BF#/μL

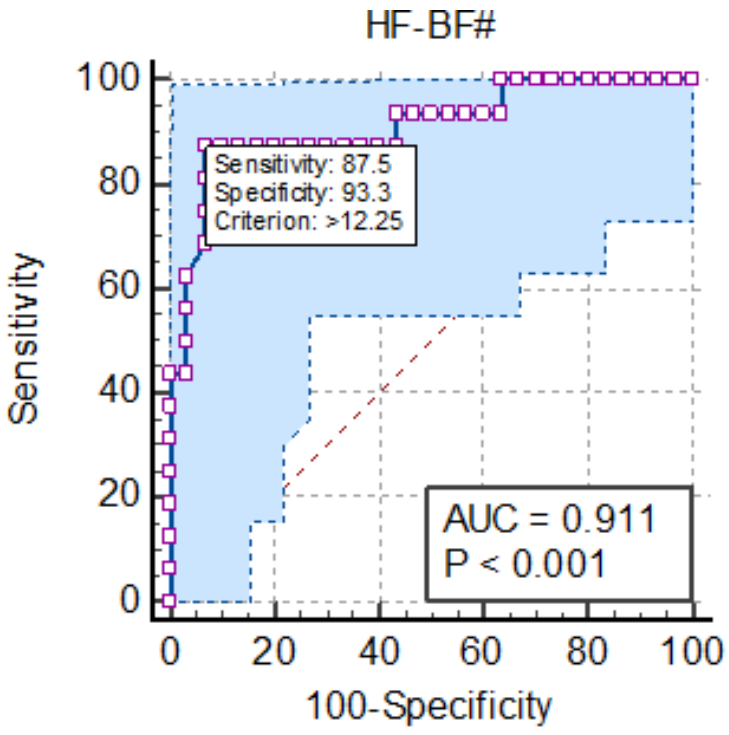

Fig 17- ROC of Pleural fluids for HF-BF#/μL

ROC ANALYSIS OF CSF FOR HF-BF# TO DIFFERENTIATE MALIGNANT FLUIDS FROM BENIGN FLUIDS (Table 24) (Fig 18)

TYPE	AUC and P value	CUT OFF (#/µL)	SENSITIVITY (%)	SPECIFICITY (%)	REMARK
CSF	AUC-0.70 P = 0.40	>1.7	100.0	47.06	Statistically significant

Table 24- ROC analysis of CSF for HF-BF#/µL

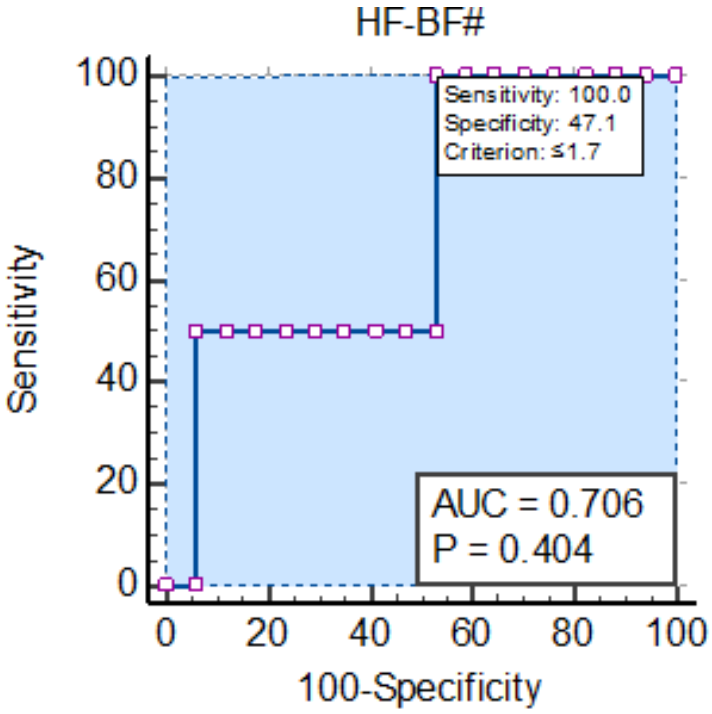

Fig 18- ROC of CSF for HF-BF#/µL

ADDITIONAL OBSERVATIONS WERE DONE IN THE PRESENT STUDY-

CORRELATION BETWEEN TOTAL COUNT BY MANUAL METHOD AND AUTOMATED METHOD AND COMPARATIVE RESULTS

The total WBC count is calculated for all body fluids manually with the help of a Neubauer chamber. TC-BF and WBC-BF of all body fluids are obtained after running the fluids in Sysmex XN 1000 hematology analyzer. The coefficient of correlation between Manual TC and TC-BF is found to be r=1.000, signifying a perfect positive correlation. The p-value of the correlation between Manual TC and TC-BF is 0.0001, which is statistically significant. (Table 25) (Fig 19)

Correlation between	Correlation coefficient	P Value	Remark
Manual TC and TC-BF	r=1.000	P=0.0001	Perfect positive correlation Statistically significant

Table 25- Correlation between manual TC and TC-BF

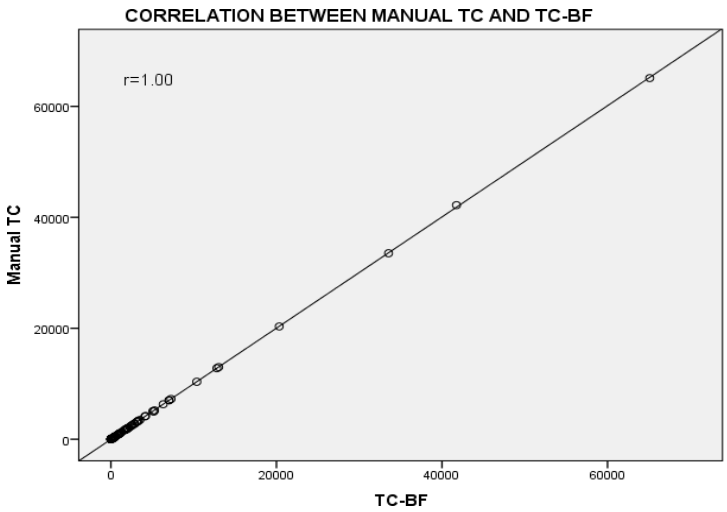

Fig 19- Co-efficient of correlation(r) between manual TC and TC-BF

The coefficient of correlation between Manual TC and WBC-BF is found to be r=1.000, signifying a perfect positive correlation.

The p-value of the correlation between Manual TC and WBC-BF is 0.0001, which is statistically significant. (Table 26) (Fig 20)

Correlation between	Correlation coefficient	P Value	Remark
Manual TC and WBC-BF	r=1.000	P=0.0001	Perfect positive correlation Statistically significant

Table 26- Correlation between manual TC and WBC-BF

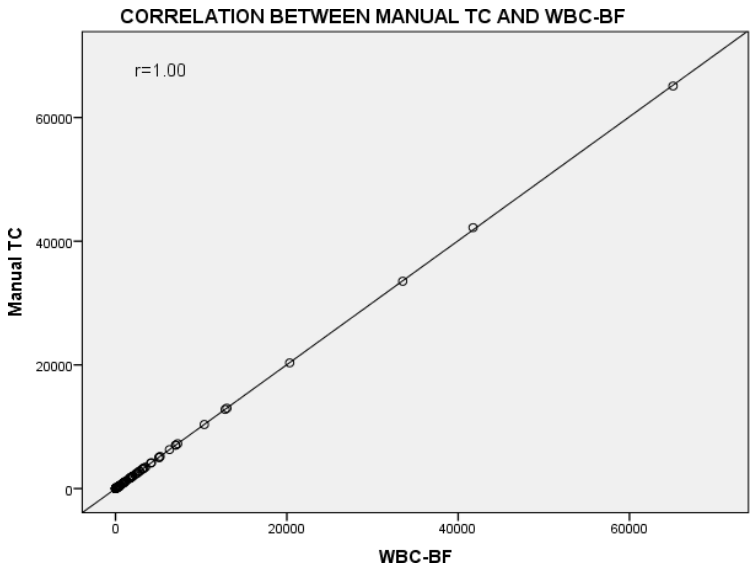

Fig 20- Co-efficient of correlation(r) between manual TC and WBC-BF

DISCUSSION

In standard laboratory practise, automated analyzers are gradually replacing manual techniques by incorporating artificial intelligence (AI) in fluid cytology. With rapid emergence and upgradation of AI, it is now possible to reduce complex practical problems arising in cytopathology to detect malignant cells.[52]

High-fluorescent cells (HF-cells), signal cells with a high N:C ratio and a high nucleic acid content. It has been suggested that the existence of HF cells, which are related to mesothelial and/or cancerous cells, is a sign that microscopic examination is necessary[38]. However, because there is no methodological standardisation[38] and there is disagreement among researchers regarding the number or proportion of HF-cells that indicate the necessity for review by conventional cytology. Many primary objectives and standards have been employed in research, the majority of these investigations used Sysmex analyzers[.32,1,39–47].

TYPE OF FLUID	HF-BF%(CUT-OFF)		HF-BF#(CUT-OFF)	
	Rastogi L, et al. [1]	**OUR STUDY**	**Rastogi L, et al. [1]**	**OUR STUDY**
ASCITIC FLUID	>3.95	>4.3	>17	>9.32
PLEURAL FLUID	>4.05	>6.32	>17	>12.25
CSF	>0.75	>1.03	>1	>1.7
ALL FLUID	>2.85	>4.56	>12	>12.25

Table 27- Comparison of Cut off obtained for HF-BF% and HF-BF# between our study and **Rastogi L, et al. [1]**

Our study results are in concordance with the study done by **Rastogi L, et al.** [1] Their study results show that for malignant BF samples, the HF- BF% and HF -BF# were higher than that of benign body fluid samples. In our study HF-BF%/100 WBCs for NFM, AUS, SFM, and MAL Body Fluid samples are (1.17± 1.69), (6.34± 1.7), (11.02 ± 6.74) and (26.65 ± 6.56) respectively. HF-BF# µL for NFM, AUS, SFM, and MAL Body Fluid samples are (5.99 ± 10.75), (13.32 ± 10.60), (70.18 ± 104.8) and (151.78 ± 134.9) respectively.

STATISTICAL PARAMETERS	HF-BF%		HF-BF#	
	Rastogi L, et al. [1]	OUR STUDY	Rastogi L, et al. [1]	OUR STUDY
SENSITIVITY	64.4	87.1	71.2	74.19
SPECIFICITY	61.4	93.8	71.2	90.12
AUC	0.7	0.92	0.76	0.81

Table 28- Comparison of sensitivity, specificity and AUC obtained for HF-BF parameters between our study and **Rastogi L, et al.** [1]

Xu W, et al [45] studied serous cavity effusion to evaluate the efficacy of Sysmex XN-1000 hematology analyzer to screen malignant cells. Both manual and automated methods for cell counting and high-fluorescent cells (HF Cells) were studied and they analysed ROC curve for collected data, which showed AUC=0.7 when the cut-off value of HF% was 4.4% and HF# was 24.5/µL. In our study cut off value for HF-BF% is found to be 4.56 with AUC=0.92 and cut off value for HF-BF# is found to be 12.25 with AUC= 0.81. Study done by **Rastogi L, et al.** [1]

showed cut off value for HF-BF% is 2.85 with AUC=0.70 and cut off value for HF-BF# is >12 with AUC= 0.76. [1]

Buoro S et al [2]. studied the XN-BF module for analysis of ascitic and pleural fluids and found Pearson's correlations, p<0.001, and high diagnostic concordance (Area Under the Curve between 0.85 and 0.99). They concluded that XN-BF data increased the sensitivity and specificity of BFs classification to 98% and 95%. According to the authors, all specimens with a count of more than 50 HF cells per millilitre are a sign of microscopic review. In our study we have analysed ROC for all body fluids as well as individual type of body fluids and derived Pearson's correlations between benign and malignant fluids. We found AUC (area under curve) = 0.926, p<0.0001 with sensitivity of 87.10% and specificity 93.83% when ROC analysis was done for HF-BF% parameter for all 112 body fluids in detecting malignancy. Similarly, AUC =0.814, p<0.0001 with sensitivity of 74.19% and specificity 90.12% was found when ROC analysis was done for HF-BF# parameter for all 112 body fluids in detecting malignancy.

Our study results are in concordance with the study done by **Cognialli RCR, et al [3].** They studied a total of 56 samples (35 ascitic and 21 pleural fluids) which were analyzed by manual microscopy and an XE-5000 automated hematology analyzer. HF-BF showed high PPV was found for both fluids [3]. In our study we have observed perfect positive correlation between TC-BF and manual cell count as well as between WBC-BF and manual cell count (r=1), with a p value <0.001.

Ai T, et al [4] studied 92 body fluids to develop a new flowcytometry-based gating analysis mode XN-BF gating algorithm to detect malignant cells using a Sysmex XN-1000 automated hematology analyzer. The XN-BF gating algorithm showed a sensitivity of 63.0% and

specificity of 87.8% with PPV-68.0% and NPV-85.1% in detecting malignancy. [4] In our study sensitivity and specificity of HF-BF parameter in detecting malignancy is in correlation with above study.

Our study results are in concordance with the study conducted by **Aulesa C et al [6],** in which they studied the reliability of the automated hematology analyzer by calculating the WBC count of 179 body fluids. The automated WBC counts of CSF are correlated with counts obtained by a manual method in a Neubauer chamber (r = 0.958; P = .0001). In our study correlation coefficient between manual TC and TC-BF is r=1.000, showing a perfect correlation. Our study shows a statistically significant correlation between automated WBC count and manual WBC count.

Labaere D et al [26] evaluated BF mode of the Sysmex XN-2000 hematology analyzer in detecting malignant cells in body fluids. 49 of 230 samples were malignant in microscopic examination and malignant samples showed significantly high HF-BF% (10.2/100WBC) than benign samples (2.6/100 WBC) and higher HF-BF# for malignant samples (65/μL) than bdnign samples (10/μL) ($p < 0.001$).

Cho YU, et al. [41] studied BF mode in XN-2000 automated hematology analyzer to detect presence of malignant cells and found that HF-BF cells were identified significantly more frequently in malignant samples (17.8/100 WBC) compared to benign samples (4.15/100 WBC)($P < 0.001$). ROC analysis gave a cut off for HF-BF% of 6.9/100 WBC for detecting malignant samples (AUC of 0.791). In our study cut off value for HF-BF% is found to be 4.56 with AUC=0.92.

AUTHOR	HF-BF%(MEAN)		HF-BF#(MEAN)	
	BENIGN	MALIGNANT	BENIGN	MALIGNANT
OUR STUDY	1.12	26.65	5.99	151.78
Rastogi L, et al. [1]	4.41	24.8	19.57	329.86
Labaere D et al [26]	2.6	10.2	10	65
Cho YU, et al. [41]	4.15	17.8	N/A	N/A

Table 29- Comparison of MEAN values obtained for HF-BF parameters between our study and other studies

The requirement for evaluation by optical microscopy has been determined by a number of methods, which include HF-BF value.[32,33,40,45,46]. According to a recent study, HF-BF count shows increased sensitivity when it is analysed in conjuction with clinical information. [46]

Manual evaluation of BFs, preferably on cytospin smears, must be used to validate automated results. [22–24] BF modes currently provide HF-BF parameters under its research parameters, thus it can be used for detecting presence of malignant cells or to screen body fluid samples as a part of routine body fluid evaluation along with cytological examination.

In addition to the existing limitations of manual technique such as imprecision, inter-observer variability and subjectivity, longer TAT and affect of cytospin may affect the proportion of cell types.[14,25] Thus combination of automation and manual technique will increase precision of diagnosis.

CONCLUSION

High Fluorescence-Body Fluid parameters, both HF- BF% and HF- BF#, given by Sysmex XN 1000 automated hematology analyzer shows excellent correlation ($p<.0001$) with conventional cytological method in the detection of malignant cells in the body fluids. It also reduces turn- around-time (TAT) and minimize labor intensive workload.

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ANNEXURE – I

B.L.D.E. (DEEMED TO BE UNIVERSITY) IEC/NO.09/2021 Date-22/01/2021

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated: 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Evaluation of high-fluorescence body fluid (HF-BF) parameter as a diagnostic tool for malignancy in body fluids using sysmex XN-1000 automated hematology analyser

Name of PG student: Dr Sultana S Talukdar , Department of Pathology

Name of Guide/Co-investigator: Dr R M Potekar , Professor Pathology

DR .S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

Inward No : 23
Date : 17/6/2021

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed-to-be-University u/s 3 of UGC Act, 1956

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE(DU)/REG/PG-Guide/2021-22/518 June 16, 2021

To,
The Professor and HOD
Department of Pathology,
BLDE (DU)'s Shri B. M. Patil Medical College,
Hospital and Research Centre,
Vijayapura

Sir,

Sub: Regarding change of PG Guide.
Ref: Your letter no. Path/2021/420 dated 1st June, 2021.

With reference to the subject and letter cited above, on approval of the Hon'ble Vice-Chancellor, the change of PG Guide is permitted in respect of PG Student of your department as per below:

Sl. No.	Name of the Student	Previous Guide	New Guide	Batch/ Year
1.	Dr. Sahitya H.	Dr. R. M. Potekar	Dr. S. U. Arakeri	2018 - 19
2.	Dr. Saswati Subhadarshini		Dr. Vijayalaxmi Patil	2019 - 20
3.	Dr. Sultana Shahnaz		Dr. S. B. Hipparagi	2020 - 21
4.	Dr. Anin Prakash		Dr. Savitri Nerune	2020 - 21

This is for your information and needful.

REGISTRAR
REGISTRAR
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Copy to:
- The Dean, Faculty of Medicine and Principal
- The Controller of Examinations
- The Concerned PG Teacher

Circulate to concerned PG guides & teachers.

Prof. & HOD
Dept. of Pathology
BLDE (Deemed to be University)
Shri B. M. Patil Medical College,
VIJAYAPUR

Smt. Bangaramma Sajjan Campus, Sholapur Road, Vijayapura - 586 103, Karnataka
University: Phone: +918352-262770, Fax: +918352-263303, Website: www.bldeu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, Website: www.bldeu.ac.in

ANNEXURE-II

B.L.D.E (DEEMED TO BE UNIVERSITY) SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYPURA-586103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT: "EVALUATION OF HIGH-FLUORESCENCE BODY FLUID (HF-BF) PARAMETER AS A DIAGNOSTIC TOOL FOR MALIGNANCY IN BODY FLUIDS USING AUTOMATED HEMATOLOGY ANALYSER"

PRINCIPAL INVESTIGATOR: Dr.SULTANA SHAHNAZ ZABIN TALUKDAR
P.G. DEPARTMENT OF PATHOLOGY

PG GUIDE : DR. SUREKHA B. HIPPARGI MD
PROFESSOR, DEPT OF PATHOLOGY.

PURPOSE OF RESEARCH:

I have been informed that the present study is a study to analyze the High Fluorescence-Body Fluid parameter(both HF-BF# and HF-BF%) given by Sysmex XN-1000 and to study its correlation with conventional cytological method to detect the presence of malignant cells in the BFs.

PROCEDURE:

I understand that I undergo detailed history and after which necessary investigations will be done.

RISK AND DISCOMFORTS:

I understand that, there is no risk involved for me being a part of the study.

BENEFITS:

I understand that my participation in the study will help to find a reliable alternate for manual technique of Body Fluid assessment.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If data is used for publications the identity of patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I might be asked for more information about my disease at any time.

REFUSAL FOR WITHDRAWL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have read and fully understood this consent form. Therefore, I agree to participate in the present study.

_____

Participant/Guardian Date:

_____

Signature of Witness Date:

I have explained the patient/patients attender the purpose of study, the procedure required and possible risk and benefit of my ability in the vernacular language.

_____

Investigator/P.G Date:

_____

Witness to Signature Date:

ANNEXURE - III

PROFORMA

Name : OP/IP No. :

Age :

Sex : D.O.A :

D.O.D :

Presenting Complaints :

Similar history in family :

Other Investigations performed:

Clinical Diagnosis:

EVALUATION OF BFs:

1. Total volume:
2. Appearance:
   Colour:
   Consistency:

AUTOMATED BODY FLUID ANALYSIS

Parameters	***Value***
HF-BF% /100 WBCs	
HF-BF #/µl	
TC-BF	
WBC- BF	
PMNs	

MNs	
PMN%	
MN%	

MANUAL CELL COUNTING:

Parameters	***Value***
Total count	
MN%	
PMN %	
Others	
Atypical cells	
Remark	

CYTOLOGIACAL EXAMINATION:

Parameters	***Value***
PMN %	
MN%	
Reactive cells	
Atypical cells	
Background	
Others	
Remark	

DIAGNOSIS

KEY TO MASTER CHART

Sl No.	Serial Number
TC-BF	Total nucleated cell count- Body Fluid
WBC-BF	White blood cells- Body Fluid
HF-BF%	High- fluroscence- body fluid%
HF-BF#	High- fluroscence- body fluid#
Manual TC	Manually calculated Total cell count
Dx	Diagnosis (cytological)

MASTER CHART

Sl no	Age (Y)	sex	Fluid	HF-BF%	HF-BF#	TC-BF	WBC-BF	Manual TC	Dx
1	39	M	CSF	1.03	5.234	488	484	350	NEGATIVE FOR MALIGNANCY
2	38	M	Pleural fluid	0.02	1.256	68	65	60	NEGATIVE FOR MALIGNANCY
3	60	F	Pleural fluid	1.05	4.002	3300	3297	3300	NEGATIVE FOR MALIGNANCY
4	80	F	Ascitic fluid	4.22	1.24	7037	7030	7030	NEGATIVE FOR MALIGNANCY
5	65	M	Ascitic fluid	1.99	38.74	5068	5065	5060	NEGATIVE FOR MALIGNANCY
6	43	F	Ascitic fluid	0.22	34.235	310	307	300	NEGATIVE FOR MALIGNANCY
7	45	M	Ascitic fluid	0.02	1.005	30	30	30	NEGATIVE FOR MALIGNANCY
8	33	F	CSF	0	0	4	4	4	NEGATIVE FOR MALIGNANCY

9	33	F	CSF	0.68	0	4	4	4	NEGATIVE FOR MALIGNANCY
10	60	F	Ascitic fluid	2.04	1.07	450	444	450	NEGATIVE FOR MALIGNANCY
11	35	M	CSF	0.001	0.01	5	5	5	NEGATIVE FOR MALIGNANCY
12	61	M	Ascitic fluid	1.61	1.035	112	109	110	NEGATIVE FOR MALIGNANCY
13	57	M	Ascitic fluid	0.88	0.325	216	211	210	NEGATIVE FOR MALIGNANCY
14	32	M	Ascitic fluid	1.005	16.23	1868	1863	1860	NEGATIVE FOR MALIGNANCY
15	40	F	CSF	0.67	0.35	33	30	30	NEGATIVE FOR MALIGNANCY
16	52	M	Ascitic fluid	1.004	3.36	927	922	920	NEGATIVE FOR MALIGNANCY
17	30	F	Pleural fluid	1.03	55.56	1764	1758	1750	NEGATIVE FOR MALIGNANCY
18	51	M	Ascitic fluid	0.36	4.26	127	125	120	NEGATIVE FOR MALIGNANCY
19	32	F	Pleural fluid	1.02	0.98	2449	2441	2450	NEGATIVE FOR MALIGNANCY
20	55	M	Ascitic fluid	0.01	1.258	20	20	20	NEGATIVE FOR MALIGNANCY
21	38	M	Pleural fluid	13.6	3.57	1840	1834	1830	NEGATIVE FOR MALIGNANCY
22	45	M	Ascitic fluid	1.05	1.25	117	115	115	NEGATIVE FOR MALIGNANCY
23	18	F	Pleural fluid	1.7	2.28	1540	1532	1550	NEGATIVE FOR MALIGNANCY
24	29	M	Ascitic fluid	1.005	3.335	167	162	160	NEGATIVE FOR MALIGNANCY
25	55	F	CSF	0.38	1.254	2	2	2	NEGATIVE FOR MALIGNANCY
26	50	M	Ascitic fluid	0.26	2.25	360	355	360	NEGATIVE FOR MALIGNANCY
27	65	M	Ascitic fluid	0.21	0.009	164	160	160	NEGATIVE FOR MALIGNANCY
28	56	M	CSF	0.004	1.298	28	25	30	NEGATIVE FOR MALIGNANCY
29	54	M	Pleural fluid	0.036	0.027	56	52	55	NEGATIVE FOR MALIGNANCY

30	61	F	Pleural fluid	1.04	0.115	149	143	145	NEGATIVE FOR MALIGNANCY
31	25	M	Ascitic fluid	1.08	9.32	309	300	325	NEGATIVE FOR MALIGNANCY
32	46	M	CSF	0.007	0.47	7	7	10	NEGATIVE FOR MALIGNANCY
33	13	F	Pleural fluid	1.69	3.24	2800	2789	2800	NEGATIVE FOR MALIGNANCY
34	65	M	Pleural fluid	1.21	1.25	4200	4192	4200	NEGATIVE FOR MALIGNANCY
35	34	M	Ascitic fluid	0.044	2.54	50	46	55	NEGATIVE FOR MALIGNANCY
36	40	F	CSF	1.009	2.98	515	509	515	NEGATIVE FOR MALIGNANCY
37	68	M	Pleural fluid	1.02	1.68	427	421	420	NEGATIVE FOR MALIGNANCY
38	55	M	Pleural fluid	1.01	1.69	150	142	140	NEGATIVE FOR MALIGNANCY
39	68	M	Pleural fluid	0.98	1.47	278	270	270	NEGATIVE FOR MALIGNANCY
40	14	M	Ascitic fluid	0.25	3.97	111	106	106	NEGATIVE FOR MALIGNANCY
41	55	M	CSF	0.067	2.69	60	54	60	NEGATIVE FOR MALIGNANCY
42	56	M	CSF	1.006	2.1002	102	97	90	NEGATIVE FOR MALIGNANCY
43	60	M	Pleural fluid	1.85	4.687	1000	989	1000	NEGATIVE FOR MALIGNANCY
44	60	F	Pleural fluid	1.1	2.25	3500	3496	3480	NEGATIVE FOR MALIGNANCY
45	65	M	Pleural fluid	1.33	6.74	389	382	380	NEGATIVE FOR MALIGNANCY
46	69	F	Ascitic fluid	0.04	5.21	49	45	45	NEGATIVE FOR MALIGNANCY
47	28	M	Ascitic fluid	0.66	6.69	525	520	520	NEGATIVE FOR MALIGNANCY
48	42	M	CSF	0.001	1.24	11	10	10	NEGATIVE FOR MALIGNANCY
49	52	F	Ascitic fluid	1.03	1.35	322	316	315	NEGATIVE FOR MALIGNANCY
50	44	F	Pleural fluid	0.94	3.54	88	81	80	NEGATIVE FOR MALIGNANCY

51	61	M	Pleural fluid	1.9	8.79	702	701	700	NEGATIVE FOR MALIGNANCY
52	47	M	Pleural fluid	0.02	6.473	220	218	220	NEGATIVE FOR MALIGNANCY
53	51	M	Ascitic fluid	1.06	3.547	205	200	200	NEGATIVE FOR MALIGNANCY
54	37	F	Ascitic fluid	1.005	2.58	209	201	210	NEGATIVE FOR MALIGNANCY
55	37	F	Ascitic fluid	1.33	2.58	209	201	200	NEGATIVE FOR MALIGNANCY
56	59	M	CSF	0.05	3.687	25	25	25	NEGATIVE FOR MALIGNANCY
57	39	M	Ascitic fluid	3.14	1.645	3303	3300	3300	NEGATIVE FOR MALIGNANCY
58	42	F	Ascitic fluid	0.08	6.25	115	109	110	NEGATIVE FOR MALIGNANCY
59	36	M	CSF	0.11	4.69	54	49	50	NEGATIVE FOR MALIGNANCY
60	25	M	Pleural fluid	0.05	0.268	43	40	40	NEGATIVE FOR MALIGNANCY
61	28	M	CSF	0.67	0.691	17	15	15	NEGATIVE FOR MALIGNANCY
62	53	M	Pleural fluid	1.15	8.57	347	340	340	NEGATIVE FOR MALIGNANCY
63	49	M	CSF	0.8	1.345	16	14	12	NEGATIVE FOR MALIGNANCY
64	41	M	Ascitic fluid	2.1	1.258	543	540	540	NEGATIVE FOR MALIGNANCY
65	61	M	Pleural fluid	1.04	4.658	155	151	150	NEGATIVE FOR MALIGNANCY
66	42	M	Pleural fluid	0.78	6.35	191	182	180	NEGATIVE FOR MALIGNANCY
67	46	F	Ascitic fluid	2.55	1.98	916	899	900	NEGATIVE FOR MALIGNANCY
68	67	M	Pleural fluid	0.22	7.69	479	470	470	NEGATIVE FOR MALIGNANCY
69	39	F	CSF	0.753	9.85	57	54	54	NEGATIVE FOR MALIGNANCY
70	45	F	Ascitic fluid	2.69	1.21	3171	3167	3180	NEGATIVE FOR MALIGNANCY
71	56	F	Pleural fluid	1.1	8.21	900	890	890	NEGATIVE FOR MALIGNANCY

72	56	M	Pleural fluid	2.5	29.006	1022	1003	1010	NEGATIVE FOR MALIGNANCY
73	48	M	Pleural fluid	2.46	8.368	33543	33532	33525	NEGATIVE FOR MALIGNANCY
80	43	F	Ascitic fluid	3.1	57.112	6310	6300	6300	NEGATIVE FOR MALIGNANCY
81	28	M	Pleural fluid	2.47	12.25	65095	65088	65100	NEGATIVE FOR MALIGNANCY
74	30	F	Ascitic fluid	4.3	35.003	2549	2544	2500	ATYPIA OF UNDETERMINED SIGNIFICANCE
75	55	F	Pleural fluid	4.56	8.23	7019	7015	7020	ATYPIA OF UNDETERMINED SIGNIFICANCE
76	50	M	Ascitic fluid	5.8	7.004	7255	7250	7250	ATYPIA OF UNDETERMINED SIGNIFICANCE
77	54	M	Ascitic fluid	8.2	6.254	10374	10365	10360	ATYPIA OF UNDETERMINED SIGNIFICANCE
78	36	F	Ascitic fluid	8.88	18.2	20334	20330	20330	ATYPIA OF UNDETERMINED SIGNIFICANCE
79	65	M	Pleural fluid	6.32	5.256	41748	41741	42200	ATYPIA OF UNDETERMINED SIGNIFICANCE
82	54	M	CSF	0.023	0.002	7	5	8	SUSPICIOUS FOR MALIGNANCY
83	46	F	Ascitic fluid	19.2	1.08	2550	2500	2500	SUSPICIOUS FOR MALIGNANCY
84	50	F	Ascitic fluid	8.01	15.01	192	188	185	SUSPICIOUS FOR MALIGNANCY
85	70	F	Pleural fluid	0.03	66.08	2075	2000	2000	SUSPICIOUS FOR MALIGNANCY
86	70	F	Ascitic fluid	19.22	276.01	2310	2260	2250	SUSPICIOUS FOR MALIGNANCY

87	55	M	Pleural fluid	23.082	61.23	308	300	300	SUSPICIOUS FOR MALIGNANCY
88	25	F	Ascitic fluid	5.21	39.002	87	80	80	SUSPICIOUS FOR MALIGNANCY
89	43	F	Pleural fluid	11.92	29.006	2074	2000	2000	SUSPICIOUS FOR MALIGNANCY
90	62	M	Pleural fluid	12.02	29.038	411	400	400	SUSPICIOUS FOR MALIGNANCY
91	40	M	Ascitic fluid	12.001	39.821	845	823	823	SUSPICIOUS FOR MALIGNANCY
92	65	F	Ascitic fluid	12.336	3.01	168	150	150	SUSPICIOUS FOR MALIGNANCY
93	43	M	Ascitic fluid	3.001	135.9	13024	13000	13000	SUSPICIOUS FOR MALIGNANCY
94	62	M	Pleural fluid	18.4	167.9	1118	1100	1100	MALIGNANT
95	50	F	Ascitic fluid	29.2	2.2	1262	1250	1250	MALIGNANT
96	60	M	Pleural fluid	30.3	187.9	5266	5200	5200	MALIGNANT
97	48	F	Ascitic fluid	28.9	245.8	1802	1750	1750	MALIGNANT
98	10	F	CSF	19.2	1.7	205	200	200	MALIGNANT
99	65	F	Pleural fluid	21.572	48.01	183	180	180	MALIGNANT
100	55	F	Pleural fluid	40.9	256.056	4100	4100	4100	MALIGNANT
101	65	F	Pleural fluid	25.006	20.06	12800	12800	12800	MALIGNANT
102	29	M	Ascitic fluid	32.02	20.001	3120	3100	3100	MALIGNANT
103	80	F	Ascitic fluid	28.82	391.128	2022	2020	2020	MALIGNANT
104	75	M	Pleural fluid	18.888	328.83	1689	1685	1680	MALIGNANT
105	51	F	Pleural fluid	11.002	3.008	762	750	750	SUSPICIOUS FOR MALIGNANCY

106	70	F	Pleural fluid	19.25	4.99	1963	1900	1900	SUSPICIOUS FOR MALIGNANCY
107	60	M	Pleural fluid	9.2	17.069	288	260	250	SUSPICIOUS FOR MALIGNANCY
108	62	M	Pleural fluid	8.982	37.298	1758	1720	1720	SUSPICIOUS FOR MALIGNANCY
109	78	F	Pleural fluid	11.03	22.92	1073	1050	1050	SUSPICIOUS FOR MALIGNANCY
110	47	M	Ascitic fluid	11.01	397.001	5187	5100	5000	SUSPICIOUS FOR MALIGNANCY
111	13	M	Pleural fluid	22.01	225.009	2890	2800	2800	SUSPICIOUS FOR MALIGNANCY
112	28	M	Ascitic fluid	2.06	1.22	192	180	180	SUSPICIOUS FOR MALIGNANCY

20BMPAT010-SULTANA-EVALUATION OF HIGH-FLUORESCENCE BODY FLUID (HF-BF)

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