Original Article

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Pathology Section

Cell Block versus Cytospin in the Detection of Malignancy in Body Fluids: A Cross-sectional Study

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ABSTRACT

Introduction: Cytological examination of body fluids is a commonly performed investigation that provides information about inflammatory and malignant lesions, aiding in the diagnosis of effusion aetiology and potential differential diagnoses. The cell block technique, combined with cytospin study, offers an additional advantage in such cases.

Aim: To compare the diagnostic utility of cell block method and cytospin method in detecting malignancy in body fluids.

Materials and Methods: This cross-sectional observational study was conducted for a period of two years at Bharati Vidyapeeth (DTU) Medical College Hospital and Research Centre in Pune, Maharashtra, India. A total of 100 body fluid samples were analysed, including 73 pleural fluids, 24 ascitic fluids, and 3 pericardial fluids. Each fluid sample was divided into two equal parts: one processed for cytospin (CYTOTEK), and the other processed to create a cell block. Four Immunohistochemistry (IHC) markers (Thyroid Transcription Factor-1 (TTF-1), Cytokeratin 7 (CK7), Cytokeratin 20 (CK20), and Calretinin) were applied as needed. Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 25.0, and results were calculated using the Chi-square test.

Results: Of the total 100 fluid samples, 65 were negative, 17 were positive, and 18 were suspicious for malignancy using the cytospin method. In comparison, the cell block method identified 63 samples as negative, 29 as positive, and 8 as suspicious for malignancy. The cell block method exhibited a higher sensitivity (100.00%) and specificity (94.03%) than the cytospin method (81.82% and 88.06%, respectively) in this study.

Conclusion: Cell block method accurately diagnosed 10 cases as positive for malignancy that were initially deemed suspicious on cytospin smear. Therefore, the cell block method proved to be a superior diagnostic tool for malignancy compared to cytospin smear. Given that cell blocks allow for longer storage and additional analysis such as IHC and microarray, they should be adopted in addition to cytospin for effusion cytology.

Keywords: Effusion, Immunohistochemistry, Malignant

INTRODUCTION

Body fluids are examined for various purposes, including verifying a diagnosis or determining the origin and nature of a fluid. Cytology of body fluids is an important diagnostic test for both malignant and benign disorders [1]. Effusions can be caused by inflammatory, infectious, benign, neoplastic, malignant, and primary or metastatic malignancies, which can present diagnostic challenges due to overlapping characteristics. In the laboratory, fluids are analysed for total count, differential count, and cytological examination to determine if the fluid is benign or malignant. Cytospin and cell block diagnoses are commonly used to identify the nature of the fluid, and based on that, clinicians may send biopsies of respective tissues for Haematoxylin and Eosin (H&E) diagnosis [2]. Thus, cytological diagnostic methods serve as valuable early diagnostic tools [3]. In diagnostic cytopathology, analysing body fluids cytologically plays a crucial role. However, the variable concentration of diseased cell populations in serous effusions can make this process challenging. Various procedures, such as a standard smear preparation, cytospin, and cell block preparation, are available for cytological evaluation [4]. Examining cytology smears and cell blocks can aid in detecting malignant effusions and provide information about inflammatory and malignant serous membrane lesions [5]. It also helps to determine the origin of effusions and consider alternative diagnoses. In such cases, the cell block approach, in conjunction with cytospin examination, has shown to be beneficial [6]. Cytospin technology is universally used to study body fluids containing malignant and non malignant cells, as well as for diagnosing tissues using the monolayer technique [7]. Cytospin is primarily designed to concentrate cells in small quantities [8]. The benefits of the cell block approach are the preservation of tissue architecture and the ability to acquire several sections from a single material for various staining. In traditional cytological smears, identifying cells as malignant or reactive mesothelial cells is a diagnostic challenge [9]. Using the cell block approach and specific IHC markers, these cells can be distinguished [10]. The plasma thromboplastin cell block method is straightforward, economical, and easily adaptable in standard pathology laboratories. Therefore, this study was conducted to compare the effectiveness of cell block preparation with cytospin in the cytodiagnosis of malignancy in serous effusions obtained in the laboratory.

MATERIALS AND METHODS

This was a cross-sectional observational study in which 100 body fluid samples were analysed between July 2020 and July 2022 (two years) in the Department of Pathology of a tertiary care hospital in western India. The study was approved by the Institutional Ethics Committee (IEC) (BVDUMC/IEC/139).

Inclusion criteria: All the specimens of body fluids which were received in the laboratory for cytological evaluation were included in this study.

Exclusion criteria: CSF samples were excluded from this study.

Sample size: Sample size was calculated based on the samples received per year for the cell block. The consent was taken for collection of the samples by the treating doctor before the procedure.

Procedure

All the fluid samples were received into two parts: one part was received in EDTA vacutainer and was processed by the cytospin method, and the other part received in plain container was processed

to prepare cell blocks. The widely used plasma thromboplastin technique was employed to prepare the cell blocks using a minimum of 100 mL of the fluid sample. For cell block- the sediments of the fluid obtained from the container were centrifuged for 15 minutes at 2500 revolutions per minute (rpm). After removing the supernatant, two drops of thromboplastin and two drops of pooled plasma were added to the sediment and allowed to stand for five minutes. The sediment at the bottom of the tube was placed on filter paper, wrapped, and fixed with 10% formalin. Cell block sections were fixed with formalin and then embedded in paraffin. Sections were cut at a thickness of 4 µm and stained using H&E. IHC staining was performed on malignant or suspected malignant cases as required. In the cytospin method, the samples were placed into an automated cytospin machine (CYTOTEK) and centrifuged at 2000 rpm for 5 to 10 minutes. Sections for the IHC assay were taken on clean glass slides coated with poly-L-lysine. The markers used in this study were CK7, CK20, TTF1, and Calretinin. Slides prepared using the cytospin technique were fixed in 95% ethyl alcohol for 20-30 minutes and then stained. Two main stains, Leishman-Giemsa stain and Papanicolaou stain, were used. The stained slides were then examined under a microscope. Histopathological diagnosis was obtained from the biopsy report, which serves as the gold standard.

STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS software version 25.0. Sensitivity and specificity were calculated using the Chi-square test.

RESULTS

In this study, a total of 100 fluid samples were examined using two methods: 1) Cytospin and 2) Cell block. The mean age of the patients was 57.44 years. The majority of the fluids were pleural fluid (73%), followed by ascitic fluid (24%) and pericardial fluid (3%). Male patients accounted for 63% of the cases, while female patients accounted for 37% of the cases. The colours of the fluids were categorised as pale yellow (48%), yellow (46%), and reddish (6%). The appearance of the fluids was slightly turbid (53%), turbid (34%), clear (12%), and hazy (1%).

Out of the total 100 fluids, 63 were negative and 29 were positive for malignancy using the cell block method, while 65 were negative and 17 were positive using the cytospin method [Table/Fig-1]. Cell block method demonstrated a sensitivity of 100% and a specificity of 94.03% [Table/Fig-2]. The gold standard method considered was histopathological diagnosis based on biopsy samples from the same patients. A comparison between the cell block technique and the cytospin technique is shown in [Table/Fig-3]. Both techniques yielded negative results for malignancy in 58 fluids, and positive results for malignancy in 12 fluids. The sensitivity and specificity of the cytospin method were 81.82% and 88.06% respectively [Table/Fig-4]. Among the 18 suspicious samples in the cytospin technique, four also showed suspicion in the cell block technique, two were negative in the cell block technique, and 12 were positive in the cell block technique. Therefore, the cell block technique clearly differentiated 14 out of 18 suspicious samples into benign and malignant categories, demonstrating its advantage over the cytospin technique. The remaining four cases were reported as suspicious for malignancy in the cell block technique and were recommended for IHC markers, but unfortunately, IHC could not be performed as the patients were lost to follow-up.

Result	Cell block	Histopath	Cytospin
Positive	29	33	17
Negative	63	63	65
Suspicious	08	04	18
Total	100	100	100

[Table/Fig-1]: A comparision of cell block and cytospin technique with histopathology.

Statistics	Value	95% CI
Sensitivity (Gold standard-HPE Biopsy)	100.00%	89.42% to 100.00%
Specificity	94.03%	85.41% to 98.35%
Positive Predictive Value	89.19%	76.13% to 95.52%
Negative Predictive Value	100.00%	-
Accuracy	96.00%	90.07% to 98.90%

[Table/Fig-2]: Screening parameters of cell block technique against histopathology.

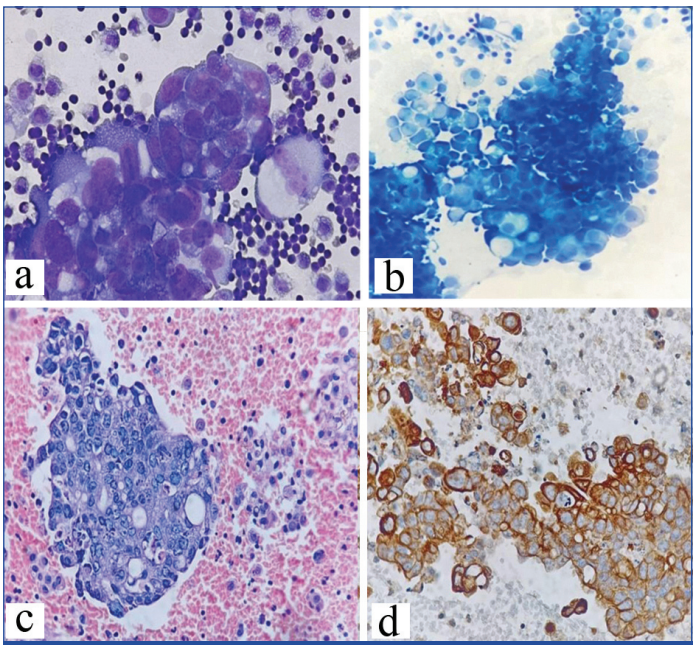
Cell block technique	Cytospin technique			Total (n=100)
	Negative	Positive	Suspicious	
Negative	58	3	2	63
Positive	5	12	12	29
Suspicious	2	2	4	8
Total	65	17	18	100

[Table/Fig-3]: A comparison of results of cell block and cytospin techniques.

Statistic	Value	95% CI
Sensitivity (Gold standard HPE)	81.82%	64.54% to 93.02%
Specificity	88.06%	77.82% to 94.70%
Positive predictive value	77.14%	63.33% to 86.83%
Negative predictive value	90.77%	82.59% to 95.32%
Accuracy	86.00%	77.63% to 92.13%

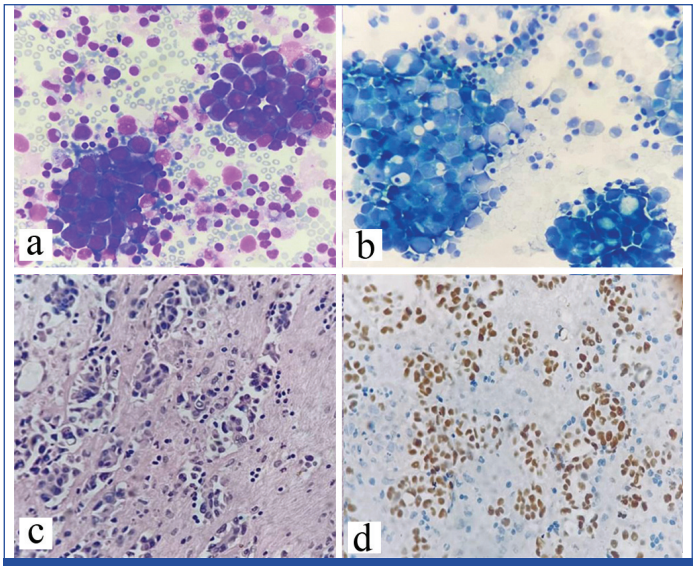
[Table/Fig-4]: Screening parameters of cytospin technique against histopathology.

[Table/Fig-5] displays an ascitic fluid sample that tested positive for malignancy using both cytospin and cell block methods, along with CK7 IHC staining. [Table/Fig-6] shows a pleural fluid sample that tested positive for malignancy using both cytospin and cell block methods, along with TTF1 IHC staining. [Table/Fig-7] presents a pericardial fluid sample that tested positive for malignancy using both cytospin and cell block methods.

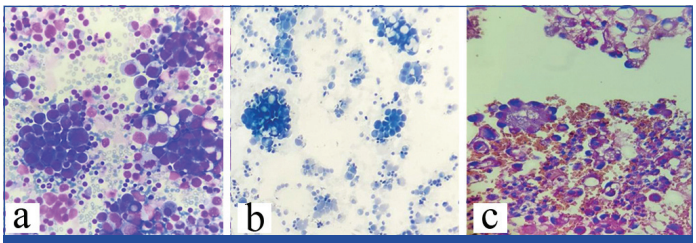


[Table/Fig-5]: a) Ascitic fluid positive for malignancy (Leishman stain 400X); b) Ascitic fluid positive for malignancy (PAP stain 400X); c) Ascitic fluid positive for malignancy cell block (H&E stain 400X); d) Ascitic fluid positive for malignancy diffuse cytoplasmic staining (CK7IHC stain 400X).

Due to limited funds, IHC markers (TTF1, CK7, CK20, and Calretinin) could only be performed on 12 out of 100 samples. The utility of IHC markers was observed in cases where there was no radiological history of a mass in any organ, as they provided clues about the possible origin of the tumour. The statistical data for IHC-TTF was found in 4% of the samples. CK7, CK20, and Calretinin were positive in 6%, 0%, and 2% of the samples, respectively.



[Table/Fig-6]: a) Pleural fluid positive for malignancy (Leishman stain 400X); b) Pleural fluid positive for malignancy (PAP stain 400X); c) Pleural fluid positive for malignancy cell block (H and E stain 400X); d) Pleural fluid positive for malignancy nuclear staining (TTF1 IHC stain 400X).



[Table/Fig-7]: a) Pericardial fluid positive for malignancy (Leishman stain 400X); b) Pericardial fluid positive for malignancy (PAP stain 400X); c) Pericardial fluid positive for malignancy cell block (H&E stain 400X).

DISCUSSION

In cytology, both cytospin and cell block techniques are essential diagnostic tools. The presence of malignant cells in effusions is usually indicative of metastasis, as primary tumours derived from mesothelial cell linings are uncommon. Cell block slides provide histopathological sections, allowing concentrated cells to be viewed in a small region. Unlike smears, which may have a bloody or soiled background, histological patterns are more discernible against a clean background. Multiple sections can be obtained and used for special staining and IHC if needed. A study by Singh M et al., found that the majority of fluids analysed were pleural (74%), followed by peritoneal effusions, which aligns with present study [11]. In present study, 73% of the samples were pleural fluid, while ascitic and pericardial fluids accounted for 24% and 3%, respectively. Another study by Vidyashree and Deepak RK received 206 fluid samples, including both ascitic fluid (50%) and peritoneal wash (50%) [12].

In present study, malignancy was detected in 33% of the fluid samples. Vidyashree VA and Deepak RK reported 49.06% of fluids as malignant [12]. Joshi A et al., found that 77.33% of effusions were non neoplastic, while 22.66% were neoplastic [4]. The proportion of malignant effusions in the study by Thapar M et al., was 36.84%, consistent with present study findings [13].

The cell block technique is a well-established method for determining the composition of bodily fluids. When using 10% alcohol and formalin as fixatives, which cause less cellular damage, the cellularity of the sample increases. This results in improved morphological details and enhanced diagnostic sensitivity. The cell block approach allows for the generation of multiple sections, which can be utilised for specific stains and immunohistochemical analyses [14].

In present study, 29% of fluid samples tested positive using the cell block technique, while 63% were negative and 8% were suspicious. Vidyashree VA and Deepak RK found that 49.06% of patients were identified as positive for malignancy on cell block analysis, and a case suspected on cytospin smear was confirmed as malignant through cell block research [12].

In the present study, a case with pleural fluid positive for malignancy and TTF1 positive was eventually confirmed as lung malignancy through biopsy. Similarly, Calretinin was also helpful in distinguishing reactive mesothelial cells from malignant cells in two cases.

The cell block technique showed 100% sensitivity and 94.3% specificity in this study, with positive and negative predictive values of 89.19% and 100% respectively.

In this study, 17% of the fluid samples were positive for malignancy using the cytospin technique. The proportion of positive and suspicious samples was 65% and 18% respectively. According to Vidyashree VA and Deepak RK, cytospin identified 47.17% as positive for malignancy, 50.94% as negative, and 1.89% as suspicious due to poor cellularity and dubious morphology on cytospin smears [12].

Present study found that cytospin alone had lower sensitivity and specificity compared to cell block in diagnosing malignant effusion. However, the combination of cytospin and cell block has advantages. It increases cellularity over standard centrifugation and ensures equal dispersion of cells [15]. Cytocentrifuge smears may cause cell flattening due to centrifugal force, resulting in higher cellular area measurement. It also enhances the recognition of irregular nuclear contours [16].

Joshi A et al., found no difference between cytospin and cell block methods for effusion analysis [4]. However, present study found that the cell block technique is superior in diagnosing malignant effusion compared to cytospin technique. The presence of malignant cells in effusion fluid presents a diagnostic challenge. Accurate identification of malignant cells and determining the type of tumour and primary site of origin is crucial for staging, prognosis, and patient management [17]. Combining the cell block treatment with conventional cytological smear improves the diagnostic yield of malignancy. Cell block has the potential to provide additional information, enhancing its sensitivity [18].

Cell block method and cytospin comparison results with other studies is given below [Table/Fig-8,9] [6,12,19-21].

Author, publication year, place	Sensitivity	Specificity	PPV	NPV	Accuracy
Present study, 2023, Bharati Vidyapeeth (DTU) Medical College Hospital and Research Centre, Pune, Maharashtra	100%	94.03%	89.19%	100%	96.0%
Vidyashree and Deepak RK, 2021 [12], SDM University, Karnataka	96.15%	100%	100%	96.42%	98.11%
Miachieo N et al., 2020 [19], King George's Medical University, Lucknow, Uttar Pradesh	100%	100%	100%	100%	100%
Gayathri MM et al., 2014 [20], Mysore Medical College, Mysore	86.7%	100%	100%	97.7%	98%
Matreja SS et al., 2017 [6], Sri Aurobindo Medical College and PG Institute, Indore	92.3%	99.2%	92.3%	99.28%	98.69%

[Table/Fig-8]: Cell block method results compared with other studies [6,12,19,20].

Author, publication year, place	Sensitivity	Specificity	PPV	NPV	Accuracy
Present study, 2023, Bharati Vidyapeeth (DTU) Medical College Hospital and Research Centre, Pune, Maharashtra	81.82%	88.06%	77.14%	90.77%	86.00%
Miachieo N et al., 2020 [19], King George's Medical University, Lucknow, Uttar Pradesh	75%	100%	100%	100%	86.54%
Joseph LM et al., 2022 [21], Government Medical College, Kottayam, Kerala	94.00%	100%	100%	96.8%	97.9%

[Table/Fig-9]: Cytospin results compared with other studies [19,21].

Limitation(s)

Although the cell block approach demonstrates high specificity, it is more sensitive than the cytospin method. Combining cytospin and cell block analysis has the potential to offer a more precise diagnosis. One major limitation of this study was the availability of a limited number of IHC markers.

CONCLUSION(S)

Present study found that cell block method was superior to the cytospin method for cytological diagnosis of body fluids. However, incorporating the cell block method as an adjunct to the cytospin method is particularly useful when there is diagnostic uncertainty. Additionally, the use of IHC markers provides an added advantage with the cell block method. IHC markers not only aid in differentiating between benign and malignant conditions but also assist in identifying the primary site of malignancy.

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