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Utility of Immunohistochemistry on Cell Blocks for Detection of Malignancies using Body Cavity Fluids in a Tertiary Care Hospital

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ABSTRACT

Objective: To determine the utility of cell block technique and immunohistochemistry in diagnosing the abdomino-pelvic and thoracic malignancies using body fluids.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Histopathology, Foundation University Medical College/Fauji Foundation Hospital, Rawalpindi Pakistan, from Jan 2023 to Dec 2024.
Methodology: Data was obtained from the Department of Histopathology. All body fluid samples which underwent cell block preparation following initial cytological evaluation were noted. Those cell blocks on which immunohistochemistry was applied were evaluated to see the final diagnosis.
Results: Cytology fluids were received from a total of 136 patients. Out of these, 76 revealed malignant cells on cytology and cell block. Immunohistochemistry was applied on 50 cell blocks, and the primary origin of malignancy were ovary (90%), breast (4%), lung (4%) and endometrium (2%).
Conclusion: In all cases showing malignant cells on cytology, cell blocks confirmed the presence of malignant cells and highlighted architectural details of carcinoma. Immunohistochemistry gave final diagnosis of the primary malignancy which eliminated the need for biopsy in patients with carcinoma of unknown primary.

Keywords: Ascites, Carcinoma of Unknown Primary, Cell Block, Cytology, Immunohistochemistry, Pleural Effusion.

How to Cite This Article: *Taj Z, Sarfraz T, Aneeqa S, Riaz R, Hanif A, Rustam G. Utility of Immunohistochemistry on Cell Blocks for Detection of Malignancies using Body Cavity Fluids in a Tertiary Care Hospital. Pak Armed Forces Med J 2026; 76(Suppl-6): S890-S893.*
DOI: https://doi.org/10.51253/pafmj.v76iSUPPL-6.13784

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INTRODUCTION

Malignant effusion is the pathologic accumulation of fluid in the abdominal and pleural cavity caused by metastasis of malignant cells. Common causes of ascites include liver cirrhosis (84.1%), cardiac failure (2.7%) and malignant neoplasms (2.4%).[1] In malignancies, ascites is caused by obstruction of lymphatic vessels by tumor cells. Malignant ascites is most frequently associated with ovarian cancer, followed by gastric, uterine, breast and colorectal cancers.[2,3] In ovarian and breast cancers which are sensitive to chemotherapy, prompt treatment may achieve good results.[4]

In routine cytology, smears are prepared from pleural fluid, and slides are viewed under microscope for any malignant cells. Cell block technique, introduced in 1896, acts as a Formalin-Fixed and Paraffin-Embedded (FFPE) tissue on which further ancillary studies can be performed such as Special stains, Immunohistochemistry (IHC) and Molecular studies.[5] It uses specimens, such as fine-needle aspirations, body cavity fluids and washings, and resembles routine hematoxylin and eosin–stained tissue sections.[6] Cell blocks are very useful in the diagnosis of malignancies because of their preserved architectural patterns as seen in the histopathology slides.[7]

In many patients with carcinoma of unknown origin (CUP), ascites or pleural effusion may be the initial presentation and cell block preparation is very helpful. Combining cytomorphology with cell block and IHC can help identify the origin of the malignant cells without the need of biopsy.[8] Local studies have evaluated the diagnostic accuracy of fine needle aspirate cell blocks in patients of breast cancer and another utilized IHC for differentiating benign vs malignant cells on cytology.[9,10] Our study focuses on tumor diagnosis using IHC on cell blocks so that the patients can be treated timely without the need of invasive tissue biopsy.

METHODOLOGY

This was cross-sectional study, carried out at the Department of Histopathology, Foundation

Correspondence: Dr Zainab Taj, Department of Histopathology, Foundation University Medical College, Rawalpindi Pakistan
Received: 30 Aug 2025; revision received: 28 Jan 2026; accepted: 29 Jan 2026

University Medical College/Fauji Foundation Hospital Rawalpindi Pakistan, after approval of the Institutional Review Board (Ref No 924/RC/FFH/RWP). We studied all cases received from Jan 2023 to Dec 2024.

Inclusion Criteria: All fluid samples for cytology received in Histopathology during the study period for which cell blocks were prepared, were included.

Exclusion Criteria: Pap smear samples (gynecological cytology) and fine needle aspiration cytology were excluded.

Sample size was calculated using the WHO calculator with anticipated population proportion of patients with ascites due to malignant neoplasia being 2.4%.[1] The minimum required sample size obtained was 63. However, a total of 136 cases were available and included, which exceeds the minimum requirement and enhances the precision and reliability of the results. Non-probability, consecutive sampling was used to collect data.

In this study 247 cell blocks were prepared from body fluids of 136 patients. Therefore, data of only 136 cell blocks was included because of repeat/ multiple effusion specimens attributed to the same disease process. Some patients had clinical suspicion of malignancy and others were known cases of a malignancy with suspected metastasis or malignant effusions. The cell blocks were made using thrombo-plastin reagent. This method is based on the principle of preparing a cell pellet from the centrifuged cell suspension by adding clotting factor to enmesh the cellular material in a clot. Fluid sample is centrifuged for 5-10 minutes at 2500-3000 rotations per minute. The tissue coagulum is then fixed for 24 hours in 10% buffered formalin. Fixed blocks of fluid samples are subjected to cutting. Four micrometer (4µm) thickness sections are prepared and stained with hematoxylin eosin stain. The IHC panel was applied according to the differential diagnosis of a particular case.

Statistical Package for Social Sciences (SPSS) version 21 was used for data analysis. To check normality, Kolmogorov Smirnov test was used, and data was found to be parametric. Quantitative variable i.e. age was expressed as Mean+SD and qualitative variables like type of sample and diagnosis given on IHC were expressed as frequency and percentages.

RESULTS

For 136 patients, total 247 cytology samples were received, as for some patients three or more consecutive samples were sent where clinical suspicion of malignancy was high. Patient's age ranged from 19 to 85 years, with a mean age of 58.90±12.621 years. Most patients were females, i.e. 132(97.1%) and only 4(2.9%) were males. Most common sample received was Ascitic fluid, followed by pleural fluid, peritoneal washings, cyst fluid and CSF. Further details are mentioned in Table-I.

Table-I: Categorization of all fluid samples (n=136)

Type of fluid samples	n(%)
Ascitic fluid	93(68.4)
Pleural fluid	37(27.2)
Peritoneal washings	4(2.9)
Cyst fluid	1(0.75)
Cerebrospinal fluid	1(0.75)

In 76(59.9%) of cases, malignant cells were seen in cytology and cell block, 53 cases (39%) showed no malignant cells, 5 cases (3.7%) showed degenerated cells, and 2 cases (1.5%) showed lymphocytic effusion.

Immunohistochemistry was applied in 51(37.5) cases, 50 of them showed malignant cells on cytology and cell block. In most cases, there was no known history of malignancy, therefore; IHC panel was applied to guide the clinician about the primary lesion. One of them had previous history of breast carcinoma and cytology was only sent for confirmation of metastasis (Table-II).

Table-II: Immunohistochemistry (IHC) panel used for Diagnosis of Carcinomas

Diagnosis	Positive markers	Negative markers
High grade serous carcinoma of ovary	CK7, WT1, Pax8, p53	Ck20, CDX2
Adenocarcinoma Lung	CK7, TTF1, Napsin A	CK20, CDX2, GATA3
Breast Carcinoma	CK7, GATA3	CK20, CDX2, TTF1, NapsinA
Serous Carcinoma of Endometrium	CK7, p53, Vimentin	CK20, CDX2, WT1, Pax8

Table-III: Diagnosis given on Immunohistochemistry (n=50)

	Diagnoses given on Immunohistochemistry				
Diagnosis	High Grade Serous Carcinoma of ovary	Adeno-carcinoma Lung	Breast Carcinoma	Serous Carcinoma of Endometrium	Total
n(%)	45(90%)	2(4%)	2(4%)	1(2%)	50(100%)

Out of 50 cases which had malignant cells, all cases were given final diagnosis on Immuno-histochemistry (IHC). Forty-five (90%) were diagnosed as high-grade serous carcinoma of ovary, out of which three were later confirmed on surgery i.e. Hysterectomy and bilateral Salpingo-oophorectomy. Other diagnosis given were metastatic adeno-carcinoma of lung and breast carcinoma in 2(4%) patients respectively and serous carcinoma of

endometrium in 1(2%), which can be seen in Table-III. The IHC was inconclusive in one case, where no malignant cells were seen on cytology and cell block.

DISCUSSION

In our study, we concluded that cell blocks act as formalin-fixed paraffin-embedded tissue in which not only architectural details of tumor are visible, but in addition they can also be utilized for ancillary testing such as immunohistochemistry as mentioned in two other recent studies.[5,6] In addition, they also highlighted its role in advanced molecular testing which could not be performed in our setup due to limited resources and funding.

High grade serous carcinoma (HGSC) was the most commonly diagnosed cancer in our study, reason being our hospital mostly deals with families of retired personals and malignancies are more common in older age group. That is why majority of the patients diagnosed in our study are older females. The ovaries are suspended within the abdominal cavity by peritoneal folds and they are not rigidly fixed. Therefore, any malignancy of ovary can easily spread in the abdomen and cause ascites. This is why malignant ascites is most frequently associated with ovarian cancers.[2] Studies stated that ovarian malignancies (specifically high-grade serous carcinoma of ovary) are the most common cause of malignant ascites, and occurs in 37% of all cases.[11,12] Also, malignant ascites is twice as common in females as compared to males. In our study, in female patients who presented with malignant ascites, the first initial panel of IHC applied was CK7, WT1, p53, Pax8, Ck20 and CDX2. First four markers are positive in HGSC of ovary and last two were applied to rule out colorectal origin. Lisio *et al.,* also concluded similar IHC positivity in HGSC of ovary, however; Nishal *et al.,* pointed out that most HGSC were positive for WT1 and p53 while negative for CK7 and CK20.[13,14]

In our study, four female patients presented with malignant pleural effusion, therefore the panel chosen in these cases in addition to CK7, CK20 and CDX2 was TTF1, Napsin A and GATA3. Two cases were diagnosed as metastasis from lung origin and two from breast. A study by Ye *et al.,* indicated that double positivity of Napsin A and TTF-1 immunostain is highly specific for lung adenocarcinomas.[15] Similarly, Cimino-Mathews *et al.,* stated that GATA3 is very useful for metastatic breast carcinoma, especially triple-negative cancers.[16]

One case diagnosed as serous carcinoma of endometrial origin was positive for Vimentin in addition to CK7 and p53 and negative for WT1 and Pax8. Zhao *et al.,* also concluded that WT1 and Pax8 are positive in majority of the cases in malignant effusions with primary ovarian origin.[17] Pax8 is a useful marker for carcinomas of uterine origin, however; WT1 is not positive in majority of serous carcinomas of endometrium.[18,19] Vimentin positivity also favors origin from uterine corpus rather than ovarian origin as seen in our study.[20]

Although a significant number (39%) of cases in our study showed negative smears, and degenerated cells (3.7%), cell block was prepared for those cases as well. IHC was also applied on one case, as the patient was a known case of breast carcinoma and clinical suspicion of malignant pleural effusion was high, the IHC results were inconclusive due to low yield on cell block. Thapar *et al.,* found that by using a combination of the cell block and smear, 13% more malignant cases were detected as compared to only smears.[21] Diagnostic yield of malignant effusion was increased by 10% in exudative pleural effusions according to Köksal *et al*.[22] However, in our study there was 100% concordance in benign vs malignant results in cytology and cell block. A recent study by Khan *et al.,* done in Pakistan showed that cell block in addition to IHC significantly increased the number of benign and malignant cases (p-value =0.001) and reduced the number of suspicious cases detected on cytology alone.[10] In our study no result was given as suspicious after cell block preparation, even without IHC.

LIMITATIONS OF STUDY

Our main limitation was that advanced molecular testing could not be performed in our setup due to limited resources and funding.

CONCLUSION

Cell block is a very useful method which can be used along with routine cytology. It highlights architectural details of a tumor akin to tissue sections. IHC was found to be a very useful adjuvant tool in diagnosis of primary malignancy in cases of malignant ascites or pleural effusions, reducing the need for tissue biopsy.

Conflict of Interest: None.

Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

ZT & TS: Data acquisition, data analysis, critical review, approval of the final version to be published.

SA & RR: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

Ah & GR: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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