Diagnostic efficiency of Cytologic Smears and Cell-blocks in body cavity effusions - A prospective study and the way forward

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Abstract

Background: Cytosmears (CS) are cytologic preparations from the centrifuged sediment of effusions. Cell-block (CB) technique is used to process tissue fragments in the residue left behind following cytosmear (CS) preparation. This residual tissue often contains valuable diagnostic evidence. The study compares the diagnostic efficiency of the two methods.

Aim: The study aimed to observe the cytomorphologic features of body cavity effusions on conventional cytology smears and cell-block preparations and to identify their sensitivity and limitations in providing a diagnosis of neoplasms.

Material and Methods: This was an observational study. Cytosmears and cell-blocks of patients with body cavity effusions were analyzed. Cytosmears were stained with Papanicolaou and Leishman stains; cell-blocks were processed by Plasma-Thrombin clot method and stained with hematoxylin and Eosin. Special stains were done on need basis. The cytology and cell-block impressions were classified as neoplastic and non-neoplastic and the agreement between the two techniques determined.

Results: 84 effusions from the pleural, peritoneal and pericardial cavities were studied. The yield with the two techniques were compared for non-neoplastic and neoplastic lesions. The agreement between the two methods was 91.3%; cell-blocks showed an improvement rate of 8.3% over cytosmears in identifying neoplastic effusions.

Conclusions: Cell-blocks were superior, but should be used as an adjunct to cytosmears, in diagnosing neoplastic cases to complement each other. Optimal utilization and processing of the sample for cytosmear and cell block preparation, diligent technical and morphological analysis and appropriate ancillary studies give the best diagnostic results.

Key-words: ancillary, neoplastic, non-neoplastic

Introduction

The etiology of effusions can be detected by different techniques. Cytosmears (CS) preparation involves processing the centrifuged sediment into smears and staining them. Any large tissue fragments in the sediment may not be amenable to morphologic examination by cytosmears. Cell block (CB) technique utilizes these large tissue fragments in the sample to process into formalin-fixed paraffin embedded blocks like conventional histopathology specimen[1]. The tissue fragments provide the histologic pattern in CBs, which may not be evident in cytology smears (CS). The histologic appearance gives diagnostic clues regarding the site of origin in cases of malignant effusions by virtue of special stains and ancillary studies[2,3]. This study was done to explore the morphological findings of conventional CS and CB by plasma-thrombin clot method preparations, and identify their sensitivity and limitations in providing a diagnosis of neoplasms in body cavity effusions.

Material and Methods:

This was a prospective observational study of patients admitted with body cavity effusions over a period of one year in a tertiary care hospital attached to the teaching institute. The study was conducted after obtaining approval from the Institutional Ethics Committee.

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Inclusion criteria: All cases of effusions with adequate sediment to process into CBs and compare with cytology smears were included in the study.

Exclusion criteria: Effusions with scanty sediment and inability to process into CBs were excluded from the study.

Sample collection: Aspirates of body cavity effusions- pleural, peritoneal and pericardial effusions obtained by thoracentesis, abdominocentesis and pericardiocentesis respectively were considered for the study. Relevant clinical data was collected from each case by studying the case records. Out of 110 cases of effusions sent to the laboratory, 84 cases satisfactory for both, CS and CB were included in the study. The aspirated fluid sample, transported within one hour to the laboratory was analyzed. The sample was centrifuged at 2000 rpm for 10 minutes. Wet fixed smears were prepared from the sediment and stained with Papanicolaou stain (Rapid Pap method). Air dried smears were stained with Leishman stain. The sediment in the tube following smear preparation was used for CB preparation by Plasma-Thrombin Clot method. Four drops of AB group plasma and four drops of thrombin were added to the fresh unfixed sediment, mixed and allowed to stand. The clot that was subsequently formed was transferred to a filter paper, and wrapped after adding eosin. In case of a spontaneously formed clot, excess fluid was wrung out from the clot against the wall of the test tube and wrapped in a filter paper. The wrapped clot was fixed in 10% formalin and processed like histology samples. The paraffin embedded sections were stained with Harris Hematoxylin and Aqueous Eosin. Special stains such as Ziehl-Neelsen (ZN), Periodic acid–Schiff (PAS), mucicarmine and relevant immunohistochemical (IHC) markers were used on the paraffin sections for various purposes- to categorize the effusion, to distinguish reactive mesothelial cells from malignant cells and to identify the site of origin of the lesions. The turn-around time for the diagnosis of CS was 24 hours and for CB 72 hours.

Effusions were classified into non-neoplastic and neoplastic based on the predominant cell type and cytomorphologic features[4]. Cases suspicious of malignancy were classified as non-neoplastic to ensure comparability between the two techniques.

Statistics: The agreement between the smear diagnosis and CB diagnosis was calculated using kappa statistics. The sensitivity, specificity, and positive and negative predictive values of conventional cytology smears in detecting neoplasms in effusions were calculated.

Results:

The CBs (n=110) were prepared from body cavity effusions, of which the samples (n=84) meeting the selection criteria were analyzed. The samples belonged to 40 males and 44 females, with an age range of 7-82 years with the highest incidence in 51-60 years age group (38.5%). (Figure 1)

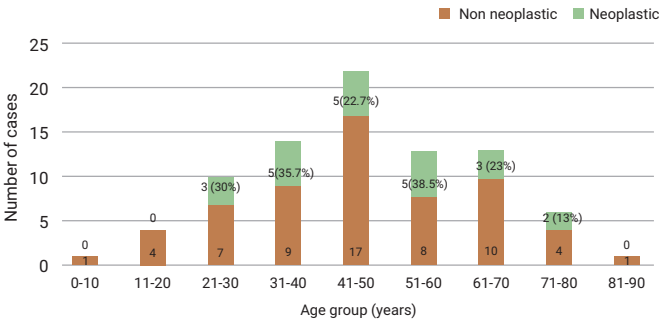

Figure 1: Age distribution of non-neoplastic and neoplastic effusions

Among all effusions, sixty-one were non-neoplastic and twenty-three neoplastic. Out of 61(72.6%) non-neoplastic effusions, 34 were from males and 27 from female patients. Of 23(27.4%) neoplastic effusions, a female preponderance (n=17, 73.9%) was observed; 6(26.1%) cases were from male patients. (Table 1)

Table 1: Gender distribution of neoplastic effusions

Site	Male	Female
Pleural effusion	2	2
Peritoneal effusion	2	15
Pericardial effusion	2	0

The causes for **non-neoplastic effusions** in the pleural cavity included tuberculosis (n=16; 26.23%), pneumonia (n=10,16.4%), nephropathy (n=02,3.28%), rheumatoid arthritis, polyserositis, anemias and alcoholic liver disease in one patient(1.64%) each. In the peritoneal cavity, cases found were chronic liver disease with cirrhosis (n=15;24.6%), tuberculosis (n=03;4.92%), nephropathy (n=03; 4.92%) and enteric fever, endometriosis, cor-pulmonale, sigmoid volvulus, post-gastrectomy, spontaneous bacterial peritonitis in one patient(1.64%) each. Non-neoplastic pericardial effusions were one case(1.64%) each of tuberculosis and ischemic heart disease.

The causes for **neoplastic effusions** in the pleural cavity included one patient (4.35%) each with melanoma, breast, cervix, and lung carcinoma. Ovarian cancer (n=12; 52.17%) was the most common neoplasm causing effusion in peritoneal cavity followed by malignancy of stomach (n=02, 8.7%), colon (n=01, 4.35%) and unknown primary (n=02, 8.7%). The increased incidence of ovarian cancer explains the female dominance in neoplastic effusions. Neoplastic

effusions in pericardial cavity were due to carcinoma lung (n=02, 8.7%).

Comparison of diagnosis: Among the **pleural** effusions diagnosed as non-neoplastic by CS, CB detected neoplasm in one case of metastases from breast carcinoma; among the **peritoneal** effusions diagnosed as non-neoplastic by CS, CB detected neoplasms in six more cases. (Table 2)

Table 2: Diagnosis of effusions by Cytology Smear and Cell Block

Effusion	Non-neoplastic effusions		Neoplastic effusions	
Site	Cytology Diagnosis	Cell block Diagnosis	Cytology Diagnosis	Cell block Diagnosis
Pleural (n=36, 42.85%)	33	32	3	4
Peritoneal (n= 44, 52.38%)	33	27	11	17
Pericardial (n= 4, 5.5%)	2	2	2	2

The overall improvement rate of CB over CS in the diagnosis of neoplasms in effusions is 7/84 (8.3%). (Table 3).

Table 3: Negative correlation of cytology with cell block

Site	Cell Block Diagnosis	Cytology Diagnosis	Number of cases
Pleural	Carcinoma breast	Non-neoplastic exudate	1
Peritoneal	Carcinoma ovary	Non-neoplastic exudate	3*
	Carcinoma stomach	Non-neoplastic exudate	1
	Neoplastic effusion	Non-neoplastic exudate	1
	Carcinoma prostate	Non-neoplastic exudate	1

*All the three cases were reported with given diagnosis in CB and CS

Statistical Analysis: The sensitivity, specificity, and positive and negative predictive values of conventional cytology smears in detecting neoplasms in effusions was found to be 69.56%, 100%, 100% and 89.71% (p=0.000). At 95% confidence level, $p < 0.05$ and the kappa value was 0.769; thus, CB fared better than smears.

Discussion

Cell block is a cyto-preparatory technique abridging cytology and histology utilizing the clots and cellular elements in the effusion specimen. Richardson et al and Dekker et al opine that CBs supplement cytology smears in the diagnosis of effusions, thus contributing to an accurate diagnosis[5-7]. The cellblock preparation is considered most useful in lesions where architecture and morphology are important and proved to be useful on tissues derived from various cystic lesions, fine needle aspirates, brushings etc[8,9]. Different methods of CB preparation have been proposed; the formalin fixation method, agar method, plasma-thrombin clot method, celloidin bag method to mention a few[9-13].

Non-neoplastic effusions: The cytology smears and CBs of effusions concluded as non-neoplastic showed benign reactive mesothelial cells and inflammatory cells, predominantly mature lymphocytes. A few cases like spontaneous bacterial peritonitis, pneumonia and sigmoid volvulus demonstrated infiltration with polymorphonuclear leukocytes. Tuberculous effusions presented with a predominant lymphocyte population. Similar cytologic findings have been reported by Reagan and Luse et al[4,14].

Neoplastic effusions: It is relatively more challenging to differentiate nonneoplastic from neoplastic effusions because of subtle differences between reactive mesothelial cells and malignant cells. Accurate morphological identification by CS remains a challenge and limitation. CBs have proved to be useful adjuncts in detecting malignancies, where CS alone often fail to give a confirmatory result[15].

A case of ductal carcinoma of the breast with metastasis to the **pleural** cavity, reported as nonneoplastic reactive mesothelial cells in CS, were not readily distinguished from the discrete malignant cells. The CB of that case showed tumor cells in acinar pattern having atypical features. Further evaluation of the patient revealed metastatic carcinoma deposits in the left axillary lymph node. Cell block offers definitive diagnosis in such cases since the histologic acinar structures are well-defined and special stains for mucin complement their findings[6]. The three-dimensional clusters of tumor cells described by Khan et al were noted in our study too[16]. The smearing had distorted the histologic architecture of tumour cells in CS. The degree of cytologic atypia in correlation with the clinical picture clinches the diagnosis.

Of 17 neoplastic **peritoneal** effusions picked up by CBs, 11 (64.7%) were diagnosed as neoplastic by cytology smears; adenocarcinoma ovary (n=03) and carcinoma stomach (n=01) were misinterpreted as reactive effusions on direct smears. One case of carcinoma prostate and a granulosa cell tumor of the ovary concluded as neoplastic by CB were interpreted as reactive effusions by cytology smears.

A case of peritoneal effusion had a tubo-ovarian mass

with deposits on the bowel loops and bladder wall. Malignancy was missed in direct smears which was picked up by the CB that showed malignant cells in acini. The tumor cells had a vesicular nucleus with prominent nucleoli and vacuolated cytoplasm suggestive of adenocarcinoma. The fluid had a blood clot in it with entrapped tumor cells. Hence, tumor cells were not discernible in the smears though they were present in the fluid. Subsequent HPE showed adenocarcinoma with metastases to adjacent organs. Naylor et al have described such situations where CBs give diagnostic information when cells are trapped in the clot and not visualized in direct smears[11].

A case of mucinous adenocarcinoma of the ovary was diagnosed as a reactive effusion with degenerative change on smears. The CB showed tumor cells in papillary fragments and confirmed malignancy by immunohistochemical studies. (Figure 2)

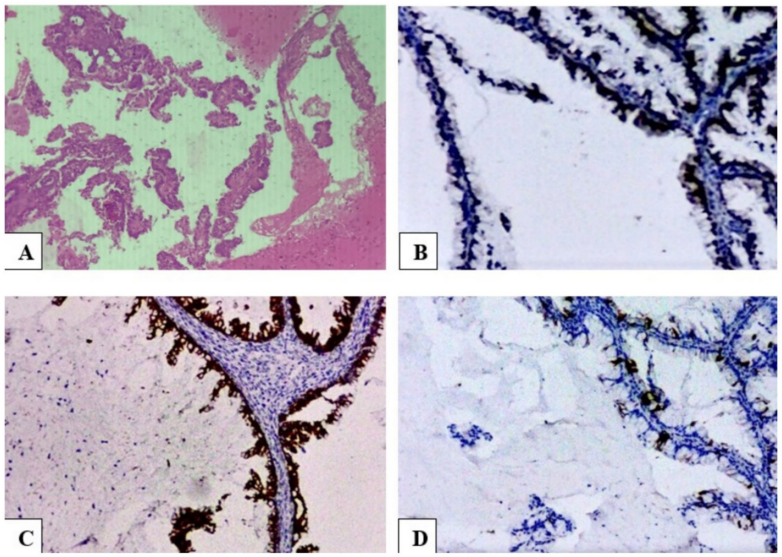

Figure 2: Cell block section of carcinoma Ovary. A: Tumor cells in papillary pattern (HE 100X). B: CDX2 positive tumor cells (100 X). C:CK7 positive tumor cells (100 X). D: CK20 (focal)positive tumor cells (100 X).

An ovarian tumor metastatic to the peritoneal cavity diagnosed as poorly differentiated carcinoma/ sarcoma in CB and on histopathological examination proved to be endometrial stromal tumor by immunohistochemistry. The tumor cells were not identified in direct smears because they were interpreted as mesothelial cells.

There were two cases in which CB helped in making a diagnosis of neoplastic effusion. Subsequent histopathological examination and ancillary studies aided the diagnosis. In a granulosa cell tumor of the ovary presenting with ascites confirmed by histopathology, the tumor cells were interpreted as reactive mesothelial cells on smears and as neoplastic effusion in the cell block. One case of carcinoma of the stomach showed malignant cells in CB mainly in singles; the corresponding CS displayed, scattered atypical cells with cytoplasmic vacuoles which were confused with degenerating changes in the mesothelial cells. It was a case of gastric signet ring cell carcinoma.

There was an effusion in a patient with mild prostatomegaly and elevated free PSA levels, but no abnormal uptake in whole body 18 F FDG PET-CT. The CS was suspicious of malignancy and could not definitely conclude it as neoplastic, but CB showed adenocarcinoma on haematoxylin-eosin and confirmed the primary as prostate on IHC. (Figure 3)

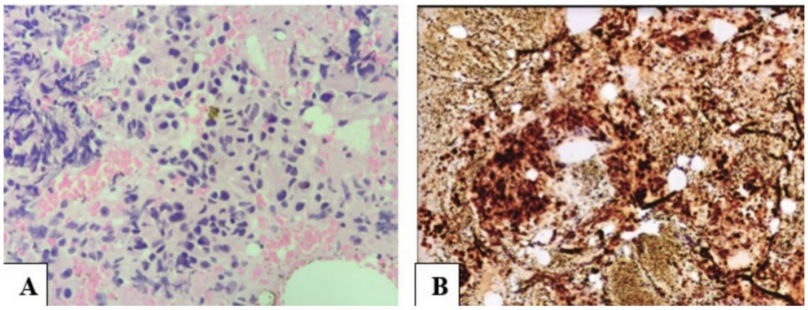

Figure 3: Cell block section of carcinoma prostate. A: Tumor cells in nests and acinar pattern (H & E 100X). B: NKX3.1 positive tumor cells (100 X)

Two malignant **pericardial** effusions were diagnosed as adenocarcinoma in both smears and cell blocks. The cells were in sheets and had prominent macronucleoli. Both were mucicarmine positive on CB sections and were diagnosed as carcinoma lung. Radiological findings also pointed towards lung as the primary source of malignancy. Similar descriptions for carcinoma of the lung have been described in reports by Reagan JW and Khan et al[4,16].

In our study, the CB preparation identified additional seven cases of neoplastic effusions which were not discernible in direct smears, yielding an improvement of 8.3%. The accuracy rate of cytology smears for diagnosing neoplastic effusions was 69.6%. Nathan, Udasimath et al and Khan et al have noted 12%,14% and 20% higher detection rate respectively for malignant cells with CB [10,15,16].

Merits of CB preparation:

1. Represent 'micro biopsies' displaying the histologic architecture which gives a lead to the diagnosis, by identifying the possible site of primary malignancy[17]. There is a concentration of cells obtained from a large surface area in the same plane[3,9,16].
2. Feasibility of special stains, ancillary studies on CBs is an added advantage[4]. Satturwar and Pantanowitz have shown that CBs are suitable for whole slide imaging and teleconsultation in challenging non-gynecologic cytology cases[17].
3. A longer storage period of CB allows further evaluation later using other techniques[18].
4. Preservation of samples for 72 hours in a refrigerator (80°C) before processing into CBs avoids the need for weekend or overtime cytotechnologist serving as an additional advantage[1].

Demerits of CB preparation and troubleshooting:

1. In this study, cell-blocks could not be prepared in six cases diagnosed malignant by smears due to inadequate sediment. Richardson et al have reported such incidences and attributed it to the nature of the procedure adapted[5]. As the material was removed from cellular sediment for smear preparation prior to CB preparation, the cellular content of the CB was handicapped. Dividing the sample into two parts before processing overcomes this issue, but it is not possible to ascertain the presence of representative cells in both techniques. Since many factors have been identified for low cellularity in CB, Saqi A has proposed measures like, development of protocols for sample collection, appropriate triage of samples and modifications in processing methodology, to optimize preparation of CB[3].
2. Another problem with CB is ensuring that the right plane of paraffin-embedded tissue containing abnormal cells is being studied. Varsegi and Shidham have demonstrated using an AV marker to monitor the depth of section cutting[19].
3. Adequacy of tissue in the CB when multiple markers are applied is another limitation. Shidham has also proposed strategies to concentrate diagnostic tumor cells in cellular and paucicellular samples in order to enable multiple ancillary tests on CB[20].
4. Time-consuming compared to CS. Studies are under way to assess the efficiency of frozen sections of CB in order to mitigate this limitation[21].

Conclusion

Cell-blocks display more evident morphological patterns like acinar and papillary which helps in identifying malignancy when compared to cytology smears. Cell block technique by Plasma-Thrombin Clot method is more advantageous than cytologic smears in the diagnosis of neoplastic and non-neoplastic effusions and complements rather than substituting cytosmears. Both methods have inherent pitfalls. Optimal utilization and processing of the sample for cytosmear and cell block preparation, diligent technical and morphological analysis and appropriate ancillary studies give the best diagnostic results.

It is recommended to have a multidisciplinary approach between the clinicians, radiologists and pathologists during sample collection thus ensuring adequate sample. Laboratory personnel needs to be trained regarding proper allocation of samples for CS and CB. Adoption of new techniques to enhance diagnostic yield and to reduce the turnaround time increases the efficiency of the CB technique.

Limitation

The limitation of this study was that a histopathological diagnosis was available in thirteen cases - two nonneoplastic and eleven neoplastic. Histopathological examination was clinically not indicated in reactive transudate effusions and hence not available in all the cases for evaluating the efficacy of cell-block or cytological diagnosis. Even in those cases where neoplastic process was diagnosed, histopathological examination was carried out only when indicated by therapeutic guidelines for different malignancies.

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