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Original Research Article

Diagnostic utility of Plasma Thromboplastin cell block preparation in cytological evaluation of serous effusions

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Abstract

Background: Cell block preparations act as a useful adjunct to smear cytology for categorization of malignant and benign effusions. Plasma thromboplastin cell block technique is simple, requires less time and offers improved cytomorphological features.
Aim: To study the utility of Thromboplastin-plasma cell block technique and its diagnostic significance in conjunction with conventional cytology smears in evaluation of serous effusions.
Material and methods: One hundred samples were included in the study. In addition to preparation of conventional smears, fluids were subjected to cell block technique. Cell blocks were prepared using Plasma thromboplastin cell block technique.
Results: Cellularity and diagnostic yield for malignancy was increased by cell block preparation.
Conclusion: Plasma Thromboplastin cell block method provides high cellularity, better architectural patterns and good preservation of cellular and nuclear details, thereby, increasing diagnostic yield in the cytological evaluation of serous effusions when compared to conventional smears alone. Moreover, it is easier to apply immunomarkers and perform molecular studies on the cell blocks that can be stored indefinitely for future testing. Thus, cell blocks can act as a useful adjunct to the conventional cytosmears for evaluation of serous effusions.
Keywords: Plasma Thromboplastin, Cell block, Immunohistochemistry

1. Introduction

The role of cell block has been well established in aspiration cytology for the diagnosis of solid tumours, however, its use in serous effusions in routine practices has been lately highlighted in few studies [1-5]. Cell block (CB) preparation with conventional techniques such as agar gel or formol- alcohol is laborious and time consuming. Therefore, in this study, Plasma –Thromboplastin cytoblock technique (PT-CB) was performed. This technique is simple, cost-effective and readily adaptable in routine hospital laboratories [6]. Morphologic examination of cell blocks and application of ancillary technique such as immunohistochemistry on cell block material provides additional information that is essential to resolve the diagnostic dilemmas.

Aims

In the current study, we assessed the utility of Thromboplastin-plasma cell block technique and its diagnostic significance in conjunction with conventional cytology smears in evaluation of serous effusions.

2. Material and Methods

The present study was conducted on 100 patients who underwent paracentesis for the cytodiagnosis of effusion fluids (pleural, pericardial and ascitic fluid) over a period of 8 months from January 2014 to August 2014. All the 100 fluid specimens were included in the study. Fluid specimens less than 10 ml, clotted samples and suboptimally preserved fluids were excluded from the study. Conventional cytological smears (CS) or cytospins were prepared from each sample. From the remaining fluid, cell blocks were prepared and immunomarkers were applied whenever needed.

2.1 Smearing technique

In conventional smear technique, 5 ml of the effusion fluid was centrifuged at 2500 rpm for 5 mins and direct smears or cytocentrifuged smears were prepared from centrifuged deposits. In selective cases, 300 µl of fluid was placed in cytospin funnel with the filter paper placed between the slide

and the funnel, then subjected to centrifugation at 700 rpm for 6 minutes. A minimum of three smears were prepared. One smear was prepared after air drying and stained with May-Grunwald-Giemsa stain. The other two smears were fixed in 95 % ethanol and stained with Haematoxylin-Eosin stain and Papanicolaou stain.

2.2 Cell block technique

Cell blocks were prepared by plasma–thromboplastin technique. 10 ml fluid was centrifuged at 2500 rpm for 10 mins. The supernatant was removed and the fresh unfixed sediment deposit was mixed with two drops of pooled plasma (pooled plasma was kept frozen and was brought to room temperature before use). Subsequently, 2 drops of thromboplastin (Himedia) were added and mixed. This mixture was allowed to stand for 2 minutes. The resultant clot was wrapped in a premoistened filter paper and placed in a cassette. The tissue cassette was fixed into a jar containing buffered formalin fixative for at least 4 hours.

Cell blocks were embedded in paraffin and sectioned at 3µm thickness. Thus, the same fluid was evaluated for a comparative analysis. Sections were stained with Haematoxylin and eosin stain. Immunostaining on Poly-L-Lysine coated slides using the standard Horseradish Peroxidase (HRP) technique was performed whenever needed.

A comprehensive panel of immunomarkers were utilized in doubtful cases to distinguish atypical mesothelial cells from metastatic malignancies and then to categorise the type of malignancy. The immunomarkers used were panCK, EMA, CK7, CK20, Calretinin, TTF-1, ER-PR, PSA, CD45, CD20, CD3, CA-125, Vimentin, and Synaptophysin. (Bio-SB, Leica, Biogenex).

2.3 Scoring and Analysis:

Two authors independently graded on a semiquantitative basis four different parameters including cellularity, morphology, degenerative changes and architecture according to Mair *et al* scoring system [7]. Scores of 0, 1 and 2 were assigned to each smear and cell block preparations [Table 1].

3. Results

All the samples analyzed were divided into three categories: positive for malignancy, suspicious and benign/reactive processes.

In the present study, a total of 100 serous effusions were included, out of which 79 were pleural, 06 were pericardial and 15 were ascitic (Fig 1). Twenty fluids were diagnosed either as positive or suspicious of malignant cells and remaining 80 were benign effusions.

Amongst the 80 benign effusions, 67(84%) cases were of pleural fluid, 10(12%) cases were of ascitic fluid and 03(04%) were of pericardial fluid. Age range was between 18 to 74 years with commonest decade being 4^{th} and 5^{th}. Males (65%) outnumbered the females (35%) with a ratio of 1.9:1. Most common cytological diagnosis was lymphocytic effusion (42/80; 53%) followed by mixed inflammation (24/80; 30%) and acute inflammation (11/80; 13%). Most common cause of benign/reactive effusions was tuberculosis (26/80;33%), followed by cardiac diseases (12/80;15%), liver diseases (03/80; 04%), lung diseases (03/80;04%), renal diseases(02/80; 03%), inflammatory bowel disease (01/80 ;01%), known cases of malignancy (15/80; 18%) and unknown cases (18/80; 22%).

Amongst all the hundred serous effusions, 28 were hemorrhagic and only fifteen (54%) of these were positive for malignancy. Twenty of the hundred cases were reported as positive or suspicious of malignancy. Amongst the malignant effusions, 12(60%) were pleural, 03(15%) were pericardial and 05(25%) were ascitic. Females (55%) outnumbered the males (45%) with a ratio of 1.22: 1. Age of these patients ranged from 31 to 81 years. Majority of the samples were from the sixth and seventh decade. Most common site of primary was lung followed by breast and ovary [Table 2]. In two of the pleural effusions, malignancy was diagnosed on subsequent samples and not on first sample sent for cytology examination.

Cytological smears and Plasma thromboplastin cell block preparations were studied independently and their score was recorded. While evaluating the cellularity, score 0 was observed in 10% of the smears and 5% of the cell blocks. Score one was noted in 55 % of the smears which decreased to 40 % in the cell blocks. Score 2 was seen in 35 % of the smears and 55 % of the cell blocks. Thus, cellularity was increased in 20 % of the effusions when cell blocks were prepared (Fig 2). Diagnostically superior result with preserved cellular morphology was noted in 60 % of the smears and 75% of the cell blocks giving a score of 2.

When assessment for retention of appropriate architecture and cellular arrangement was performed, score 1 was noted in 70 % of smears and 60 % of cell block while score 2 was observed in 20% and 40 % of the smears and cell blocks respectively. Cell block preparations revealed better cytoplasmic and nuclear details.

Cytological examination of the smears revealed 81 benign cases, 4 suspicious cases and 15 positive cases. Out of 100 effusion samples, cell block preparations revealed 80 benign cases, 2 suspicious and 18 positive cases. Thus, cell block preparations increased the diagnostic yield by 15% [Table 3]. This discrepancy was observed due to three cases wherein final diagnoses was changed from suspicious to positive in two and from negative to positive in one after cell block preparation and application of immunomarkers.

One of these cases was of carcinoma prostate that was benign on smears but was diagnosed positive on cell block sections with PSA positive malignant clusters (Fig 3A-3D). Another case was of carcinoma rectum wherein ascitic fluid revealed occasional atypical cells on cytosmears. However, cell block sections showed presence of few atypical clusters that exhibited strong expression for CK20 and CEA but CK7 was negative. Third case was of a 50 years old woman who presented with massive pleural effusion. Her first

effusion sample was benign while occasional malignant cells were observed in her second sample. Cell block preparations in this case revealed presence of several malignant clusters showing acinar arrangement. These cells were positive for Pan-cytokeratin and negative for calretinin. The patient was subsequently lost to follow up and primary site of tumor could not be ascertained.

Amongst other malignant effusions, there were four pleural fluid specimens with unknown primary. Immunohistochemical markers were applied in these cases on cell blocks and/ or cytosmears. In one of the cases malignant cells showed diffuse positivity for both CK7 and TTF-1 and later a mass lesion was detected in lung by the pulmonologist which was diagnosed as adenocarcinoma on biopsy. Diagnosis in three of the cases that were suspicious of malignancy on smears was considered as positive after application of immunomarkers like pan-cytokeratin, and calretinin on cell blocks. Out of these three cases, one case was CA-125 positive indicating ovary as the primary site of tumour where sonography revealed bulky and enlarged ovary. The histopathology report was serous cystadenocarcinoma (Fig 4A-4D). The other two cases showed positivity for CK7 and TTF-1 possibly suggesting primary in the lung. These two cases were followed up. These patients had advanced metastasis with irregular lesions in the lung.

Comparison of expression of immunocytochemical antibodies was more homogenous on cell blocks with intense staining pattern whereas some of the cytosmears showed heterogeneous and erroneous results (Fig 5).

Table 1: Mair et al point scoring system

Criteria / Point score	Volume of Blood/ clot Obscuring background	Amount of Diagnostic cellular Material present	Degree of Cellular degeneration And cellular trauma	Retained architecture /Cellular Arrangement
Score 0	**Large** Diagnosis greatly compromised	**Minimal** Diagnosis not possible	**Marked** Diagnosis possible	**Minimal** diagnosis not possible
Score 1	**Moderate** Diagnosis possible	**Moderate** Sufficient for diagnosis	**Moderate** Diagnosis possible	**Moderate** Some preservation
Score 2	**Minimal** Diagnosis easy, textbook quality specimen	**Abundant** diagnosis simple	**Minimal** Good preservation	**Excellent** Architectural display

Table 2: Sites of primary malignancy in serous effusions

SN	Site of cancer	Pleural	Pericardial	Ascitic	Total
1.	Ca Lung	5	-	-	5
2.	Ca Breast	-	3	-	3
3.	Ca Ovary	-	-	2	2
4.	Ca Gall Bladder	-	-	1	1
5.	Ca Prostate	1	-	-	1
6.	Ca Rectum	-	-	1	1
7.	Gastric Ca	-	-	1	1
8.	Lymphoma	1	-	-	1
9.	Not Known	5	-	-	5
	Total	12	3	5	20

Table 3: Comparison of diagnostic yield on smears and cell blocks

Techniques	Negative for malignancy	Suspicious for malignancy	Positive for malignancy	Total
Cytosmears	81%	4%	15%	100
Cell blocks	80%	2%	18%	100

Table 4: Comparative analysis of diagnostic yield in other studies

Author	Year	Increased diagnostic yield
Richardson et al [14]	1955	12 %
Dekker and Bupp et al [15]	1978	38%
Bodele et al [28]	2003	7%
Khan et al [17]	2006	20%
Thapar et al [2]	2009	13%
Shivkumarswami et al [1]	2012	15%
Shubhada et al [29]	2013	6.33%
Singh et al [30]	2015	41.7%
Present study	2015	15%

Figure 1: Relative distribution of serous effusions during the study period

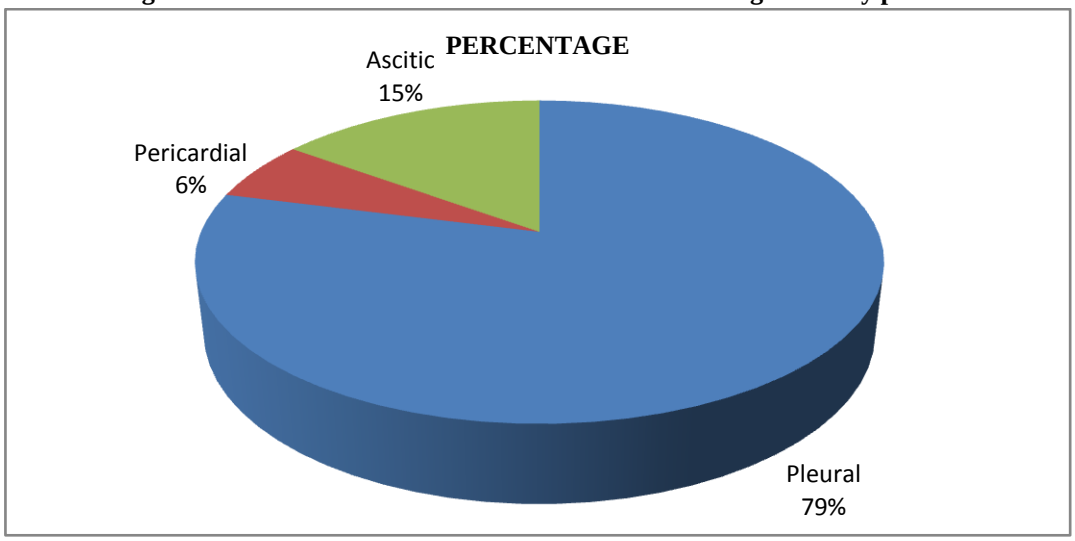


Figure 2: Comparative assessment of cellularity on smears and cell blocks

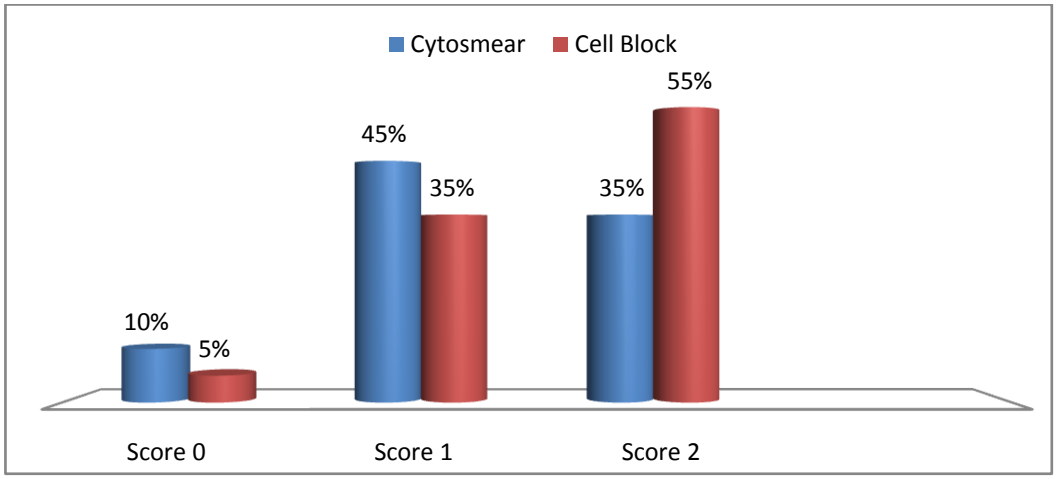
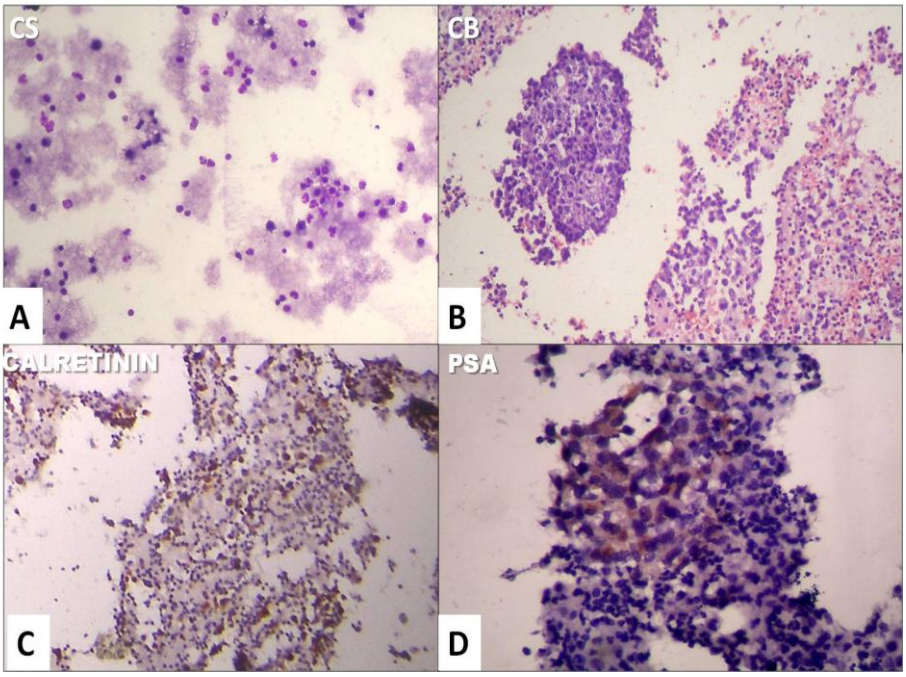
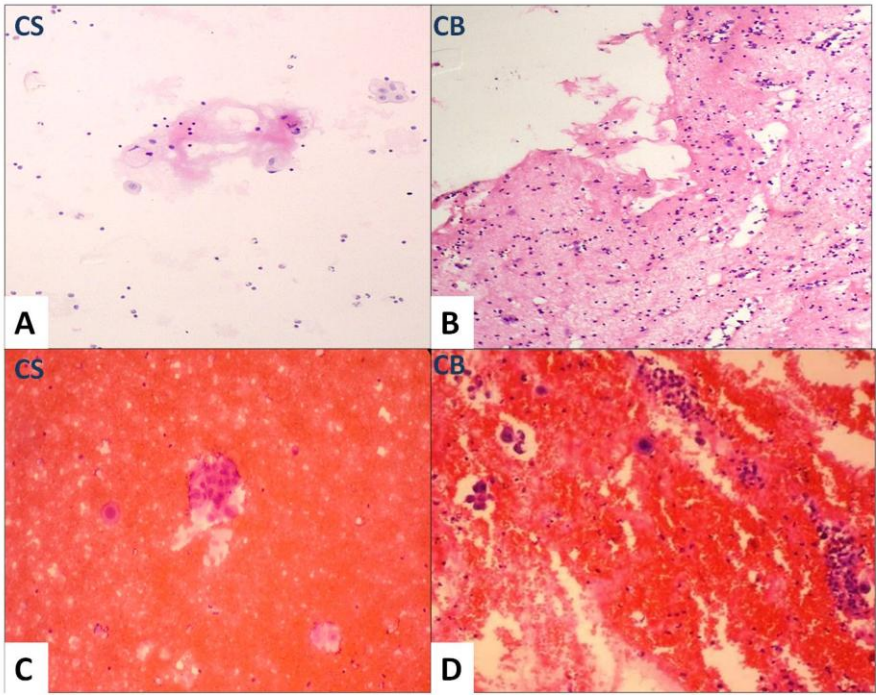


Figure 3: Known case of carcinoma prostate



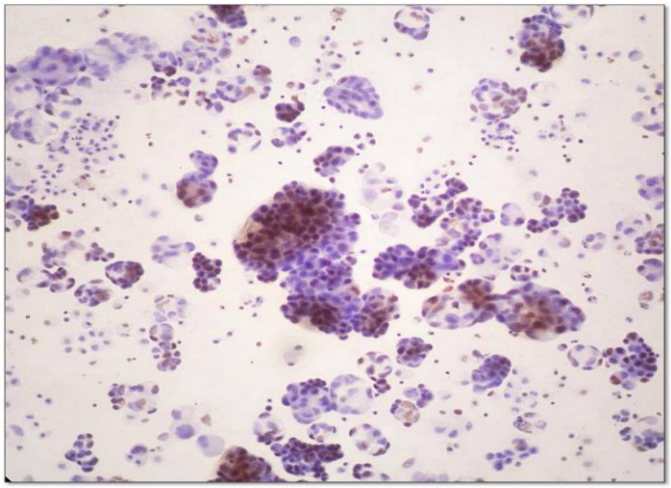
(a) Cytosmears showing presence of lymphocytes and neutrophils and were negative for malignancy (b) section from cell block showing presence of cluster of atypical epithelial cells (c) Calretinin positive in mesothelial cells and negative in atypical cells (d) Prostate specific antigen expression in atypical epithelial cells

Figure 4: Case of carcinoma Ovary. Comparison of cytosmears versus cell block



(a) Cytosmears showing mild cellularity (b) section from cell block of the same case showing increased cellularity and presence of cell clusters in a limited area (c) cytosmears show haemorrhagic background obscuring the morphology of tumour cells (d) section from cell block of the same case showing haemorrhagic background but preserved nuclear morphology and chromatin details

Figure 5: Immunostain for TTF-1 on cytosmear showing heterogenous expression among tumour cells



4. Discussion

Although use of cell block technique in cytology can be traced back as early as 1896 [8], it is now being recommended in most of the laboratories by the experts as a routine practice [9]. Cytological examination of serous effusions is an essential part of clinical medicine and is important not only for diagnosis but also plays a vital role in staging, prognosis and further management of the patient. Cell block preparation has been utilized as a useful adjunct to conventional cytology. A variety of techniques are being used for cell block preparation like bacterial agar method, cell block from Millipore, Histogel method, compact technique, albumin method, automated preparations etc [10],[11]. Crapanzano *et al* [12] conducted an electronic survey to assess the methods used worldwide for cell block preparation and their satisfaction rate with the technique employed. They found that approximately ten different methods were being used and many respondents were either unsatisfied or sometimes satisfied with their CB quality, with low-cellular yield being the leading cause of dissatisfaction. They concluded that most of the institutions were using plasma thromboplastin technique (30%) for cell block preparation as it was simple, safe, cost effective and less time consuming. It did not require any special equipment and training. All the other methods required an additional material, most of them were time consuming or tedious whereas few others were expensive.

In the present study, amongst the 100 serous effusions, pleural fluid (79/100) was the commonest followed by ascitic fluid (15/100) and pericardial fluid (06/100). Eighty

percent of the serous fluids were benign. Majority of these effusions were lymphocytic and tubercular (26/80; 33 %) in origin. Bhanvadia *et al* [13] also reported pleural fluid (79/150; 53%) as the commonest fluid among all the effusions with tuberculosis and acute infections being the major cause. Thapar *et al* [2] studied 190 cases of serous effusions where the most common fluid was peritoneal (92/190) followed by pleural (88/190) and pericardial (8/190) and the commonest cause of reactive effusion was tuberculosis (18.3%).

A single variable that strongly favours malignancy is "hemorrhagic effusion". In the present study, out of 100 fluid samples, 28 % were hemorrhagic and only fifteen (54%) of these were positive for malignancy. In a study by Kushwaha *et al* [14], there were 31.19% hemorrhagic fluids and 68.97% of hemorrhagic effusions were positive for malignancy.

On the basis of Mair *et al* point scoring system, 35 % of the smears and 55 % of the cell blocks achieved a score of 2 when cellularity was assessed, thereby, providing an additional increase of 20 % by cell block preparations. This showed that cell blocks prepared from plasma thromboplastin technique produced abundant amount of diagnostic cellular material. In a study by Thapar *et al* [2], 54% of the cytosmears and 67% of the cell blocks scored 2 with increased cellularity in 13 % of the cases.

Preserved cellular morphology was noted in 60 % of the smears and 75% of the cell blocks giving a score of 2. Thus, cell blocks showed good preservation by plasma thromboplastin technique with less cellular trauma and degeneration. Singh *et al* [15] and Shubhada *et al* [16] were also able to appreciate clear morphology with similar results.

On evaluating the architectural and cellular arrangement, score 2 was observed in 20% of the smears and 40 % of cell blocks. Cell block preparations revealed excellent architectural display with clearly recognizable malignant cells arranged in acini, papillae, clusters and 3D cell balls with minimal shrinkage and aberration. Morphological features including both cytoplasmic and nuclear details were sharp and distinct. These findings were in concordance with the findings of Bhanvadia *et al* [13].

Haemorrhagic fluids posed a greater diagnostic difficulty by obscuring the background on conventional smears but cell blocks prepared by PTCB technique revealed minimal volume of background blood with recognizable cellular details (Fig 4C- 4D).

In the present study, four cases were diagnosed as suspicious on smears but on cell block preparations, diagnosis changed from suspicious to positive in two of the cases. The other two cases remained suspicious even on cell blocks due to scanty cellularity. One of the cases was negative on cytological smears but diagnosed as positive for malignancy on cell blocks as it demonstrated few clusters of malignant cells. Therefore, the diagnostic yield increased by 15 % using cell block technique. Thus, the number of suspicious and positive fluids obtained with the combined smear and cell block technique increased the diagnostic accuracy than that of specimens examined by smears alone. This finding is in agreement with the findings of Thappar *et al* [2] who showed 20 % and Richardson *et al* [17] who showed 12 % increase in the diagnostic yield. In another study by Dekker and Bupp *et al* [18] additional yield of malignancy was noted in 38 % of the cell blocks [Table 4].

As described in other studies, it was found in the present study that with the TP-CB technique the cellular elements were better preserved and concentrated in a small area, making their evaluation less time-consuming and producing an accurate diagnosis.

In the present study, amongst the malignant effusions, five of the twelve pleural fluids were known cases of lung cancers, 1 case was of Prostate cancer, 1 case of Non Hodgkin's lymphoma and 5 cases with unknown primary. Out of five ascitic effusions, 2 cases were of ovarian cancers and 1 case each of gall bladder cancer, rectal cancer and gastric cancer. All the three pericardial effusions were due to breast cancers as all the samples were obtained from female patients. Shivkumarswamy *et al* [1] studied 60 pleural fluid samples where 10 of these fluids were malignant and primary was not known in three of the cases. In a study by kushwaha *et al* [14], out of 28 samples with malignancy, the primary site could be confirmed on cytology in 16 (57.14%) of cases while in remaining 12 cases, primary was not known.

Of the five pleural effusions with unknown primary, probable primary could be ascertained in four cases while one patient was lost to follow up. Therefore, detection of primary was possible in 80 % of the cases. These results are consistent with khan *et al* [19] who determined the primary site in 81.3 % of the serous fluids of unknown origin.

In the present study, immunomarkers used to distinguish between reactive mesothelial cells and malignant cells were PanCK, EMA and Calretinin as per our routine institutional practise. The next generation of markers employed to categorise the malignancy based on differential diagnosis were CK7, CK20, TTF-1, ER-PR, PSA, CD45, CD20, CD3, CA-125, Vimentin, and Synaptophysin. These markers helped to resolve the diagnostic dilemma in most cases and showed homogenous expression of various antibodies on cell blocks.

ICC can be performed on various cytologic preparations including cytospins, smears, and ThinPrep preparations as the situation may be but when performed on formalin fixed, paraffin embedded cell blocks of serous effusions, it is considered ideal because it simulates surgical pathology preparations most closely [20]. Cytosmears pose a great difficulty in malignant effusions due to overcrowding of cells and fixation artefacts. On the contrary, cell blocks allow application of immunocytochemical markers with fairly good results assuring its superiority over smears [21]. Flens *et al* [22] found heterogeneous expression of immunomarkers on cytosmears due to different protein expression profiles between the tumor cells suspended in serous effusion than those fixed in tissue, difference in fixation and sample

preparation between ICC and IHC. Cell-block preparations are considered superior to ThinPrep for many of the immunomarkers markers more specifically nuclear markers as their frequency and intensity of reaction with ThinPrep were significantly lower than with the cell-block preparation [23].

5. Conclusion

To conclude, PT-CB preparation in cytological evaluation of serous effusions offers following advantages: (1) Better preservation and better morphology of cell clusters, (2) Concentration of diagnostic material in a limited area and (3) Benefit to study multiple sections and application of IHC markers and special stains if required. It also offers an additional advantage for preservation of cell blocks for future molecular pathology.

This study reports an increase in diagnostic yield with the aid of PT-CB cell blocks. Thus, PT-CB preparations play a significant role in resolving the gray zone that a cytopathologist encounters while determining the nature of cells on effusions whether reactive, atypical or beyond doubt malignant.

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