ORIGINAL RESEARCH

A prospective study of plasma thromboplastin cell block preparation and conventional smears on serous effusions and its classification based on newly proposed international system for reporting serous fluid cytopathology

[1]Dr. Kamlesh Kumar, [2]Dr. Mamta Dwivedi, [3]Dr. Sneha Chauhan, [4]Dr. Astha Chaudhury, [5]Dr. Veenu Jain

[1]Associate Professor, Department of Pathology, Maharshi Vashistha Autonomous State Medical College, Basti, Uttar Pradesh, India
[2]Assistant Professor, Department of Pathology, Autonomous State Medical College, Basti, Uttar Pradesh, India
[3]Senior Resident, Autonomous State Medical College, Basti, Uttar Pradesh, India
[4]MD, Autonomous State Medical College, Basti, Uttar Pradesh, India
[5]Professor, Autonomous State Medical College, Basti, Uttar Pradesh, India

Corresponding author
Dr. Kamlesh Kumar
Associate Professor, Department of Pathology, Maharshi Vashistha Autonomous State Medical College, Basti, Uttar Pradesh, India
Email: drkumarasmc2020@gmail.com

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ABSTRACT

Introduction: Plasma thromoplastincytoblock technique is a simple , cost effective and readily adaptable in routine hospital laboratories as compared to cell block preparations made from conventional techniques such as agar gel or formol alcohol , which is much laborious and time consuming . We applied the recently proposed International system for reporting serous cytopathology (TIS) on the serous effusions and reported our experience. **Aim**: To study the diagnostic utility of Thromboplastin cell block technique in conjunction with conventional smears and its classification according to TIS. **Material and methods**: 114 samples were included in the study over a period of 8 months, both conventional and cell block preparations were made. **Result**: A total of 114 cases were studied: 64 pleural fluid, 45 ascetic fluid and 05 pericardial fluid. 04 (3.5%) were diagnosed as non diagnostic, 69 (61%) as Negative for malignancy, 10 (8.8%) as Atypia of underterminedsignificance, 12 (10.52%) as Suspicious for malignancy, 19 (16.66%) as Malignant. Cellularity and diagnostic yield for malignancy was increased by cell block preparation. **Conclusion:** Plasma thromoplastin cell block method provides high cellularity, better architectural patterns and a good preservation of nuclear details. TIS is auser friendly reporting system.

INTRODUCTION

Cytological evaluation is often the first line of investigation in the clinical setting of serous effusions. [1] Effusions may be caused by a wide variety of stimuli and not uncommonly are harbingers of an underlying malignancy. [2] Accurate identification and typing of tumor cells in effusion samples serves as a guide to patient management. On the other hand, exclusion of malignancy allows for appropriate management in case of non-malignant effusions. Cytological examination of serous fluids is one of the commonly performed investigation. The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. The cell block (CB) technique is one of the oldest methods for the evaluation of body cavity fluids. [3] However, a new method of cell block preparation by using 10% alcohol-formalin as a fixative was used, to identify the sensitivity of the diagnosis in comparison with the conventional smear (CS) study. The main advantages of the CB technique are preservation of tissue architecture and obtaining multiple sections for special stains and immunohistochemistry. [4]

Serous effusion indicates accumulation of excess fluid in the body cavities, namely, pleural, pericardial, and

peritoneal, the latter also referred to as ascites. Effusion invariably indicates an underlying pathology and constitutes an important diagnostic sample in clinical practice, including oncology. [5] Specimen from various anatomic sites can be evaluated by cytology. The techniques for collection, transportation, and preparation of specimen are of prime importance, as an adequate, well-prepared, well-stained smear helps in the ultimate goal of an accurate cytopathological diagnosis. [6]

Thoracentesis is a diagnostic procedure for patients with pleural effusion. Pleural fluid (PF) obtained from the procedure should be submitted for biochemical, microbiological, and cytological study (CS). In cases of suspicion of malignant pleural effusion (MPE), CS is extremely useful as it provides a diagnostic rate of 60%, ranging from 40% to 87%. [7-10] CS is important not only in diagnosis but also in staging and further guiding treatment for malignancy. Many widely used guidelines, such as those of the American College of Chest Physicians (ACCP) and the British Thoracic Society (BTS), recommend CS of two samples of pleural effusionn. [7,10] If the procedures turn out to be non-diagnostic, further invasive investigations such as imaged-guided pleural biopsy or thoracoscopic biopsy are recommended for a definitive diagnosis. The challenges of obtaining a diagnosis from CS include indistinct morphological details, overlapping or overcrowding of cells, abundance of inflammatory cells, paucity of representative cells, and cell losses or changes. [11]To overcome these limitations, cell block (CB) method was developed to provide better tissue architecture and morphological features for differentiating between malignant and non-malignant cells and also for further processing via special stains and immunohistochemistry. [12]

Various methods for preparing CBs have been reported and the techniques are in a state of continuous improvement. Different methods include usage of various adjuvants such as agar, thrombin, gelatine, and egg albumin. The ideal method should be simple, faster, reproducible, and able to concentrate cells in a limited field without loss of cellular material and cost-effective. All traditional methods of CB require overnight formalin fixation and processing and subsequent manual embedding similar to histological techniques. This would cause the delay in diagnosis. Shandon Cytoblock (Thermo) and Cellient Automated Cell Block System (Hologic) are the two automated cellblock preparation systems available. For these systems, the manufacturer's recommendations should be followed. [13-15] Hence, this study was conducted to study the diagnostic utility of Thromboplastin cell block technique in conjunction with conventional smears and its classification according to TIS.

RESULTS

A total of 114 cases were studied :64 pleural fluid , 45 ascetic fluid and 05 pericardial fluid .

04 (3.5%) were diagnosed as non diagnostic ,69 (61%) as Negative for malignancy , 10 (8.8%) as Atypia of undertermined significance , 12 (10.52%) as Suspicious for malignancy , 19 (16.66%) as Malignant . Cellularity and diagnostic yield for malignancy was increased by cell block preparation. The maximum estimated rate of return of malignancy (ROM) was 25% for category 1 (non-diagnostic), 12% for category 2 (indicating no malignancy), 50% for category 3 (showing atypical characteristics), 90% for category 4 (suggesting suspicion of malignancy), and 100% for category 5 (confirming malignancy).

Table 1: Frequency of cases in categories in serious effusion cytology.

Body fluid	Non-diagnostic	Negative for malignancy	Atypia of undetermined significance	Suspicious for malignancy	Malignant
Pleural fluid	1	40	6	7	10
Ascitic fluid	3	26	4	4	8
Pericardial fluid	0	3	0	1	1
Total	04 (3.5%)	69 (61%)	10 (8.8%)	12 (10.52%)	19 (16.66%)

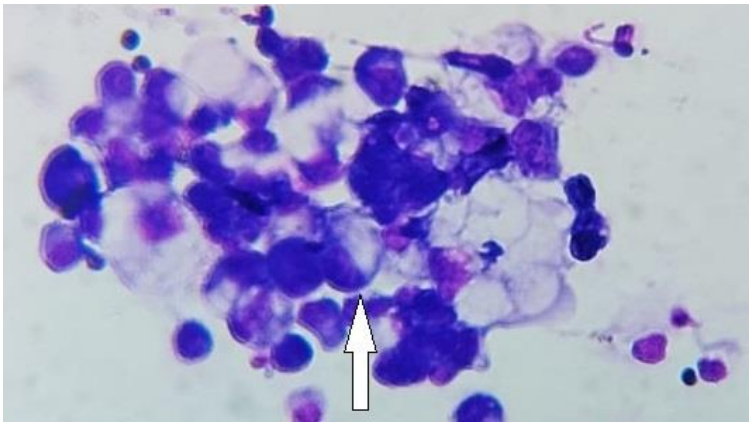

Figure 1: Signet ring cells in pleural fluid.

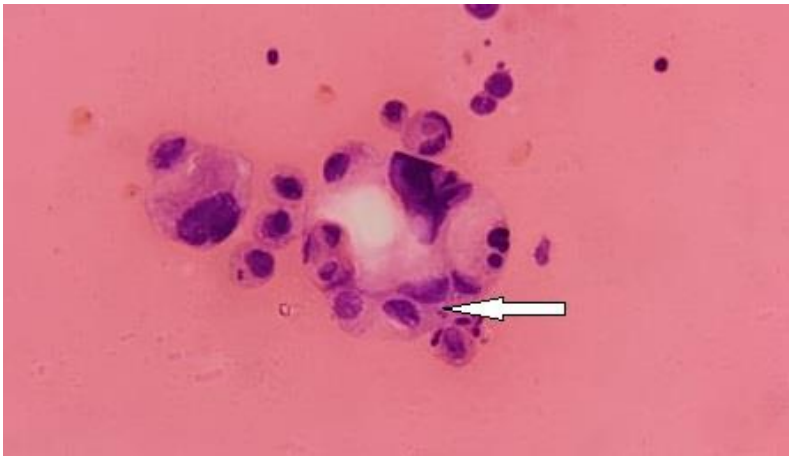

Table 2: Estimated risk of malignancy in the IAC effusion diagnostic categories

Category	Cases	Estimated risk of malignancy (upper bound)
Non –diagnostic	4	25%
Negative for malignancy	69	12%
Atypia of undetermined significance	10	50%
Suspicious for malignancy	12	90%
Malignant	19	100%

DISCUSSION

The cytological examination of serous effusions has increasingly gained acceptance in clinical medicine, to such an extent that a positive diagnosis is often considered the definitive test and obviates explorative surgery. It is important not only in the diagnosis of malignant lesions, but also helps in staging and prognosis. [16] The development of malignant pleural effusion is a common complication of cancers like pulmonary and gastric carcinomas. [17] Examination of fluids from the serous cavities of the body is an essential component of management in adult patients. Malignant neoplasms, especially lymphoid neoplasms, represent a major cause of death in children and in these cases cytological examination is very useful in their management. [18] Hence, this study was conducted to study the diagnostic utility of Thromboplastin cell block technique in conjunction with conventional smears and its classification according to TIS.

In the present study, a total of 114 cases were studied :64 pleural fluid , 45 ascetic fluid and 05 pericardial fluid. 04 (3.5%) were diagnosed as non diagnostic, 69 (61%) as Negative for malignancy, 10 (8.8%) as Atypia of undertermined significance, 12 (10.52%) as Suspicious for malignancy, 19 (16.66%) as Malignant . Cellularity and diagnostic yield for malignancy was increased by cell block preparation. A study by Kundu R et al, assess the feasibility of applying the IAC reporting categories to effusions, determine the frequency, and provide an estimate of the risk of malignancy (ROM) for individual diagnostic categories. All cases of serous effusion fluids reported in the year 2019 were retrieved from the archives and reassigned as per the IAC diagnostic categories. The clinical and histopathological follow-up information was obtained wherever possible. A total of 1340 effusion samples were received from 1085 patients. There were 561 (51.7%) males and 524 (48.3%) females. Majority were pleural (1066, 79.5%), followed by peritoneal (187, 14%) and pericardial (87, 6.5%) effusions. The age ranged from 7 months to 92 years. There were 35 (2.6%) samples in category 1 (non-diagnostic), 954 (71.2%) in category 2 (benign), 17 (1.3%) in category 3 (atypical), 59 (4.4%) in category 4 (suspicious for malignancy) and 275 (20.5%) in category 5 (malignant). The estimated ROM in serous effusion samples was 20% for category 1, 16.7% for category 2, 50% for category 3, 94.4% for category 4 and 100% for category 5. The categorization of serous effusion cytology samples as per the IAC diagnostic categories and as per the reporting format developed by the IAC is feasible and the management recommendations are mostly appropriate. [19]

In the present study, the maximum estimated rate of return of malignancy (ROM) was 25% for category 1 (non-diagnostic), 12% for category 2 (indicating no malignancy), 50% for category 3 (showing atypical characteristics), 90% for category 4 (suggesting suspicion of malignancy), and 100% for category 5 (confirming malignancy). A study by Rekhi B et al, assess and validate the diagnostic utility of cell blocks (CBs) and compare its results with the corresponding conventional smears, prepared from effusion samples. CBs were prepared by thromboplastin technique in 220 cases. In 208 cases, diagnostic concordance between results obtained from smears and corresponding CBs was evaluated. Various antibody markers were tested, as per individual case. The average age of patients was 52.2 years. Positive immunohistochemical (IHC) staining for various

markers was observed in 182 cases (82.7%) The most frequently positive antibody marker was PAX8 (101/134), followed by p53 (85/92) [mutation type (either diffusely positive or completely negative)], WT1 (tumor cells) (80/112), calretinin (2/87) (diffuse), BerEP4 (21/49), CA125 (21/24), CK7 (31/39) and CK20 and CDX2, together (5/16). Various other IHC markers utilized, including their positive expression, were TTF1 (1/10), p40 (3/3), p63 (2/4), ER (21/29), HBME1 (1/7), GATA3 (1/4), and MIC2 (1/1). Complete diagnostic concordance between CBs and smears was observed in 170/208 cases (81.7%). There were 20 major discordances, 10 minor and 8 cases with sampling errors. IHC was useful in classifying 158/182 (86.8%) cases, including serous or Müllerian adenocarcinoma (n = 123), mostly high-grade (121); metastatic squamous carcinoma (3); gastrointestinal-type adenocarcinoma (8); pulmonary adenocarcinoma (1); breast adenocarcinoma (1); Ewing sarcoma (1); and mesothelioma (2). CBs are complementary to smears in the detection of gynecological malignancies, mostly high-grade serous adenocarcinomas. These provide an opportunity for testing several IHC markers, for a precise diagnosis, including in various uncommon case scenarios, associated with significant therapeutic implications.[20]Shivakumarswamy U et al, compare the morphological features of the CS method with those of the cell block (CB) method and also to assess the utility and sensitivity of the CB method in the cytodiagnosis of pleural effusions. The study was conducted in the cytology section of the Department of Pathology. Sixty pleural fluid samples were subjected to diagnostic evaluation for over a period of 20 months. Along with the conventional smears, cell blocks were prepared by using 10% alcohol–formalin as a fixative agent. Statistical analysis with the 'z test' was performed to identify the cellularity, using the CS and CB methods. Mc. Naemer's χ2test was used to identify the additional yield for malignancy by the CB method. Cellularity and additional yield for malignancy was 15% more by the CB method. The CB method provides high cellularity, better architectural patterns, morphological features and an additional yield of malignant cells, and thereby, increases the sensitivity of the cytodiagnosis when compared with the CS method. [21] One of the most common problems in CS cytology is to distinguish reactive mesothelial cells from metastatic neoplasms. The difficulty is either secondary to marked atypia of mesothelial cells caused by the microbiological, chemical, physical, immunological, or metabolic insults to the serous membranes or to the subtle cytomorphological features of some malignant neoplasms, particularly well-differentiated adenocarcinomas. The problem may become compounded by artefacts from poor fixation, preparation, or staining techniques. [22] Although the preparation of CS is a much simpler procedure than that of paraffin sections, it has limitations, that is, lack of tissue architecture. In some cases, appreciation of tissue architecture make diagnosis easier. [23] Another limitation of the conventional cytological examination of effusions is that it has a sensitivity of only 40–70% for the presence of malignant disease due to overcrowding of cells, cell loss and different laboratory processing methods. Others like reactive mesothelial cells, abundance of inflammatory cells and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional smears. [24] Malignant pleural/peritoneal effusion from an ovarian primary were confirmed as high grade serous carcinoma by cell-block immunocytochemistry using a panel comprising CK7, WT1, PAX8 and p53. A minimum panel of PAX8, WT1, and p53 allowing for specific pre-neoadjuvant chemotherapy diagnoses of ovarian high grade serous carcinoma in effusions is advocated by Bansal et al. [25] All our cases received neoadjuvant chemotherapy following immunophenotyping as high grade serous carcinoma of ovarian origin.

CONCLUSION

Plasma thromoplastin cell block method provides high cellularity, better architectural patterns and a good preservation of nuclear details . TIS is a user friendly reporting system.

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