Concordance between conventional cytology and cell block of suspected malignant pleural effusion: A record-based study in a tertiary care center of eastern India

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ABSTRACT

Background: Cytological study of pleural fluid helps establishing the underlying cause of pleural effusion. It is considered the most important tool in diagnosing malignancy in effusions. However, accurate identification of cellular morphology can be a problem in conventional cytology. Cell block preparation from pleural effusion aids in this diagnosis due to the preservation of tissue architecture, and multiple sections can be obtained for further studies. **Aims and Objectives:** The objectives of this study were to compare the diagnostic results of conventional cytology and cell block studies of suspected malignant pleural effusions and find out the diagnostic agreement or concordance between the two methods. **Materials and Methods:** A record-based study was conducted at the Department of Pathology on conventional cytology and cell block study reports of 150 suspected malignant pleural effusion cases over 35 months. The diagnoses were expressed in five categories – non-diagnostic, negative for malignancy, atypia of undetermined significance, suspicious for malignancy, and malignant. χ^2 test was used to identify the percentage of malignancy reported. Agreement of both methods was assessed through Kappa statistics. **Results:** About 4.67% of cases were reported as malignant by conventional cytology, whereas cell block study diagnosed malignancy in 19.33% of cases. Relative diagnostic agreement between the two methods was 0.73 and concordance by Cohen's Kappa was 0.34, which signifies fair concordance. **Conclusion:** Cell block study, though having a fair concordance, is much superior to conventional cytology smears in detecting malignant cells and diagnosing a pleural effusion as malignant.

Key words: Cell block; Cytology; Malignancy; Pleural effusion

INTRODUCTION

Cytological examination of pleural effusion fluid is a routine laboratory test. It helps establishing the cause of pleural effusion, which can be of underlying malignancy, inflammatory, or other conditions.[1-3] Cytologic techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant cells in pleural effusions.[1] Accurate identification of the exact nature of cells is often a practical problem in conventional cytology smears due to indistinct morphological details, overlapping or overcrowding of cells, abundance of inflammatory cells, paucity of representative cells, and cell losses or changes.[1,2] It has been seen in various studies that the cytological examination of fluids by means of smears, however carefully prepared, leaves behind a large residue that is not further investigated but that might contain valuable diagnostic material. This residual material can be evaluated in a simple and expedient fashion by treating it as a cell block, embedded in paraffin, and examined in addition to the routine smears.[1-3] The main advantages of the cell block technique are preservation of tissue

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architecture and obtaining multiple sections for special stains, immunohistochemistry, and molecular studies.[2-4] Because of its safe and easy collection, pleural fluid cell block is considered an alternative to pleural tissue, especially if the patient ineligible for surgery or biopsy.[4,5]

Although the cell block technique has been long known and well established, there have been few reports and a limited number of samples involving the direct comparison of conventional cytology and cell block on consecutive patients for diagnosis of pleural effusion. Studies have identified the cell block techniques as an underutilized and overlooked method for diagnosis of malignant pleural effusions.[6,7] Studies on the concordance between conventional cytology and cell block methods are rarer still. This study is expected to minimize the gap in knowledge in the cytodiagnosis of pleural effusion by conventional cytology and cell block methods.

Aims and objectives

To compare the diagnostic results of conventional cytology and cell block studies of suspected malignant pleural effusions and find out the diagnostic agreement or concordance between the two methods.

MATERIALS AND METHODS

A record-based cross-sectional study was conducted at the Department of Pathology, College of Medicine and Sagore Dutta Hospital, Kolkata from January 2021 to November 2023 on reports of conventional cytology and cell block study of 150 suspected malignant pleural effusion cases. An Institutional Ethics Committee endorsed the study protocol at College of Medicine and Sagore Dutta Hospital, Kolkata.

Inclusion criteria

The study included reports of suspected malignant pleural effusion samples examined by both conventional cytology and cell block study methods within the study period from the records.

Exclusion criteria

Records of suspected malignant pleural effusion samples examined by only conventional cytology method but where no cell block study was done were excluded from the study.

Methodology

Reports of conventional cytology and cell block studies of suspected malignant pleural effusions following the inclusion and exclusion criteria were obtained from the records of the Department of Pathology, College of Medicine, and Sagore Dutta Hospital, Kolkata. Within the study period (January 2021–November 2023, i.e., 35 months), a total of 150 cases were found suitable, and all were included in the study. No randomization was done for case selection.

The diagnoses of conventional cytology and cell block studies of the 150 cases were expressed in the following five categories as per The International System for reporting serous fluid cytopathology[8] and Indian Academy of Cytologists Guidelines:[9]

- Non-diagnostic (ND): Specimens with insufficient cellular elements for a cytologic interpretation
- Negative for Malignancy (NFM): specimens with cellular changes completely lacking evidence of mesothelial or non-mesothelial malignancy
- Atypia of Undetermined Significance (AUS): Specimens that lack quantitative or qualitative cytologic features to be confidently diagnosed as either benign or malignant and that exhibit sufficiently clear morphologic features to exclude the possibility of classifying them as ND
- Suspicious for Malignancy (SFM): Specimens showing cytological features usually found in malignant lesions, but that are insufficient either in quality or quantity to make a definitive diagnosis of malignancy
- Malignant (MAL): Specimens showing cytomorphological features that, either alone or in combination with ancillary studies are diagnostic of malignancy.

Ethics

An Institutional Ethics Committee endorsed the study protocol at College of Medicine and Sagore Dutta Hospital, Kolkata. Anonymity and confidentiality of the patients have been maintained.

Statistics

Data were collected, entered in MS Excel, and checked for consistency. Epi Info version 7 was used for data analysis. The numerical variables in the descriptive analyses were reported in the form of means and SDs; categorical data were expressed in proportion. χ^2 test was used to identify the percentage of malignancy reported. Agreement of both methods was assessed through Kappa statistics.

RESULTS

One hundred and fifty pleural fluid reports from patients aged 2 to 85 years were included in this study. The maximum number of patients belonged to the age group 61–70 years (28%), with a mean age of 54.76±15.26 years. Male population constituted 64.67% and females 35.33% of total with a ratio of 1.83. About 57.33% of the pleural effusions were right sided, and 42.67% were left sided.

After analysis of the above samples, by conventional cytology and cell block methods, the diagnostic findings of each method were categorized into ND, NFM, AUS, SFM, and MAL (Figure 1).
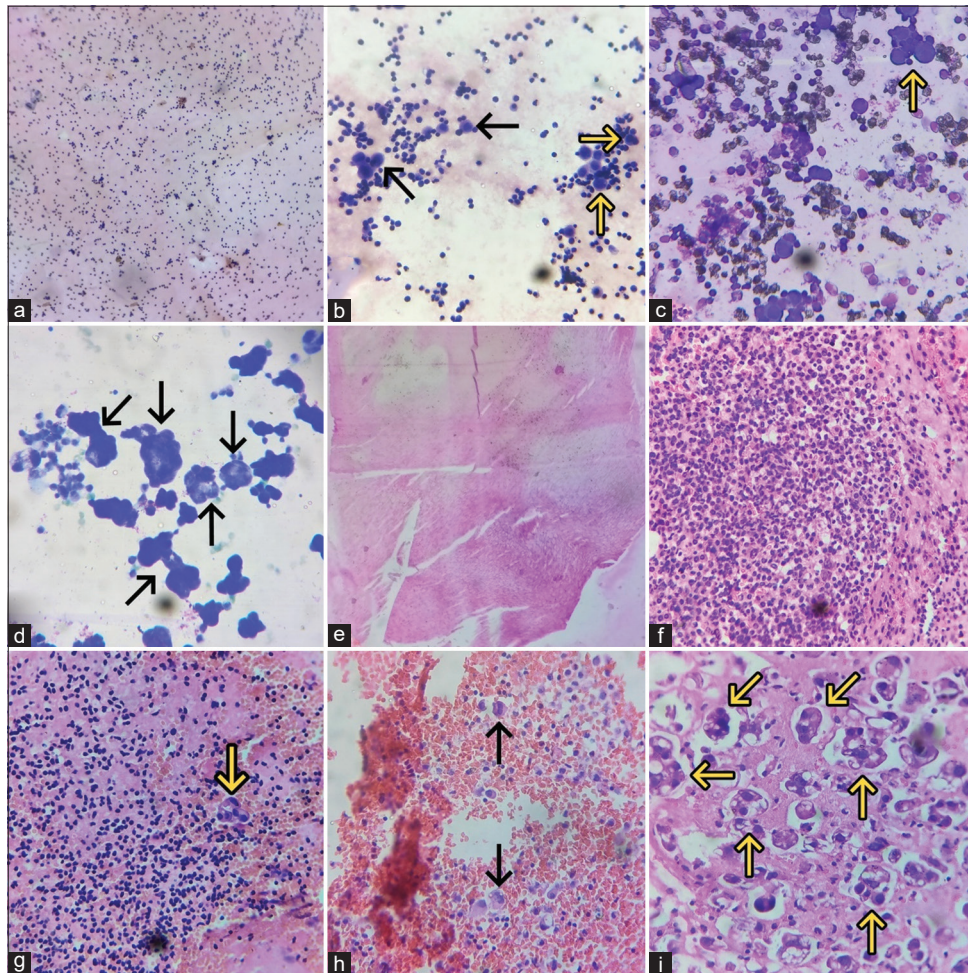
Figure 1: Composite image of photomicrograph of conventional cytological smear (CS) (a-d) and cell block sections (CB) (e-i) of pleural fluid showing: (a) Only inflammatory cells and reactive mesothelial cells in CS – Negative for Malignancy (NFM) (Papanicolaou stain, ×100); (b) Inflammatory cells and reactive mesothelial cells (black arrows) with few atypical looking cells (yellow arrows) in CS – atypia of undetermined significance (AUS) (Papanicolaou stain, ×400); (c) Few clustered suspicious looking atypical cells (yellow arrow) in a background of inflammation in CS – Suspicious for malignancy (SFM) (Leishman stain, ×400); (d) Clusters of cells with high N: C ratio, pleomorphic nuclei, irregular nuclear membrane (black arrows) in CS – Malignant (MAL) (Leishman stain, ×400); (e) Only fibrinous and hemorrhagic elements, no cellular elements in CB – Non-diagnostic (ND) (H&E stain, ×40); (f) Only inflammatory cells and reactive mesothelial cells in CB –NFM (H&E stain, ×400); (g) Inflammatory cells with few atypical looking cells (yellow arrow) in CB –AUS (H&E stain, ×400); (h) Many discrete suspicious looking atypical cells (black arrows) in a background of inflammation and haemorrhage in CB –SFM (H&E stain, ×400); (i) Clusters of cells with high N: C ratio, pleomorphic nuclei, irregular nuclear membrane containing mucinous material and some forming acini like structures (yellow arrows) in CB –MAL (H&E stain, ×400)

In conventional smear, there were no ND samples. 123 (82%) samples were NFM, 9 (6%) AUS, 11 (7.33%) SFM, and 7 (4.67%) samples were found to be MAL. However, in cell block studies, 3 (2%) samples came out to be ND, 105 (70%) were NFM, 5 (3.33%) AUS, 8 (5.33%) SFM, and malignancy was found in 29 (19.33%) samples (Figure 2).

Out of the 123 samples diagnosed NFM by conventional cytology, in cell block 101 samples had the same diagnosis, while malignancy was found in 10 samples; AUS was found in 5 samples, and cells SFM were found in 4 samples; and the rest 3 samples were classified as ND. Out of the nine AUS diagnosed samples in conventional cytology, 2 turned out to be NFM, 3 were SFM, and 4 were found to be positive for malignancy by cell block method. In
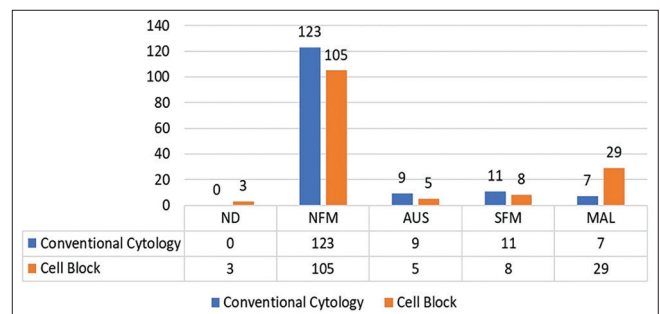
Figure 2: Bar diagrammatic representation of diagnostic findings of pleural effusion by conventional cytology and cell block methods

conventional cytology, suspicious cells were found in 11 samples, and by cell block study, 8 were found to be MAL, 2 were found to be NFM, and 1 sample had the

same diagnosis. Of the 7 samples diagnosed MAL by conventional smear, malignancy was found in cell blocks of all the samples (Table 1).

Hence, of the 29 samples found to have malignant cells by cell block, only 7 could be positively deemed malignant by conventional smear, whereas atypical and suspicious cells were found in 4 and 8 samples, respectively, whereas malignancy could not be detected in 10 samples by conventional cytology (Table 1).

Two cases of samples showing AUS in conventional smears, but later classified into negative or positive for malignancy by cell block studies, respectively, are shown in Figure 3.

The overall relative diagnostic agreement between the two methods is 0.7267. The concordance with Cohen's kappa is 0.3352. However, taking the ND samples out of account, the concordance increases slightly, with the relative agreement being 0.7413 and the kappa value being 0.356 (Table 1).

The malignant effusions were almost the same in the case of male and females (15 in males and 14 in females). Out of the 29 malignant cases, primary was known in 22 cases, out of which 19 were carcinoma of the lung, and all of which were Non-Small Cell Lung Carcinoma; in three cases, the primary was Intraductal Carcinoma of the Breast, and one case of Ovarian serous carcinoma as primary. Final clinical diagnoses for the rest of the cases could not be known as these were unavailable in the records.

DISCUSSION

In our study of 150 cases, there were more male than female patients, which is similar to other studies like Shivakumarswamy et al.,[2] Thapar et al.,[3] Rani et al.,[4] Poon et al.[10] The majority of the patients are over 40 years of age. This may be explained by higher smoking prevalence in the male population, along with the practice of delayed health seeking behaviour.[10]

In our study, combined conventional cytology and cell block studies yield a diagnosis of 2% ND, 70% NFM, 3.33% AUS, 5.33% SFM, and 19.33% as MAL effusions. The findings are close to other studies like Poon et al.,[10] and Pinto et al.[11]

The rate of inadequacy of samples was higher for cell block preparation (2%) than conventional cytology (0%). According to current studies, the rate of insufficiency in pleural effusion studies combining conventional smear and cell blocks is reported to be 1–6%.[10,11] Our results fall within that range.

Table 1: Concordance between diagnostic findings of conventional cytology and cell block methods

Conventional cytology	Cell block					Total
	ND	NFM	AUS	SFM	MAL	
ND	0	0	0	0	0	0
NFM	3	101	5	4	10	123
AUS	0	2	0	3	4	9
SFM	0	2	0	1	8	11
MAL	0	0	0	0	7	7
Total	3	105	5	8	29	150
Relative diagnostic agreement						0.7267
Cohen's Kappa						0.3352
Relative diagnostic agreement ND						0.7413
Cohen's Kappa excluding ND						0.356
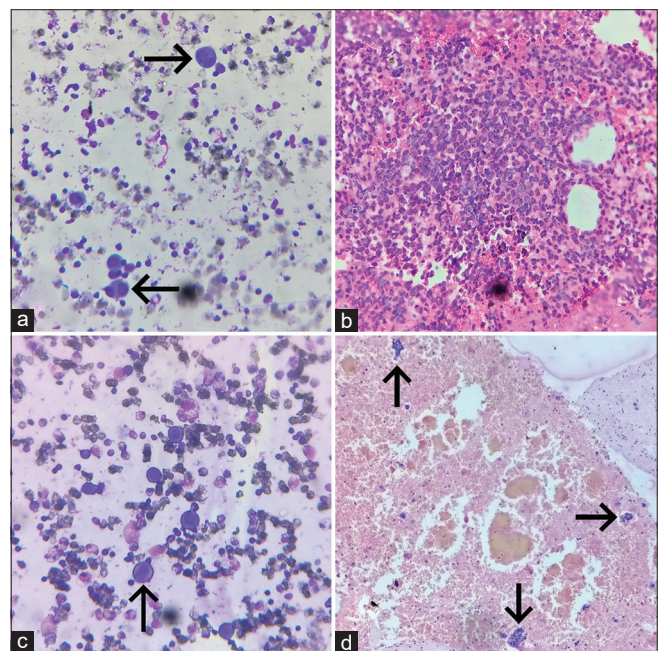
Figure 3: Composite image showing photomicrographs of: (a) conventional smear of pleural fluid (Leishman stain, ×400) showing few atypical binucleate cells (Black arrows) in a background of inflammatory cells and reactive mesothelial cells - diagnosed as Atypia of Undetermined Significance (AUS), and (b) cell block study from the same pleural fluid sample of Figure 3a (H&E stain, ×400) showing only reactive mesothelial cells without any atypical features, finally diagnosed as negative for malignancy (NFM); (c) conventional smear of a different pleural fluid sample (Leishman stain, ×400) showing few discrete atypical cells (Black arrows) in a background of inflammatory cells – diagnosed as AUS, and (d) cell block study from the same pleural fluid sample of Figure 3c (H&E stain, ×100) showing a few clusters of cells (Black arrows) with high N: C ratio, irregular nuclear membranes, and nuclear pleomorphism – diagnosed as MAL

One of the major problems that arise in the case of conventional smear is the difficulty to distinguish between malignant cells and reactive mesothelial cells.[4] This is primarily due to indistinct morphological details, overlapping, overcrowding or paucity of cells, and obscuring by hematological elements in conventional smears.[1,2] Cell block studies are much better in diagnosing the nature of cells

and detecting malignancy because of the better-preserved architecture, less dispersal of the cells which aid in recognition of histological patterns despite the presence of blood in the background.[1,2] In our study, detection of malignancy was increased manyfold using cell block, with only 4.67% of cases showing malignancy in conventional smear, which increased to 19.33% in case of cell block.

The concordance with kappa value comes to 0.34, which slightly increases, if inadequate samples are excluded, to 0.36. Hence, in our study, the conventional cytology and cell block methods are in fair diagnostic agreement.

Limitations of the study

Since our study was a departmental record-based study, the final clinical diagnosis of all the cases could not be established and complete correlation between conventional smear and cell block could not be done. Immunohistochemical studies also could not be done due to unavailability of resources.

CONCLUSION

The study shows that cell block study, though having a fair concordance, is much superior to conventional cytology smears in detecting malignant cells and diagnosing a pleural effusion as malignant. Hence, it is better to make it a routine adjuvant to conventional smear cytology for diagnosis of pleural effusions.

ACKNOWLEDGMENT

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Source of Support: Nil, **Conflicts of Interest:** None declared.