Original Research Article

Diagnostic yield of pleural fluid cell block and fluid cytology in malignant pleural effusion: Study of 200 cases in tertiary care

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Abstract

Background: malignant pleural effusion missed routinely because of less diagnostic yield of conventional fluid cytology. **Materials and Methods:** The study included 200 cases of unexplained, exudative pleural effusion with ADA ≤30/IU/liter and pleural fluid cytology is either positive for malignant cell with or without cell type differentiation, or cytology suspicious for malignant cell. All cases were subjected to cell block preparation. Statistical analysis was done by using chi-test. **Observation and analysis:** In study of 200 cases, mean age of group was 68±9.5 years and adenocarcinoma was predominant malignancy in 72% cases, mesothelioma in 10% cases, squamous cell carcinoma in 7% cases and 9% cases were having primary tumor outside the thoracic cavity. In study cases pleural fluid cytology was positive in 42% cases (84/200) and pleural fluid cell block was positive in 96% cases (192/200) in detecting malignant pleural effusion ($p<0.0001$). Remaining six and two cases were diagnosed by using image guided and thoracoscopy guided pleural biopsies respectively. IHC was done in all pleural fluid cell block preparation for calretinin, cytokeratin and EGFR. **Conclusion:** Pleural fluid cell block is sensitive, superior, cost effective and specific diagnostic method over conventional pleural fluid cytology. 'Cell block' specimens are enough for primary diagnosis and IHC analysis necessary for cell typing. It will decrease need for more invasive and costlier diagnostic methods like thoracoscopy and image guided pleural biopsies. We recommend cell block for every exudative pleural fluid samples with ADA<30 IU/liter.
Key Word: Malignant pleural effusion, Pleural fluid cell block, cytology, Lung cancer

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INTRODUCTION

Lung cancer is a leading cause of cancer-related mortality world wide, with non-small cell lung cancer (NSCLC) accounting for around 80%-85% of lung cancers.[1]Although pleural effusion is one of the clinical signs of malignant disease, its accurate diagnosis is sometimes difficult. Determining the diagnosis of pleural effusion is important in planning the appropriate management and in the prognostication of the malignant disease.[2-4]Thoracentes is and/or closed pleural biopsy are generally considered as the first step for diagnosis of pleural effusion because these procedures can be easily performed even in outpatients. Some studies have reported that the diagnostic yield of cytology by thoracentesis was 62% to 90%and that of closed pleural biopsy was 40% to 75%.[4] Cytologic techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumors in effusions.[5] Accurate identification of the exact nature of cells (benign/ malignant/reactive) is often a practical problem in conventional cytology smears (CS), due to overcrowding of cells, cell loss and different laboratory processing methods.[6] On the other hand, cell block is also a useful method to evaluate pleural effusion by enabling observation of tissue architecture and providing additional sections that are easily available for special

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stains and immunochemistry.[7,8] Quincke in 1882, first published detailed description of cancer cells in abdominal and pleural fluids using cell films from sediment[9] while Bahrenburg first introduced cell block technique or paraffin embedding of sediments in 1896.[10] Many techniques for CB are described like the plasma thromboplastin method[11] bacterial agar method,[5,11] simplified cell block technique[11,12] compact cell block technique[13] histogel technique[14] and Fixed sediment method (FSM).[11] In this study we assessed diagnostic yield of pleural fluid cell block in comparison to conventional fluid cytology. We also assessed utilization of cell block specimens for immunohistochemistry analysis.

MATERIALS AND METHODS

Prospective multicentric study conducted in Internal Medicine, Pulmonary Medicine, MIMSR medical college Laturand Venkatesh chest Hospital, Latur during Jan 2014 to June 2016, to find out diagnostic yield of conventional pleural fluid cytology in malignant pleural effusion and its comparison with pleural fluid cell block specimens. We also analyzed immunohistochemistry analysis of cell block specimens. Total 200 cases of unexplained, exudative pleural effusion were enrolled in study after IRB approval and written informed consent of patient.

Inclusion criteria

1. Unexplained, exudative pleural effusion
2. Exudative pleural effusion with pleural fluid ADA ≤30/IU/liter
3. Hemorrhagic or reddish colored pleural effusion with ADA ≤30/IU/lite
4. Pleural fluid cytology is either positive for malignant cell with or without cell type differentiation, or cytology suspicious for malignant cell
5. Clinical and radiological feature suggestive of malignant pleural effusion.(Radiological features of malignant pleural effusion-massive pleural effusion, pleural effusion with fixed mediastinum or central mediastinum)

Exclusion criteria

1. Transudate pleural effusion
2. Exudative pleural effusion with ADA>44 IU/Liter and high index of suspicion for tuberculosis.
3. Bilateral pleural effusion with co-morbidity like heart failure, kidney disease, or hypoproteinemia, anemia.
4. Cases not willing to participate in study or not willing for pleural fluid aspiration

METHODOLOGY

Cases attending outdoor unit after scrutinizing inclusion and exclusion criteria with high index of suspicion of malignancy on clinical and radiological criteria were enrolled in study. All 200 study cases undergone pleural fluid aspiration and at least 100 ml pleural fluid is aspirated as per standard guidelines for thoracentesis. Pleural fluid aspiration was done under ultrasound guidance and aspirated fluid was divided in to two aliquots, one sent for cytology and second for cell block preparation. All study samples were evaluated by two different cytopathologists and oncopathologists having expertise in field of thoracic oncology. Those cases not diagnosed by fluid cytology or cell block were undergone image guided pleural biopsy and thoracoscopy guided pleural samplings to confirm the diagnosis.

Procedure of pleural fluid cytology: 20 ml pleural fluid was were centrifuged at 2500 rpm for 10 minutes. A minimum of 3 smears were prepared from the sediment. One smear was prepared after air drying and it was stained with the May-Grunewald-Giemsa stain. The other twos mears were immediately fixed in 95% alcohol, and were stained with Haematoxylin-Eosin stain.

Cytology results were categorized as-

1. Cytology suspicious for malignant cells or malignant cells with undifferentiated morphological type
2. Cytology showing clearmorphological malignant cells differentiation
3. Cytology negative for malignant cell or showing benign cellularity

Procedure of 'pleural fluid cell block': Cell block processing for serous effusion- Modified Thromboplastin method is used. After centrifugation at 2500 rpm for 10 minutes, drain the supernatant or pipette out the supernatant cell and residual sediment was formed. Excess supernatant was blotted out, 2 drops of plasma added to the tube and then 4 drops of thromboplastin added and allowed to clot for 20 minutes. Then inert the tube and collect the cell block on filter paper. The cell blocks were embedded in paraffin and sectioned at 4 µmthickness (process clot as any tiny biopsy specimen). Finally, paraffin blocks were cut into 3-µm sections for Haematoxylin-Eosin stain. All cell block specimens were send for Immunohistochemistry (IHC) analysis.

Technical considerations for cell block preparation as we specifically recommend are:

1. Pooled plasma remains well in a freezer up to one month
2. Thromboplastin is to be kept in the refrigerator
3. Reagents should be brought to room temperature before processing.

Cell block preparation results were categorized as-

1. Histology showing malignant cell undifferentiated type
2. Histology showing malignant cells with exact differentiation
3. Histology negative for malignant cell or showing benign cellularity

Cell block specimens after primary evaluation and confirmation as malignancy was send for IHC analysis for reanalysis of primary diagnosis by cell block method and mutation analysis in tumor cells to avail exact treatment to have excellent treatment outcome. In IHC analysis, we specifically recommend for EGFR, ROS, Calretinin, Carcinoembryonic antigen and ALK analysis.

Cell block immunohistochemistry specimen's results were categorized as-

1. Confirmatory and sample sufficient
2. Confirmatory and sample insufficient

The statistical analysis was done using chi-squared test(three methods of chi-squared test such as independence, goodness of fit, and proportion test). Significant values of$\chi 2$ were seen from probability table for different degree of freedom required. *P* value was considered significant it was below 0.05 and highly significant in case if it was less than 0.001.

OBSERVATION AND ANALYSIS:

Total 200 patients between age group 31 to 90 years, with mean age 68±9.5 years, male population constitutes 66 % and females 34 % of total. In study cases only 9 % cases were smoker. Commoner symptoms were shortness of breath in (91.33 %), cough in (54.00 %) and chest pain (46.66 %) cases, and massive pleural effusion 42%, mass with effusion 28 %, effusion with fixed mediastinum in 21% and bilateral pleural effusion 9 % were commoner radiological abnormalities.

Table 1: Yield of pleural fluid cytology in study cases (n=200)

	Yield positive (n=200)	Percentage
Cytology suspicious for malignant cells or malignant cells with undifferentiated type	36	18
Cytology malignant cells differentiation	48	24
	84/200	42

In study of 200 cases with malignant pleural effusion, 84 cases were diagnosed by conventional cell cytology; out of which only 48 cases were diagnosed with clear histological type. Sensitivity of conventional cell cytology in detecting malignant pleural effusion is 42%. (Table 1)

Table 2: Yield of pleural fluid 'cell block' in study cases

	Yield positive (n=200)	Percentage
Histology showing malignant cell undifferentiated type	34	17
Histology showing malignant cells with exact differentiation	158	79
	192/200	96

In study of 200 cases with malignant pleural effusion, 192 cases were diagnosed by cell block histology technique; out of which 158 cases were diagnosed with exact histological type. Sensitivity of 'cell block' in detecting malignant pleural effusion is 96%.(Table 2)

Table 3: Comparison of pleural fluid cytology and 'cell block' in confirmed cases by these techniques in study cohort (n=192/200)

	Pleural fluid cytology Positive yield (n=84/200)	Pleural fluid 'cell block' Positive yield (n=192/200)
Histology showing malignant cell undifferentiated type	36	34
Histology showing malignant cells with exact differentiation	48	158
Total	**84**	**192**

$\chi 2$ =19.52, df= 1, P < 0.00001

In study of 200 cases with malignant pleural effusion, 192 cases were diagnosed by pleural fluid cell block; while only 84 cases were diagnosed by pleural fluid cytology. Pleural fluid cell block has very significant yield as compared to conventional pleural fluid cytology (p<0.00001). (Table3)

Table 4: Comparison of overall yield of pleural fluid cytology and 'cell block' in study cohort

	Positive yield	Negative yield
Pleural fluid Cytology (n=200)	84	116
Pleural fluid 'Cell block' (n=200)	192	08

$\chi 2 = 136.32$, df= 1, $P < 0.00001$

Pleural fluid cell block has 96% (192/200) diagnostic yield as compared to conventional cytology having 42% (84/200) diagnostic yield. Pleural fluid cell block has 2.28 times more detection rate than cytology ($p<0.00001$). (Table 4)

Table 5: IHC analysis on pleural fluid 'cell block' specimens

	Cell block (n=192)	Percentage
Confirmatory and sample sufficient	180	93.75
Confirmatory and sample insufficient	12	6.25

Immunohistochemistry analysis in pleural fluid cell block specimens were confirmatory and sample was sufficient for diagnosis in 93.75% cases. (Table 5)

DISCUSSION

In present study of 200 cases with malignant pleural effusion, 84 cases were diagnosed by conventional cell cytology; out of which only 48 cases were diagnosed with clear histological type. Sensitivity of conventional cell cytology in detecting malignant pleural effusion is 42%.Studies by Rivera *et al*., McGrath et zl., Gupta *et al*., and Hooper *et al*. documented that in malignant pleural effusion, cell cytology from pleural fluid provides a diagnostic rate of 60%, ranging from 40% to 87%. Various studies by Köksal D *et al*., Jing X *et al*. Ugurluoglu C *et al*. and Bhanvadia VM *et al*. observed that improper smear, fixation, and staining techniques in pleural fluid cell cytology can cause cell overlapping or overcrowding, cell loss, artifacts, and poor background staining, while these are less frequent in cell block. Studies by Köksal D *et al*. Ugurluoglu C *et al*, Bhanvadia VM *et al*, Dekker A *et al*., andShivakumarswamy U *et al*. mentioned that discrimination of reactive mesothelial cells and malignant cells is a major challenge in cytology smear, as reactive mesothelial cells may express large irregular nucleoli, coarse chromatin, and enlarged nuclei, mimicking malignancy.

In study of 200 cases with malignant pleural effusion, 192 cases were diagnosed by cell block histology technique; out of which 158 cases were diagnosed with exact histological type. Sensitivity of 'cell block' in detecting malignant pleural effusion is 96%. Pleural effusion cell block is a useful alternative because collection is easy and better morphologicpreservation of the architectural pattern may be obtained, compared with conventionalcytology. In previous studies by Nathan NA *et al*, Kern WH *et al*., Axe SR *et al*., Wojcik EM *et al*., Leung SW *et al*., and Norimatsu Y *et al*. documented sensitivity of cell block varied widely from 60% to 89.4%, probably because of differences in sampling type, size, type of specimens, and aspirationtechniques. Thapar M *et al* observed 65.7% positivity for malignancy on cell block while in Nathan *et al*. study cell block confirmed malignancy in 92.7% of cases. Shion Miyoshi *et al*[30] showed that pleural biopsy using flex-rigid pleuroscopy had a significantly higher diagnostic yield (94.2%) than pleural effusion cell block (71.4%) for malignantpleural disease. Cellularity is higher by cell block compared with fluid cytology and is concentrated in one small area that can be evaluated at a glance, with all cells lying in the same focal plane of the microscope. In addition, cell block provides better cellular morphological details, such as better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin; there is also less difficulty in microscopic observation, in spite of the presence of excess blood in the background.

In this study, we observed pleural fluid cell block has 96% (192/200) diagnostic yield as compared to conventional cytology having 42% (84/200) diagnostic yield with 100 ml pleural fluid sent for analysis. Baumann MH *et al*[46] observed that malignant cells are considered to be present heterogeneously within the pleural effusion and can be precipitated by gravity. Position of the patient's body and the site of puncture may affect the diagnostic yield of cytology or cell block from thoracentesis. Shion Miyoshi *et al* documented that heterogeneity of pleural fluid cells probably did not affect the diagnostic yield of the cell block preparations because pleural fluid was collected under direct vision by flex-rigid pleuroscope. However, scarcity of free malignant cells in pleural fluid samples remains a serious challenge. A prospective study by Swiderek J *et al* demonstrated that at least 150 mL of pleural fluid is needed for analysis with both cytology and cell block. Shion Miyoshi *et al* in their study analyzed 150 mL pleural was routinely collected in all patients; and they also mentioned that, the volume of pleural fluid can be increased if assessment by cell blocks requires more cell volume.

CONCLUSION

Pleural fluid cell block is more sensitive, superior, cost effective and specific diagnostic method over conventional pleural fluid cytology in malignant pleural effusion. 'Cell block' specimens are enough for primary diagnosis and IHC analysis necessary for cell typing. Results of cell block are comparable to more invasive and costlier diagnostic methods like thoracoscopy and image guided pleural biopsies. Additionally it will decrease need for thoracoscopy guided techniques, especially in resource limited setting like India where availability and cost factor makes more difference. We recommend cell block for every exudative pleural fluid samples with ADA<30 IU/liter to have early diagnosis. More emphasis should be given to pleural fluid cell block analysis training.

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