

Original Article

Pleural Fluid Analysis - Role in Diagnosing Pleural Malignancy

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Abstract

This study aimed to assess the role of pleural fluid analysis in diagnosing pleural malignancy. Thoracentesis and closed needle biopsy of parietal pleura, using Cope's needle, were performed in 45 cases of pleural effusion coming to Department of Pulmonary Medicine. Samples were processed and studied as per standard methods. The male to female ratio was 4.6:1. Age range was 18–74 years. Two pleural effusions were transudates. Amongst the 43 exudates, 17 cases were malignant on pleural fluid analysis. Cyto-histological correlation was 68.4% for malignancy. Adenocarcinoma was the most common malignancy. Pleural fluid showed good sensitivity, specificity and accuracy. In developing countries, where investigations and health facilities are inadequate and cost of treatment is un-affordable, careful analysis of pleural fluid still remains a very convenient, low-cost and safe investigation that helps in diagnosing cases of malignant pleural effusion. Its combination with pleural closed needle biopsy can further enhance its usefulness in diagnosing pleural malignant lesions.

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Key Words : Malignant pleural effusion, thoracentesis, Cope's needle, pleural malignancies.

Introduction

Pleural effusion is an abnormal accumulation of fluid in the pleural cavity. Conservative estimates suggest that around 25% of the cases seen by a pulmonologist involve diseases of pleura.¹ Although a variety of clinical conditions may be the cause of a pleural effusion and the radiographical detection of pleural abnormalities may be obvious, determination of a specific diagnosis can be a major challenge.¹ The possibility of a malignant involvement of pleural cavity should always be considered in difficult to diagnose cases.

Since percutaneous access of the pleural space is relatively simple, techniques like pleural biopsy and thoracoscopy have become very popular, especially in developed countries, pushing pleural fluid analysis to a 'back seat'. However in a developing country like ours, where such specialized facilities are available only

in select advanced pulmonary medicine centers, pleural fluid analysis and cytology remains the mainstay for diagnosing the various pulmonary diseases. Thoracentesis can be safely performed to collect pleural fluid sample if the thickness of the layering fluid is at least 10 millimeters.²

The aim of this study was to obtain pleural fluid samples by thoracentesis in patients of suspected malignant pleural effusion and determine the efficacy of pleural fluid analysis in diagnosing malignant lesions of pleural cavity. Closed needle biopsy of parietal pleura was performed in all these patients to confirm the malignant lesions.

Materials and Methods

Amongst the patients of pleural effusion who sought treatment at the Department of Pulmonology, Himalayan Institute of Medical Sciences from January 2003 to December 2003, a total of 45 cases were

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selected, where adequate samples of pleural fluid as well as pleural closed needle biopsy (CNB) could be obtained for comparison. Details of clinical history, physical examination, radiological investigations and routine hematological investigations, including ESR, platelet count and coagulation profile, as per our routine protocol, were noted.

Consent was taken from all the patients before doing the procedure. All patients were placed in sitting position for the procedure except six patients, who were placed in the prone position for US-guided biopsy and in either the supine or prone position for CT-guided biopsy. Under aseptic measures, a 22 gauge needle attached to 10 ml syringe, filled with 2 ml of local anaesthetic (2% xylocaine), was advanced slowly into the chest wall through the intercostal space superior to seventh rib, periodically attempting to aspirate fluid and then injecting a small amount of xylocaine, to clear the needle and to anesthetize the deeper tissues. The entry to the pleural space was indicated by a sensation of "giving way" and the aspiration of fluid. Two samples of five milliliters each of pleural fluid were collected in sterile vials.³ Next, Cope's needle,⁴ which is a reverse-bevel needle used for performing CNB of parietal pleura, was inserted through a nick superior to the upper edge of the seventh rib, in the mid-axillary line. For diffuse pleural thickening, the cutting needle was advanced along the plane of maximal pleural thickness to allow the majority of the biopsy specimen notch to lie within the abnormal pleura. Three to four bites of pleura were taken and immediately transferred to vials containing 10% formal-saline before sealing the puncture site. The two vials containing the pleural fluid were sent for analysis to cytology and biochemistry sections, while pleural biopsies were sent to histopathology section for processing. All cases tolerated the procedure well.

Pleural fluid was analyzed for physical appearance, proteins, glucose, leucocytes and erythrocytes. Cytology smears were prepared after centrifuging the pleural fluid and were stained by Leishman, hematoxyline and eosin and Papanicolaou stains. Standard tissue processing procedures were followed for the pleural biopsies. The histological sections were stained with hematoxyline and eosin stain; and Zeihl Neelsen (Z-N) stain for demonstration of acid-fast bacilli (AFB).

Observations

Amongst the 45 cases of pleural effusion, in which thoracentesis and pleural closed needle biopsy (CNB) were done simultaneously, 37 were males and eight were females, the male to female ratio being 4.6:1. Their ages ranged from 18-74 years.

On the basis of pleural fluid analysis, samples from two out of 45 cases, turned out as transudates while 43 samples were exudates. Of these 43 exudates, a provisional diagnosis of malignancy was made in 17 cases. Similarly, 20 cases were diagnosed as tuberculous, two cases as acute inflammation and four cases as chronic inflammation (Table 1).

Correlation of pleural fluid cytology was done with histological findings of CNB samples. We found 68.4% (n=13/19) cyto-histological correlation for malignant cases (Table 2).

On the basis of pleural biopsy histology, out of the 45 cases of pleural effusion, 13 cases were true positive (TP), 22 cases were true negative (TN), while four cases were false positive (FP) and six cases were false negative (FN) for malignancy (Table 3).

Pleural fluid analysis showed a sensitivity of 68.4%, specificity of 84.6% and overall accuracy of 77.8%, in diagnosing pleural malignant lesions (Table 4).

Of the 19 malignant cases diagnosed by pleural

Table 1: Provisional diagnosis of pleural diseases by pleural fluid analysis (n=45)

Gross appearance	Total WBC Count/cmm	Predominant Leucocyte Type	Erythrocyte	Proteins g/dl	Glucose g/dl	No.of Cases	Impression
Turbid to bloody. Serous in few	> 1000	Mononuclear	Present++++	> 3	Normal or < 60	17	Malignant
Serous to sero-sanguinous	500 to 1000	Mononuclear	Present +	> 3	Normal or <60	20	Tuberculosis
Turbid to purulent	2000 to 10,000	Polymorphs	Present +	> 3	< 60	2	Acute Inflammation
Serous clear to slightly turbid	> 1000	Mononuclear & Polymorphs	Occasional	> 3	Normal or < 60	4	Chronic Inflammation
Serous clear to slightly turbid	< 500	Mononuclear & Polymorphs	Occasional	< 3	Normal	2	Inconclusive

Table 2 : Correlation of pleural fluid cytodiagnosis with pleural biopsy histology (n = 45)

Pleural fluid	Pleural biopsy histology					Total
	Malignancy	Tuberculosis	Acute Inflammation	Chronic Inflammation	Non specific pathology	
Malignancy	13	3	-	1	-	17
Tuberculosis	4	14	-	2	-	20
Acute Inflammation	-	-	2	-	-	2
Chronic Inflammation	2	1	-	1	-	4
Transudate	-	-	-	-	2	2
Total	19	18	2	4	2	45
Correlation	(13/19) 68.4%	(14/18) 77.8%	(2/2) 100%	(1/4) 25%	(2/2) 100%	

Table 3 : Positivity of pleural fluid for malignancy (n=45)

Sample	Test Result				Total
	TP	TN	FP	FN	
Pleural fluid	13	22	04	06	45

TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative

Table 4: Diagnostic indices of pleural fluid

Indices	Formula x 100	Pleural Fluid
Sensitivity	TP / (TP+FN)	68.4
Specificity	TN / (TN+FP)	84.6
Positive Predictive value	TP / (TP+FP)	76.5
Negative Predictive value	TN / (TN+FN)	78.6
False Negative index	FN / (FN+TP)	31.6
False Positive index	FP / (FP+TN)	15.4
Accuracy	$\frac{(TP+TN)}{(TP+TN+FP+FN)}$	77.8

TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative

Table 5: Histological spectrum of malignancies diagnosed in the preset study (n = 19)

Histodiagnosis	No. of Cases	Percentage
Malignant mesothelioma	3	15.7
Adenocarcinoma	6	31.6
Small cell carcinoma	5	26.3
Squamous cell carcinoma	2	10.5
Non-small cell carcinoma	1	5.3
Neuroendocrinal tumour	1	5.3
Malignant lymphoma	1	5.3

biopsy, three cases (15.7%) were of malignant mesothelioma. Amongst the cases showing invasive or metastatic involvement of pleural cavity, six cases

(31.6%) were of adenocarcinoma followed by five cases of small cell carcinoma (26.3%) (Table 5).

Discussion

Pleural effusion is a frequently encountered problem in patients suffering from pulmonary or cardiac problems. Pleural effusion can be a transudate if the cause is increased hydrostatic or decreased oncotic pressure in the pleural cavity, or an exudate, if there is abnormal pleural capillary permeability, reduced lymphatic clearance of accumulating fluid, infection, or bleeding, into the pleural space.² Hence discrimination of the pleural fluid as transudate or exudate remains the basic diagnostic algorithm. For exudates, malignancy, bacterial pleurisy and tuberculous effusions are the principal differential diagnoses.²

More often than not, a tuberculous pleural effusion is impossible to differentiate from a malignant pleural effusion on clinical grounds alone.⁵ Diagnostic methods traditionally used to diagnose tuberculosis and malignancy are either not absolutely precise or more invasive than they should be.⁵

Medical thoracoscopy, in trained hands of a pulmonologist is a safe and effective procedure for the diagnosis and therapy of various pleural diseases, but it is an invasive and expensive procedure, with a risk of complications like pneumothorax.¹ Moreover, it is available at very select centers, and its cost is beyond the reach of an average person, in a developing country like India. Moreover, a recent survey revealed that even in USA, only 6% of pulmonologists are currently trained in and perform this valuable procedure.¹ By contrast; thoracentesis has been a very popular diagnostic as well as therapeutic procedure for tapping pleural effusions and has very few procedural complications.

In our study, pleural fluid analysis gave sufficient information to categorize the samples as transudates

or exudates on the basis of biochemical tests and cell count (Table 1).² Moreover, we could provisionally categorize the samples as malignant, tuberculous etc. by the cytological findings (Table 1).²

When compared with the pleural biopsies, 68.4% (n = 13/19) correlation was found between pleural fluid samples diagnosed as malignant, cytologically; and histological diagnosis of corresponding pleural biopsies (Table 2). Besides these 13 True Positive (TP) malignant pleural fluid samples, 22 samples were confirmed as True Negative (TN) histologically. Thus out of the 45 cases included in our study, a correct diagnosis was established in 35 (13TP + 22TN) cases by pleural fluid cytology and confirmed by pleural biopsy, its accuracy turning out to be 77.8% (Table 3 and 4). The sensitivity of 68.4%, observed for pleural fluid in our study was in agreement with 60-90% sensitivity reported by other researchers.^{1,6-9} The specificity, positive predictive value and negative predictive value of pleural fluid cytology in our study were 84.6%, 67.5% and 78.6% respectively (Table 4).

In our study, we found four False Positive (False Positive Index =15.4%) and six False Negative (False Negative Index =31.6%) malignant pleural fluid samples (Tables 2, 3 and 4). Several factors influence the diagnostic potential of pleural fluid cytology. The sensitivity of pleural fluid depends primarily upon the free-floating malignant cells. These malignant cells have to be morphologically well-preserved with enough intact cytological features to be first diagnostic of malignancy and hopefully also diagnostic of a specific malignant cell type (Fig. 1).¹ These features, however, may not be present, either with a malignancy that does not shed-off malignant cells in

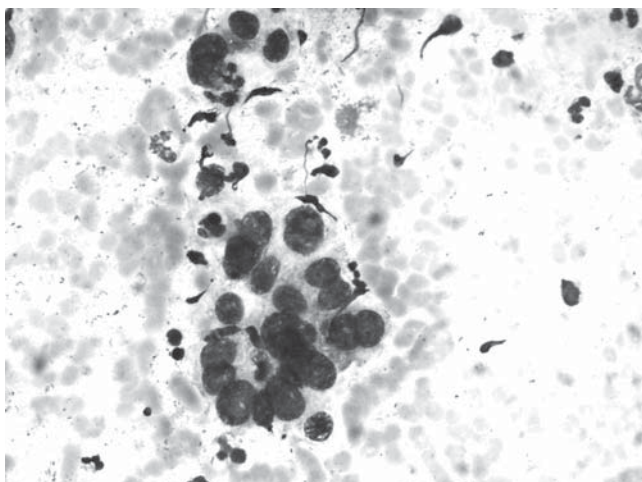


Fig. 1 : Pleural fluid: Atypical epithelial cells showing acinar pattern and morphology of adenocarcinoma (MGG, x 400).

to the pleural fluid; or a malignancy that is largely necrotic and releases cells that are non-diagnostic.^{1,8,9} Moreover, the extent of spread of malignancy is also very important. If a malignancy is confined till the visceral pleura of the pleural cavity, both pleural fluid as well as closed needle biopsy of parietal pleura will fail to obtain diagnostic samples of that malignant lesion, leading to false negative results.^{1,7-9} Reactive mesothelial cells pose a major problem during pleural fluid analysis, often leading to a diagnosis of false positive malignant pleural effusions, as their morphology can often deceptively resemble adenocarcinoma or atypical malignant cells, especially when their cytoplasm also shows large vacuolations, giving them 'signet-ring' appearance (Fig. 2).⁶ Pleural biopsy helps in differentiating such false positive and false negative cases. The chances of getting higher diagnostic yield are more in cases with a malignancy in advanced stage rather than cases where the extent of malignancy is still confined to lung parenchyma.¹ The combined diagnostic yield of pleural fluid cytology and closed needle pleural biopsy has been reported to be as high as 90%.^{1,6}

Thoracoscopy is the ideal procedure available to directly explore the pleural cavity and visualize the malignancies in early stages, which are confined up to visceral pleura of lung. However, even after thoracoscopy, around 10% of effusions remain undiagnosed.^{10,11} The reasons for false-negative thoracoscopy include insufficient and nonrepresentative biopsies that depend largely on the experience of the thoracoscopist;^{1,12} and the presence of adhesions that prevent access to neoplastic tissue which often are a consequence of repeated

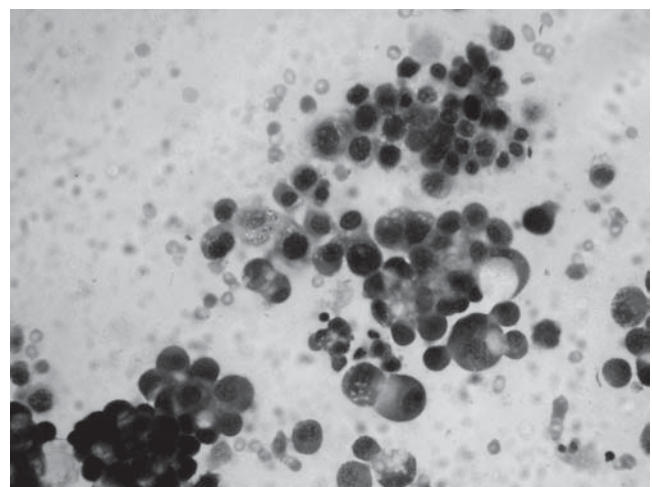


Fig. 2 : Pleural fluid: 'Signet-ring' like appearance of some reactive mesothelial cells misleads to diagnosis of adenocarcinoma (MGG, x 400).

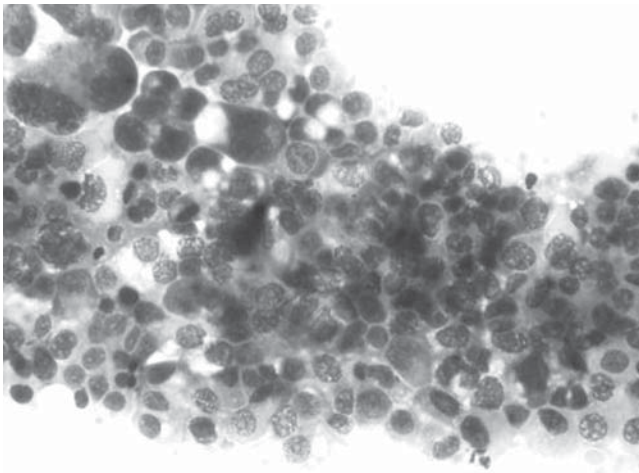


Fig. 3 : Pleural fluid: Mesothelial cells showing atypical morphological features (MGG, x 400).

therapeutic thoracentesis.^{12,13} The invasive nature of thoracoscopy, besides its lack of availability and high cost 'tilts the balance' in favour of less invasive and less costly procedures like thoracentesis and closed needle biopsy.¹

In our study, pleural biopsy helped in morphological diagnosis of the malignant pleural effusion cases (Table 5). Malignant mesothelioma, the primary malignancy of pleura was 15.7% (n= 3/19) of all malignancies detected (Fig 3 and 4). The most common malignancy to invade or metastasize pleural cavity was adenocarcinoma (n= 6/19 i.e. 31.6%) followed by small cell carcinoma (n= 5/19 i.e. 26.3%). Adenocarcinomas and small cell carcinomas are the most common malignancies that extend to pleural cavity.^{14, 15} Squamous cell carcinoma was diagnosed in two cases (10.5%). This predominance of adenocarcinoma was probably due to its more peripheral origin in lung parenchyma in comparison to squamous cell carcinoma which arises most often from the main tracheo-bronchial region.¹⁴ In addition, few less common malignancies like lymphoma (5.3%); non-small cell carcinoma (5.3%); and neuroendocrinal tumour (5.3%) were also diagnosed by the pleural biopsy.

Combining pleural fluid analysis with pleural needle biopsy can help in the diagnosis and morphological classification of majority of malignant pleural lesions, leaving around 7-12% undiagnosed cases.¹⁶ Those remaining may ultimately be subjected to more invasive and expensive investigations.

Conclusion

In developing countries like ours, where investigations and health facilities are inadequate and

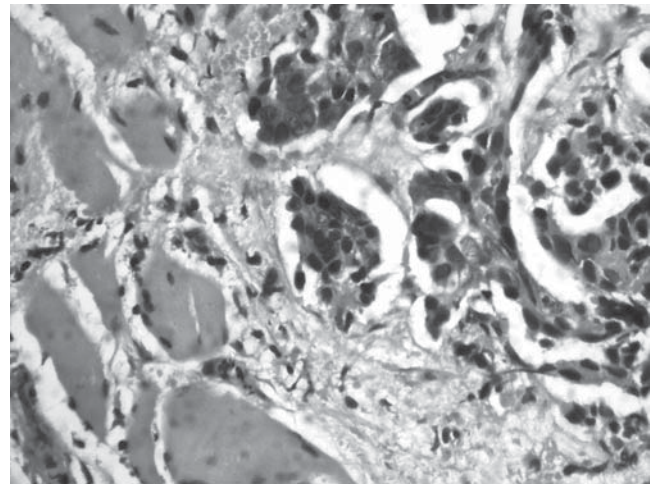


Fig.4 : Pleural biopsy: Malignant mesothelioma (MGG, x 400).

cost of treatment is often un-affordable, pleural fluid analysis and cytology should continue to be a first-line investigation to screen out the suspiciously malignant pleural effusion cases, as it is a very convenient, cost-effective and safe investigation. It shows good sensitivity, specificity and accuracy in diagnosing primary as well as metastatic pleural malignancies. Its combination with pleural closed needle biopsy can further enhance its usefulness in diagnosing pleural malignant lesions.

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