The Value of Cytology in the Diagnosis of Pleural Effusions

Cuneyt Tetikkurt[1*], Bilge Yılmaz Kara[1], Seza Tetikkurt[2], Nail Yılmaz[1], Ilknur Yasar[1] and Rian Disci[3]

[1]*Department of Pulmonary Diseases, Cerrahpasa Medical Faculty, Istanbul University, Turkey.*
[2]*Department of Pathology, Bagcılar Training and Research Hospital, Istanbul, Turkey.*
[3]*Department of Biostatistics and Medical Informatics, Istanbul Medical Faculty, Istanbul University, Turkey.*

Authors' contributions

This work was carried out in collaboration between all authors. Author CT designed and performed the study and wrote the first draft of the manuscript. Author BYK wrote the protocol. Author ST conducted the pathology evaluation. Author NY performed cytologic analysis. Author IY designed the patient files and managed the literature searches. Author RD performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The aim of this study is to assess the value of cytology in the diagnosis of pleural effusions. It is a retrospective review of the patients with pleural effusions admitted at our clinic in a 8-year period. We evaluated the cytological and diagnostic results of 470 patients. Male to female ratio was 3:1 with a mean age of 38.6 years (range 18-76 years). Samples were processed and evaluated according to the standard methods. Cytology results were reviewed and the patients were stratified according to the their final diagnosis of the 470 effusions, 40 were transudates. Among 430 exudates, 190 (74.8%) were malignant on pleural fluid analysis alone. Adenocarcinoma was the most common malignancy. Tuberculosis was the second most frequent etiology for the exudative effusions. Cells in benign or malignant effusions were easily recognized without further invasive evaluation, contributing to diagnosis and therapeutic decisions. Diagnostic power of cytology was high and showed a good correlation with the eventual

Corresponding author: Email: tetikkurt@gmail.com;

pathologic diagnosis, with an overall 70.6% sensitivity. Cytologic examination of the pleural fluid is a simple minimally invasive procedure as an initial step in the diagnostic work-up of patients with pleural effusions. It does not only provide high diagnostic sensitivity but also leads the clinician in the correct algorithm as the most informative and leading test even when not diagnostic on its own.

1. INTRODUCTION

Pleural fluid cytology is a simple and a minimally invasive technique as the preliminary step for the diagnostic evaluation of pleural effusions, assisting the clinician in establishing the differential diagnosis. It may lead to final diagnosis and provide useful information for treatment. The diagnostic yield of the cytologic analysis may be attributable to the cell population present in the sediment that is representative of a much larger surface area than the pleural biopsy [1]. In malignant effusions, it may be a fast, efficient and minimally invasive procedure to reach a diagnosis. Almost all adenocarcinomas are diagnosed with cytology. It may also providecrucialclues for the identification of both non-malignant pleural and transudative effusions. Sensitivity will be higher if the clinical, radiologic and laboratory results are collaborated. This study was carried out to determine the diagnostic yield of pleural fluid cytology.

2. MATERIALS AND METHOD

Cytologic smears from pleural effusions of 470patients admitted between 2003 January and 2011 September were reviewed retrospectively.The study has been approved by the IRB/Ethics Committee of Cerrahpasa Medical Faculty. A routine blood biochemistry with complete blood count was performed simultaneously with thoracocentesis. Pleural fluid was analyzed for appearance, protein, LDH, albumin, leucocyte and differential cell count. Chest x-ray and computed tomography of the thorax, excluding patients with transudative effusions were performed in every subject. Informed consent was taken from all the patients in whom thoracocentesis, closed or thoracoscopic pleural biopsy was performed for histopathologic tissue sampling. The patients were placed in the sitting position for thoracocentesis or closed pleural biopsy. A 22 gauge needle filled with 2 ml 2% xylocaine was inserted through the intercostal space into the subcutaneous and intercostal tissues while periodically injecting small amount of xylocaine and aspirating fluid. Two samples of ten mililiters pleural fluid were collected in sterile vials. The samples were sent for biochemistry and microbiology evaluation. A minimum of 50 mL pleural fluid was sent for cytologic examination with submission of a maximum of three samples from the same patient in case of undiagnosed effusions. Abrams needle was used for closed pleural biopsy and four samples of parietal pleural tissue were taken at different sites for histopathologic examination. A final pathological and/or clinical diagnosis was obtained in each case.

Transudative and exudative effusion differential diagnosis was done according to the Light's criteria and serum-pleural fluid albumin gradient [2,3]. Cytological smears were prepared after centrifuging the pleural fluid for 10 minutes at 2000 rpm. Smears were prepared from the sediment. Differential cell count was done to determine the possible etiologic causes of the effusion according to the predominant cell type.It was useful to characterise and diagnose the cause of a pleural effusion by identifying a predominant cell type. Neutrophil-

rich effusions were often encountered in cases of pneumonia, pulmonary embolism, viral infection, or benign asbestos-related pleural disease. Lymphocytic effusions were typically found in tuberculosis, neoplasia, and collagen vascular disease. An eosinophilic effusion (>10% cell count) was found in neoplasia but also occured in infection, pulmonary infarction, Churg-Strauss and benign asbestos-related pleural disease. Giemsa, Hematoxyline eosin and Papanicolaou were used for staining cytologic smears. Smear and culture for microbiolgical analysis were performed to identify infection. Ziehl-Neelsen stain was used for identifying acid-fast bacilli. Serum biochemistry and urine analysis were done in all patients. ADA, ACE, PET/CT and MR was performed whenever indicated.

The adenocarcinoma cells were identified according to the increased nuclear/ cytoplasm ratio, large nucleoli, irregular nuclear borders, sharply defined cytoplasmic boundaries, the presence of three dimensional aggregates or cell balls. The cell balls may have smooth or less often scalloped edges. Criteria for small cell carcinomas was existence of small hyperchromatic cells with high nuclear/cytoplasm ratio, scant basophilic cytoplasm often with extensive cellular and nuclear molding. Squamous cell carcinoma criteria were dense hyperchromatic and jaggedly irregular basophilic nuclei. The cells usually contain abundant cytoplasm and some may have keratinization seen as eosinophilic/orangephilic on Pap stain. For mesothelioma, cytopathologic features of atypical mesothelial cells were defined as cellular molding, nuclear hyperchromasia, high nuclear/cytoplasm ratio, prominent nucleoli, extensive morphologic variability, and coarse chromatin clumping [4].

Malignant lymphocytes in serous effusions are isolated from each other and most often they are single or in small loosely adherent clusters. These effusions contain a distinct population of discohesive mononuclear cells. If true tissue aggregates are present, lymphoma and leukemia can be excluded. The cells of lymphoma/leukemia often have scant cytoplasm. Karyorrhexis is particularly common in lymphomas. The cells of diffuse B large cell lymphomas are larger than histiocyteswith aprominent nucleoli. Nuclei are round or highly irregular and the chromation is coarsely textured. Cytoplasm is often abundant, pale, and vacuolated. Follicular lymphomas have irregular and cleaved nuclei and scant cytoplasm. Cell population has a monomorphic nature in small cell lymphomas and low-grade lymphomas. Pleural effusion of acute myeloid leukemia includes blasts and karyorrhectic cells. Blasts are round, two to three times the diameter of lymphocytes and dispersed as isolated cells. The nuclei is round or irregularly shaped. The chromatine is pale and the nucleoli are usually prominent [5,6].

Criteria for a reactive pleural effusion were moderate number of mesothelial cells singly or in small clusters, mild nuclear variability, prominent nucleoli, normal nuclear/cytoplasm ratio, variable inflammatory background, and absence of malignant cells. Diagnostic criteria for tuberculous pleural effusions were predominance of lymphocytes (>50%) and small lymphocytes with variable morphology. Granular, amorphous, particulate, and acellular debris was diagnostic for rheumatoid effusions. Other criteria were numerous multinucleated, elongated, spindled giant cells, and degenerating leukocytes. For SLE associated effusions presence of LE cells, HE bodies, tart cells, reactive mesothelial cells, a background of nuclear fragments and necrotic cells were diagnostic. In parapneumonic effusions, numerous polymorphonuclear leukocytes and necrotic material with reactive mesothelial cells constituted the diagnostic criteria. Transudative effusions were diagnosed by the presence of low cellularity of different origin consisting of lymphocytes, reactive mesothelial cells in sheets or clusters, and macrophages [4,7].

Statistical analysis was done by calculating sensitivity test with a 95% confidence interval. Correlation of the pleural cytology findings was done with the final clinical diagnosis by calculating the sensitivity within 95% confidence intervals. A p value less than 0.05 was considered significant.

3. RESULTS

A total of 470 patients, 360 males (77%) and 110 females were included in the study. The age of the patients ranged from 18 to 76 (38.6±18.4) years. Overall, 440 (94%) of the effusions were exudative. Seventy-two patients (15.3%)had bilateral pleural effusions.Eighteeneffusionswere due to collagen vascular disease, 8 from lung carcinoma metastasis, 6 from breast carcinoma metastasis and 4 from hematologic malignancies metastasis. Forty effusions (8.5%) were transudative type. Cytologic examination of the pleural fluid was diagnostic in 70.6% (332/470 patients). Diagnosis was confirmedby histopathologic examination by closed and thoracoscopic biopsy specimens in 29% (138/470) and 54% (256/470) of the patients respectively. In the remaining 76 (16%) patients, the final diagnosis was clinical. All cases tolerated the thoracentesis well. Vasovagal reactions occurred in 32 (6.8%) subjects. Pneumothorax developed in 20 (4.3%) patients and 6 (1.3%) of them required chest tube drainage.

Correlation of pleural fluid cytology was done with the final clinical diagnosis in equivocal cases. Cytologic analysis of the pleural fluid according to various disease groups with the sensitivity results is shown in Table 1. Cytologic findings pointed out to the etiology and were highly suggestive of the final diagnosis in 42 malignant, 16 tuberculous, 8 infectious, 5 collagen vascular disease, and 4 transudative effusions. The overall initial cytology was positive in 312/470 (66.3%) patients while second and third samples were positive in 12/470 (2.5%) and 7/470 (1.4%) more cases. When cytology was combined with the clinical data including the laboratory results (serum biochemistry, urine analysis, ADA and ACE) and the radiologic findings (CT, PET/CT, MR, and US), the overall sensitivity was higher than cytology alone Table 1, reaching 88.9%.

The specific features of a single cell or a group of cells were very useful from the diagnostic point of view. The adenocarcinoma cells were single, formed clusters or adenoid groups. They had large nuclei with a pale vacuolated cytoplasm Fig. 1.All samples were exudative and 28 were hemorrhagic. Metastatic small cell carcinoma cells were seen in the pleural fluid samples of42 (22%) patients; it was exudative in 40(15.6%) and hemorrhagic in 37(14.5%) patients. The cells appeared discretely or in loose cohesive clusters of small atypical epithelial cells like lymphocytes, all with large dense nuclei, coarse chromatin and scant cytoplasm. Malignant squamous cells were present in 38 (14.9%) samples, all of which exudative and 30(11.7%) were hemorrhagic. Squamous cells were discrete and showed evidence of keratinization. They tended to have a more flat, two-dimensional appearance than those of adenocarcinoma. Thirty four (13.3%) effusions were positive for hematologic malignancies like lymphoma and leukemia. Mesothelioma was diagnosed in 18 patients by cytology.They were exudative and viscous in appearance. The mesothelial cells varied considerably in size and were arranged as single cells or in an articulated fashion forming large clusters with lumpy borders Fig. 2. Cytologic analysis was diagnostic in190/254 (74.8%) of the patients with a malignant effusion. The diagnostic sensitivity increased to 92.5% when cytology was combined with other laboratory results. The first effusion sample was diagnostic in 168/254 (66.3%) of the patients. Analysis of second and third samples increased the diagnostic sensitivity to 68.9% and 74.8%, respectively.The primary site was diagnosed in 63.7% (162/254) of the patients by cytology.
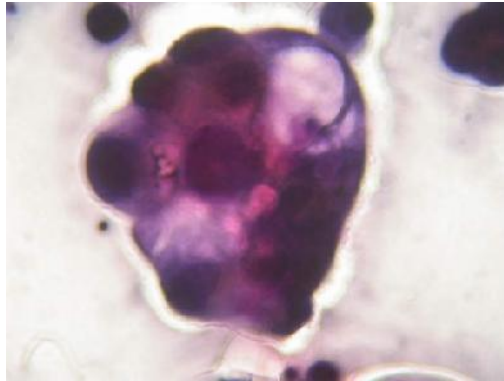
Fig. 1. Metastatic cells from gastric adenocarcinoma with a vacuolated cytoplasm forming adenoid groups (Papanicolaou stain; x400)
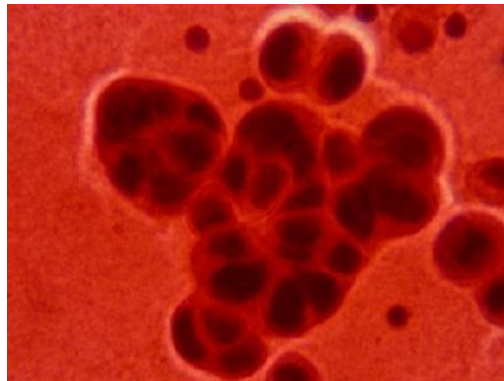

Fig. 2. A large cluster of mesothelioma cells in pleural fluid (Papanicolaou stain; x400)

Clinically, in 86 cases the final diagnosis wastuberculosisand tuberculosis was confirmed in 62 cases (72%) cytologically. When cytology was combined with other laboratory findings sensitivity was higher and reached to 94%. Almost all of these effusions were rich in small lymphocytes with variable morphologies ranging from 50% to 96% of the total cell count. These effusions also contained small numbers of neutrophils, macrophages, plasma cells or red blood cells. Eighteen (20.9%) samples were hemorrhagic. None of the pleural effusions with an eosinophil count more than 10% or mesothelial cell count over 1% were due to tuberculosis. Of the clinically diagnosed 49parapneumonic samples, 38 (65%) correlated with cytology. Diagnostic sensitivity increased up to 91.7% with combination of cytology and laboratory findings Table 1. All the cells in parapneumonic effusions were mostly neutrophils. In purulent effusions, a high proportion of neutrophils had undergone degeneration appearing as smudgy anucleated blobs. Microorganisms either in dispersed fashion or in cloud-like colonies were also frequently encountered. CVD was cytologically diagnosed in 28 of 42 (66.7%) the pleural effusion samples due to rheumatoid disease (27 SLE, 15 RA effusions). Rheumatoid arthiritiscells were observed in 11, lupus cells were encountered in 21 and tart cells were seen in 12 effusions. None of the transudative effusions had a cell count over 1.000/mm^3.

Sensitivity of cytology was high Table 1 and statistically significant in malignant and tuberculous pleural effusions ($p<0.05$). Sensitivity showed significant ($p<0.05$) increase if cytology and laboratory were collaborated. In contrast, diagnostic yield of cytology alone was low in infectious, CVD and transudative pleural effusions. Combination of cytology with laboratory data increased sensitivity significantly ($p<0.01$) up to 91.7, 90.4, and 95.0%, respectively.

Table 1. Sensitivity of pleural fluid cytology for malignant, tuberculous, collagen vascular disease associated, parapneumonic and transudative effusions

	n	% Sensitivity	%95 CI
Total (+) cytology	332/470	70.6	66.3 - 74.7
Cyt+clin+lab	418/470	88.9	85.8 - 91.6
M (+) cytology	190/254	74.8	68.6-80.9
Cyt+clin+lab	235/254	92.5	88.6 - 95.4
Tbc (+) cytology	62/86	72.0	62.5-81.4
Cyt+clin+lab	81/86	94.2	87.0 - 98.1
Inf (+) cytology	29/48	60.4	49.9-70.8
Cyt+clin+lab	44/48	91.7	80.0 - 97.7
CVD (+) cytology	28/42	66.7	50.5 - 80.4
Cyt+clin+lab	38/42	90.4	74.4 - 96.0
Trn (+) cytology	17/40	42.5	27.0-59.1
Cyt+clin+lab	38/40	95.0	83.0- 99.3

CI: confidence interval, M: malignancy, Tbc: tuberculosis, Inf: infection, CVD: collagen vascular disease, Trn: transudate, Cyt: cytology, clin: clinic, lab: laboratory

4. DISCUSSION

Pleural effusions are caused by pulmonary or non-pulmonary diseases. Although the etiologic spectrum is wide, most effusions occur due to malignancy, heart failure, tuberculosis or bacterial infection [8,9,10]. Because the effusions develop as amanifestation of an underlying disease, it is difficult to determine the precise incidence. However, it is estimated to be 1.5 million cases in the United States annually [11]. Cytologic examination is often the initial diagnostic step for its etiologic identification. It represents the whole pleural surface due to the existence of exfoliated cells in the fluid. The major limitation is the false negative or false positive results [1,12]. Even if the initial examination is negative, the cellular profile of the fluid leads the clinician in the correct diagnostic pathway, as the results of our study suggests. Consequently, this minimally invasive method appears to be the best preliminarystep for the assessment of pleural effusions.

In our study, the highest positive results were obtained in malignant pleural effusions, followed by tuberculous and parapneumonic fluids. In patients with a malignant pleural effusion, cytological examination is a fast, efficient and minimally invasive procedure to establish the diagnosis. Almost all adenocarcinomas were diagnosed with cytology, but the yield was less with with squamous cell carcinomas, Hodgkin's disease and sarcomas. This may be due to the fact that the adenocarcinoma cells are more easily identified cytologically and large tissue biopsies are usually needed for the diagnosis of lymphoma and sarcomas. The initial pleural cytologic examination is positive in approximately 60% of the patients. If three separate pleural fluid specimens are submitted to an experienced cytologist, nearly 80% of the subjects will have positive results by cytology alone [13,14]. The first sample was diagnostic in 66.3% of the patients while with the analysis of the second and third samples

the sensitivity increased to 68.9% and to 74.8% in our study. Although the increase insensitivity was not statistically significant, it was crucial from the clinical point of view. Our initial diagnostic rates based on cytology alone, were higher than Bueno and Jarvi[14,15]. This may be due to the presence of different diseases, tumor type, tumor burden in the pleural space and the cytologist's skills.

Cytologic diagnosis of malignant effusions has been reported to be between 40% and 87% in different studies [12,14,15]. Several factors may influence its sensitivity. The effusion may develop secondary to other factors such as infection, pulmonary emboli or lymphatic blockade. The incidence of positive pleural cytology is higher in patients with a large tumor burden in the pleural surface by causing more exfoliated malignant cells in the effusion [16,17,18]. In our study, positive cytology for malignant effusions was 74.8%. The primary site was identified by cytologic examination alone in 62.8% of the patients with metastatic malignant effusions. The overall sensitivity was 70.6%, reaching 88.9% if it was integrated with other laboratory findings.

No diagnosis is established in approximately of 15% of patients with exudative effusions [19,20,21]. Even after thoracoscopy, 10% of the pleural effusions may remain undiagnosed [22,23]. In our study, the sensitivity of pleural cytology was highestfor malignant effusions which may be explained by the presence of different specific cell types present in such type of effusions. Diagnostic yield was low in infectious, CVD, and transudative pleural effusions. The low diagnostic yield in our study is attributed to the lack of specific cells or specific cell patterns in these type of effusions. Collaboration of laboratoryresults with cytology for final diagnosis was very efficient in the identification of such effusions.

A tuberculous pleural effusion is frequently a diagnostic challenge for the pulmonary clinician. It is impossible to differentiate a tuberculous effusion from a malignant pleural effusion on clinical grounds alone and usually more invasive diagnostic interventions are needed. As the results of our study suggests, the absence or the scarcity of mesothelial cells along with the presence of more than 50% small lymphocytes should be regarded as a strong evidence for tuberculosis. The absence of mesothelial cells is attributed to the deposition of fibrin on the pleural surface, either sealing off the mesothelialcells, destroying them or both [16,24]. Although the mechanism of eosinophil accumulation in the pleural space is unknown, eosinophils play an important role in idiopathic, allergic diseases, or drug reactions. The presence of air in the pleural space may also cause eosinophilia.It is well known that tuberculous pleural effusions rarely contain more than 10%eosinophils [25,26,27,28]. The presence of numerous mesothelial cells or eosinophils was useful to exclude tuberculosis in the differential diagnosis of exudative pleural effusions.In the present study, pleural fluid analysis was not diagnostic in approximately 30% of the patients and thereby indicating its limits. The incidence of iatrogenic pneumothoraxwas verylow in our patients and almost all the patients tolerated the procedure well without any other significant complication which appeared to be another advantage in regard to other invasive diagnostic modalities.

As the results of our study suggests,cytology of the pleural fluid is the most informative and definitive initial diagnostic step in pathologic states involving the pleura. This simple and minimally invasive technique may be considered as the best initial diagnostic tool in the hands of an experienced cytologist without any serious complications of thoracocentesis. Examination of the pleural fluid can narrow the differential diagnosis considerably. Cytology can be the key to direct diagnosis or can indicate the next step leading the clinician in the correct pathway for final diagnosis even when not diagnostic on its own, and thus precluding

unnecessary invasive interventions. In most diseases related to pleural effusion, the pleural fluid analysis yields important diagnostic information and in certain cases it provides the final diagnosis.

5. CONCLUSION

Pleural fluid cytology is a useful, rapid and a highly sensitive method for the diagnosis of pleural effusions. The sensitivity may be attributable to the presence of exfoliated cell population representative of a large pleural surface. Integration of pleural fluid cytology with clinical and laboratory findings further increases the diagnostic yield. Cytologic features of malignant cells may establish the primary site in metastatic effusions andthereby provide useful data for patient prognosis. The low sensitivity of cytology for infectious, CVD and transudative effusions may be overcome by collaborating cytology with other laboratory findings. Other advantages of cytologic analysis include low incidence of complications associated with thoracocentesis and ease of reaching diagnosis without unnecessary invasive interventions.

CONSENT

All authors declare that "written informed consent" was obtained from the participants of this study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

We declare that our data or presentation of information is not influenced by any personal or finanacial relationship with other people or organizations. We have no financial interests to declare.

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