**Comparison of Pleural Fluid Cytology Before and After Pleural Needle Biopsy in Diagnosis of Malignant Pleural Effusion: A Cross-sectional Study**

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**Authors' contributions**

The authors confirm their contributiono in this paper as follows:

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**Conflict of Interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Availability of Data and Materials**

All of the data in excel is available for sharing. Also, the bronchoscopic report and pathologic report are available in Shahid Sadoughi Hospital, Yazd, Iran.

**Ethical Considerations**

We confirm that our trial was performed in accordance with the Declaration of Helsinki and subsequent revisions and the study protocol was approved by the Ethics Committee of the Shahid Sadoughi University of Medical Science in Yazd and received the ethics code no.: IR.SSU.MEDICINE.REC.1397.120. Written informed consents were obtained before entering into the study.

**Patient consent**

Not applicable

**Informed consent:**

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article. The manuscript does not contain any individual person’s data in any form.

**Abstract**

**Background & Objective:** Correct diagnosis of the causes of exudative pleural effusions is important for the treatment and prognosis of these patients. Diagnosis of the malignant pleural effusion is made possible by the detection of malignant cells in a cytological examination of pleural fluid or by observing these cells in a pleural biopsy sample prepared through needle biopsy, thoracoscopy, or thoracotomy. We observed that the diagnostic yield of bronchial fluid cytology increases after bronchial biopsy, therefore we decided to compare the cytology of pleural fluid before and after the pleural needle biopsy in the diagnosis of malignant pleural effusion.

**Materials & Methods:** In this descriptive-analytical study, sampling was performed by simple consecutive method on patients presenting with lymphocytic exudative pleural effusion. Before pleural needle biopsy, two separate 20ml of pleural fluid were taken and sent for cytological examination and another for culture and smear of mycobacterium tuberculosis. after pleural biopsy, two separate 20ml of pleural fluid were sent again for cytological examination, and mycobacterium tuberculosis assay. Finally, the data were analyzed by SPSS statistical software ( version 21, Chicago, IL, USA )

**Results:** The present study was performed on 100 patients with lymphocytic exudative pleural effusion and the mean age of patients was 57.38±8.4 years. Also, 61 (61%) were male and 39% were female. In 24% of cases, the cytology result was positive before biopsy and after biopsy, this percentage increased to 33%. The sensitivity, specificity, positive predictive value, and negative predictive value of cytology before biopsy were 59%, 98.4%, 95.8%, and 78.9%, respectively, which reached 79%, 96.7%, 93.9%, and 88.1% after biopsy.

**Conclusion:** The result of the present study showed that the diagnostic yield of pleural cytology increases if a pleural fluid sample was taken after the pleural biopsy. Based on the results of the study, it can be concluded that cytological examination after a pleural biopsy can be used as an easy, low-cost, and non-invasive method with acceptable sensitivity in the first stage of diagnosis of pleural malignancies and we can use this method instead of repeating thoracentesis and cytological examination in consecutive days.

**Introduction**

Pleural effusion occurs for a variety of reasons. Although the condition is easy to diagnose,  sometimes it is not possible to determine the cause. The first step in identifying the cause of a pleural effusion is to perform thoracentesis. Based on the Light’s criteria it is classified into two categories: exudative effusion commonly caused by malignancies and infectious causes, and transudative effusion caused by systemic causes such as cirrhosis and heart failure (1-4). Appropriate assessment of the causes of pleural effusion in patients with malignancy is important for the treatment and prognosis of these patients. Detection of these effusions is possible by examining malignant cells in cytological examinations of pleural fluid or by observing these cells in pleural biopsy through needle biopsy or thoracoscopy and thoracotomy (5,6). It should be kept in mind that according to thoracoscopic studies, the first areas involved in metastatic malignancies of the pleura are the diaphragmatic and mediastinal surfaces of the pleura, and the involvement is focal so that in 50% of cases, the costal pleura is not involved. On the other hand, a needle biopsy takes a sample from the pleural surface of the costal pleura. Yet, in cytological examinations, cells shed into the fluid from any surface of the pleura are studied (7,8). Thus, it is logical that cytological examination of pleural fluid is more effective in confirming the diagnosis of malignant effusion with appropriate diagnostic accuracy, and if due to any process (such as pleural biopsy), the shedding of cells in pleural fluid increases, it can multiply this diagnostic value (repeated cytology increases test sensitivity). In most articles, simultaneous cytology and sampling by needle biopsy have the highest diagnostic sensitivity (9,10). This study aimed to compare the cytology of pleural fluid before and after pleural biopsy in the diagnosis of malignancies. Given that this issue has been studied sporadically in this form so far, it can be of great help in early diagnosis and faster treatment at a lower cost.

**Methodology**

This cross-sectional and descriptive-analytical study included the patients who were referred to Yazd Shahid Sadoughi Hospital from 2016 to 2017 due to lymphocytic exudative pleural effusion, and who were candidates for pleural biopsy. Exclusion criteria included patients' dissatisfaction with biopsy and participation in the study, coagulation disorders, exudative pleural effusion with parapneumonic effusion diagnosis, transudative pleural effusion, and history of exudative pleural effusion with a specific cause. Before performing pleural needle biopsies, thoracentesis was performed with a 21 gauge syringe, and 20 ml of pleural fluid was sent for cytology and another 20 ml for culture and smear of Mycobacterium tuberculosis. After the pleural biopsy, 20 ml of pleural fluid was taken for cytology and another 20 ml for culture and smear of Mycobacterium tuberculosis through an Abrams needle. The samples were sent blindly to a pathologist so that the pathologist did not know which sample was in before and after pleural biopsy. Pleural Needle biopsy was performed by Abrams needle under sterile conditions and local anesthesia with 15 ml of 2% xylocaine. The variables of age, gender, and results of the biopsy and cytology of patients were recorded in a questionnaire.

**Ethical Considerations**

While maintaining patients' confidentiality under the Helsinki Convention, patients were assured that their information would be confidential and would only be used for research purposes, and written informed consent was obtained from all participants using their physical examination, medical history, and hospital records in this study. No additional costs were charged to patients in this study. The study protocol was approved by the Ethics Committee of the Shahid Sadoughi University of Medical Science in Yazd and received the ethics code no.: IR.SSU.MEDICINE.REC.1397.120.

**Statistical Analysis**

The data were analyzed using IBM SPSS statistics software (version 21, SPSS, Chicago, IL, USA). We used descriptive statistics to assess the percentage frequency distribution, mean and standard deviation. In all cases, P-values less than 0.05 were considered statistically significant.

We used the chi-squared test, the Fisher’s exact test, or ANOVA for comparing groups.

**Results**

This study compared the cytology of pleural fluid before and after pleural needle biopsy in the diagnosis of malignancies in 100 patients with lymphocytic exudative pleural effusion. The mean age of patients was 57.38 ± 8.4 years with a range of 41 to 80 years. 61 (61%) patients were male and 39 (39%) were female. In this study result, 60% of the patient's pleural biopsy and pleural fluid cytology were not malignant. Of these, 14 cases were tuberculosis and the rest were referred for thoracotomy or VATS. Adenocarcinoma was the most type of malignancies reported (23%), and then 10% showed metastasis, 3% had lymphoma, 2% showed small cell lung cancer (SCLC), and 1% had pleuritis, and 1% squamous cell carcinoma (SCC).

Generally, 24% of cytology cases were positive before pleural needle biopsy; but this percentage increased to 33% after biopsy, indicating that pleural fluid cytology was more sensitive after biopsy in diagnosing malignancies. The results of the study of the diagnostic nature of pleural fluid cytology before and after pleural biopsy according to the type of histopathology of pleural biopsy in the studied patients are given in Table 1. As can be seen in table 1, in cases where the diagnosis of pleural biopsy has been adenocarcinoma, metastasis, and SCLC, positive pleural fluid cytology cases increased after the biopsy.

Comparing the results of pleural fluid cytology before pleural needle biopsy with the results of pathology, in 59% of cases where the result of pathology was positive, the result of cytology was positive, but in 98.4% of cases where the result of pathology was negative, the result of cytology was also negative. Therefore, according to Table 2, before the biopsy, the cytological sensitivity was 59% and its specificity was 98.4% due to the reference to the pathology report.

Comparing the results of pleural fluid cytology after pleural needle biopsy with the results of pathology, in 79.5% of cases where the result of pathology was positive, the result of cytology was positive, but in 96.7% of cases where the result of pathology was negative, the result of cytology was also negative. Therefore, according to Table 3 after pleural needle biopsy, the sensitivity of pleural fluid cytology due to the reference of pathology report was 79.5% and its specificity was 96.7%, which compared to the previous table shows an increase in cytological sensitivity after biopsy.

According to Table 4, the diagnostic sensitivity of pleural fluid cytology after a pleural needle biopsy has increased significantly (from 59% to 79.5%) while its specificity shows a slight decrease. After pleural needle biopsy the negative predictive value(NPV) of the pleural fluid cytology has also increased significantly (from 78.9% to 88.1%), but the positive predictive value(PPV) of cytology after the biopsy has decreased slightly compared to before.

In this study, the kappa agreement coefficient between the pleural needle biopsy result with the pleural fluid cytology before and after the biopsy was 0.616 and 0.784, respectively. both of which were significant with a P-value = 0.001. As a result, the pleural fluid cytology after the pleural needle biopsy was more valuable.

**Discussion**

In various studies, cytological sensitivity of pleural fluid in the diagnosis of pleural malignancy has been reported to be 40-87% (11-14). According to Johnston’s study, one cytology session can diagnose 60% of lung carcinomas and 20-30% of mesothelioma cases (15). In the study by Loveland et al., 153 samples of pleural fluid cytology were examined and in 39.9% of cases, malignant cells were reported. The sensitivity and NPV of cytology were 67.2% and 82.1%, respectively (16). In the study by Bhattacharya et al., out of 66 patients with malignant pleural effusion, pleural fluid cytology was positive in 69% of cases. Also, in this study, cytology repetition increased the percentage of positive cases, so in the first round cytology was positive in 52% of the samples, and in the second and third rounds, 15% and 1.5%, respectively, contributed to the positive cytological result (17). However, in Johnston's study, repetition of thoracentesis and examination of pleural fluid cytology did not cause a significant increase in positive cases (15). In 2011, Solooki et al. examined the diagnostic value of pleural fluid cytology and pleural needle biopsy in the diagnosis of pleural malignancy in 318 patients (18). In this study, the sensitivity and NPV of cytology in the diagnosis of pleural malignancies were 32.4% and 65.6%, respectively, which was much lower than our results. In Solooki’s study, it was found that repetition of thoracentesis and pleural fluid cytology on separate days increased the sensitivity of this test from 11.6% in the first turn to 23.8% in the third turn. In our study, the sensitivity and NPV of the pleural fluid cytology after the pleural needle biopsy were determined to be 79.5% and 88.1%, respectively. In both studies, the diagnostic sensitivity of pleural fluid cytology increased with the repeated cytological examination.

Findings of a 2015 study by Moghimi et al. In 61 patients with malignant pleural effusion showed that pleural fluid cytology results were positive in 63.9% of cases and pleural needle biopsies in 91.8% of cases, which was higher than our study. In their study, the pleural needle biopsy and the pleural fluid cytology were both positive for malignancy in 55.7% of cases, and are consistent with the pre-pleural biopsy results in our study (59%). In the study of Moghimi et al. repeated thoracentesis and sending pleural fluid cytology increased its diagnostic percentage. In their study, in the first turn, the cytology result was positive in 50.8% of cases and after repetition, it reached 63.9%. In our study, this number increased to 79.5% after pleural biopsy, and this indicates an improvement in the diagnostic efficiency of pleural fluid cytology with repetition after pleural needle biopsy (19).

In a study by Bielsa et al. performed on 466 patients with malignant pleural effusion, the first pleural fluid cytology sample was diagnostic in 48.5% of cases, and by repeating the cytology sample for the second and third time, 28.6% and 10.3%, respectively, were added to diagnosed cases(20).In the study of Ong et al. out of 103 patients, a total of 56.3% of cytological samples were positive for malignancy, which in the first turn was 48.5% positive, and by repeating cytological samples in the second and third rounds, 15.8% and 15.4%, respectively, positive cases of pleural fluid cytology have been added (21). In our study, the sensitivity of the pleural fluid cytology before the biopsy was 59%.

The reasons for the difference in the results of this study with previous studies are the volume of pleural fluid tested, inclusion and exclusion criteria, the pathologist's expertise in the study of pleural fluid cytology, and the type of cancer involving the pleura. According to most researchers, increasing the frequency of thoracentesis and cytology can increase the diagnostic value of this method, and believe that the reason for the positive results of cytology next time is the entry of new and non-degenerative malignant cells into the pleural space. In contrast to the studies that examined the repetition of cytology specimens on different days, in this study, we compared the cytology of pleural fluid before and after a pleural needle biopsy in one day, which showed a significant increase in the diagnostic value of cytology up to 79.5% after pleural needle biopsy. Especially in cases where the pathology involved adenocarcinoma pleura and metastasis, this increase in cytological sensitivity was greater after the biopsy. Increased diagnostic sensitivity of pleural fluid cytology after the pleural biopsy was more in cases where the pathology of pleural biopsy was adenocarcinoma and metastasis. This increase in positive cytology reports may be due to abrasion of pleural surfaces during the biopsy or it may be due to cell shedding by stimulation of the pleura after a needle biopsy, or the fact that the sedimented cells float in the pleural space and more are removed during the second thoracentesis.

**Conclusion**

Based on the results of the study, it can be concluded that cytological examination of pleural fluid after a pleural needle biopsy can be used as an easy, low-cost, and non-invasive method with acceptable sensitivity in the first stage of diagnosis of pleural malignancies and we can use this method instead of repeating thoracentesis and cytological examination in consecutive days.

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