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ORIGINAL ARTICLE

The Predictive Role of Second and Third Fluid Cytology in the Diagnosis of Malign Plevral Fluids in Patients with Negative First Fluid Cytology

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Abstract

Introduction: In this study, we wanted to investigate the role of pleural fluid cytology, which is examined for the second and third times in malignant pleural fluid cases that cannot be diagnosed by first pleural fluid cytology.

Methods: A total of 116 patients were evaluated in this retrospective study. 2nd samplings of pleural effusions were evaluated via thoracentesis in 105 patients (90.51%)with negative cytology results on the first thoracentesis attempt, and 3rd samplings were evaluated for 34 patients (29.31%). Pleural biopsy using a cope needle was performed on patients who could not be diagnosed upon pleural fluid cytology. If pleural biopsy did not yield any diagnosis, VATS (video assisted thoracoscopy) was performed, and pleural decortication was performed in some patients who could not be diagnosed with VATS. Pleural fluid cytology results suspected to be malignant were considered to be negative and the same procedure was performed as above.

Results: 7 out of the 116 cases (6.03%) were found to be cytologically positive on the first thoracentesis. Out of the cases found to be negative on first attempt, 29 (26.6%) were diagnosed positive upon second attempt. 34 cases which were not diagnosed at first and second attempt underwent a third thoracentesis attempt and were evaluated cytologically. Out of the 34 cases, 11 (32%) were diagnosed in the 3rd attempt. A total of 47 cases (40.5%) were diagnosed with pleural fluid cytology. In the presence of primary lung cancer, the rate of diagnosis by 2nd fluid cytology was statistically significant compared to other types of malignancy ($p<0.05$).

Discussion and Conclusion: In cases with negative pleural fluid cytology at the first examination, examination of fluid cytology for the second and third times should be considered with a high contribution to the diagnosis, especially in patients who are unable to apply more invasive diagnostic methods.

Keywords: Pleural effusion; thoracentesis; liquid cytology.

Malignant or paramalignant pleural effusions may occur during the course of many malignant diseases or in recurrent conditions after completion of treatment for the primary malignant disease. While cancer cells are detected in the cytological evaluation of the pleural fluid or in the pleural biopsy in malignant pleural effusions (MPE), the

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fluid in paramalignant effusions is usually due to secondary causes such as bronchial obstruction, lymphatic invasion or pulmonary embolism, and no malignant cells are detected in cytological evaluation [1].

Malignant pleural effusions (MPE) occurs in pleural tumor spread in malignant diseases, asa result of impaired secretion and absorption of pleural fluid. They constitute approximately 30-60% of all pleural fluids [2]. During the course of lung, breast, ovarian tumors and lymphomas, malignant pleural effusions develop with a rate of more than 75%. Metastatic adenocarcinoma is the most common cause of MPE. While lung cancer is the most common primary cause of MPE in men, breast cancer is the most common cause in women [3-4]. It is stated that despite all the examinations, the diagnosis could not be reached in 5-14% of the cases [1]. The presence of MPE indicates widespread or advanced disease, which is associated with shortened survival. Although the average survival following the diagnosis of MPE varies depending on the organ of origin of the primary tumor, histological type, and the stage of the disease, it can be said that it is between 3-12 months. Survival was found to be the shortest in lung cancer and the longest in ovarian cancer. It has been shown that carcinomas of unknown primary progress with moderate survival [2].

Malignant cells are usually not seen in the first cytological examination in MPE. Therefore, invasive methods are increasingly needed to reach a definitive diagnosis. Especially in malignant effusions, the deterioration of the general condition of the patients and shortened survival complicate the use of invasive methods. However, we think that the probability of showing malignant cells in repeated thoracentesis increases.

Therefore, in our study, we wanted to draw attention to the contribution of repeated thoracentesis to the diagnosis and how many times we need to perform thoracentesis in these cases where invasive methods are difficult to perform.

The aim of this study is to determine the contribution of repeated pleural fluid cytology sampled by thoracentesis, which is a minimally invasive method in the diagnosis of MPE, before performing other invasive methods, in cases in which the result is found negative in the first sampling, and to determine how many times we have to sample pleural fluid cytology.

Materials and Methods

A total of 116 patients diagnosed with malignant pleural effusion and examined in our center between January 2014 and December 2015 were analyzed. The files of these patients and their records in our hospital database were reviewed retrospectively. Diagnostic cytological examination of pleural fluid was performed at least once and at most 3 times from all patients included in the study. The amount of samples taken for cytological examination ranged from 10 ml to 50 ml. Malignant pleural effusion was diagnosed by pleural biopsy, video-assisted thoracoscopic surgery (VATS) or decortication methods in cases that could not be diagnosed with pleural fluid cytological examination.

The data were transferred to the IBM SPSS Statistics 22 program. Descriptive statistics were used for categorical variables (n, %) and for numerical variables (Mean, Std. Deviation). While evaluating the study data, compliance with the normality assumption was tested initially, and in examining the difference between categorical variables with two groups, the independent sample t-test was applied for the variables suitable for the normality assumption, and the Mann-Whitney U test was applied for the variables that were not suitable for the normality assumption. Chi-square test was used to examine the relationship between two categorical variables.

Results

Of the 116 patients included in the study, 70 (60.3%) were male and 46 (39.7%) were female. The mean age of the patients included in the study was 64.08 years. Pleural fluid cytological examination was performed at least once for diagnostic purposes. Cases that could not be diagnosed with the first fluid cytology were diagnosed using one or more of the 2nd fluid cytology examination, 3rd fluid cytology examination, pleural biopsy, VATS, and decortication methods.

While the first fluid cytology result of 6.0% of the patients included in the study was positive for malignancy, the second fluid cytology was positive for 27.6%, the third fluid cytology was positive for 32.4%, the results of 63.5% of those who underwent pleural biopsy method were positive, the results of 94.3% of those who underwent VATS and 100% of those who underwent decortication were positive for malignancy (Fig. 1).

While 72.4% of the patients in the study had primary lung cancer, 16.4% had malignant mesothelioma, 6.9% had metastatic tumors (renal cell carcinoma metastasis, ovarian carcinoma metastasis, pancreatic cancer metastasis and sarcoma metastasis) and 4.3% had other malignancies (spindle cell malignant mesenchymal tumor, carcinoma metastasis, malignant epithelial tumor, round cell tumor) (Table 1).

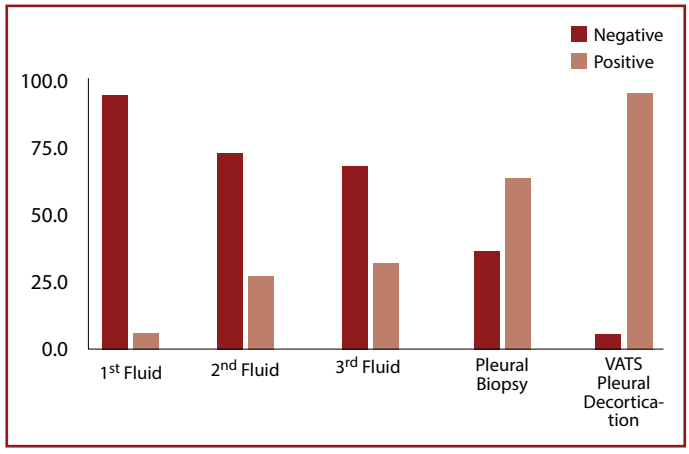

Figure 1. Positivity Rates of Diagnostic Methods.

Table 1. Distribution by Types of Malignancies

Type of Malignancy	n	%
Primary Lung Cancer	84	72.40
Malignant Mesothelioma	19	16.4
Metastatic Tumors	8	6.9
Others	5	4.3

Of the patients with primary lung cancer, 82.1% had adenocarcinoma, 8.3% had other cell type carcinoma, 4.8% had small cell carcinoma and 2.4% had squamous cell carcinoma and non-small cell carcinomas.

As a result of chi-square analysis, the relationship between the applied methods and cell types was examined and no statistically significant relationship was found between cell types and first fluid cytology, 3rd fluid cytology, pleural biopsy and VATS ($p>0.05$), while a statistically significant relationship was found between the second fluid cytology and cell types ($p<0.05$). Accordingly, the rate of positivity in patients with primary cell type lung cancer among the patients who were performed 2nd fluid cytology method is significantly higher than the rate of positivity in patients with other cell types (Table 2). When we look at the diagnostic rates of all cases according to the different methods applied, we see that 47 of 116 cases were diagnosed by fluid sampling with thoracentesis, which is a much more minimally invasive method compared to other methods (Table 2).

Table 2. Examining the Relationship Between Methods and Cell Types (Chi-Square Analysis)

	Cell Type		Chi-Square	p
	Primary Lung Cancer	Others		
1ST FLUID				
Negative				
n	77	32	2.838	0.187
%	91.7	100.0		
Positive				
n	7	0		
%	8.3	0.0		
2ND FLUID			9.223	0.002
Negative				
n	48	28		
%	64.0	93.3		
Positive				
n	27	2		
%	36.0	6.7		
3RD FLUID			3.234	0.113
Negative				
n	14	9		
%	58.3	90.0		
Positive				
n	10	1		
%	41.7	10.0		
Pleural Biopsy			2.728	0.099
Negative				
n	7	12		
%	25.9	48.0		
Positive				
n	20	13		
%	74.1	52.0		
VATS			2.519	0.202
Negative				
n	0	2		
%	0.0	12.5		
Positive				
n	19	14		
%	100.0	87.5		

Discussion

The ideal situation for cytological diagnosis is to use the least invasive method. Thoracentesis is an accessible and valuable method because it is very easy to apply and does not require an additional preparation period before its application. However, studies in recent years have revealed that the diagnostic value of fluid cytology is controversial. Many factors affect the diagnostic value of the fluid, such as the amount of fluid sampled, immunohistochemical study, tumor type, and the experience of the cytopathologist. The ideal examination time of the collected sample is within two hours, but cells can be preserved for 72 hours at 2-8°C. It is stated in the pleural diseases guide of the British Tho-

racic Society (BTS) that 50 ml of fluid is the ideal amount for diagnosis [4]. Diagnostic thoracentesis can be easily performed with a small needle and syringe [5,6].

In the study of RW ; Porcel et al., [7] it is recommended that 10 ml of pleural fluid sample is sufficient for cytological diagnosis, and that the second thoracentesis should be performed in the presence of suspected malignancy and when the first pleural fluid cytology is negative. In our study, pleural fluid samples ranging from 10 ml to 50 ml were taken from the patients and sent to the cytology laboratory. No statistically significant difference was found between the diagnostic value of these different amounts of fluid (10 ml vs 50 ml).

It has been reported that the diagnosis of malignant pleural effusion by cytology can be made with a rate of 40% to 87% in different series [8-10]. However, the number of times diagnostic thoracentesis should be performed before pleural biopsy and thoracoscopic biopsy is a matter of debate. In cases with a negative first fluid cytological examination, a second cytological examination is recommended at the level of evidence B [4].

In the retrospective study of JM Porcel et al. [11] with 831 cases, they obtained 51% positive results in terms of malignancy in the first sample. The second sample added 7%, and the third sample 2% additional diagnostic value. In the study of LW Garcia et al., [12] 55 of 215 patients could be diagnosed with pleural fluid cytology examination. Of these cases, 36 (16.7%) patients were diagnosed with malignancy in the first sample, 27 (6.9%) in the second sample, 3 (2.6%) in the third sample, and 1 (1.81%) in the fifth sample.

In the retrospective study of Prakash et al. [13] with 414 cases, malignancy was detected in 281 (68%) of these patients. Of the cases with malignancy, 162 (57.6%) were diagnosed by pleural fluid cytology examination.

In the study of Salyer et al., [14] the diagnostic rate of pleural fluid cytology was 72.6%, in the study of Nance et al. [15] this rate was 71%, and in the study of Hirsch et al., [16] it was 53.8%. In Johnston's study with 472 cases, only one pleural fluid sample was taken from 375 patients, 342 of which were found to be malignant, and 33 were suspicious for malignancy. Two pleural fluid samples were taken from 81 patients, malignancy was found in the first pleural fluid sample in 72, the first fluid sample was suspicious in 9, while the second fluid sample was found to be significant in terms of malignancy. Three pleural fluid samples were taken from 15 patients. While malignancy was detected in the first fluid sample in 13 patients, malignancy was found in the second and third fluid samples in 2 patients. In one patient, pleural fluid samples were taken four times and malignancy was found in all of them. In summary, malignancy was detected in the first pleural fluid sample in 427 (90.5%) of 472 cases, while malignancy was detected in 11 (2.3%) of the patients whose pleural fluid sample was taken for the second time. It was determined that the third fluid sample cytology did not contribute to the diagnosis [17].

In our study, 47 (40.5%) of 116 cases were diagnosed with cytological examination of pleural fluid samples, 33 (28.5%) with closed pleural biopsy, 33 (28.5%) with video-assisted thoracoscopic surgery (VATS), and 3 (2.5%) could be diagnosed with decortication. Of 47 cases diagnosed by pleural fluid examination, 7 (14.8%) could be diagnosed as malignancy with the first pleural fluid sample, 29 (61.7%) with the second pleural fluid sample, and 11 (23.5%) with the third pleural fluid sample (Table 3).

The distribution of 116 cases according to cell type was as follows: 84 cases (72.4%) with primary lung cancer, 19 cases (16.4%) with malignant mesothelioma, 8 cases (6.9%) with metastatic tumors and 5 cases (4.3%) with other tumors (adenoid malignant tumor, epithelioid malignant tumor).

Considering the relationship between the applied methods and cell types, there is no statistically significant relationship between cell types with the 1st fluid cytology, 3rd fluid cytology, pleural biopsy and VATS methods ($p>0.05$), while there was a statistically significant relationship between the 2nd fluid cytology method and cell types ($p<0.05$). Accordingly, the rate of positivity in patients with primary cell type lung cancer in whom the 2nd fluid cytology method was used, was found to be significantly higher than the rate of positivity in patients with other cell types.

When the cases with primary lung cancer were analyzed according to their cell types, 82.1% had adenocarcinoma, 4.8% had small cell carcinoma, 2.4% had squamous cell carcinoma, 2.4% had non-small cell carcinoma (with unspecified subtype) and 8.3% had other tumors (adenoid malignant tumor and epithelioid malignant tumor). In the

Table 3. Distribution of All Cases by Diagnostic Methods

Diagnostic Method	n	Diagnostic Rate (%)
First fluid	7	6.03
Second fluid	29	25
Third fluid	11	9.48
Pleural Biopsy	33	28.45
VATS	33	28.45
Pleural Decortication	3	2.59
Total	116	100

study of CE Escudero Bueno et al. [10] with 99 cases with MPE, adenocarcinoma was found in 72 cases (73%). In the study of B Naylor et al., [18] the probability of diagnosis of adenocarcinoma by examining pleural fluid cytology was found to be 66.9%.

In the study of Hooper C et al., [4] ovarian carcinoma (6%) and sarcomas (4%) were also found to cause malignant pleural effusions. Primary tumor could not be detected in 6-7% of the patients. In our study, metastatic tumors, including ovarian carcinoma and sarcoma metastasis, were detected at a rate of 6.9%.

The fact that the cytological evaluation of the fluid has a higher diagnosis rate than biopsy in malignant pleurisy is explained by the focal spread of the tumor in the pleura and the cell population in the sediment representing a much larger pleural area than the pleural area obtained by closed needle pleural biopsy [18,19].

In general, sampling of pleural fluid with thoracentesis is considered as the first step in the MPE diagnostic algorithm because it is a minimally invasive and easily applicable method. In cases where the diagnosis cannot be made by pleural fluid cytology examination, the more invasive methods are used: closed needle pleural biopsy, VATS, and thoracotomy, respectively.

In conclusion, it is still controversial how many times thoracentesis and pleural fluid sampling should be performed before other invasive methods in the diagnosis of MPE. In the literature, it is stated that the first fluid cytology is valuable in terms of diagnosis, the second fluid cytology should be evaluated in cases where the diagnosis cannot be made with the first fluid cytology, and if the diagnosis cannot be made with these 2 attempts, it is stated that performing a third cytological examination does not contribute to the diagnosis. Although the contribution of third fluid cytology to the diagnosis is limited both in the literature and in our study, if the cytology result can be reached in a reasonable time in the center, since the next step in the diagnostic algorithm is invasive methods such as closed needle pleural biopsy and VATS, we think that we should not ignore the limited contribution of the third cytological examination of pleural fluid obtained by thoracentesis, which is a more minimally invasive method. Again, we think that closed needle pleural biopsy should be chosen before VATS because it has a higher contribution to the diagnosis compared to fluid cytological examination and is a more minimally invasive method.

The limitations of our study are its retrospective nature, limited number of patients, and being a single-center study. However, we believe that similar studies should be conducted with a larger patient group in the coming years.

The authors have no conflict of interest.

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