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Original Article

Cytology of Pleural Effusions

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ABSTRACT

Objective: Thoracentesis is a simple bedside diagnostic method to collect samples of pleural effusion (PE) readily. In this study, we aimed to investigate and define the etiology of pleural effusion with routine cytological examination along with the examination of cytology and cell blocks.

Methods: Patients (404 cases), who underwent primary thoracentesis in the period from 2018 to 2022, were included in the study retrospectively. All collected patient samples were submitted to the pathology laboratory for the cytological examination.

Results: The vast majority of the PE specimens (320 cases; 79.2%) were classified as benign. Sixty-six (16.3%) cases were diagnosed as malignant. Ten cases were considered as non-diagnostic and 8 cases were considered as suspected for malignancies. No statistically significant differences were found in the age and sex between the serous and hemorrhagic effusion groups. Statistically significant differences were identified based on the cytopathologic diagnosis (p=0.001).

Conclusion: Etiologic classification of pleural effusion as benign or malignant can be made with high diagnostic accuracy by examining the cell blocks in combination with the cytological examination of the pleural fluid.

INTRODUCTION

Thoracentesis is a simple bedside diagnostic method to collect samples of pleural effusion (PE) readily, allowing for the examination of the collected fluid under the microscope.[1]

The most common causes of pleural effusion are heart failure, malignancy, pneumonia, tuberculosis, and pulmonary embolism.[2] Malignant PE account for approximately 15-35% of all pleural effusions. Of all cases, more than one-third often results from lung and breast pathologies. If the person has a history of exposure to asbestos, mesothelioma may be considered in the third place. Furthermore, it should suggest lymphoma and ovarian tumors, too.[3] Overall sensitivity is approximately 60% in the diagnosis of malignant PE. This rate may be increased by performing a repeat thoracentesis.[1,4] The diagnostic sensitivity of identifying a pleural malignant disease depends on the type of underlying malignant disease. In lung cancer patients; the sensitivity for diagnosing adenocarcinoma, small cell carcinoma, and squamous cell carcinoma are 78%, 53%, and 25% respectively.[1] The type of the tumor, tumor burden, and the effusion volume are the cytology determinants of the diagnosis.[5-7] Moreover, a hemorrhagic appearance strengthens the possibility of malignancy causing the PE.[8] Neoplastic cells are present in malignant PE. The major cytologic challenge is to distinguish neoplastic cells from reactive mesothelial cells. The cytomorphological characteristics of the reactive mesothelial cells may be variable. Because of their atypical features, there is an increased likelihood of making a misdiagnosis of malignancy.[9] Examination of cytology cell blocks and immunohistochemical testing are used for identifying the primary site and making a definite diagnosis to avoid misdiagnosis.[10]

In this study, we aimed to investigate and define the etiology of pleural effusion with routine cytological examination along with the examination of cytology cell blocks.

MATERIALS AND METHODS

Patients, who underwent primary thoracentesis in the period from 2018 to 2022, were included in the study retrospectively. Iatrogenic pleural effusion patients were excluded. All collected patient samples were submitted to the pathology laboratory for the cytological examination and the examination of the cell blocks.

Pathology

Fresh pleural fluid (PF) specimens were accepted in the pathology laboratory. The specimens were centrifuged for 15 minutes at 1500 revolutions per minute. A drop of sediment was collected and placed in the cytospin chamber. Two cytospin slides and a cell block were prepared for each specimen. Of the two cytospin slides, one was air-dried and the other was fixed in 95% alcohol. Air-dried slides were stained with May Grunwald Giemsa (MGG) stain. The slides fixed in 95% alcohol were stained with Papanicolaou (PAP) stain. Immunohistochemical staining was performed on the cell blocks.

Interpretations of the microscopic findings in the cytology specimens were recorded under four general categories as follows: "Benign", "malignant", "non-diagnostic", and "malignancy suspected".

The presence of reactive mesothelial cells, acute and chronic inflammatory cells, and/or blood without any evidence for the presence of malignant cells was considered to indicate a benign pathology.

The benign category included the presence of nonspecific (neutrophilic, lymphocytic and/or eosinophilic) inflammation and reactive mesothelial cells.

The presence of malignant cells with/without reactive mesothelial cells, inflammatory cells, and or blood was considered to indicate a malignant pathology.

For the diagnosed malignancies, the tumor type and/or the favored primary tumor site were identified and recorded based on the cytomorphological characteristics and the immunohistochemical test results performed on the cell blocks.

Statistical Method

The continuous variables were presented as mean ± standard deviation or median (interquartile range). The categorical variables were presented as numbers and percentages. Categorical variables were compared using X 2 or Fisher's Exact tests where appropriate. A two-sided p-value of less than 0.05 was considered significant for all statistical analysis tests. Collection and statistical analysis of the data were performed using SPSS 11.5 (SPSS Inc, Chicago, Ill., USA) program.

Table 1. Cytopathologic diagnosis of the pleural effusion

	Numbers of patients (%)
Benign	320 (79.2%)
Malignant	66 (16.3%)
Non-diagnostic	10 (2.5%)
Suspected malignant tumor	8 (2%)

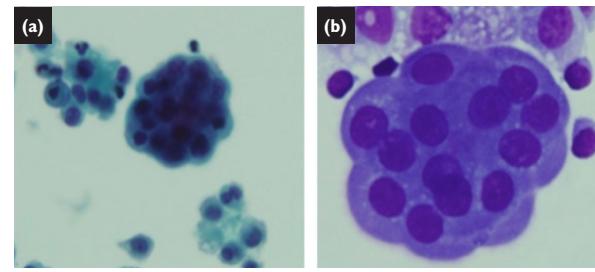

Figure 1. Benign mesothelial cells in pleural fluid cytology. **(a)** Cytospin preparation shows benign mesothelial cells (Papanicoloau staining x200) **(b)** Cytospin preparation shows mesothelial cells (Giemsa stainingx400).

RESULTS

The study included 404 patients. The mean age of the study patients was 64.1±16.4 years. Of the patients included in the study, 249 were males (61.6%) and 155 were females (38.4%).

Cytopathologic diagnoses of the patients are listed in Table 1. The vast majority of the PF specimens (320 cases; 79.2%) were classified as benign. Sixty-six (16.3%) cases were diagnosed as malignant. Ten cases were considered as non-diagnostic and 8 cases were considered as suspected for malignancies.

The subtypes of benign and malignant cytopathologic diagnoses made in the PE specimens are listed in Table 2. The mesothelial cells (Fig. 1) were most commonly found in the benign group (70.6%). This was followed by the

Table 2. The benign and malignant cytopathologic diagnosis of the pleural effusion

	Numbers of Cytopathologic diagnosis
1. Benign (n:320)	Mesothelial cells 226 (70.6%)
	Eosinophil rich cells 16 (5%)
	Lymphocyte rich cells 39 (12.1%)
	Neutrophil rich cells 39 (12.1%)
2. Malignant (n:66)	Lung adenocarcinoma 24 (36.3%)
	Malignant mesothelioma 3 (4.5%)
	Lymphoid neoplasm 3 (4.5%)
	Breast cancer 17 (25.7%)
	Mesenchymal tumor 1 (1.5%)
	Leukemia 1 (1.5%)
	Epithelial bladder cancer 1 (1.5%)
	Gastrointestinal system adenocarcinoma 5 (7.5%)
	Lung squamous cell carcinoma 8 (12.1%)
	Ovarian serous carcinoma 2 (3%)
	Mucoepidermoid carcinoma1 (1.5%)

lymphocyte-rich group (12.1%), the neutrophil-rich group (12.1%) and the eosinophil-rich group (5%) respectively. The most common diagnoses in the malignant PE were the lung adenocarcinoma (36.3%) followed by the breast carcinoma (25.7%).

Diagnostic grouping based on whether the fluid was serous and hemorrhagic is shown in Table 3. In the serous group; the mesothelial cell-rich benign pleural effusion was on the first rank, followed by the lymphocyte-rich benign pleural effusion and neutrophil-rich benign pleural effusion on the second and third ranks respectively. In the hemorrhagic PE group; the mesothelial cell-rich benign pleural effusion was on the first rank, followed by the lymphocyte-rich benign pleural effusion and the lymphocyte-rich benign pleural effusion on the second and third ranks respectively. Lung adenocarcinoma (Fig. 2) that was positive for TTF1 and NAPSİN A was the most common malignan-

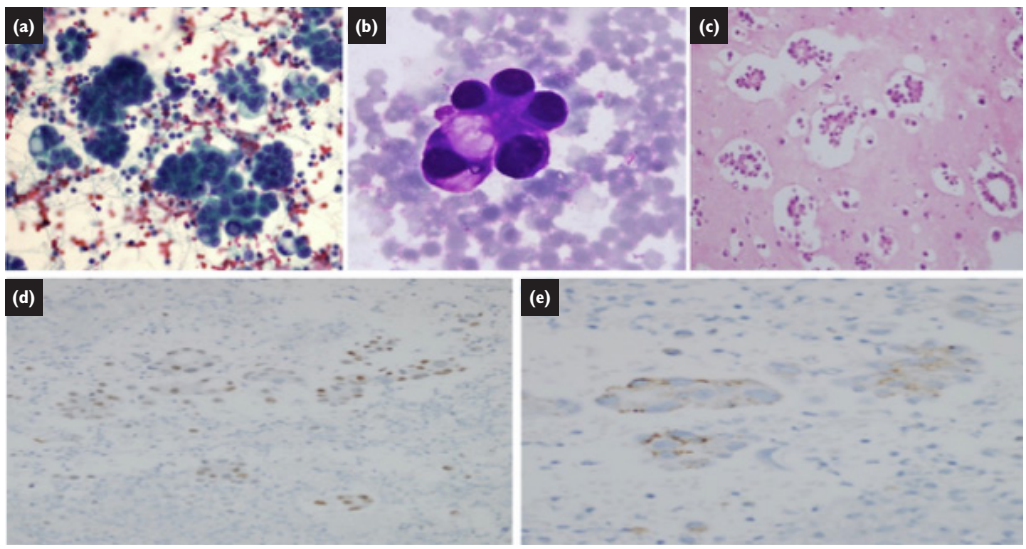

Figure 2. Lung adenocarcinoma metastasis in pleural fluid cytology. A- Cytospin preparation shows metastatic adenocarcinoma (Papanicoloau staining x200) B- Cytospin preparation shows tumor cells (Giemsa stainingx400) C- Cell block of the metastatic lung adenocarcinoma (H&Ex200) D-Immunohistochemistry for TTF1 is positive (IHC staining x200) E- Immunohistochemistry for Napsin A is positive (IHC staining x200).

Table 3. Diagnostic grouping of the pleural effusion based on whether the fluid was serous and hemorrhagic

	Total (n:404)	Serous (n:277)	Hemorrhagic (n:127)
Mesothelial cells	226 (55.9%)	179 (64.6%)	47 (37%)
Eosinophil rich cells	16 (3.9%)	11 (3.9%)	5 (3.9%)
Lymphocyte rich cells	39 (9.6%)	24 (8.6%)	15 (11.8%)
Lung adenocarcinoma	14 (3.4%)	15 (5.4%)	9 (7%)
Malignant mesothelioma	3 (0.7%)	2 (0.7%)	1 (0.7%)
Lymphoid neoplasm	3 (0.7%)	2 (0.7%)	1 (0.7%)
Non-diagnostic	10 (2.4%)	5 (1.8%)	5 (3.9%)
Breast cancer	17 (4.2%)	7 (2.5%)	10 (7.8%)
Neutrophil rich cells	39 (9.6%)	21 (7.5%)	18 (14.1%)
Mesenchymal tumor	1 (0.2%)	1 (0.3%)	0 (0%)
Leukemia	1 (0.2%)	0 (0%)	1 (0.7%)
Epithelial bladder cancer	1 (0.2%)	0 (0%)	1 (0.7%)
GIS adenocarcinoma	5 (1.2%)	3 (1%)	2 (1.5%)
Lung SCC	3 (0.7%)	1 (0.3%)	7 (5.5%)
Suspected malignant tumor	8 (1.9%)	4 (1.4%)	4 (3.1%)
Ovarian serous carcinoma	2 (0.4%)	1 (0.3%)	1 (0.7%)
Mucoepidermoid carcinoma	1 (0.2%)	1 (0.3%)	0 (0%)

GIS: Gastrointestinal system; SCC: Squamous cell carcinoma.

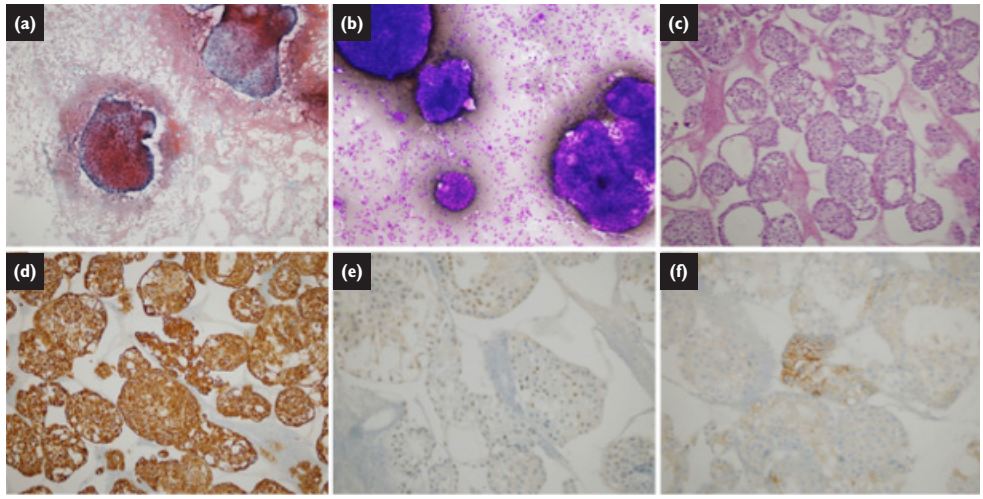

Figure 3. Metastatic breast carcinoma in pleural fluid cytology. A- Cytospin preparation shows metastatic breast carcinoma (Papanicoloau staining x100) B- Cytospin preparation shows tumor cells (Giemsa stainingx100) C- Cell block of the metastatic breast carcinoma (H&Ex100) D-Immunohistochemistry for CK7 is positive (IHC staining x100) E- Immunohistochemistry for GATA3 A is positive (IHC staining x200) F- Immunohistochemistry for MOC31 is positive (IHC staining x200).

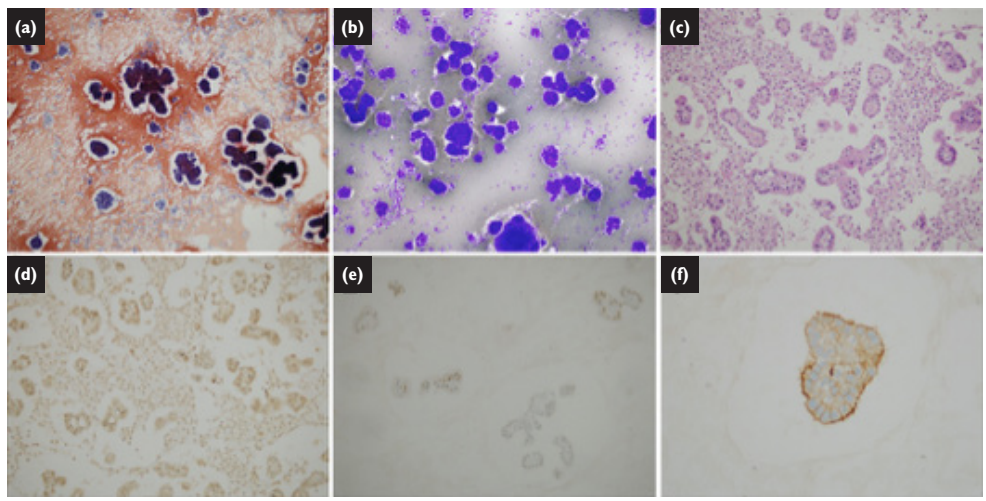

Figure 4. Mesothelioma in pleural fluid cytology. A- Cytospin preparation shows malignant cells that formed papillary clusters (Papanicoloau staining x100) B- Cytospin preparation shows malignant cells (Giemsa stainingx100) C- Cell block of the mesothelioma (H&Ex200) D-Immunohistochemistry for Calretinin is positive (IHC staining x200) E- Immunohistochemistry for WT1 A is positive (IHC staining x200) F- Immunohistochemistry for D2-40 is positive (IHC staining x400).

cy in the serous group; whereas, breast carcinoma (Fig. 3) that was positive for CK7, GATA3 and MOC31 was the most common in the hemorrhagic group. 2 cases of malignant mesothelioma (Fig. 4) were in the serous group, whereas 1 case of malignant mesothelioma was in the hemorrhagic group. Immunohistochemistry for kalretinin, WT1 and D2-40 was positive for malignant mesothelioma.

No statistically significant differences were found in the age and sex between the serous and hemorrhagic effusion groups. Statistically significant differences were identified based on the cytological diagnosis (p=0.001) (Table 4). The likelihood of a benign cytological diagnosis was significantly higher in the serous group compared to the hemorrhagic group and the likelihood of a malignant cytologic diagnosis was significantly higher in the hemorrhagic group compared to the serous group.

DISCUSSION

Our study found out that the cytological diagnosis was most commonly benign in the PE specimens. In the benign group, the mesothelial cell-rich group was the most common. In the malignant PE specimens, lung adenocarcinoma was the most common, followed by the breast carcinoma on the second rank. The comparison of serous and hemorrhagic groups revealed that benign pathologies were statistically significantly more common in the former and the malignant pleural effusions were statistically significantly more common in the latter group. Etiologic classification

Table 4. Age, sex, and cytopathologic diagnosis between the serous and hemorrhagic effusion groups

	Total n:404	Serous n: 277	Hemorrhagic n:127	p value
Age	64.1±16.4	64.3±16.6	63.5±16	0.66
Sex (male)	249 (61.6%)	169 (61%)	80 (62.9%)	0.39
Benign	320 (79.2%)	235 (84.8%)	85 (66.9%)	0.001
Malignant	66 (16.3%)	33 (11.9%)	33 (25.9%)	
Non-diagnostic	10 (2.4%)	5 (1.8%)	5 (3.9%)	
Suspected malignant tumor	8 (1.9%)	4 (1.4%)	4 (3.1%)	

of the pleural effusion, whether benign or malignant, can be made with high diagnostic accuracy by undertaking a cytological examination ancillary to assessing the pleural fluid cytology. Thoracentesis is a simple bedside diagnostic method that allows collecting samples readily and undertaking both microscopical examinations and biochemical tests.[1] The most common causes of pleural effusion are heart failure, malignancy, pneumonia, tuberculosis, and pulmonary embolism.[2] Serous effusions are mostly benign and the respective cytological findings are usually non-specific, excluding connective tissue diseases. Cytological examination of the effusion yields sensitivity and specificity rates of 80% and 98%, respectively, for malignant diseases. The yield of these two parameters can be further improved using ancillary tests.[3] Our study determined the most common type of pathology of the pleural effusions are in the benign category. Cytological diagnosis of benign pleural effusions is non-specific and may not help to make the diagnosis of primary disease in contrast to making the diagnosis of malignant diseases. In the benign group, the mesothelial cell-rich group was the most common.

Especially, the presence of reactive mesothelial cells can be misdiagnosed as malignancy due to the atypical appearance of the cells. Cytologically, the most important challenge is to distinguish the reactive mesothelial cells from the neoplastic ones. The cytomorphological structure of the reactive mesothelial cells may vary, increasing the likelihood of making a false-positive diagnosis of malignancy due to the atypical appearance of the cells. Adenocarcinomas are the most commonly diagnosed type. The presence of intracytoplasmic vacuoles in adenocarcinomas is their cytological hallmarks. However, intracytoplasmic vacuoles may also be present in degenerated mesothelial cells and histiocytes. Despite the well-described cytomorphologic features suggestive of certain primary sites, immunohistochemistry (IHC) is frequently used for confirming the primary site of the neoplastic cells. The rationale of this practice is the presence of overlapping cytomorphological findings in malignant neoplasms.[9] Examination of cell blocks and IHC tests are used for identifying the primary site of origin and making a definite diagnosis. BER-EP4 and MOC 31 are the epithelial markers used for differentiating the neoplastic cells from the mesothelial ones.[10] In the study, we combined the cytological examination with the cell block cytology to avoid making a misdiagnosis and to increase the diagnostic accuracy. Definitive diagnosis was achieved through immunohistochemical staining applied to cell blocks. Adenocarcinoma cells observed in cell blocks were positive for TTF1 and NAPSİN A, while mesothelioma cells showed positivity for Calretinin, WT-1 and D2-40.

Malignant pleural effusions account for approximately 15-35% of all pleural effusions. More than one-third of the cases originate from the lung and breast. If the person has a history of exposure to asbestos, mesothelioma may be considered in the third place. In addition, lymphoma and ovarian tumors should be considered, too.[11] In the study performed by Tetikkurt et al.,[12] 600 exudative effusions were examined retrospectively and 240 malignant effusions were identified. Adenocarcinoma was found to be the most common malignancy. A diagnosis of tuberculosis held the second rank and they were followed by the infections and collagen vascular diseases on the third and fourth ranks respectively. Exfoliative cytological examination yields sensitivity and specificity rates of 70.1% and 62.5%, respectively, along with a positive predictive value of 95.9% in making the final diagnosis.[12] In this study, the final diagnosis was made by the histopathological diagnosis based on the examination of biopsy specimens.

The largest prospective study performed by Arnold et al.[13] found out malignancies in 515 out of 921 patients during an 8-year follow-up period. The overall sensitivity for malignancy was 46% (95% CI 42-58). This finding showed variations especially based on the types of cancer. The rates were reported to be 6% for mesothelioma and 40% for hematologic malignancies, compared to the 79% rate found in adenocarcinomas. Malignant PEs secondary to ovarian cancer had high pick-up rates (95%). While the risk of malignant PE is 60% in men with a history of asbestos exposure, its cytological sensitivity is 11%.[13]

In a retrospective study performed by Paula Loveland et al.,[14] the malignancy rate was 39.9%, originating from lung cancer, mesothelioma, ovarian carcinoma, and lymphoma at rates of 44.3%, 18%, 11.5%, and 8.2%, respectively. The most common causes of benign effusions were cardiac (16.3%) and parapneumonic etiologies (13%). While the diagnostic sensitivity is reported to be 67.2% for all malignant PEs, the diagnostic sensitivities for adenocarcinoma and mesothelioma were reported to be 87.9% and 45.5%, respectively.

In our study, we detected lung adenocarcinoma as the origin of malignant effusions. We found out that the difficult-to-diagnose tumor types by cytology only, such as mesotheliomas and the squamous cell carcinoma of the lung, could be diagnosed more commonly when cytology and examination of cell blocks were employed in combination. Although it is known that mesothelioma is difficult to diagnose and should be included in the differential diagnosis in patients with a history of asbestos exposure, we detected 3 mesothelioma cases.

The cytological examination is particularly difficult in mesothelioma. IHC tests can increase the diagnosis rates, using Bap 1 Nuclear Expression and P16 deletion.[15] However, in experienced centers, mesothelioma can be diagnosed at rates of up to 73% in the hands of experienced cytologists.[16]

Although all adenocarcinomas are readily diagnosed by cytology, the difficulty in diagnosing other tumor types can be overcome by using cytology and cell block examination in combination. Conventional pleural effusion cytology may yield 40% negative results approximately in malignant effusions. This rate is even higher in lung mesothelioma and squamous cell carcinomas (70-85%).[1,13] Cytological yield is not only related to the tumor type but also to the experience of the cytopathologist, the purpose of the analysis, and the number and volume of specimens accepted.[17] Although the optimal volume of the sample fluid has not been established, volumes from 20 to 40 ml will suffice.[4] Moreover, taking more than 2 separate samples does not increase the sensitivity.[18]

Notably, standard cytological techniques should include the preparation of smears (Papanicolaou or May- Grünwald-Giemsa stainings) and cell blocks (CBs) (hematoxylin and eosin staining), since both are complementary. In a series of 414 malignant pleural effusion cases performed by Porcel et al.,[18] 11% of the negative cytological smears of the pleural fluid had malignant cells in the CB, while 15% of the cases with a negative CB had positive smear results. That study included 632 cytological smears and 554 CBs from 414 patients with malignant effusion. While the diagnostic rate of the first specimen was reported to be 44% in the first examination, regardless of the specimen type being smear or CB, the use of subsequent separate specimens increased the malignancy diagnosis rate to 56%.

Examination of cell blocks of pleural effusion in combination with the smears may lead to higher rates of diagnostic accuracy based on microscopic findings in the cellular solid portion of the specimens.[4] However, cell blocks are not used routinely in every center as it is demanding in regards to time and labor. In some studies, cell block examinations performed in addition to routine cytological examinations have increased the diagnostic rates of malignancies by 5-15%. However, the number of patients in these studies is small, insufficient to provide comprehensive information.[19,20] In our study, we determined that the diagnostic rate was significantly increased by ancillary cell block examination performed in combination with conventional cell cytology. Cell block examination provides some advantages over the examination of smears. These include the likelihood to appreciate the tissue architecture, the opportunity to readily identify reactive mesothelial cells, the likelihood of the cytomorphological distinction between mesothelioma and metastatic adenocarcinoma, and the potential to process multiple sections for immunocytochemistry.[21] In our study, we determined the types of tumors by applying IHC tests on the cell blocks. The macroscopic appearance of the pleural fluid is very important as it provides a clue in making the diagnosis. A milky consistency of the fluid is characteristic for chylothorax. The presence of purulence is typical for empyema. A hemorrhagic effusion more commonly characterizes malignancies.[22] In the present study, we detected a high rate of malignancy in the hemorrhagic pleural effusion group. A malignant pleural effusion is characterized by the presence of neoplastic cells. The most important cytological challenge is to distinguish neoplastic cells from reactive mesothelial cells. The reactive mesothelial cells can show variable cytomorphologic features and increase the likelihood of a diagnosis of a malignancy because of their atypical properties. Adenocarcinomas are the most commonly diagnosed diseases.

The presence of intracytoplasmic vacuoles in adenocarcinomas is a cytological hallmark. However, intracytoplasmic vacuoles may also be present in degenerated mesothelial cells and histiocytes. Although the cytomorphologic features suggestive of certain primary sites have been well-established, IHC is frequently used for confirming the primary site of the neoplastic cells due to the overlapping cytomorphological characteristics of malignant neoplasms.[9]

Examination of cell blocks are employed in combination with the IHC tests to identify the primary site, make the definite diagnosis, and avoid false-positive and incorrect diagnoses. BER-EP4 and MOC 31 are epithelial markers and they allow distinguishing neoplastic cells from the mesothelial ones.[10]

Limitations

The main limitation was that our study was conducted at a single-center and it was not a randomized or prospective study. Unavailability of the clinical features of the patients and the lack of follow-up findings are the other limitations.

Conclusion

Our study found out that the cytological diagnoses were most commonly benign in the PE specimens. In the benign group, the mesothelial cell-rich group was the most common. In the malignant pleural effusions, the most common diagnosis was lung adenocarcinoma and the second most common one was breast carcinoma. The comparison of the serous and hemorrhagic groups revealed that benign pathologies were more common in the serous group and rates of malignant pleural effusion were higher in the hemorrhagic group statistically and significantly. Etiologic classification of pleural effusion as benign or malignant can be made with high diagnostic accuracy by examining the cell

blocks in combination with the cytological examination of the pleural fluid.

Ethics Committee Approval

The study was approved by the Kartal Dr Lutfi Kirdar City Hospital Ethics Committee (Date: 29.04.2024, Decision No: 2024/010.99/3/18).

Informed Consent

Retrospective study.

Peer-review

Externally peer-reviewed.

Authorship Contributions

Concept: G.G.G.; Design: G.G.G.; Supervision: N.O.B.; Fundings: G.K.A.; Materials: D.E.I.; Data: G.G.G.; Analysis: G.G.G.; Literature search: D.E.I.; Writing: G.G.G.; Critical revision: R.D.

Conflict of Interest

None declared.

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Plevral Efüzyonlarin Sitolojisi

Amaç: Torasentez yatakbaşı yapılabilen plevral efüzyonları örneklemek için kullanılan basit bir yöntemdir. Bu çalışmada, sitospin ve hücre bloğu hazırlanarak plevral efüzyonların rutin sitolojik incelemesi ile efüzyonların etiyolojisini araştırmayı ve belirlemeyi planladık.

Gereç ve Yöntem: 2018 ile 2022 yılları arasında primer torasentez uygulanan hastalar retrospektif olarak çalışmaya dahil edildi. Hastalardan alınan tüm örnekler patoloji labaratuarına sitolojik inceleme için gönderilmişti.

Bulgular: Plevral efüzyon spesmenlerinin büyük çoğunluğu (320 olgu; %79.2) benign olarak sınıflandırıldı. 66 (%16.3) olgu malign olarak sınıflandırıldı. 10 olgu yetersiz, 8 olgu ise malignite kuşkulu olarak değerlendirildi. Plevral efüzyonların seröz ve hemorajik olması ile hastanın yaşı ve cinsiyeti arasında istatiksel olarak farklılık bulunmadı. Ancak sitopatolojik tanı ile anlamlı ilişki bulundu (p=0.001).

Sonuç: Plevral sıvıların hücre bloğu ve sitospin ile birlikte incelenmesi, plevral efüzyonların benign ve malign olarak etiyolojik sınıflamasında yüksek tanı değerine sahiptir.

Anahtar Sözcükler: Plevral efüzyon; sitopatolojik inceleme; torasentez.