

RESEARCH ARTICLE

Importance of the Cell Block Technique in Diagnosing Patients with Non-Small Cell Carcinoma Accompanied by Pleural Effusion

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Abstract

Background: Cytological examination of pleural effusions is very important in the diagnosis of malignant lesions. Thoracentesis is the first investigation to be performed in a patient with pleural effusion. In this study, we aimed to compare traditional with cell block methods for diagnosis of lung disease accompanied by pleural effusion. **Materials and Methods:** A total of 194 patients with exudative pleural effusions were included. Ten milliliters of fresh pleural fluid were obtained by thoracentesis from all patients in the initial evaluation. The samples gathered were divided to two equal parts, one for conventional cytological analysis and the other for analysis with the cell block technique. In cytology, using conventional diagnostic criteria cases were divided into 3 categories, benign, malignant and undetermined. The cell block sections were evaluated for the presence of single tumor cells, papillary or acinar patterns and staining with mucicarmine. In the cell block examination, in cases with sufficient cell counts histopathological diagnosis was performed. **Results:** Of the total undergoing conventional cytological analyses, 154 (79.4%) were reported as benign, 33 (17%) as malignant and 7 (3.6%) as suspicious of malignancy. With the cell block method the results were 147 (75.8%) benign, 12 (6.2%) metastatic, 4 (2.1%) squamous cell carcinoma, 18 (9.3%) adenocarcinoma, 5 (2.6%) large cell carcinoma, 2 (1%) mesothelioma, 3 (1.5%) small cell carcinoma, and 3 (1.5%) lymphoma. **Conclusions:** Our study confirmed that the cell block method increases the diagnostic yield with exudative pleural effusions accompanying lung cancer.

Keywords: Non-small cell lung cancer - cell block - pleural effusion - immunohistochemistry

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Introduction

Our century is a century that struggle with cancer is continuing. In this struggle, along with effective treatment methods, developing early and easy diagnosis techniques are crucial. Cytological examination of the pleural effusion fluid is the most common diagnosis technique (Rodriguez et al., 1989; Biesterfeld et al., 2014). The cytological examination of the pleural effusion fluid is important in terms of diagnosis, prognosis and stating of malignant lesions (Nance et al., 1991; Cusumano et al., 2007). In conventional cytology, discrimination of the reactive mesothelium cells and malignant cells is the most important diagnostic problem (Carpagnano et al., 2012). The different techniques that different laboratories use, the overlapping of the cells, overcrowded cell communities, loss of cells are all reasons why conventional technique has a low sensitivity. Cell-block technique is one of the oldest techniques that is used in evaluating serous fluids (Basnet et al., 2001). Today, in cell-block preparation, 10% formalin is used in fixation. The superiority of this

technique is that it is easy, inexpensive and does not require a special team or instruments (Koksall et al., 2013).

With this method, cellularity is increased, the morphological details are better observed, special histochemical stainings and immune histochemical studies can be done (Ensani et al., 2011). Thus, sensitivity in diagnosis increases. Compared with other conventional techniques, this technique is a more sensitive model (Ikeda et al., 2011).

The most common reason of exudative pleural effusions is the metastatic disease of the lymph nodes of the pleura or mediastinum and the incidence increases with age. Lung cancers, breast cancers and lymphomas are responsible for 75% of pleural effusions (Rodriguez et al., 1989). Any organ cancer may metastasis to the pleura but the most common cancer with pleural involvement and that causes malign effusion is lung cancer. Malign pleural effusion is seen in 23.1% of patients with lung cancer. In every type of lung cancer, pleural effusions are possible (Mandal et al., 2013). None the less, because of its peripheral localization and dissemination way,

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adenocarcinoma is the most common tumor type that causes malignant plural effusion (Soini et al., 2006). Cell-block technique is particularly helpful in discriminating cytological abnormalities such as reactive mesothelium cells or well differentiated adenocarcinoma which can sometimes be deceptive.

In this study, evaluating the program of cell block preparation with increased cytological diagnosis sensitivity, identifying malignant effusions and demonstrating the superiority of cell block technique compared to conventional technique in identifying the primary region are aimed.

Materials and Methods

This study includes 194 patients clinically and radiologically proven to have pleural effusion in Konya Education and Research Hospital between 2008 and 2013. The fluids gathered from the patients were divided to two equal parts, while in one half conventional cytological analysis was done, in the other half the analysis was done with the cell block technique. Half of the specimens were centrifuged for 5 minutes with 2000 rpms. The sediment acquired was applied on the slide and stained with routine Giemsa and Hematoxilen Ezoin stains. The other halves of the specimens were centrifuged for 5 minutes with 2000 rpms. The residual fluid over the tube was emptied. The sediment in the bottom was gathered on the blotting paper by turning the tube upside down. The material obtained was fixed with 10% formalin solution and then routine histological follow up was done. After follow up paraffin blocks were formed and paraffin blocks were cut in 4 μ sections. These sections were stained with routine Hemotoxilen Eosine. After light microscope examination, in required cases special histochemical (Figure 1) and immunohistochemical (Figure 2) studies were done. In cytological diagnosis the conventional diagnosis criterias were divided in to 3 categories as benign, malignant and undetermined. Every preparate's cellularity, the cytoplasm and nuclear details of the cells, the arrangements (acini, papillary structure, two or three dimensional cell clusters) were analyzed (Figure 3). In the cell block examination, in cases with sufficient cell counts histopathological diagnosis was done (Figure 4). In this study, the cell block technique compared to the conational method is compared and the procedure of the cell block technique is discussed.

Results

194 patients gone through conventional cytological analyses were reported as, 154 (79.4%) benign, 33 (17%) malignant and 7 (3.6%) suspiciously malignant (Table-1). The group where the examination was done with cell block method the results were: 147 (75.8%) benign, 12 (6.2%) metastasis, 4 (2.1%) squamous cell carcinoma, 18 (9.3%) adeno carcinoma, 5 (2.6%) large cell carcinoma, 2 (1%) mesothelioma, 3 (1.5%) small cell carcinoma, 3 (1.5%) lymphoma (Table 2). Breast cancer, with 8 patients, was the most common in the metastasis group the second was over carcinoma metastasis with 2 patients. 1 with stomach carcinoma and 1 carcinoma metastasis were detected as

well. The 194 patients included to the study were 57.2% (111) men and 42.8% (83) were women. 12 (0.8%) were affirmed benign with the conventional technique was detected malignant with cell block technique. Besides, the 2 (0.6%) of the 31 cases affirmed malignant with the conventional technique, were detected benign with the cell block technique and also the 3 (42%) of the 7 cases affirmed suspiciously malignant with the conventional technique was determined as benign with the cell block technique (Table 3). In our study, the specificity of conventional cytology method is 96%, the sensitivity

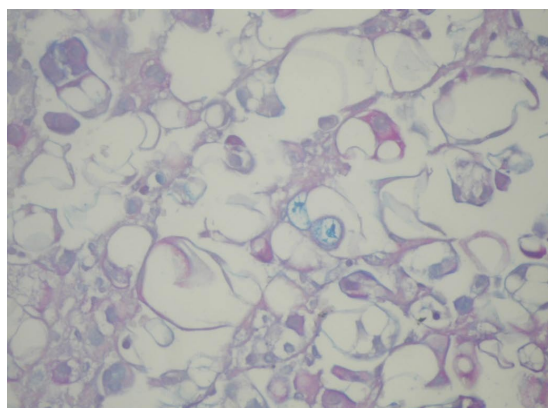


Figure 1. Focal Intracytoplasmic Mucin Existence with Pab Staining in Cell Block

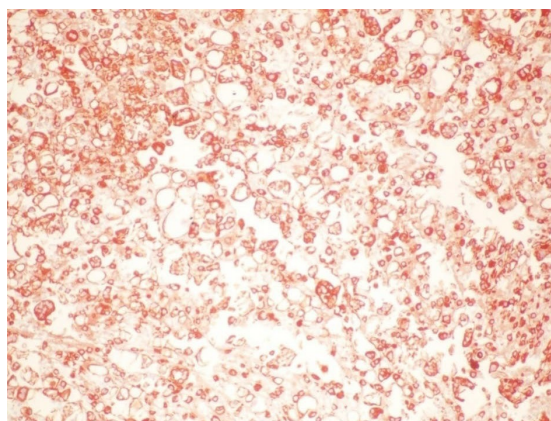


Figure 2. Intracytoplasmic Positive Staining with CK7 with Immune Histochemical Staining Under 40x10 Magnification in Cell Block

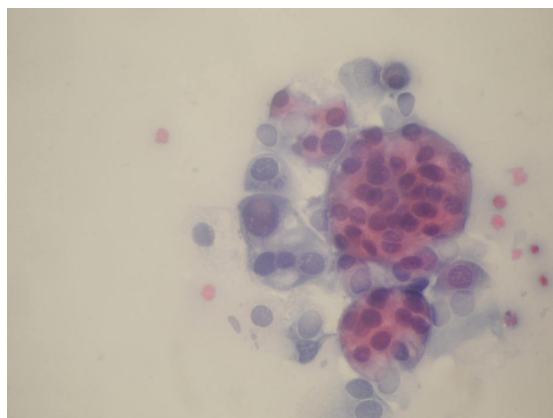


Figure 3. Malignant Cells with Giemsa Staining Under 40 x10 Magnification

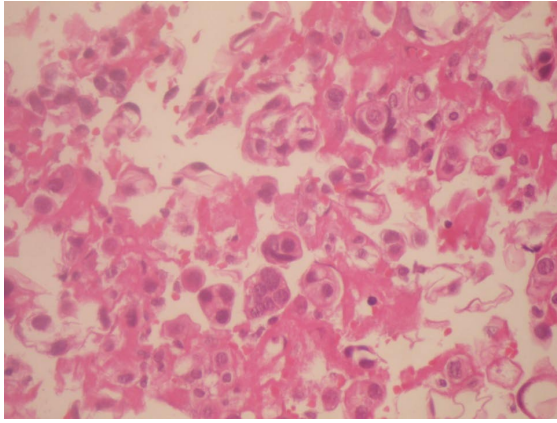


Figure 4. Fibrin and Malignant Cells among Erythrocytes with Hematoxylin and Eosin Staining Under 40x10 Magnification in Cell Block

Table 1. Conventional Cytology Smear and Cell Block Technique Diagnostic Ratios

	Conventional Cytology Smear	Cell Block
Benign	154 (79.4%)	147 (75.8%)
Malignant	33 (17%)	47 (24.2%)
Suspiciously Malignant	7 (3.6%)	

Table 2. Features of Patients Diagnosed with the Cell-Block Technique

Benign	147 (75.8%)
Metastasis	12 (6.2%)
Squamous Cell Carcinoma	4 (2.1%)
Adenocarcinoma	18 (9.3%)
Large Cell Carcinoma	5 (2.6%)
Mesothelioma	2 (1.0%)
Small Cell Carcinoma	3 (1.5%)
Lymphoma	3 (1.5%)

Table 3. Case Analyzes were Performed with the Cell Block Method after Conventional Cytological Analysis

	Benign	Malign	Suspicious for malignancy	Total
Benign	142	2	3	147 (75.8%)
Malignant	12	31	4	47 (24.2%)
Total	154	33	7	194 (100%)

is 50%. However, sensitivity and specificity of the cell block method is 100%. The positive predictive value of conventional cytology method is 96%, negative predictive value is 33%. The predictive value of the cell block method is 100%. Test the validity of the conventional cytology method is 90% and test the validity of the cell block is 100%.

Discussion

Malignant pleural effusion which is described as malignant cell existence in the pleural fluid or parietal pleura is not only seen in the progress of lung cancer but also in other malignancies. Effusion, which is mostly developed after the diagnoses of cancer may sometimes be the first finding of the tumor's dissemination or relapse

(Ghosh et al., 2012). In patients who have lung cancer suspicion and pleural effusion, the first process is to do is thoracentesis (Rivera et al., 2013; Zhang et al., 2014). For the disease's staging and treatment strategy the discrimination of malignant effusion from Para malignant effusion, the examination of this fluid is important (Dagli et al., 2011; Porcel et al., 2014). The examination of the pleural fluid cytology is crucial for the pleural involvement of the malignancy of the lungs or the visceral or parietal pleural metastatic involvement of an extra pulmonary malignancy (Biesterfeld et al., 1985; Porcel et al., 2014). While repeated thoracentesis increases the diagnostic ratio, the pleural fluid's cytological examination's contribution ratios to the diagnosis are different (Nance et al., 1991; Carpagnano et al., 2012; Bhanvadia et al., 2014).

Pleural biopsy guided with thoracoscopy of course may provide important results. Since biopsy is an invasive procedure, the priority which is to do a cytological examination to the fluid obtained with thoracentesis is very important (Gao et al., 2014).

In this purpose, even though conventional techniques have been used generally till this day, cell block technique is also being used recently (Shivaskumarswamy et al., 2012). In this study we aimed to compare cell block technique with the conventional techniques and emphasize the importance of it.

With this method, cellularity is increased, the morphological details are better observed, special histochemical stainings and immunohistochemical studies can be done (Ensani et al., 2011). Thus, sensitivity in diagnosis increases. Compared with other conventional techniques, this technique is a more sensitive model. (Ikeda et al., 2011)

Conventional smear cytology technique is a longstanding easy technique that is used. The main hardship of this technique is to discriminate the malignant cells from the reactive mesothelium cells (Soini et al., 2006; Khan et al., 2012). Also, the staining techniques, bad fixation and artifacts due to the preparation contribute to the difficulty in the diagnosis (Price et al., 1992).

It is demonstrated in the study of Basnet and his friends that in the diagnosis of neoplastic lesions cell block technique is superior to the smear technique in terms of better staging of the tumor and rapid identification (Basnet et al., 2012).

A parallel study to ours, which Koksai and his friends has done, it is shown that in a group that was considered benign with the Conventional smear cytology technique, there was malignant cases proven with the cell block technique. Besides, they also detected that it was possible to type the cancer with cell block technique in the cases considered malignant with the Conventional smear cytology technique. They have diagnosed 7 cases as adenocarcinoma (Koksai et al., 2013). Correspondingly, in our study, 12 cases considered benign with Conventional smear cytology technique was found out that they were actually malignant with the cell block technique and again with the cell block technique adenocarcinoma was the most common diagnosis with 18 cases among all of the malignant cases.

In another study that Atalay and his friends have

concluded, it was detected that, in malignant pleural effusion typing in patients with lung cancer, adenocarcinoma was the most common tumor type with a ratio of 50% (Atalay et al., 2001). In our study, with the cell block technique, adenocarcinoma was detected 47.5% as well. In a study that Grandhi and his friends have recently concluded, with the cell block technique, 5 more cases were diagnosed with malignancy, parallel with our findings (Grandhi et al., 2014). In our study 47 more cases were diagnosed with malignancy or metastasis. The reason why our numbers are high may be because of the greater numbers of cases involved in our study. Compared to thoracoscopy, which is a more invasive technique than cell block technique, using the cell block technique, which is also known for the high positive and negative predictive values, in daily practice, is important also for the patient comfort. Since sub typing the lung cancer histological is important in choosing the right chemo therapeutic agents, the cell block technique should be used more common than the conventional smear cytology technique (Wang et al., 2007; Scagliotti et al., 2011; Sanz et al., 2012; Kossakowski et al., 2014).

In conclusion, in patients with lung cancer accompanied with pleural effusion, the fluid obtained from thoracentesis, should be examined with both the Conventional smear cytology and cell block technique cytologically, in order to make the benign or malignant discrimination and increase the right diagnosis rate. It is crucial to popularize the usage of the cell block technique, which is very important in making a cancer diagnosis in patients with pleural effusion.

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