Introduction

Malignant pleural mesothelioma (MPM) is the most common type of primary tumour of the pleura. It usually develops diffusely in the visceral and parietal pleura leading to their thickening, and it then envelops the entire lung in the form of a thick mantle. It is mostly unilateral, and positioned on the right lung. Depending on the type of the predominant cell, there are three histological types of mesothelioma: epithelioid, sarcomatoid and biphasic [1]. Recent references suggest different subtypes of primary malignant mesothelioma histological types such as desmoplastic, microcellular and rhabdoid mesothelioma [2].

Malignant mesothelioma, like other peripheral lung lesions, is most commonly manifested by dyspnea, chest pain or back pain, persistent cough, weakness, weariness, anorexia, weight loss, and in some cases, flu-like symptoms, such as mild fever, myalgia, etc., may develop [3]. However, the most important manifestation of malignant pleural mesothelioma is the occurrence of pleural effusion [4].

When it comes to malignant pleural mesothelioma, the radiographic image most often shows a unilateral pleural effusion, which develops due to the progression of inflammatory process [5]. The effusion is exudative in 95% of the cases, and contains a higher concentration of protein, inflammation and, rarely, malignant cells [2]. References show only individual cases, which were accompanied by the appearance of transudative pleural effusion [6].

The cytological examination of pleural fluid in the patients with epithelioid malignant mesothelioma showed the presence of atypical epithelial cells organized in clusters, and the sample was usually hypercellular. Sarcomatoid mesothelioma is manifested in the pleural effusion as the presence of single atypical cells with oval nucleus, which is less noticeable than in the epithelioid type, and the sample of pleural fluid does not contain malignant cells. Biphasic mesothelioma cells are either rarely present in the pleural effusion or there is a small number of them [7-9].

The aim of our study was to evaluate the significance of the cytological analysis of the pleural fluid in the diagnostic algorithm in patients with malignant pleural mesothelioma.

Material and methods

A retrospective analysis was made using clinical data on 33 patients with malignant pleural mesothelioma, who were diagnosed histopathologically to have malignant pleural mesothelioma at the Institute for Lung Diseases in Sremska Kamenica in the period from 2004 to 2009. Having analyzed the clinical and demographic data obtained from the medical records of the patients, x-rays confirmed pleural effusion, thoracentesis was performed and specimens of effusion were cytologically examined at the Department of Pathology of the Institute for Lung Diseases of Vojvodina.

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records of the Institute, we found that 24 of the patients had radiologically confirmed pleural effusion, which was punctuated, technically processed and stained using the May-Grünwald-Giemsa staining method (MGG), and then analyzed cytologically.

The sample was considered cytologically negative if there was no cellular atypia. On the other hand, what spoke in favour of the positive cytological findings was the hypercellularity of the analyzed sample and significant atypia of mesothelial cells which showed the tendency towards organizing into the so-called three-dimensional groups, i.e., clusters, the nuclei of which were enlarged and irregular (Figure 1). The samples considered cytologically suspicious were the ones in which the number of mesothelial cells was increased (but never to such an extent as in malignant effusion) and in which the cells showed medium atypia (Figure 2). Since this finding was insufficient for the diagnosis of malignant mesothelioma, the cytological analysis of the pleural fluid was repeated in these patients. The sample was not considered representative if there was a significant amount of blood, or artifacts formed during the preparation.

The χ² test was used for the statistical analysis; the mean values, as well as the percentage of occurrence and sensitivity and predictive values, were calculated. Data are presented in tables and graphs.

**Results**

The presence of pleural effusion was radiologically confirmed in 24 (73%) of 33 patients with diagnosis of MPM, which had been made histopathologically and confirmed immunohistochemically.

The patients included in our study were aged between 43 and 81, their average age being 59.5 years. Thirteen respondents (54%) were females and 11 (46%) males. Eleven respondents (46%) were smokers. All patients denied having cases of malignant mesothelioma in the family history.

The most common clinical manifestations of malignant pleural mesothelioma in our patients were dyspnea, chest pain, cough and symptoms of common infectious syndrome. Dyspnea was present in 14 patients (58%), the same number of patients complained of chest pain, cough was reported in 7 patients (29%), and symptoms of common infectious syndromes were present in 6 patients (25%).

Pleural biopsy was performed in all 24 patients by Video-Assisted Thoracoscopic Surgery (VATS) after which an immunohistochemical analysis was performed on the obtained biopsy material, which definitely confirmed the diagnosis of malignant pleural mesothelioma (Cytokeratin 5/6, calretinin and Anti-Human Mesothelial Cell HMBE-1 positive and Carcinoembryonic antigen and Thyroid transcription factor 1 negative). Seventeen (71%) patients had epitheloid MPM, 4 (16.5%) sarcomatoid, and the remaining 3 patients (12.5%) had biphasic malignant pleural mesothelioma. All patients had unilateral MPM - the tumour was positioned on the right lung in 18 of them (75%), whereas in 6 of them (25%), it was on the left one.

After thoracocentesis, 49 cytological analyses were performed. Initially, only 2 cytological findings were positive for malignancy, while 6 of the findings had suspected malignancy. The repeated analyses of the six initially suspected cytological findings confirmed malignancy in 5 of them; whereas 1, in which the presence of malignant cells had not been proven, was labelled as negative. Of the 7 positive cytological findings of malignancy, 5 had epitheloid MPM, and 2 had biphasic MPM. As for the remaining initially negative cytological smears, the pleural fluid was of mixed cellular composition in 9, and lymphocytes

**Abbreviations**

MPM – malignant pleural mesothelioma  
VATS – Video Assisted Thoracoscopic Surgery  
MGG – May Grunwald Giemsa

**Fig. 1.** Cytologically positive specimen: Hypercellularity and significant mesothelial cell atypia, MGGx200  
**Fig. 2.** Cytologically suspicious specimen: Mild degree of mesothelial cell atypia, MGGx200
dominated in 4, neutrophilic granulocytes were dominant in one, and in 2 findings the effusion was of hemorrhagic type (Graph 1).

Graph 1. Results of initial cytological analyses

Upon testing the hypothesis using $\chi^2$ test, the null hypothesis was discarded and a working hypothesis was accepted because the presence of malignant cells was confirmed in the pleural effusion in only 7 patients, whereas in other patients the effusion was inflammatory in character, of mainly mixed cellular composition with a mass of altered reactive mesothelial cells, rarely with neutrophil granulocytes, lymphocytes and macrophages.

Since the cytological analysis of pleural fluid proved malignancy in only 7 patients with MPM but had no diagnostic significance in 17, the sensitivity of cytological analysis in the diagnosis of MPM in our study was 29% and the positive predictive value was 1 i.e. 100%.

Discussion

Malignant mesothelioma is a rare primary malignant tumour of mesothelial cell origin that appears in the pleura, pericardium and peritoneum. The most frequent localization of this tumour is the pleura, which also represents the most common primary malignity [2,4].

Our study included 33 patients who had been diagnosed with malignant mesothelioma of the pleura by histopathological and immunohistochemical analysis, and the presence of pleural effusion was determined by radiological imaging in 24 patients (73%). According to literature data, 54-85% of patients with MPM have a pleural effusion at the time of the onset of symptoms [2,4,6].

According to literature data, the most common type of MPM is epitheloid MPM, while the biphasic sarcomatoid one is less frequent [2,4,9], which is consistent with our data according to which 71% of patients had epitheloid malignant mesothelioma, whereas it was sarcomatoid in 16.5%, and biphasic in 12.5%.

Of the 24 initially analyzed cytological findings, 2 were malignant, and 6 had suspected malignancy. After the repeated analyses of the six initially suspected cytological findings, malignancy was proven in five of them, whereas 1, in which the presence of malignant cells was proven, was labelled as negative. Of the 7 cytologically positive pleural punctures, 5 were epitheloid, 2 belonged to the biphasic type of MPM, and in all seven cases, cytological findings did correlate with the histopathologically determined type of MPM. The results correlate with the results of Renshaw and Dean [10], who found that 4 of the 29 initial cytological findings were malignant, and 5 had suspected malignancy. The sensitivity of the cytological analysis in the diagnosis of MPM in their study was 32%, whereas in our study the sensitivity was 29%. Rakhi et al have found in their study that the sensitivity of cytological analysis was 51.3%, and that the sarcomatoid MPM shows the lowest sensitivity rate (20%), while epitheloid MPM has a sensitivity of 55.4% [11]. According to other studies, the sensitivity of cytological analysis in MPM diagnosis ranges from 26-76% [12,13]. Some authors suggest an extremely high degree of sensitivity of cytological analysis of 90% [14]. Some other authors are even questioning the reliability of this method because of the obtained degree of sensitivity of 10% [15]. However, the importance of cytological analysis of the pleural effusion should not be belittled. Most of all, it is a fast, easy-to-perform and very cost-effective diagnostic procedure. Maskell et al [16] have shown in their years-long, extensive study that this analysis is of great importance in the clarification of the aetiology of malignant pleural effusion, and that the correct diagnosis was made by cytological examination of the pleural fluid in 60% of cases.

Unfortunately, cytological examination of pleural effusion can usually help diagnose epitheloid MPM as this type of mesothelioma cells are found in large numbers in the pleural fluid, unlike the cells of sarcomatoid and biphasic MPM, which are either rarely present in the pleural effusion or there is only a small number of them [7-9].

With this research, we have shown that the cytological analysis of the pleural effusion on malignant cells in patients with MPM gives positive results only in a small number of cases, which is explained by the so-called “walls off” phenomenon. This phenomenon is responsible for the low positivity on the malignant cells and results from covering the surface of tumour with layers of fibrinous inflammatory exudate which does not allow the desquamation of tumour cells and their transfer to the pleural effusion fluid [8,9,12].

Conclusion

Given the low sensitivity of the cytological analysis in the diagnosis of malignant pleural mesothelioma, it is necessary to do a biopsy of the pleura in order to make a definitive diagnosis in all patients suspected to have malignant pleural mesothelioma on the basis of the clinical and radiological findings (and in whom the cytological evaluation of pleural fluid gave a negative result). Pleural biopsy should be performed in all positive cytological findings as well to confirm the diagnosis of malignant pleural mesothelioma.

In addition, the sensitivity of cytological analysis should be improved with other techniques such as...
cytogenetic analysis, but until this technology becomes part of the routine diagnostic algorithm, cytological analysis of the pleural effusion should con-
tinue to be applied in all patients who have pleural ef-
fusion or a recurrent exudate and are under the clini-
cal suspicion of having malignant mesothelioma.

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