

**IMMUNOHISTOCHEMICAL DIAGNOSIS OF MALIGNANT PLEURAL MESOTHELIOMA: A RETROSPECTIVE STUDY OF 47 PATIENTS.**

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**Abstract.**

**Background.** Malignant pleural mesothelioma is a pleiomorphic relatively rare tumor, which explains the challenge for the pathologists in making this diagnosis.

**The aim** of this study is to present our 7-year practice in immunohistochemical diagnosis of malignant pleural mesothelioma.

**Material and Methods.** We present a group of 47 patients with malignant pleural mesothelioma (MPM). Pleural specimens were obtained by pleural biopsy at the time of video-assisted thoracoscopy. Light microscopic examination (hematoxylin-eosin staining) and immunohistochemical staining (with three positive markers - ck 5/6, calretinin, D2-40 and three negative markers - CEA, TTF-1, ck-20) were performed.

**Results.** On the base of immunohistochemical staining 32 (68,08 %) cases were determined as epitheloid, 13 (27,66 %) as biphasic and 2 (4,26 %) as sarcomatoid mesothelioma.

**Conclusions.** According to our experience three positive and three negative markers are needed for the exact immunohistochemical diagnosis of MPM.

**key words:** *malignant pleural mesothelioma, video-assisted thoracoscopy, immunohistochemical staining.*

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**Introduction.**

Malignant pleural mesothelioma (MPM) is an aggressive tumor, which originates from mesothelial cells of the pleura. Diagnosis of mesothelioma, a malignant tumor stemming from cells lining the serous cavities, is essentially pathological. Histologic examination of hematoxylin and eosin (H&E)-stained tissue section remains the primary method by which the diagnosis of malignant mesothelioma is established (3,4,9,12).

Malignant mesothelioma can have many different histologic patterns. However, it is frequently difficult to distinguish malignant mesothelioma from metastatic adenocarcinomas on the H&E-stained slides. Currently, immunohistochemical procedures have gained widespread acceptance as valuable adjuncts in establishing the diagnosis of malignant mesothelioma (1,2,10,11,12).

**The aim** of this study is to present our 7-year practice in immunohistochemical diagnosis of malignant pleural mesothelioma.

**Material and Methods.**

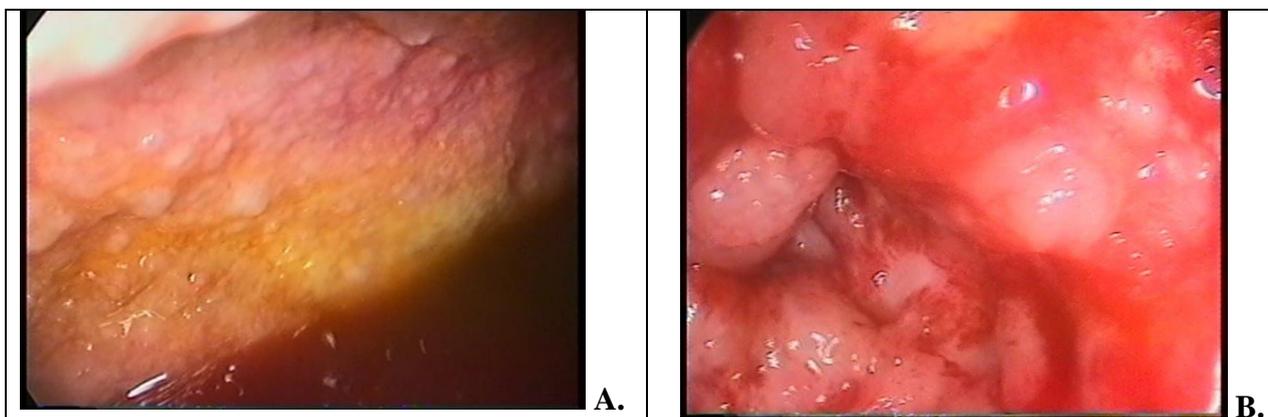
We present a group of 47 patients with malignant pleural mesothelioma (MPM) / [gender: male – 32 (68,08 %); female – 15 (31,92 %); age (in years): range: 44 - 77; mean: 61,56 (SD: 7,35)]. Pleural effusion was established as a clinical presentation in every one of our patients.

Video-assisted thoracoscopy was performed in every patient. Pleural cavity was inspected, pleural effusion was collected for cytological examination and pleural specimens (from at least three different places) were obtained by pleural biopsy at the time of video-assisted thoracoscopy.

Light microscopic examination (hematoxylin-eosin staining) and immunohistochemical staining were performed. For the immunohistochemical staining was used three positive for MPM markers: cytokeratin (ck) 5/6, calretinin, podoplanin (D2-40) as well as three negative markers: carcinoembryonic antigen (CEA), thyroid transforming factor (TTF)-1, cytokeratin (ck)-20.

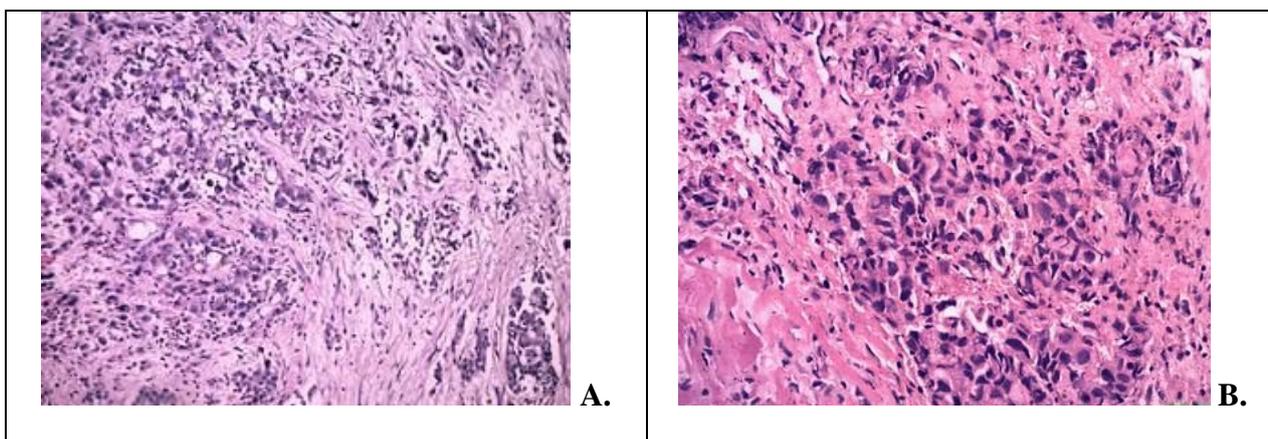
**Results.**

Trough the inspection of the pleural cavity by video-assisted thoracoscopy, for every one of the cases, was established: serous-hemorrhagic pleural effusion, diffuse spreading of the mesothelioma with affection of the parietal and visceral pleura and trapped lung (fig. 1A; fig. 1B).



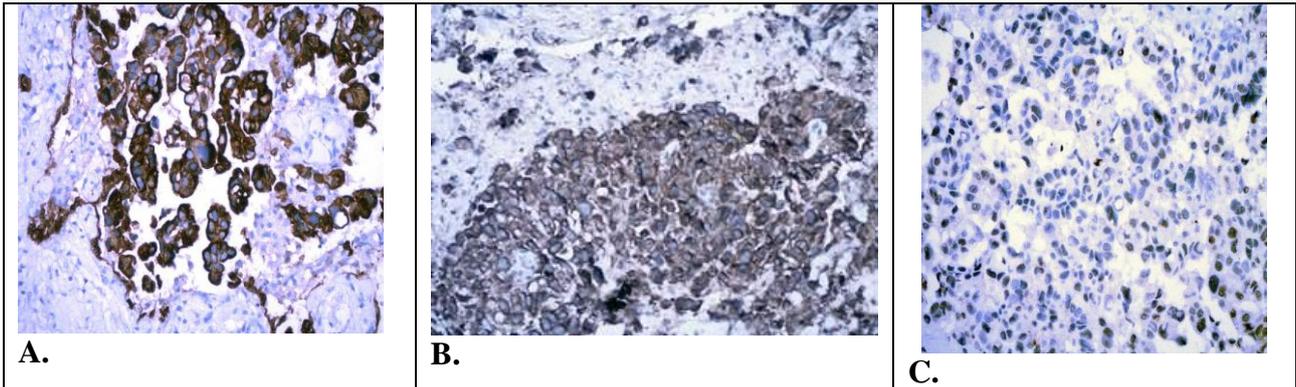
**Figure 1.** Video-assisted thoracoscopy - gross appearance of diffuse malignant pleural mesothelioma: A – diffuse involvement of the parietal pleura and hemorrhagic pleural effusion; B – diffuse involvement of the visceral pleura with trapped lung.

Light microscopic examination had put the diagnosis “malignant pleural mesothelioma” in 39 cases of the presenting group (sensitivity of the method - 82,97 %). 29 of them were determined as “epithelioid” and 10 as “biphasic” mesothelioma (fig. 2A; fig.2B). Light microscopic examination determined the rest of eight cases as “pleural metastasis by adenocarcinoma”.

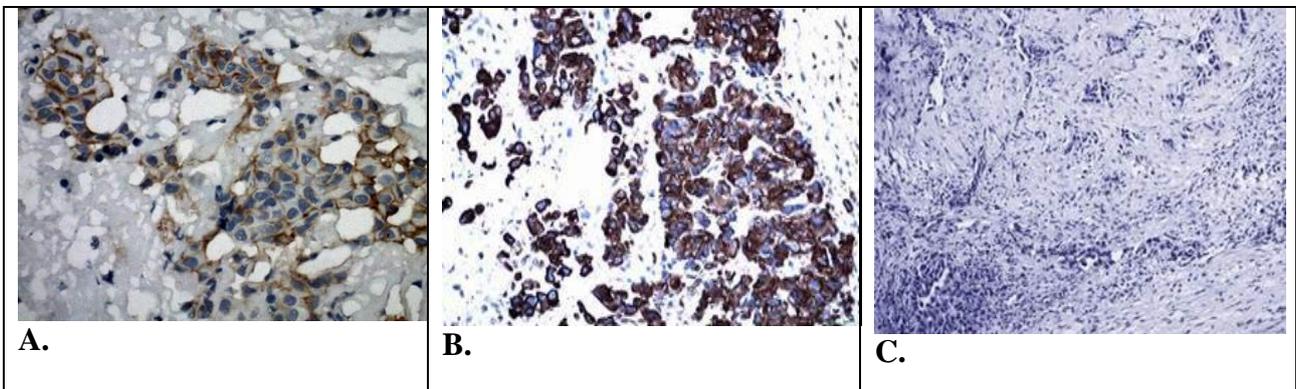


**Figure 2.** Light microscopic examination (hematoxylin-eosin staining x100): A – epithelioid MPM; B – biphasic MPM.

On the base of immunohistochemical staining, MPM cases were divided in three groups: epithelioid – 32 cases (68,08 %), biphasic – 13 cases (27,66 %) and sarcomatoid – 2 cases (4,26 %) / (fig. 3 A,B,C; fig. 4 A,B,C).



**Figure 4.** Epithelioid MPM - immunohistochemical staining: **A:** CK 5/6 positive (x200); **B:** calretinin positive (x200); **C:** CEA negative (x100).



**Figure 4.** Biphasic MPM - immunohistochemical staining: **A:** podoplanin positive (x200); **B:** CK 5/6 positive (x100); **C:** CEA negative (x100).

**Discussion.**

Malignant pleural mesothelioma (MPM) is an aggressive tumor, which originates from mesothelial cells of the pleura and its diagnosis is essentially pathological. The new 2004 WHO international classification divides MPM into four histological subtypes:

- epithelial forms known as epithelioid,
- sarcomatous appearing forms known as sarcomatoid,
- desmoplastic form being fibrous and containing few fusiform cells (often misdiagnosed as organizing pleurisy),
- biphasic form containing both epithelioid and sarcomatoid appearing cells (diagnosis of the latter subtype requires that each type of cells represent at least 10% of the tumor) / (2,3,9,10,12).

Histologic examination of hematoxylin and eosin (H&E)-stained tissue section remains the primary method by which the diagnosis of malignant mesothelioma is established (11,12). As mentioned above MPM can have many different histologic patterns. However, it is frequently difficult to distinguish malignant mesothelioma from metastatic adenocarcinomas and benign pleural lesions on the H&E-stained slides (9,10,12).

In our practice, the histologic examination on the H&E-stained slides is made by at least two experienced and independent pathologists. Even though, we report 83 % sensitivity of the method. Our data confirm that only immunohistochemical procedures have gained widespread acceptance as valuable adjuncts in establishing the diagnosis of malignant mesothelioma.

The immunohistochemical diagnosis of MPM is dependent on specimen size and its frequency increases with increasing number and size of biopsy specimens. At this point of view, medical thoracoscopy is essential in MPM diagnosis: it can show parietal and visceral pleural involvement, it allows performing biopsy from different places of the pleural surfaces in a size of biopsy specimens enough for immunohistochemical staining. Sensitivity of pleuroscopic examination exceeds 95%. Failure to obtain a diagnosis is most often secondary to inability for the operator to obtain an adequate pleural space because of adhesions (8,9,10,11,12). We successfully performed video-assisted thoracoscopy in every case of the presenting group, with obtaining biopsy specimens for immunohistochemical diagnosis of MPM.

The International Mesothelioma Interest Group has proposed guidelines for the diagnosis of MPM. They recommend validating the diagnosis with the presence two positive markers for mesothelioma and the absence of staining for two markers that are typically negative in MPM. Currently, the best markers for mesothelioma appear to be calretinin 5/6 and cytokeratin, whereas the best markers for metastatic adenocarcinomas are CEA, MOC-3, B72.3, Ber-EP4 and BG-8. The sarcomatous type of mesothelioma usually does not stain positively with calretinin. For sarcomatoid forms, two types of wide-spectrum anticytokeratin antibody markers should be used paired with absence of staining for two typically negative markers (anti-CD34, BCL2, anti-desmin, or anti-PS100) (5,6,7,8). Strategy of using two positive and two negative markers is cost effective in the immunohistochemical diagnosis of MPM (4,8,9,11,12).

In our practice we established that with two positive and two negative markers is extremely difficult to distinguish some cases of MPM with pleural metastatic disease as well as to determine the subtype of the MPM. That's why we accept the panel of three positive and three negative markers in immunohistochemical diagnosis of MPM.

### Conclusions.

The diagnosis of malignant mesotheliomas is usually established by the combination of the histology and the immunohistochemical stains. According to our experience three positive and three negative markers are needed for the exact immunohistochemical diagnosis of MPM.

### References.

1. Abutaily AS., Addis BJ., Roche WR. Immunohistochemistry in the distinction between malignant mesothelioma and pulmonary adenocarcinoma: a critical evaluation of new antibodies. *J Clin Pathol* 2002;55:662–668.
2. Alberto MM. Application of Immunohistochemistry to the Diagnosis of Malignant Mesothelioma. *Arch Pathol Lab Med* 2008;132:397-401.
3. Allen TC. Accurate Diagnosis of Mesothelioma: More Important Than Ever. *Arch Pathol Lab Med* 2013;137:601-602.
4. Barreiro TJ., Philip JK. Malignant Mesothelioma: A Case Presentation and Review. *Joa* 2006;106(12): 699-704.
5. Comin CE. Expression of thrombomodulin, calretinin, cytokeratin 5/6, D2-40 and WT-1 in a series of primary carcinomas of the lung: an immunohistochemical study in comparison with epithelioid pleural mesothelioma. *Tumori* 2014; 100:559-567.
6. Dainese E, Pozzi B, Milani M, et al. Primary pleural epithelioid angiosarcoma. A case report and review of the literature. *Pathol Res Pract.* 2010; 206:415–419.
7. Facchetti F, Lonardi S, Gentili F, et al. Claudin 4 identifies a wide spectrum of epithelial neoplasms and represents a very useful marker for carcinoma versus mesothelioma diagnosis in pleural and peritoneal biopsies and effusions. *Virchows Arch.* 2007; 451:669–680.
8. Greillier L, Arnaud C, Anne F, Arnaud S, et al. Accuracy of Pleural Biopsy Using Thoracoscopy

## Science & Technologies

for the Diagnosis of Histologic Subtype in Patients With Malignant Pleural Mesothelioma. *Cancer* 2007; 110:2248-2252.

9. Gueugnon F., Sabrina L., Christophe B., et al. Identification of Novel Markers for the Diagnosis of Malignant Pleural Mesothelioma *The American Journal of Pathology* 2011; 178:1-10.

10. King JE, Thatcher N, Pickering CA et al. Sensitivity and specificity of immunohistochemical markers used in the diagnosis of epithelioid mesothelioma: a detailed systematic analysis using published data. *Histopathology* 2006; 48: 223–232.

11. Noppen M. Pleural biopsy and thoracoscopy. *Interventional Pulmonology. Eur Respir Mon* 2010;48:119-132.

12. Scherpereel A., Astoul P., Baas P et al. Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. *Eur Respir J* 2010; 35: 479–495.