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ORIGINAL ARTICLE

Diagnostic procedures of pleural malignant mesothelioma: our experience

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Summary

Purpose: *The morphology of the epithelioid malignant mesothelioma (MM) of the pleura is similar to lung adenocarcinoma involving pleura. The aim of this study was to evaluate the value of immunohistochemistry in the accurate diagnosis of MM, especially of the epithelioid type with needle biopsy of the pleura.*

Materials and methods: *The diagnosis of MM was established with pleural needle biopsy and tumor immunophenotyping in 30 patients. A broad spectrum of monoclonal antibodies was applied: HBME-1, E-cadherin, calretinin, cytokeratin 5/6, vimentin, thyroid transcription factor (TTF-1) and surfactant apoprotein A (SP-A).*

Results: *We diagnosed 24 epithelioid, 2 biphasic and 4 sarcomatoid MM. HMBE-1 was the most sensitive tumor marker of the epithelioid type, being positive in 100% of the cases. Calretinin, E-cadherin and cytokeratin 5/6 were positive in 70%, 73%, and 50% of all tumors, respectively. TTF-1 and SP-A were negative in all MM. Vimentin was positive in spindle cells of all sarcomatoid and biphasic MM (20%).*

Conclusion: *The accurate diagnosis of MM is mandatory for appropriate treatment decision (surgical or nonsurgical). Our results demonstrate that HMBE-1 is a most useful diagnostic antibody for epithelioid MM, and TTF-1 for lung adenocarcinoma (its thyroid origin excluded) involving pleura.*

Key words: adenocarcinoma, immunohistochemistry, lung, malignant mesothelioma, pleura

Introduction

Before the era of immunohistochemistry histological diagnosis of MM was frequently dependent on clinical and radiological findings. MM is classified by the current WHO classification into epithelioid, sarcomatoid, and biphasic type, according to morphologic and immunophenotypic pattern [1]. The differential diagnosis between the epithelioid type of MM and lung adenocarcinoma involving pleura is a known problem, as well as it is between the sarcomatoid type and extensive pleural fibrosis, or metastatic sarcoma, especially in samples taken by percutaneous pleural needle biopsy. The morphology of epithelioid MM and lung adenocarcinoma is similar. The epithelioid type shows mostly tubulo-papillary pattern composed of well-differentiated, uniform cuboidal cells with acidophilic cytoplasm and less pleomorphic nuclei with prominent nucleoli. Some cuboidal cells contain vacuoles mimicking adenocarcinoma. Extensive fibrosis, sometimes with numerous blood vessels, is present among neoplastic epithelioid cells. The sarcomatoid pattern is characterized by spindle-shaped cells with nuclear pleomorphism and mitoses, sometimes associated with extensive fibrosis and desmoplastic pattern.

Nowadays, immunohistochemistry has resolved most of the aforementioned dilemas; as one antibody does not show absolute specificity and sensitivity for either tumor, a panel of antibodies must be applied in diagnosing MM. Our study was carried out in order to assess the diagnostic value of immunohistochem-

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istry in MM by defining a standard panel of available commercial monoclonal antibodies according to the literature and our abilities.

Materials and methods

The histological diagnosis of MM was established on tissue samples of the pleura obtained by percutaneous needle biopsy and pleuroscopy from 30 patients. All of them had clinical and radiological evidence of pleural tumor: multinodular or diffuse growth of the pleural surface, with or without infiltration of the pulmonary tissue, lung atelectasis and pleural effusion.

Immunohistochemical staining

Immunohistochemical staining was performed on 4 μm thick sections cut from formalin-fixed, paraffin wax-embedded tissue, using microwave antigen retrieval and a standard streptavidin-biotin based-technique. A panel of monoclonal antibodies was selected according to the tumor morphology: epithelioid type was stained by mesothelial cell antibody (HBME-1), calretinin, E-cadherin and cytokeratin 5/6, and sarcomatoid and biphasic type by vimentin. TTF-1 and SP-A were used to exclude adenocarcinoma of the lung. Dilution rates of primary antibodies were carried out according to the manufacturers' instructions or the literature [2]. Negative and positive controls were added in each test. Diffuse and local cytoplasmic, membrane, and nuclear staining was labelled as positive reaction [2].

Results

We diagnosed 30 MMs applying immunohistochemistry, including 24 cases of epithelioid, 4 of sarcomatoid, and 2 of biphasic type, according to the WHO classification.

The overall incidence of the immunopositivity of the various antibodies used in 30 MMs is presented in Figure 1.

The most sensitive monoclonal antibody was HBME-1. It was expressed in all cases of the epithelioid type MM, differentiating it from lung adenocarcinoma involving pleura (Figure 2). In the whole series this marker was positive in 83% of the cases. One of 2 biphasic MMs also exhibited HBME-1 immunostaining. The other one was HBME-1-immunonegative, both in needle biopsy samples and in operative tissue after pleuropneumonectomy; its nature was confirmed by calretinin, E-cadherin and cytokeratin 5/6 immunopositivity. Four sarcomatoid MMs reacted in the same way.

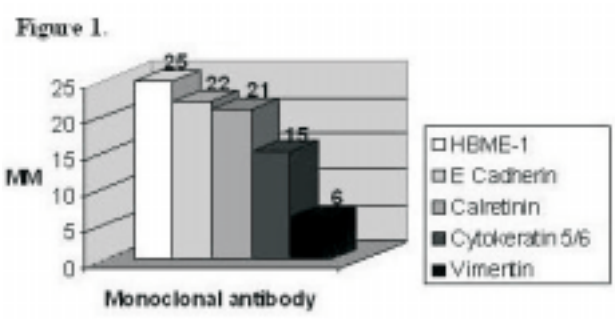


Figure 1. Immunoreactivity of the tested antibodies in malignant mesothelioma.

Calretinin was immunopositive in 21 epitheloid and in 2 biphasic MM, ie. in 77% of all cases. Fifteen pleural biopsies of all 3 types of MMs exhibited cytokeratin 5/6 staining in 50%.

E-cadherin was positive in 22 (91%) epithelioid MMs, and in 73% overall. It manifested as membrane staining, which excluded adenocarcinoma.

Vimentin was positive in the cytoplasm of pleomorphic spindle cells in 4 sarcomatoid and in 2 biphasic MMs (20%).

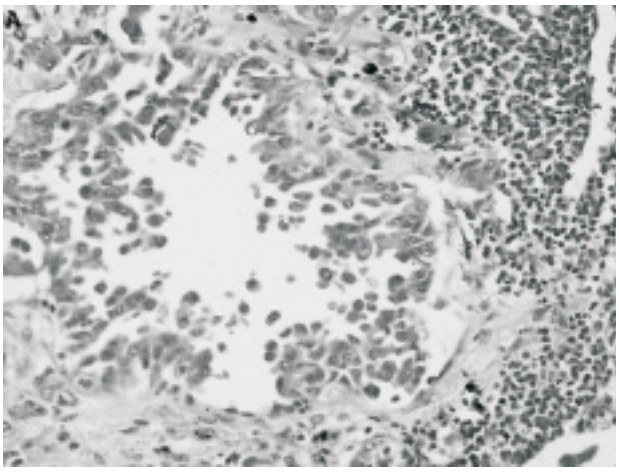
TTF-1 was not expressed in the nuclei of MM cells; this finding along with SP-A negativity excluded lung adenocarcinoma.

Pleuropneumonectomy was performed in 6 operable cases according to the clinical stage (Figure 3). Histological findings in the postoperative biopsies were the same as in needle biopsy material which confirmed the preoperative diagnosis of MM in all cases.

Discussion

MM of the pleura and pleural carcinomatosis share similar clinical manifestations of tumor spread in a form of diffuse, multinodular growth on pleural surface, with or without pleural effusion. Histologically, there are difficulties in distinguishing MM from lung adenocarcinoma involving pleura in H&E sections of percutaneous and pleuroscopic needle biopsy. Both tumors have a similar tubulopapillary pattern. The differentiation is possible only with the use of immunohistochemistry.

The panel of various antibodies used was designed according to the ability of the antibody to dif-



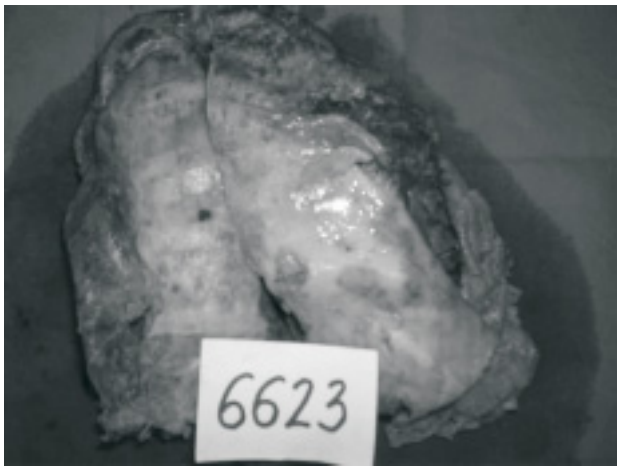
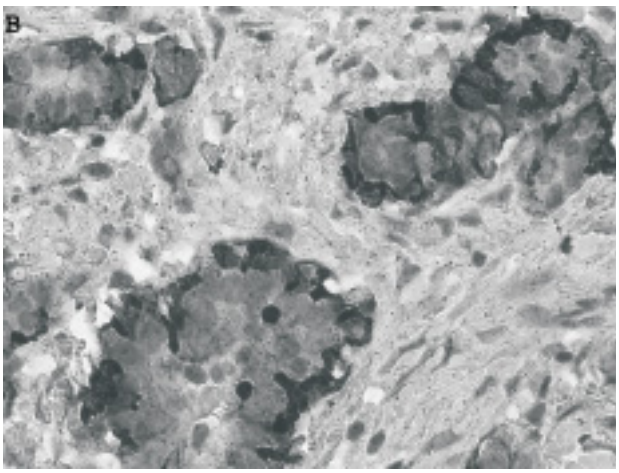


Figure 3. Malignant mesothelioma: diffuse, nodular thickening of costal pleura; right pleuropneumonectomy.



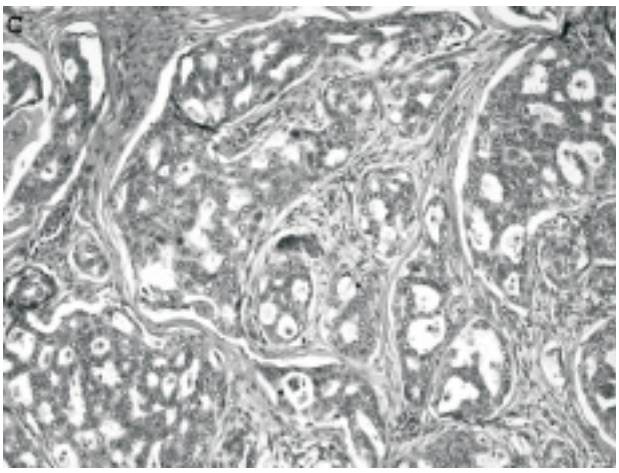


Figure 2. a) Epithelioid type of malignant mesothelioma is difficult to distinguish from lung adenocarcinoma (H&E X20). b) HBME-1 immunopositivity in epithelioid malignant mesothelioma (X40). c) Lung carcinoma involving pleura (H&E X20). Figure 2a and 2b come from the same patient.

ferentiate between an adenocarcinoma, especially of lung origin, and MM, and taking into consideration that no single antibody is of absolute and high sensitivity or specifity to determine the kind of a given tumor and its origin [3].

The mesothelial cell antibody HBME-1 is a helpful antibody, characteristically present on the membrane of the mesothelial cells. It gives a specific thick membrane pattern. HBME-1 also shows reactivity in adenocarcinoma cells of the kidney, ovary, pancreas and thyroid gland, but usually with cytoplasmic staining [4-6]. In our study HBME-1 was completely reliable in diagnosing the epithelioid type of MM by specific, thin, linear immunostaining under apical membrane of mesothelial cells. It was highly sensitive, positive in 96% of the cases, more than in the study of Abutaily et al. (63%) [4].

Immunohistochemically, MM cells showed strong positivity for calretinin. This antibody is expressed in about 80% of epithelioid MMs [5,7,8], which is comparable to our findings (70%). Calretinin is localized in the cytoplasm and the nuclei, and does not react with normal and reactive mesothelial cells. It can also be positive in about 6% of adenocarcinomas, showing cytoplasmic staining [5,7,8].

Cytokeratin 5/6 is also sensitive in MM cells, exhibiting pericellular and cytoplasmic staining in 72% of the cases; it is also expressed in 6% of adenocarcinomas in the same pattern [9]. This antibody was not of much diagnostic significance in our series, being positive only in 50% of the cases, less than in other reports [4,9]. But the high rate of cytokeratin 5/6 immunoexpression in epithelioid MM, and the low rate in adenocarcinoma makes this antobody useful for differential diagnosis [9].

Mesothelial cells sometimes express E-cadherin. It is a sensitive cell membrane antibody, seen in 22% of epithelioid MMs, and in 100% of adenocarcinomas [4,10,11]. In the latter, the staining pattern is more strong and consistent than in MM cells. If E-cadherin is negative, a tumor cannot be adenocarcinoma [4,10,11]. E-cadherin was immunoexpressed in 73% of our cases of MMs as a membrane antibody.

Vimentin labels mesenchymal cells. In MM, vimentin is localized in pleomorphic spindle cells of the mesenchymal component in the biphasic and sarcomatoid types [12,13]. It can also be coexpressed with cytokeratin in normal mesothelial cells as a sign of their immaturity. Vimentin positivity was associated with the sarcomatous component in 2 biphasic and 4 sarcomatoid MMs of our series.

TTF-1 is a specific marker for lung adenocarcinoma, if its thyroid origin is excluded. As a nuclear staining, it is positive in 100% of lung adenocarcinomas, as well as in neuroendocrine lung tumors. TTF-1 is never expressed in MM [14-16], a finding corresponding with our results.

SP-A is demonstrated in lung adenocarcinomas and bronchioloalveolar carcinomas. In adenocarcinoma it displays mostly cytoplasmic staining pattern, less membranic. If positive in MMs, it shows a membranic staining. In some cases, TTF-1 and SP-A, together with E-cadherin, are able to establish the diagnosis of adenocarcinoma of lung origin [4,17]. No MM in our series exhibited SP-A.

In conclusion, HMBE-1 is a diagnostic antibody for the epithelioid type of MM, and TTF-1 for lung adenocarcinoma involving pleura (excluding thyroid carcinoma). No single antibody is of absolute and high sensitivity to determine the precise diagnosis of MM and to differentiate between MM and adenocarcinoma, or sarcoma [18,19]. It is necessary to apply a broad spectrum of antibodies to diagnose a pleural tumor.

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