The role of immunohistochemistry in the diagnosis of neoplastic pleural effusions

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Abstract
Identification of the origin of neoplastic pleural effusion is a major concern in lung pathology. This study followed the diagnostic role of a panel of antibodies that included calretinin, HBME1, D2-40, Ber-EP4, CK5/6, CEA and TTF1, in a total of 37 cases of pleural and lung cancer with tumor-type cytology, later confirmed by histopathology. For mesothelioma, positive staining for calretinin, D2-40 and CK5/6 and negative for CEA and TTF1 were characteristic. For lung adenocarcinomas, we found Ber-EP4, CEA and TTF1 positivity, and calretinin, D2-40 and CK5/6 negativity. Squamous lung carcinomas were positive for Ber-EP4, CK5/6 and CEA and negative for HBME1, D2-40 and TTF1. The panel of antibodies used in this study provides a differential diagnosis between mesotheliomas and lung carcinomas as well as between lung adenocarcinomas and squamous carcinomas.

Keywords: pleural effusion, malignant tumors, immunohistochemistry.

Introduction
Pleural effusion is a common diagnostic problem in pulmonary medicine, often interdisciplinary, due to the numerous benign or malignant disorders that can cause them. Using a great variety of conventional biochemical investigations, cytological, histological, immunological and microbiological ones, together with thoracentesis, leads to substantial improvements in the diagnosis of the exudate in about 70% of cases, and in up to 90% of them with the help of new biological markers (immunocytochemistry, immunohistochemistry, cytogenetics) [1].

An important feature is that mesotheliomas can take a variety of cyto-histopathological aspects, which often raises many problems of differential diagnosis [2–4].

Over the past two decades, numerous studies have been published on the value of immunohistochemistry as auxiliary technique in the diagnosis of mesothelioma, reporting a general agreement on the fact that it is a very useful method in the accurate diagnosis of these tumors [5].

This study focused on the diagnostic role of a panel of antibodies that included calretinin, HBME1, D2-40, Ber-EP4, CK5/6, CEA and TTF1, in a total of 37 cases of pleural and lung cancer with tumor-type cytology, later confirmed by histopathology.

Materials and Methods
The study included a total of 37 cases of serofibrinous pleurisy with malignant etiology, which were histologically and immunohistochemically investigated. Biopsy fragments were fixed in 10% formalin, processed by the usual technique for paraffin inclusion and stained with Hematoxylin and Eosin. The immunohistochemical study was performed using the LSAB soluble immuno-enzymatic complex method, with the LSAB2 System HRP kit (Universal Dako Labeled Streptavidin-Biotin System, Horseradish Peroxidase). The panel of antibodies used is shown in Table 1.

Table 1 – Antibodies used for the immunohistochemical study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calretinin</td>
<td>Polyclonal antibody (rabbit)</td>
<td>1:20</td>
<td>Citrate buffer, pH 6</td>
<td>1 hr</td>
</tr>
<tr>
<td>HBME1</td>
<td>HBME1 Monoclonal antibody</td>
<td>1:50</td>
<td>Citrate buffer, pH 6</td>
<td>1 hr</td>
</tr>
<tr>
<td>D2-40</td>
<td>D2-40 Monoclonal antibody</td>
<td>1:200</td>
<td>Citrate buffer, pH 6</td>
<td>Overnight</td>
</tr>
<tr>
<td>Ber-EP4</td>
<td>Ber-EP4 Monoclonal antibody</td>
<td>1:30</td>
<td>Proteinase K</td>
<td>1 hr</td>
</tr>
<tr>
<td>CK5/6</td>
<td>D5/16 B4 Monoclonal antibody</td>
<td>1:50</td>
<td>Tris EDTA, pH 9</td>
<td>1 hr</td>
</tr>
<tr>
<td>CEA</td>
<td>Polyclonal antibody (rabbit)</td>
<td>1:175</td>
<td>–</td>
<td>1 hr</td>
</tr>
<tr>
<td>TTF1</td>
<td>8G7G3/1 Monoclonal antibody</td>
<td>1:25</td>
<td>Citrate buffer, pH 6</td>
<td>1 hr</td>
</tr>
</tbody>
</table>
Interpretation of immunohistochemical reactions primarily aimed at highlighting the chromogen in antigenic targets. Quantitative assessment of immunohistochemical expression of antibodies used was performed according to the score listed below (Table 2).

<table>
<thead>
<tr>
<th>Score</th>
<th>1</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cells [%]</td>
<td>1–25</td>
<td>26–50</td>
<td>51–75</td>
<td>&gt;75</td>
</tr>
</tbody>
</table>

Table 2 – Interpretation of immunohistochemical reactions

Image acquisition was performed using a Nikon Eclipse E600 microscope equipped with a digital camera and the Lucia 5 software.

Results

The 37 cases analyzed histologically corresponded in five cases to epithelial mesothelioma and in 32 cases to lung carcinoma with metastasis in the pleura. Of these, 13 were adenocarcinomas and 19 were squamous cell carcinomas.

For all 37 malignant neoplasms, we used the immunohistochemical study in order to identify the most specific markers with diagnostic value (Table 3).

Table 3 – Marker reactivity according to tumor score and type

Analysis of calretinin immunolabeling revealed a positive reaction in 10 (27%) of the cases with malignant neoplasms analyzed, both cytoplasmic and nuclear, present in all mesothelioma cases with scores 3 and 4 (Figure 1a), and in five (26.3%) of the cases with squamous carcinomas with scores 1 or 2, but in none of the adenocarcinoma cases.

HBME1 labeling showed positivity in 10 (27.02%) of cancer cases studied with membrane pattern, and five (100%) cases of mesothelioma (Figure 1b) as well as in five (38.4%) cases of adenocarcinoma, both with scores 3 and 4, while squamous carcinomas were all negative for this marker.

The study of the D2-40 immunostaining revealed the presence of a high intensity (score 3 and 4) membrane marking pattern in all mesotheliomas analyzed, in more than half of the tumor cells (Figure 1c). None of lung carcinomas were positive for this marker.

Ber-EP4 immunostaining showed the presence of a cytoplasmic and membrane-staining pattern, in varying proportions for all three-tumor types studied. We recorded positive staining in 15 (40.5%) of the tumors analyzed, of which 12 (37.5%) lung carcinomas with score 3 and 4 (Figure 1d) and three (60%) mesotheliomas with score 1.

CK5/6 labeling was present at cytoplasmic level in 23 (62.2%) of the tumors studied. We observed a positive reaction in four (80%) of the investigated mesotheliomas and in all squamous lung carcinomas (100%) (Figure 1, e and f). In contrast, none of the lung adenocarcinomas expressed CK5/6.

CEA labeling was seen in 21 (56.73%) of the lung carcinomas with score 4 (Figure 1g), but in none of the mesotheliomas. The labeling showed a cytoplasmic pattern, but more pronounced along the cell membrane.

TTF-1 immunostaining was present in all lung adenocarcinomas with a nuclear pattern and scores 3 or 4, but in none of the mesotheliomas or those cases diagnosed with squamous cell carcinoma (Figure 1h).

Discussion

Differentiation of epithelial pleural mesothelioma from lung carcinoma with secondary pleural involvement is a major diagnostic problem in surgical pathology and is the main topic of numerous studies. The main goal of most investigations is related to the differentiation of epithelioid mesotheliomas from adenocarcinomas or squamous lung carcinomas. The diagnosis of certainty for the cause of pleural effusion in such a patient is essential for treatment and prognosis, differentiating mesothelioma from lung carcinoma metastases in the pleura often being difficult and requiring further studies.

Currently, there is no individual mesothelial immunohistochemical marker that provides 100% specificity and sensitivity for the diagnosis of epithelial mesothelioma, or an epithelial marker that provides high sensitivity and negative predictive value of 100% for this diagnosis [3–6].

Due to the lack of a specific marker, the immunohistochemical diagnosis of tumors depends largely on the use of a panel of markers that are commonly expressed in mesothelioma (positive markers) associated with the most frequently expressed markers in carcinomas (negative markers). These panels of antibodies are constantly changing because of the discovery of new useful markers for the diagnosis of tumors [3].

In this study, we found that the best positive markers for mesothelioma were D2-40 and calretinin. Thus, immunostaining for D2-40 was positive in all mesotheliomas analyzed, with high intensity and in more than half of the tumor cells, while none of the lung carcinomas were positive for this marker.
The role of immunohistochemistry in the diagnosis of neoplastic pleural effusions

Figure 1 – (a) Epithelioid mesothelioma, calretinin immunolabeling, score 4 (×100); (b) Mesothelioma, HBME1 immunolabeling, score 4 (×100); (c) Mesothelioma, D2-40 immunolabeling, score 3 (×100); (d) Squamous cell carcinoma, Ber-EP4 immunolabeling (×100); (e) Mesothelioma, CK5/6 immunolabeling, score 2 (×100); (f) Squamous cell carcinoma, CK5/6 immunolabeling, score 2 (×100); (g) Squamous cell carcinoma, CEA immunolabeling (×100); (h) Lung adenocarcinoma, TTF1 immunolabeling (×100).
Calretinin was diffusely and intensely expressed by all mesotheliomas, slightly in over a quarter of squamous carcinomas (26.3%) and in none of the adenocarcinomas. In addition, another useful marker for differentiating mesotheliomas from lung adenocarcinomas was found to be cytokeratin 5/6, which was expressed by mesotheliomas and squamous carcinomas, but not lung adenocarcinomas. We also noticed the utility of CEA and TTF1 as negative markers for mesothelioma, as these two markers were expressed in all adenocarcinomas but in none of the mesotheliomas and squamous cell carcinomas.

For the diagnosis of mesothelioma, various panels of antibodies were recommended but there is no consensus on the number or type of markers that should be used. French CA et al. investigated the utility of a great number of immunohistochemical markers in distinguishing epithelioid mesothelioma from lung adenocarcinoma [7]. The conclusion of this study was that the best positive markers for mesothelioma are calretinin, cytokeratin 5/6, and WT1, and best negative markers are CEA, MOC-31, Ber-EP4 and B72.3. However, these markers do not have the same value in distinguishing epithelioid mesothelioma from lung squamous carcinoma [5, 6]. P63, MOC-31, Ber-EP4, CEA and BG-8 are among the negative markers for mesothelioma but which are expressed by the majority of squamous carcinomas [3–5].

Studies in the literature that sought to identify a minimum group of antibodies for the diagnosis of pleural mesothelioma suggested the utility of D2-40 and calretinin as positive markers, and of CEA and TTF-1 as negative markers. The panel of four antibodies indicates a high sensitivity and specificity in terms of differentiating epithelioid mesothelioma from lung adenocarcinoma [8]. Thus, in the case of mesothelioma, D2-40 and calretinin sensitivity were 84.8% and 87.9% respectively, with a specificity of 95.5%, while in the case of adenocarcinoma, CEA and TTF-1 sensitivity were 95.5% and 92.4% respectively, with a specificity of 100%.

More recent studies concerning the differentiation of epithelial mesothelioma from lung adenocarcinomas and the selection of positive or negative markers for mesothelioma communicate an association of calretinin with BG-8 and CD15 as a first step for the diagnosis of epithelial mesothelioma [9]. Other large studies report a group of three antibodies including calretinin, BG8 and MOC-31, with a sensitivity and specificity of 96% for differentiating epithelioid mesothelioma from adenocarcinoma [10], or the combination of CEA, calretinin and WT1 or thrombomodulin [11]. If this approach fails it is recommended that the panel of antibodies also includes D2-40 [3, 6, 9, 12, 13], or podoplanin, because they seem to be highly sensitive markers for epithelioid mesotheliomas [4].

Conclusions

The immunohistochemical study indicated calretinin, D2-40 and CK5/6 as specific for mesothelioma, while Ber-EP4, CEA and TTF1 were characteristic for adenocarcinomas, and Ber-EP4, CK5/6 and CEA for lung squamous carcinoma. The panel of antibodies used in this study ensures the differential diagnosis between mesothelioma and lung carcinomas as well as between and adenocarcinomas and squamous lung carcinomas.

References


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Received: May 25th, 2012     Accepted: October 30th, 2012