Sensitivity and specificity of immunohistochemical markers used in the diagnosis of epithelioid mesothelioma: a detailed systematic analysis using published data

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Aims: Immunohistochemistry is frequently employed to aid the distinction between mesothelioma and pulmonary adenocarcinoma metastatic to the pleura, but there is uncertainty as to which antibodies are most useful. We analysed published data in order to establish sensitivity and specificity of antibodies used to distinguish between these tumours with a view to defining the most appropriate immunohistochemical panel to use when faced with this diagnostic problem.

Methods and results: A systematic analysis of the results of 88 published papers comparing immunohistochemical staining of a panel of antibodies in mesothelioma with epithelioid areas, and pulmonary adenocarcinoma metastatic to the pleura. Results for a total of 15 antibodies were analysed and expressed in terms of sensitivity and specificity. The most sensitive antibodies for identifying pulmonary adenocarcinoma were MOC-31 and BG8 (both 93%), whilst the most specific were monoclonal CEA (97%) and TTF-1 (100%). The most sensitive antibodies to identify epithelioid mesothelioma were CK5/6 (83%) and HBME-1 (85%). The most specific antibodies were CK5/6 (85%) and WT1 (96%).

Conclusions: No single antibody is able to differentiate reliably between these two tumours. The use of a small panel of antibodies with a high combined sensitivity and specificity is recommended.

Keywords: adenocarcinoma, biopsy, immunohistochemistry, mesothelioma, pleural diseases, pleural neoplasm

Abbreviations: EM, epithelioid mesothelioma; IHC, immunohistochemistry; MPM, malignant pleural mesothelioma; PACA, pulmonary adenocarcinoma

Introduction

Histopathological confirmation of the diagnosis of malignant pleural mesothelioma (MPM) can be difficult. Epithelioid mesothelioma is capable of exhibiting many different histological patterns, and the pleura is a common site for metastatic spread of both pulmonary and non-pulmonary tumours. The differential diagnosis of an epithelioid pleural neoplasm is therefore broad, and potentially encompasses tumours that are uncommonly encountered outside of a specialist thoracic pathology practice. However, the most common diagnostic scenario usually involves differentiating between epithelioid mesothelioma (EM) and a pulmonary adenocarcinoma (PACA) with extensive pleural involvement.

Currently, there is no diagnostic gold standard for the diagnosis of mesothelioma. For most pathology departments, immunohistochemistry (IHC) is used as
the most reliable diagnostic arbiter. Although many authors have previously attempted to identify the most accurate, reliable and reproducible IHC antibodies currently available to distinguish between mesothelioma and adenocarcinoma, there is still uncertainty as to which are most useful in everyday practice. The aim of this paper was to clarify which antibodies were the most robust in terms of diagnostic sensitivity and specificity for distinguishing between EM and PACA metastatic to the pleura.

Materials and methods

Papers that specifically compared the results of IHC staining in formalin-fixed samples of malignant pleural mesothelioma and adenocarcinoma metastatic to the pleura were identified from the PubMed and Medline electronic literature databases for a 25-year period (1979–2004). Single case reports and papers that analysed results in less than 10 tumours were excluded, as were those primarily examining IHC in cytology specimens. The IHC staining results in epithelioid areas of each tumour classified as a mesothelioma (pure epithelioid mesothelioma and the epithelioid areas of biphasic mesotheliomas) were recorded. Results for adenocarcinoma were included only if the site of original tumour was confirmed as the lung.

There was significant variation between papers as to percentage of staining considered positive. For the purpose of this study we considered only diffuse staining involving ≥30% of tumour cells as a positive result. The number of positive and negative staining results from all papers for both EM and PACA were combined for each antibody and the diagnostic sensitivity and specificity calculated using standard statistical methods (see Table 2 in Appendix 1). These results are summarized in Figures 1 and 2 at the end of the Results section.

Results from a total of 88 papers were incorporated in this analysis. For ease of presentation the diagnostic antibodies were separated into two groups according to whether they are usually positive in PACA (‘carcinoma markers’) or mesothelioma (‘mesothelioma markers’). These antibodies are listed in Table 1.

Results I

Carcinoma markers

Eight different diagnostic antibodies were studied within this group: carcinoembryonic antigen (CEA), Ber-EP4, B72.3, Leu-M1, MOC-31, E-cadherin, thyroid transcription factor-1 (TTF-1) and Lewisy (BG8).

Table 1. Antibodies used to distinguish between pulmonary adenocarcinoma and epithelioid mesothelioma

<table>
<thead>
<tr>
<th>‘Carcinoma markers’</th>
<th>‘Mesothelioma markers’</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>CK5/6</td>
</tr>
<tr>
<td>Ber-EP4</td>
<td>Vimentin</td>
</tr>
<tr>
<td>B72.3</td>
<td>Calretinin</td>
</tr>
<tr>
<td>Leu-M1 (CD15)</td>
<td>HBME-1</td>
</tr>
<tr>
<td>MOC-31</td>
<td>Thrombomodulin (CD141)</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>N-cadherin</td>
</tr>
<tr>
<td>TTF-1</td>
<td>Wilms tumour product-1</td>
</tr>
<tr>
<td>Lewisy (BG8)</td>
<td></td>
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</tbody>
</table>

Carcinoembryonic antigen

CEA is an oncofetal glycoprotein first identified by Gold and Freeman. Mesothelial cells do not normally express CEA, yet it is commonly expressed by adenocarcinomas of lung origin. With the advent of diagnostic IHC, CEA was one of the first antibodies studied for its ability to discriminate between mesothelioma and PACA. Early studies used polyclonal CEA, which can give false-positive results in mesothelioma due to non-specific cross-reacting antigen. The introduction of monoclonal CEA has increased its diagnostic sensitivity and specificity, and it remains one of the most robust and useful antibodies in the diagnostic armamentarium.

Fifty-one studies reported CEA staining in EM and PACA. Their combined results included 1524 cases of PACA and 1818 cases of EM. Diagnostic sensitivity and specificity of CEA for identifying PACA were 83% and 95%, respectively. Twenty-four of these studies used monoclonal CEA (MoCEA), which was examined in a total of 949 PACA and 1007 EM cases.

Sensitivity was slightly reduced at 81%, but specificity increased to 97%.

Ber-EP4

Ber-EP4 is an antibody that targets a glycoprotein expressed by most epithelial cells, except those with squamous differentiation. Early studies suggested that it had a high sensitivity and specificity for adenocarcinoma; positive reactions were reported in less than 1% of mesotheliomas. Seventeen studies have compared Ber-EP4 staining in PACA and EM. A total of 702
cases of PACA and 899 cases of mesothelioma were examined. The overall sensitivity and specificity of Ber-EP4 for distinguishing between PACA and EM were 80% and 90%, respectively.

**B72.3**

B72.3 is a monoclonal antibody raised in mice against a tumour-associated glycoprotein complex expressed in breast carcinoma cell lines. As with Ber-EP4, initial studies suggested that B72.3 could be an important tool for differentiating between PACA and EM. Further investigation led to a dampening of enthusiasm, with some authors reporting positive reactions in up to 47% of EM.16 Sixteen studies that included 769 cases of PACA and 700 cases of EM showed it had an 80% sensitivity and 93% specificity for adenocarcinoma, figures comparing favourably with more established IHC antibodies.6,7,11,14,21–25,27,31,36,50,52,57,58

**Leu-M1**

Leu-M1 (CD15) is a monomyelocytic marker reported as showing positive staining in up to 80% of PACAs. Twenty-six studies evaluated Leu-M1 expression in EM in comparison with PACA.5–7,9,11,14,17,21–25,27,28,31,35–37,49,50,52,56–59

These included 1473 adenocarcinomas and 1204 mesotheliomas. Overall sensitivity for the diagnosis of adenocarcinoma was 72%, but specificity was better at 93%.

**MOC-31**

MOC-31 reacts with an epithelium-associated transmembranous glycoprotein (epithelial glycoprotein-2) raised from a small cell lung cancer cell line. The epitope is similar to that targeted by the monoclonal antibody to epithelial membrane antigen (EMA). Its ability to distinguish between PACA and EM in tissue block preparations has been reported in only seven studies, and in smaller numbers of tumours than the more commonly used IHC antibodies already discussed (PACA = 213, EM = 276).49,51–53,59–61 However, MOC-31’s sensitivity and specificity for PACA are both 93%, suggesting that it could be a useful addition to an IHC panel.

**E-cadherin**

The cadherins are a group of heterodimeric calcium-dependent, membrane-associated glycoproteins within the family of cell adhesion molecules. The type of cadherin expressed by a cell reflects its embryological origin: E-cadherin is typically expressed in epithelia. N-cadherin is expressed by cells originating from mesodermal and neural crest tissue. Seven studies evaluated E-cadherin as a discriminator between PACA (n = 183) and EM (n = 218).36,52,62–66 E-cadherin has reasonable diagnostic value with an overall sensitivity and specificity for PACA of 86% and 82%, respectively.

**Thyroid transcription factor-1**

TTF-1 is a member of a homeodomain transcription factor family that is selectively expressed in thyroid and lung epithelium. Five papers have reported the differential expression of TTF-1 in a total of 366 cases of PACA and 240 cases of EM.32,63,66–68 None of the mesotheliomas was positive for TTF-1, whilst 85 carcinomas were negative (28%). Sensitivity and specificity of TTF-1 for identifying PACA are therefore 72% and 100%, respectively.

**Lewis**

Blood group antigens have been examined as potential discriminators between PACA and EM. Lewis is the only one of these that appears to have some merit, when the BG8 clone is used. BG8 is raised from the SK-LU-3 lung cancer line and its ability to distinguish between PACA and EM was first reported by Jordon and colleagues in 1989.69 Three groups have since reported its results.27,52,63 These studies included 231 cases of PACA and 197 cases of EM. Sensitivity and specificity were both 93%.

**Results II**

**Mesothelioma markers**

The other group of IHC antibodies studied were those preferentially identifying cells of mesothelial origin, the ‘mesothelioma markers’. This group included CK5/6, vimentin, calretinin, HBME-1, thrombomodulin, N-cadherin and Wilms’ tumour product-1. These antibodies are relatively new, compared with some of the established carcinoma markers, and have been studied in smaller numbers.

**CK5/6**

Cytokeratins (CK) are intracytoplasmic intermediate filaments expressed in mesothelia, epithelia, and tumours derived from these tissues. Broad-spectrum low-molecular-weight cytokeratins are expressed in both mesothelioma and PACA, and therefore have little discriminatory value. However, the CK5/CK14 pair is almost exclusively expressed in mesothelial derivatives and therefore has the potential to distinguish between PACA and EM. The antibody CK5/6 specifically targets the CK5 moiety of this cytokeratin pair.
Eight studies have evaluated CK5/6 staining in EM (n = 402) and PACA (n = 402). The combined sensitivity and specificity were 83% and 85%, respectively.

**Vimentin**
The other intermediate filament studied in mesothelioma is vimentin. Vimentin is a group III intermediate filament that primarily identifies cells of mesodermal origin. It is expressed in both benign and malignant connective tissue, as well as in mesothelial cells and mesothelioma. The coexpression of low-molecular-weight cytokeratin and vimentin within a cell is suggestive of a mesothelial origin, particularly if the filaments are prominent and in a perinuclear distribution. Vimentin can be demonstrated in PACA, both within tumour-associated stroma and the tumour cells themselves. However vimentin expression is usually distributed diffusely within the cytoplasm, rather than in a perinuclear pattern, and staining is often much weaker than that seen in mesothelioma.

Seventeen studies compared vimentin expression in EM (n = 773) with PACA (n = 815). Overall sensitivity and specificity of vimentin for mesothelioma were 62% and 75%, respectively.

**Calretinin**
Calretinin is a 29-kDa calcium-binding molecule that is a member of the EF-hand protein group. It is related to S100 and calbindin, and is thought to be involved in the calcium-dependent intracellular signalling mechanisms that control the cell cycle. Although characteristically expressed in central and peripheral nervous system tissue, it can also be demonstrated in mesothelium. The first study of calretinin as a marker for mesothelioma was undertaken by Doglioni and colleagues in 1996. To date, a total of 17 studies have assessed the value of calretinin in distinguishing between EM and PACA. These comprise a combined total of 885 cases of EM and 912 cases of PACA. Overall calretinin sensitivity was 82%, whilst specificity was 85%.

**HBME-1**
HBME-1 is a monoclonal antibody raised from the mesothelioma cell line SPC111 that is suitable for paraffin-embedded tissue. The target epitope is located in microvilli but its exact nature is uncertain. We identified a total of 14 studies evaluating HBME-1, including 769 cases of EM and 676 cases of PACA. Overall sensitivity and specificity were 85% and 43%, respectively.

**Thrombomodulin**
Thrombomodulin is a glycoprotein expressed by endothelium, mesothelium, synovium and placental syncytiotrophoblasts. It was first described in 1982 and its value in the recognition of vascular tumours was soon recognized. Collins and colleagues were the first to investigate thrombomodulin expression in mesothelioma. They found it to have excellent sensitivity (100%) and specificity (92%) for EM compared with adenocarcinoma. Thrombomodulin has subsequently been evaluated in a further 15 studies, but with less impressive results.

Combining the results of these studies indicates that the sensitivity and specificity of thrombomodulin for mesothelioma are relatively poor, at 61% and 80%, respectively. This is based on a total of 964 cases of PACA and 831 cases of EM.

**N-cadherin**
The ability of E-cadherin to identify epithelium-derived tumours has been previously mentioned. By contrast, mesothelium and mesotheliomas expresses N-cadherin. Antibodies to N-cadherin suitable for use in paraffin-embedded tissue have become available relatively recently, and have therefore been studied only in small numbers. The combined result of five studies that included 151 cases of EM and 121 cases of PACA gave an overall sensitivity of 78% and specificity of 84%.

**Wilms Tumour Product-1**
The Wilms tumour product-1 (WT1) is expressed in fetal spleen, mesothelium and mesonephric ridge derivatives such as the kidney. In the adult WT1 continues to be expressed by mesothelium, spleen, the glomerular cells of the kidney, testicular Sertoli cells, uterine decidual cells, granulosa cells of the ovary and myoepithelial cells in the breast. WT1 positivity can also be seen in stromal cells and blood vessels. The value of WT1 as a diagnostic marker for mesothelioma in tissue samples has been investigated by eight groups. These included a combined total of 264 cases of EM and 213 cases of PACA. Overall sensitivity and specificity were 77% and 96%, respectively.

**Miscellaneous immunohistochemical markers**
Several other antibodies have been investigated for their potential to differentiate between EM and PACA. Epithelial membrane antigen and human milk fat globulin (HMFG) are related members of a family of high-molecular-weight trans-membranous glycoproteins. Epithelial membrane antigen and HMFG are positive in both EM and PACA. It is the pattern of
distribution that differs between them: cytoplasmic in PACA, and associated with surface microvilli in mesothelioma. Almost 50% of anaplastic (CD30+) large cell lymphomas are positive for EMA, and EMA positivity has also been described in the epithelioid areas of synovial sarcomas and epithelioid sarcomas.

Although EMA and HMFG-2 have been evaluated as discriminators between EM and PACA, it is difficult to make direct comparisons between papers because of differences in methodology and assessment of positive staining. Hence, they have not been included in this analysis.

Other antibodies that have been assessed as discriminants between PACA and EM include the hyaluronic acid receptor CD44S, IOB-3, OV6, SM3, hepatocyte growth factor/scatter factor, antibodies to the antityrosine kinase met and erbB-2, mesothelin (K1), 44–3A6, and AMAD-1 and -2. None of these has been shown to have reproducible diagnostic value above and beyond the antibodies already discussed, and have been studied in small numbers only.

Discussion

The exponential rise in cases of malignant pleural mesothelioma in the UK over the last 30 years has made the need for an accurate, reliable and reproducible diagnostic test imperative. The diagnosis of mesothelioma has medico-legal implications, and accurate diagnosis and tumour subtyping are essential if the results of trials assessing new therapies for mesothelioma are to be of value.

Many different tumours can theoretically involve the pleura, thereby entering into the differential diagnosis of mesothelioma. Indeed, the incidence of non-mesothelioma-related malignant pleural effusions is much higher than those caused by mesothelioma. Lung cancer and lymphoma account for the majority of cases. The presence of a mass lesion within the lung parenchyma, histological features commonly seen in small cell or non-small cell lung cancer, or the presence of extrathoracic lymphadenopathy may alert the pathologist to these diagnostic possibilities. Extrapulmonary tumours that commonly metastasize to the pleura include breast and ovary in women, prostate in men, and colon and kidney in both sexes. The presence of diffuse diastase periodic acid–Schiff expression and the judicious use of appropriate carcinoma and hormone markers will often resolve any diagnostic difficulty if the patient is known to have a personal history of carcinoma at another site. More problematic is the presence of a malignant pleural effusion without an obvious extrathoracic primary site. In these cases the combination of clinical, radiological and occupational information may be of great value and should always be considered before making a final diagnosis.

Although we have primarily focused on the distinction between EM and PACA, there are other aspects of mesothelioma diagnosis that should be remembered. Malignant mesothelioma is categorized into three main subtypes: epithelioid, biphasic and sarcomatoid. However, it is capable of exhibiting many different patterns of differentiation that may cause diagnostic confusion, and each subtype has its own list of potential differentials. It can be difficult to distinguish between EM with a prominent stromal reaction and biphasic mesothelioma, and reliance on small needle biopsy specimens has been shown to have limited diagnostic accuracy. Useful histological
features to aid mesothelioma diagnosis have been well described.110–112

The experiences of the Joint US/Canadian Mesothelioma registry serves as a good illustration of the most common problems that a pathologist has to face when correctly classifying pleural tumours.1 The most common problem was that of differentiating between EM and carcinoma involving the pleura. The differentiation between benign reactive pleural disease and mesothelioma was another problem area, accounting for approximately 26% of cases. The next largest group included cases in which confirmation of a diagnosis of mesothelioma was requested from the panel. The differentiation between sarcoma and sarcomatoid mesothelioma was addressed in 9% of cases, and the remaining referrals comprised a mixture of other diagnostic problems, such as unusual variants of mesothelioma. The extent of agreement within the panel for any given case was variable. A consensus opinion (> 75%) was achieved in 70.5% of all cases overall. An even split was seen in < 5%. The categories that afforded the least disagreement were those involving simple confirmation of mesothelioma, and the distinction between EM and carcinoma. Consensus was achieved in 70–83% of these cases. Least agreement was seen in the sarcomatoid mesothelioma versus sarcoma (46%) and benign pleural disease versus mesothelioma (59%) categories.

The ability of IHC to distinguish between mesothelioma and other pleural tumours, particularly PACA, has been the subject of numerous publications over the last three decades. Although the accumulated number of cases of mesothelioma and pulmonary tumours studied is impressive, individual papers are often limited by small numbers and diagnostic uncertainty. Because we do not yet have a gold standard for identifying mesothelioma, there is always the possibility that results could be skewed by the inclusion of even a small number of other tumours. Studies often state that the diagnosis of mesothelioma was made using standard diagnostic criteria, without stating what those were. It is therefore still unclear which antibodies are the most reproducible and reliable for everyday practice.

It is less than ideal to combine the results of several different studies when trying to evaluate a diagnostic test. Numerous factors influence the results of IHC staining. Differences in antibody source and clone, tissue preparation, fixation and processing can critically affect epitope sites, and some antibodies require antigen retrieval techniques to ensure reproducible results.27 Sampling errors can be introduced by the inclusion of non-representative areas, especially when the specimen is small, and tumour de-differentiation may be associated with loss of characteristic epitope sites.113,114 Interpretation of the percentage, pattern and intensity of staining is prone to both inter- and intraobserver variation and may be overly influenced by clinical factors such as a history of asbestos exposure.115 It can also be difficult to ascertain whether papers published by the same research group at different times have included the same cases, thereby duplicating results and artificially increasing the number of cases.

Publication bias is another factor that needs to be considered. When a new diagnostic technique is first reported, it often represents the fruits of a long period of research by an enthusiastic and committed group, who may have spent a long time ensuring best possible results. Subsequent groups will not have the advantage of familiarity with the test and allied reagents, and may not be able to reproduce similar results.

By including a large number of cases, and by excluding those studies in which the methodology, case selection or diagnostic criteria are a concern, we hope that some of these reservations can be ameliorated. It is possible to obtain a ‘good feel’ for the overall utility of those antibodies in common use, and select the most appropriate to help in a specific diagnostic dilemma. However, it is essential that the limitations of this sort of study are remembered when extrapolating our results to one’s personal practice. It may well be that an antibody that is less sensitive or specific is preferable, if it is known to be reliable and reproducible in your local laboratory.

When considering combined sensitivity and specificity, our analysis suggests that MOC-31, BG8, CK5/6 and WT1 would be the most useful panel for distinguishing between EM and PACA. It must be remembered that most of these antibodies will not distinguish between benign and malignant mesothelial cells; identification of the features of invasion is a critical part of confirming the diagnosis of mesothelioma.

Appendix 1

Diagnostic sensitivity and specificity were calculated using the following method.116

<table>
<thead>
<tr>
<th>Test result</th>
<th>Disease present (number of cases)</th>
<th>Disease absent (number of cases)</th>
<th>Total number of cases tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>a</td>
<td>b</td>
<td>a + b</td>
</tr>
<tr>
<td>Negative</td>
<td>c</td>
<td>d</td>
<td>c + d</td>
</tr>
</tbody>
</table>

Sensitivity = a / (a + c). Specificity = d / (b + d).
References


Immunohistochemical markers used in the diagnosis of EM


91. Fink L, Collins C, Schaefer R. Thrombomodulin expression can be used to differentiate between mesotheliomas and adenocarcinomas. Lab. Invest. 1992; 66: 111A.


