

HBME-1 and antithrombomodulin in the differential diagnosis of malignant mesothelioma of pleura

A D Kennedy, G King, K M Kerr

Abstract

Aims—To determine the usefulness of antibodies HBME-1 and antithrombomodulin in the differential diagnosis of malignant mesothelioma of the pleura.

Methods—Using microwave antigen retrieval and streptavidin-biotin complex horseradish peroxidase immunohistochemistry the above antibodies were used to stain sections of 57 malignant mesotheliomas, 17 reactive pleural hyperplasias, 23 cases of carcinoma metastatic in pleura, 20 primary ovarian cell carcinomas, and 20 primary renal cell carcinomas.

Results—Eighty six per cent of mesotheliomas and 82% of reactive mesothelial hyperplasias stained strongly with HBME-1. However, 48% of carcinomas metastatic to pleura also stained, as did all serous ovarian carcinomas. Seventy two per cent of mesotheliomas and 24% of reactive mesothelial hyperplasias stained strongly with the antithrombomodulin antibody; 86% and 88%, respectively, of these cases showed staining of any type. While 26% of metastatic carcinomas showed some staining with antithrombomodulin, only one third of these (9%) showed strong, yet focal, staining. Of 40 ovarian and renal carcinomas only two (5%) showed any staining with antithrombomodulin.

Conclusions—HBME-1, although a sensitive mesothelial marker, is not sufficiently specific to be useful diagnostically, as almost half of carcinomas metastatic to pleura also stained positive. Antithrombomodulin is also a sensitive mesothelial marker and is sufficiently specific to be a useful discriminator, positively identifying, in appropriate circumstances, the mesothelial nature of a cell population.

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Distinguishing between malignant mesothelioma and metastatic carcinoma is a well recognised problem in surgical histopathology. A particular issue has been the lack of a specific marker for mesothelial cells in formalin fixed, paraffin wax embedded sections, although several reports have claimed variable success.¹⁻³ Many of the markers currently in routine use

such as carcinoembryonic antigen (CEA),⁴⁻⁷ Leu M1 (CD15),^{4,8} and Ber-EP4⁹ stain carcinomas, particularly adenocarcinoma, but not mesotheliomas, while epithelial membrane antigen (EMA)⁶ and human milk fat globulin 2 (HMFG-2)⁶ stain both, although perhaps in a different pattern. Malignant mesothelioma is, in practice, usually a diagnosis of exclusion rather than positive identification and the use of a panel of immunohistochemical markers is recommended,^{10,11} in addition to a mucin stain. Antibodies to CEA are among the most useful in this area, staining carcinomas of tissues that derive embryologically from the foregut. However, ovarian and renal tubular epithelium have a similar embryological derivation to mesothelium and carcinomas of these tissues are often CEA negative.

Recently, two immunohistochemical markers have been claimed to have some mesothelial specificity: HBME-1 (an antibody raised against a suspension of human mesothelioma cells), which reacts with an antigen present in the cell membrane, and thrombomodulin, a transmembrane glycoprotein with a molecular weight of 75 kDa, produced in insect cells and found in mesothelial and endothelial cells, among others. This study assessed the value of HBME-1 and antithrombomodulin antibodies in the diagnostic differentiation of pleural malignant mesothelioma from carcinoma. Staining in malignant mesothelioma was compared with staining in cases of reactive pleural mesothelial hyperplasia and carcinoma metastatic to the pleura. Staining of primary ovarian and renal cell carcinomas was also examined, given the problem of marking many of these tumours using traditional techniques and the possibility, due to their embryological relation, that such tumours may express "mesothelial" antigens.

Methods

Fifty seven cases of malignant mesothelioma were identified from the surgical histopathology files in the department of pathology, Aberdeen Royal Infirmary. Forty seven cases showed purely epithelioid differentiation; the remaining 10 had a sarcomatous appearance, and in three of these this was in addition to epithelioid differentiation—that is, biphasic malignant mesothelioma. Seventeen cases of reactive mesothelial hyperplasia, 23 cases of carcinoma metastatic to the pleura (13 with an origin in the lung and 10 others, including metastases from the breast and gastrointestinal

Department of Pathology, Aberdeen Royal Infirmary and University Medical School, Foresterhill, Aberdeen AB25 2ZD, UK

Correspondence to: Dr Kennedy.

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Table 1 Immunohistochemical results with HBME-1

	-ve	W+	W++	W+++	S+	S++	S+++	Total
Malignant mesothelioma	4	3	1	0	4	8	37	57
Reactive mesothelial hyperplasia	1	1	1	0	1	4	9	17
Metastatic carcinoma in pleura	12	0	0	0	3	6	2	23
Serous ovarian carcinoma	0	0	0	0	0	0	10	10
Mucinous ovarian carcinoma	7	0	0	0	1	1	1	10
Renal cell carcinoma	9	1	0	0	0	0	0	10
Sarcomatoid renal carcinoma	4	0	0	0	0	1	0	5
Papillary renal carcinoma	3	1	0	0	1	0	0	5

W, weak; S, strong.

Table 2 Immunohistochemical results with thrombomodulin

	-ve	W+	W++	W+++	S+	S++	S+++	Total
Malignant mesothelioma	8	5	3	0	12	20	9	57
Reactive mesothelial hyperplasia	2	5	6	0	2	2	0	17
Metastatic carcinoma in pleura	17	4	0	0	2	0	0	23
Serous ovarian carcinoma	10	0	0	0	0	0	0	10
Mucinous ovarian carcinoma	9	0	0	0	0	0	1	10
Renal cell carcinoma	10	0	0	0	0	0	0	10
Sarcomatoid renal carcinoma	5	0	0	0	0	0	0	5
Papillary renal carcinoma	4	1	0	0	0	0	0	5

W, weak; S, strong.

tract), 20 cases of primary cystadenocarcinoma of the ovary (10 serous, 10 mucinous), and 20 cases of primary renal cell carcinoma (10 clear cell carcinoma; five with sarcomatoid differentiation, and five of papillary architecture) were also selected. Each case was reviewed and 5 µm sections were cut from representative paraffin embedded blocks and mounted on 3-aminopropyltriethoxysilane coated slides. All tissue had been fixed in 10% neutral buffered formalin. Microwave heating as the method of antigen unmasking was used.¹²

Immunohistochemistry was carried out using the streptavidin-biotin complex horse-radish peroxidase (sABC HRP) method¹³ with mouse monoclonal antibodies against HBME-1 (Dako M3505; High Wycombe, Bucks, UK), dilution 1/80, and antithrombomodulin (Dako M0617), dilution 1/10, using appropriate positive controls. The negative control for each case used Tris buffered saline.

Immunoreactivity was scored by two observers as negative (no immunostaining) or positive. Positive results were evaluated as strong or weak. The percentage of immunostained cells in the population of interest was recorded as follows: + (focal, < 5% of cells); ++ (moderate, 5–70% of cells); +++ (widespread, > 70% of cells).

Results

The immunohistochemical results with HBME-1 and antithrombomodulin are shown

in tables 1 and 2, respectively. No cases demonstrated weak staining in a widespread distribution (W+++).

HBME-1

Fifty three of 57 malignant mesotheliomas (93%) stained with HBME-1, 49 (86%) stained strongly and three quarters of those, 65% of the total, showed widespread staining. In 10 mesotheliomas with a sarcomatoid element, almost all of the sarcomatoid areas were negative or only weakly and/or focally positive, differing from the positive epithelioid regions in the three biphasic mesotheliomas. In these cases, the positivity of the epithelioid areas was recorded as the result. Sixteen out of 17 cases of reactive mesothelial hyperplasia (94%) stained with HBME-1, with a wide range of strength and number of cells positive; 14 of 17 (82%) showed strong staining, similar to the pattern seen in mesothelioma.

Twelve of 23 carcinomas metastatic to the pleura were negative with HBME-1, while the 11 positive cases (48%) showed a variable amount of strong staining. The metastases from primary lung tumours followed no particular staining pattern, and were as variable in their immunohistochemical staining results as the other metastatic carcinomas. Staining of the primary carcinomas from different sites was variable. All 10 of the serous cystadenocarcinomas of the ovary were strongly positive in a widespread distribution with HBME-1. Seven of the 10 mucinous cystadenocarcinomas of the ovary were negative but three cases demonstrated strong staining with a range of distribution. Nine of 10 renal clear cell carcinomas did not stain with HBME-1, but one case showed weak, focal positivity. Four of five renal carcinomas with a sarcomatoid pattern were negative but one case showed strong, moderate staining. The five renal carcinomas of papillary architecture also demonstrated a mixed result with three being negative and two showing degrees of positive staining.

ANTITHROMBOMODULIN

Forty nine of 57 malignant mesotheliomas (86%) stained with antithrombomodulin. As with HBME-1, the largest proportion (41 of 57, 72%) were strongly positive but the density of strongly stained cells was more variable, with the strong, moderate pattern being the most common (fig 1). The sarcomatoid areas of the malignant mesotheliomas showed similar results to those seen with HBME-1; most did not stain. Nearly all cases of reactive mesothelial hyperplasia stained (15 of 17, 88%) with a range of positive cell density. None showed strong, widespread staining, compared to nine out of 17 (53%) cases staining strongly in a widespread distribution with HBME-1.

Overall, the carcinomas showed much less staining with antithrombomodulin than with HBME-1. Seventeen of the 23 metastatic carcinomas did not stain with antithrombomodulin. Those that did (six cases, 26%) were only focally positive (strong and weak staining). All 10 ovarian serous cystadenocarcinomas were negative, as were nine of 10 mucinous

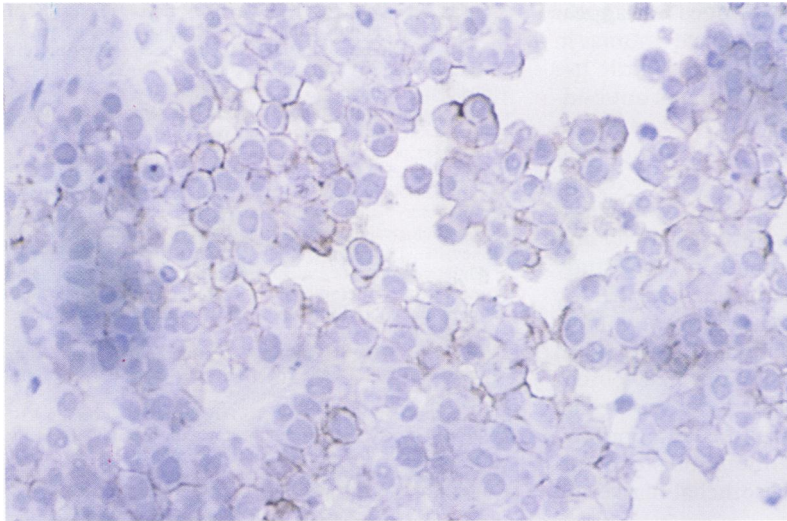


Figure 1 An example of strong, moderate staining by antithrombomodulin. Note that, typically, not all of the cells show staining (sABC method).

cystadenocarcinomas, with only one demonstrating a strong, widespread pattern. Nineteen of 20 renal carcinomas were also negative with antithrombomodulin, with only one carcinoma (of papillary architecture) weakly and focally positive.

The pattern of staining with both antibodies was membranous, with occasional additional cytoplasmic staining. There appeared to be no difference in the cellular localisation of staining between mesothelioma, carcinoma, or reactive hyperplasia.

Discussion

This study has shown that HBME-1 strongly stained 86% of malignant mesothelioma cases and 82% of reactive mesothelial cases. However, HBME-1 was not mesothelial specific because 48% of carcinomas metastatic to the pleura were also stained, each of them strongly. All serous ovarian carcinomas stained strongly in a widespread distribution.

In comparison, antithrombomodulin was slightly less sensitive for malignant mesothelioma (72% of cases stained strongly positive) and had a tendency for moderate or focal rather than widespread distribution. However, it was more specific because 74% of cases of metastatic carcinoma were negative and none showed strong and moderate or widespread staining. Of the cases of renal and ovarian carcinoma, 95% of the combined total were negative with antithrombomodulin. Antithrombomodulin also stained 88% of cases of reactive mesothelial hyperplasia.

In 1992, the original report on HBME-1 by Sheibani *et al*¹⁴ described staining in the majority of cases of epithelial-type malignant mesothelioma, often with a membranous quality, and a few adenocarcinomas also showed staining. This painted an optimistic future for HBME-1, but the lack of specificity for mesothelioma has been documented in our study and in several others,¹⁵⁻¹⁷ where up to 72% of pulmonary adenocarcinomas were positive with HBME-1.¹⁷ Miettinen and Kovatich¹⁵ also found ovarian serous carcinomas to be positive consistently, but all renal

carcinomas that they studied were negative. These authors suggested, with some reservations, that HBME-1 would be useful in an antibody panel. Our results and those of other studies^{16, 17} would not support this view.

Attanoos and coworkers¹⁷ found that antithrombomodulin had a 52% sensitivity for mesothelioma and only two out of 32 adenocarcinomas were positive, while Collins *et al*¹⁸ found antithrombomodulin to have a 100% sensitivity and 92% specificity for mesothelioma. We have shown an 86% sensitivity for mesothelioma and agree that thrombomodulin has a high enough specificity to differentiate between epithelial-type malignant mesothelioma and adenocarcinoma. In another study, thrombomodulin staining of mesothelioma and renal cell carcinoma was compared.¹⁹ Again, sensitivity for mesothelioma was lower than ours (11 of 20 cases were positive) but they showed only one case of renal carcinoma to be positive with thrombomodulin, as we did. This study concurs with our view that antithrombomodulin does not have a role in distinguishing between sarcomatoid renal carcinoma and sarcomatoid malignant mesothelioma, as both entities fail to stain.

Thrombomodulin is mesothelial rather than mesothelioma specific, because most cases of reactive and malignant mesothelium are labelled by antithrombomodulin. We agree with Collins and colleagues,¹⁸ who suggest that this antibody has no discriminating role in separating benign from malignant mesothelial cells. The tendency for strong staining in malignant mesothelial cells and weaker staining in reactive cells is probably not useful in the diagnostic situation.

In a study of serous effusions, different patterns of staining with antithrombomodulin were found for malignant mesothelioma, reactive mesothelial cases, and carcinomatous fluids; staining was described as thick membranous staining, thin membranous, and cytoplasmic, respectively.²⁰ Thirty nine per cent of carcinomatous fluids were positive in this study, a lower specificity than we demonstrated. The differences in specificity and staining patterns may be due to the medium in which the cells are presented and differences in antigen unmasking techniques.

Antithrombomodulin stained vascular endothelium. Consequently, tumours such as renal cell carcinoma, which has a prominent vascular pattern, were rather difficult to score. In addition, antithrombomodulin may show only focal or weak staining, which is difficult to identify on low power scanning. This could lead to false negative scoring on small tissue samples. Furthermore, in malignant mesotheliomas demonstrating strong but focal staining with antithrombomodulin, there was often a moderate but weak underlying pattern of staining in tumour cells not showing strong staining. Haemosiderin and occasional necrotic cells also stained positively with antithrombomodulin, as did renal tubules which had a granular positivity.

We conclude, therefore, that although HBME-1 is sensitive at decorating benign and

malignant mesothelial cells, it does not appear to be specific, because 44% of all carcinomas in this study stained positively. There is little use for HBME-1 as a marker in a panel of antibodies designed for the differential diagnosis of malignancy in the pleura. We consider that thrombomodulin is a useful marker for distinguishing malignant mesothelioma from carcinoma, given that it has an 86% sensitivity and only 13% of all carcinomas in this study stained; its usefulness is enhanced further by the fact that it does not significantly label tumours of organs with a similar embryological derivation to that of mesothelium that, potentially, might have expressed "mesothelial" markers. Antithrombomodulin is an appropriate antibody for use in an immunohistochemical panel as a "positive" mesothelial marker.

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