ORIGINAL PAPER

Value of antimesothelioma HBME–1 in the diagnosis of inflammatory and malignant pleural effusions

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Abstract

Pleural effusions occur in many benign and malignant conditions. The differentiation of mesothelial hyperplasia, malignant epithelial mesothelioma and metastatic adenocarcinoma in cytologic specimens is often difficult. Because many immunohistochemical studies had suggested that HBME–1 has a high sensibility but a low specificity for mesothelial differentiation, the authors investigate its utility in cytological specimens. In this study, immunostaining was performed on 30 smears from seven patients with inflammatory pleural effusions, 21 patients with metastatic pleural effusions and two patiens with malignant epithelial mesothelioma. The immunoreactivity was evaluated by two independent observers. Benign mesothelial cells expressed HBME–1 in 13 (46.43%) cases with thick and thin membrane pattern and with thin membrane and cytoplasmic pattern in 11 (39.29%) cases. One of the malignant mesothelioma was positive for HBME–1 with thick and thin membrane pattern. Metastatic tumor cells were positive for HBME–1 in seven (33.33%) cases; the staining pattern in metastatic adenocarcinoma cells was thin membrane and focal cytoplasmic. HBME–1 has a moderate sensibility and specificity for mesothelial cells and can be used as part of a panel for differentiation of malignant and reactive mesothelial cells from adenocarcinoma in pleural effusions.

***Keywords*:** HBME–1, immunocytochemistry, mesothelioma, pleura, effusion.

Introduction

Pleural effusions may be the first manifestation of metastatic disease or of malignant mesothelioma. In the same time, many benign conditions can due to pleural effusions. Cytologic evaluation of serous effusions is a routine diagnostic procedure and it had been estimated that when malignant cells are present in a fluid, the diagnosis of malignancy can be made in approximately 90% of the cases [1].

In some cases the differential diagnosis between reactive mesothelial cells, malignant mesothelial cells and metastatic adenocarcinoma cells is difficult [2–4].

Particularly, difficult to recognize is the florid mesothelial hyperplasia that occur in association with cirrhosis or following a pulmonary infarct [5, 6].

Cytology alone does not always allow the distinction between reactive mesothelial cells, malignant mesothelioma and metastatic adenocarcinoma.

Immunocytochemistry is the most commonly employd technique and involves the use of panel of antibodies. This panel includes antibodies like carcinoembrionic antigen (CEA), LeuM1, B72.3, BerEP4 and epithelial membrane antigen (EMA) with markers for keratin and vimentin in some laboratories [7–9].

Mesothelial positive antibodies had been introduced as part of the panel, including CK5/6, thrombomodulin, calretinin, HBME–1, N–cadherin and WT1, with varying results [8, 10].

This study evaluated the contribution of HBME–1 in differential diagnosis between reactive mesothelial cells, malignant epithelial mesothelioma and metastatic adenocarcinoma.

Material and methods

The authors studied 30 pleural effusions: seven inflammatory pleural effusion, two pleural malignant epithelial mesothelioma, and 21 metastatic pleural effusions (seven lung adenocarcinomas, one breast carcinoma, three ovary adenocarcinomas, two gastric adenocarcinomas, four small cell carcinomas, and four unknown primary) for immunoreactivity with HBME–1.

The diagnosis of each case was confirmed by computer-tomography, bronchoscopy or surgical excision and histologic examination. We used unstained smears, fixed in neutral 0.1% formaldehyde, 5 minutes and 10 minutes in 95% ethanol. The smears were immunostained using the three steps labeled streptavidin–biotin–immunoperoxidase technique (LSAB2, DAKO, Glostrup, Denmark).

Antibody used for immunocytochemical analysis was clone HBME–1 (prediluted, DAKO). The reaction product was visualized with DAB as chromogen and the nuclei were counterstained with Mayer's Haematoxylin. The mesothelial cells and the adenocarcinoma tumor cells were evaluated for cytoplasmic and membrane staining. Positive staining was defined as a thick membrane, thin membrane and cytoplasmic pattern.

Results

The staining pattern was thick and thin membrane and thin membrane and cytoplasmic. Reactive mesothelial cells were immunostained with thick and thin membrane pattern (Figures 1–4) in 13 (46.43%) cases and with thin membrane and cytoplasmic pattern in 11 (39.29%) cases.

One of the malignant mesothelioma was positive for HBME–1 with thick membrane and thin membrane pattern and cytoplasmic pattern (Figures 5 and 6). The other malignant mesothelioma was negative for HBME–1; also the malignant mesothelial cells have long, thin and bushy microvili. The metastatic tumor cells were positive with thin membrane and focal cytoplasmic pattern (Figures 7 and 8) in seven (33.33%) cases. Metastatic cells of small cell carcinoma were negative for HBME–1 in all cases; metastatic cells of ovarian adenocarcinoma were positive in 66.67% (2/3) of cases, metastatic cells of lung adenocarcinoma were positive in 42.86% (3/7) of cases and metastatic cells from gastric adenocarcinoma were positive in one case (50%); metastatic cells of breast carcinoma expressed HBME–1; the malignant pleural effusions with primary unknown site were negative in all cases for HBME–1 (Table 1).

Table 1 – ***Immunoreactivity of HBME–1 in reactive mesothelial cells, malignant mesothelioma and adenocarcinomas***

	Positive	Negative	Total
Benign mesothelial cells	24	4	28
Malignant mesothelioma	1	1	2
Malignant pleural effusions with primary unknown site	0	4	4
Breast carcinoma	1	0	1
Ovary adenocarcinoma	2	1	3
Lung adenocarcinoma	3	4	7
Gastrointestinal adenocarcinoma	1	1	2
Small cell carcinoma	0	4	4

Immunostaining was graded on a sliding scale of +1 to 3+ according to the percentage of positive cells: +, <10%; ++, 10–50%; +++, >50%) for both patterns of immunostaining: thick and thin membrane pattern and thin membrane and cytoplasmic pattern. Statistical analysis of results emphasized that positive immunostaining with thick and thin membrane pattern, in 10–50% and >50% of cells, was highly specific for mesothelial cells; contrary, positive immunostainig with thin membrane and cytoplasmic pattern in <10% of cells was found in both, mesothelial cells and adenocarcinoma metastatic cells (Figure 9).

Sensibility of immunostaining with HBME–1 for mesothelial cells was 80% and specificity 77%.

Discussions

Many studies evaluated the expression of mesothelial markers including HBME–1, Thrombomodulin, Calretinin and CK5/6, with different results [7, 8].

We studied the expression of HBME–1 and its value in differential diagnosis between reactive mesothelial cells, malignant epithelial mesothelioma and metastatic adenocarcinoma. HBME–1 is a mouse monoclonal antibody prepared from human mesothelial cells from patients with malignant epithelial mesothelioma [11].

Using immunohistochemical techniques, HBME–1 stained normal mesothelial cells, epithelial mesothelioma and various adenocarcinoma. It is non-reactive with sarcomatous mesothelioma and with sarcomatous components of the biphasic variants [11].

Originally, the staining pattern had been described as thick membrane in malignant mesothelioma and cytoplasmic with occasional thin membrane staining in adenocarcinoma [12, 13]. Other studies do not find such a distinctive pattern [7, 11].

Previous studies report approximately 89% positive malignant mesothelioma, with 82% of cases showing a thick bushy membrane staining and 18% a thin membrane pattern. Cytoplasmic pattern was present in 16% of malignant mesothelioma in conjunction with either bushy or thin membrane pattern. Adenocarcinoma reacted positively in 65% of cases with 64% of cases exhibiting a thick/bushy membrane pattern [7].

In our study we find positive immunoreactivity for HBME–1 in one malignant mesothelioma (50%) with thick and thin membrane pattern. HBME–1 was negative in another case of malignant mesothelioma also the malignant mesothelial cells exhibit long, bushy and thin microvili. Reactive mesothelial cells had a thin membrane and cytoplasmic pattern in 11 (39.29%) cases and thick and thin membrane pattern in 13 (46.43%) cases. HBME–1 was positive in seven (33.33%) cases of metastatic pleural effusions. The metastatic adenocarcinomatous cells exhibit a thin membrane and focal cytoplasmic pattern whereas the positive malignant mesothelioma had a thick membrane and thin membrane and cytoplasmic pattern.

Previous studies report positive immunoreactivity with HBME–1 not only in malignant mesothelioma but also in lung and ovarian adenocarcinoma whereas breast and gastrointestinal adenocarcinoma were less commonly positive [7, 11, 14].

In our study, we find positive immunoreactivity in 66.67% of ovarian adenocarcinoma, in 50% of gastric adenocarcinoma, in 42.86% of pulmonary adenocarcinoma; breast adenocarcinoma was also positive for HBME–1. Small carcinoma cells exhibit negative immunoreactivity in all cases. We did not find thick membrane pattern in metastatic adenocarcinoma cells. Statistical analysis revealed that positive immunostaining with thick and thin membrane pattern in 10–50% and >50% of cells was highly specific for mesothelial cells.

Conclusions

HBME–1 is a sensitive antibody but with low specificity for mesothelial cells; it had a limited utility alone but can be used as part of the panel for differential diagnosis between reactive mesothelial cells, malignant mesothelioma and metastatic adenocarcinoma.

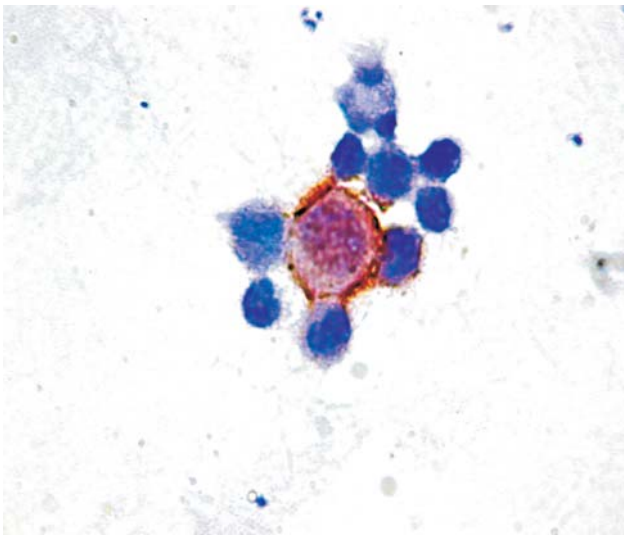

Figure 1 – *HBME–1 positive reactive mesothelial cell with thick membrane pattern, inflammatory pleural effusion, ×400*

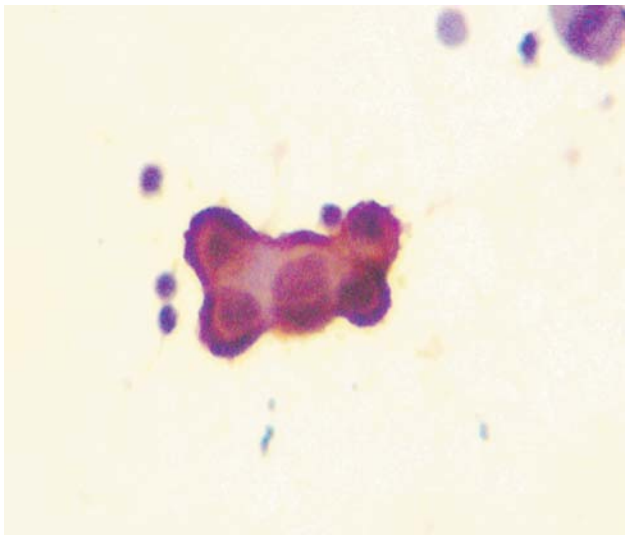

Figure 2 – *HBME–1 positive cohesive group of reactive mesothelial cells with thick membrane pattern, inflammatory pleural effusion, ×400*

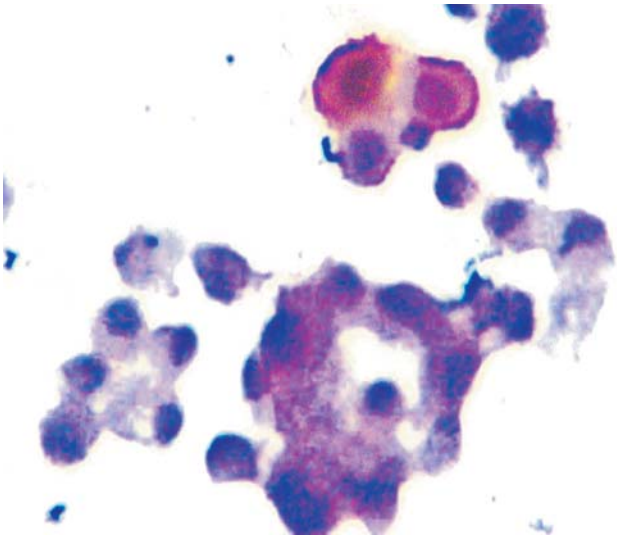

Figure 3 – *HBME–1 positive reactive mesothelial cells and a group of malignant negative cells, malignant pleural effusion with primary unknown, ×400*

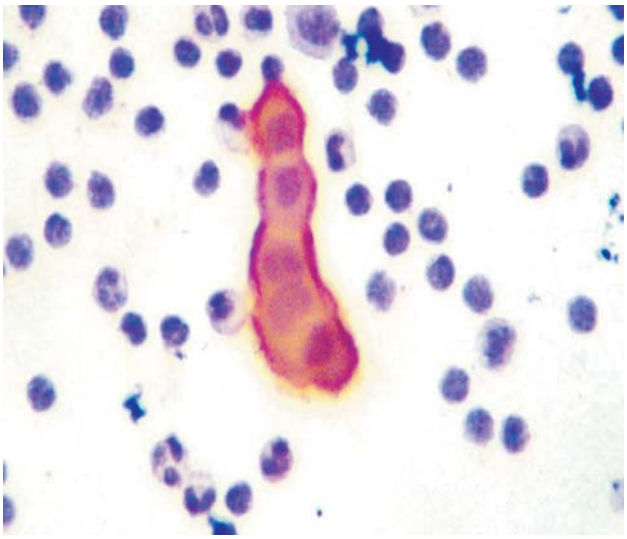

Figure 4 – *HBME–1 positive reactive mesothelial cells with thick membrane pattern, inflammatory pleural effusion, ×400*

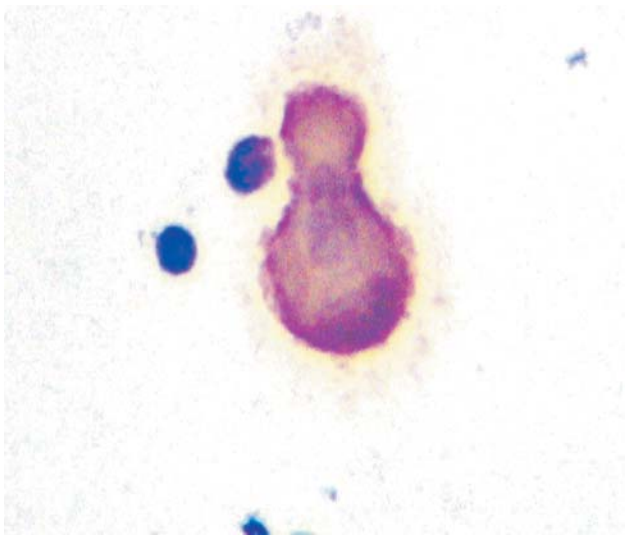

Figure 5 – *HBME–1 positive malignant mesothelial cells with thick membrane pattern, malignant mesothelioma, ×400*

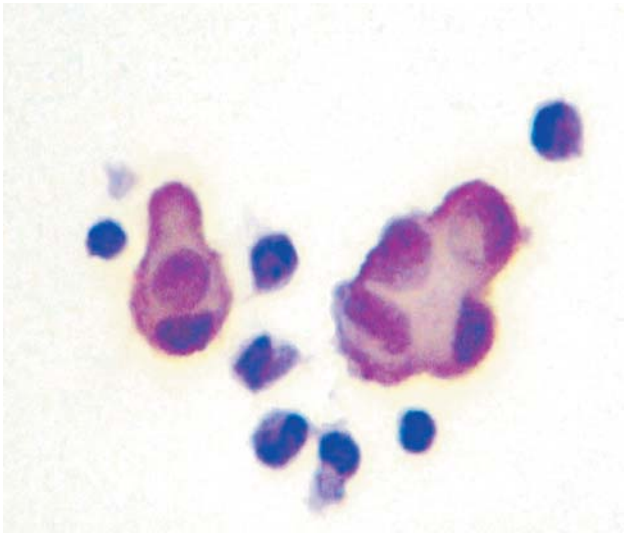

Figure 6 – *HBME–1 positive cohesive malignant mesothelial cells with thick membrane pattern, malignant mesothelioma, ×400*

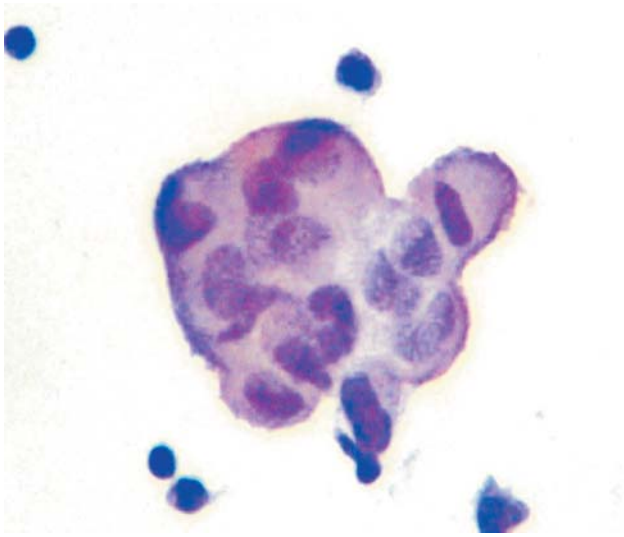

Figure 7 – *HBME–1 positive cohesive group of malignant adenocarcinoma cells with thin membrane and cytoplasmic focal pattern, malignant pleural effusion with primary unknown, ×400*

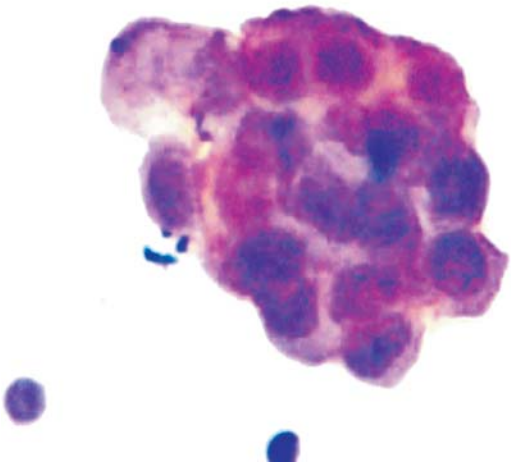

Figure 8 – *HBME–1 positive cohesive group of malignant adenocarcinoma cells with focal, cytoplasmic pattern, malignant pleural effusion with primary unknown, ×400*

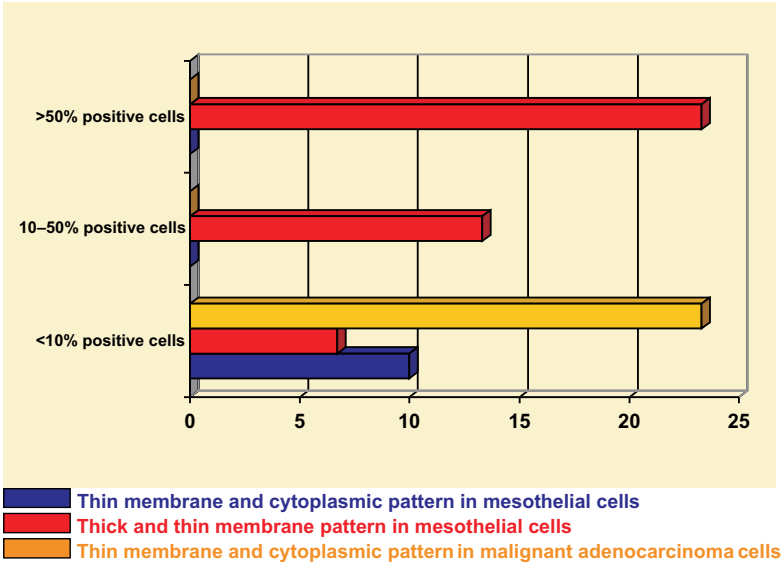

Figure 9 – *Intensity weight of immunostaining with HBME–1 in mesothelial and adenocarcinoma cells*

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