Original Article

Expression of Mesothelial Markers in Malignant Mesotheliomas: an Immunohistochemical Evaluation of 173 Cases

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Abstract

Objective: To see the distribution of Calretinin, thrombomodulin, CK5/6 and HBME-1 markers in various subtypes of mesotheliomas and extend the published data on this topic. The positivity of adenocarcinoma specific markers (CEA and BerEP4) in malignant mesotheliomas have also been evaluated.

Methods: Various markers in 173 cases of malignant mesotheliomas received over a period of 8 years were evaluated by immunohistochemistry.

Results: In majority of malignant mesotheliomas i.e., epithelioid and biphasic types, the positive staining patterns complement the gold standard histologic diagnosis. However, in a small minority mainly sarcomatoid variant, heavy reliance cannot be placed on these markers. CEA and BerEP4 are useful negative markers of mesotheliomas, although occasionally these are positive in clear cut mesotheliomas.

Conclusions: Specificity of various markers in malignant mesotheliomas should be assessed according to histologic subtypes. The existing generation of markers is not reliable in diagnosis of sarcomatoid mesotheliomas. Fortunately this forms only a small group of mesothelial malignancy. In common epithelioid and biphasic variants calretinin, thrombomodulin, CK5/6, HBME-1 are sensitive positive markers whereas CEA and BerEP4 are negative markers of malignant mesotheliomas (JPMA 55:205;2005).

hydrochloride (Myonal) on headache caused by muscle tension. Med Consult N Remedy 1985;22:1-20.

Introduction

Gold standard for the diagnosis of malignant mesothelioma is histology[1] since other tumours can diffusely infiltrate the pleura and mimic malignant mesotheliomas clinically, radiologically and macroscopically, so called pseudomesotheliomas.. However, in a significant number of cases diagnostic aids are required to distinguish between malignant mesotheliomas and adenocarcinomas, and between benign and malignant mesothelial proliferations. Techniques used are histochemistry, electron microscopy, morphometry, in situ hybridization and immunohistochemistry.[2-6] Most studies in assessing efficacy of immunohistochemistry have compared immuno-reactivity patterns in mesotheliomas and adenocarcinomas and as a result, wide robust panels of antibodies are available.[7-10] The most reliable mesothelioma specific markers suggested are CK5/6, calretinin, thrombomodulin and HBME-1.[8,9] We introduced these antibodies in for the mesothelioma panel over the last few years. This has provided us with an opportunity to review the immunoprofile of cases diagnosed as malignant mesotheliomas in our laboratory.

The results of the analysis of malignant mesotheliomas diagnosed in our Department along with suspected clinicopathologic diagnosis of malignant mesothelioma are presented. These cases were diagnosed according to current morphological criteria.[11] Patients had undergone a pleural biopsy at pleuroscopy, FNA, VATs and open surgical biopsies. A total number of 173 cases comprise this report.The aim of the study was to determine the specificity of various markers in the diagnosis of malignant mesothelioma, in particular proposed specific markers in various histologic types.

Material and Methods

Patients with clinico-pathological diagnosis of malignant mesothelioma were included in this study. During the period between January 1995 and December 2002, we received 173 cases of malignant mesotheliomas. Immunohistochemical analysis was performed as follows:

Prior to 2000 all immunostaining was carried out using an Avidin Biotin Complex method (Data Duet) in the Shandon Sequenza staining system. In 2000 all immunostaining was transferred to a fully automated immunostainer (Dako Cytomation Techmate 500+) and dedicated LSAB II (Chemmate) reagents are now used.

In all cases the preparation of sections and relevant pre-treatments remained the same. Briefly, 3 - 4[paraffin sections were mounted on either APES coated (pre 2000) or positively charged slides and dried overnight at 37^{o}C. Following heating at 60^{o}C for 10 to 15 minutes, slides were dewaxed and rehydrated. Endogenous peroxidase was blocked using either 3% hydrogen peroxide in methanol for 10 minutes or Chemmate Blocking solution (Dakocytomation) for 15 minutes. Where antigen retrieval

was required, sections were placed in one litre of 0.1M citrate buffer, pH6.0 and boiled for a total time of 20 minutes. (10 minutes full power, 10 minutes lower power) followed by rapid cooling in running cold water.

Normal swine serum diluted 1;5 was used as diluent for all antibody solutions in the ABC technique, dedicated solutions are now used currently.

The source and dilutions at which antibodies were used is as follows:

Antibody	Source	Dilution	HIER
Calretinin	Novocasma	1:100	YES
Thrombomodulin	Dakocytomation	1:60	NO
Cytokeratin 5/6	Dakocytomation	1:100	YES
HMBE-1	Dakocytomation	1:100	NO
BerEP4	Dakocytomation	1:50	YES
CEA	Serotec	1:300	YES
EMA	Dakocytomation	1:400	YES
CK5	Binding Site	1:200	YES
CK7	Dakocytomation	1:100	YES
CD15	Beeton-Dickinson	1:20	YES
Vimentin	Dakocytomation	1:250	YES
MNF116	Dakocytomation	1:100	YES
CK20	Dakocytomation	1:100	YES

HIER: Heat induced epitope retrieval.

Results

A total number of 173 malignant mesotheliomas were received in the period between 1995 - 2002. Of these 98 were epithelial, 26 sarcomatoid and 29 biphasic. In 20 cases the histologic type could not be ascertained due to various reasons such as superficial tissue, scanty material, diagnosis from cytology or cell block and artefacts. The epithelial type most commonly had a tubulo-papillary pattern.

Among the specific group of markers cytokeratin CK5/6 was positive in 66 out of 77 (86%) cases (Figure 1). Calretinin tested in 61 cases showed nuclear and granular cytoplasmic positivity in 50 (82%) cases (Figure 2). Thrombomodulin was positive in 86 out of 103 (84%) cases tested. This marker mostly showed thin membrane positivity. This membrane positivity may not be obvious on low power examination hence high power examination is essential (Figure 3). HBME-1, another cell membrane associated marker, was positive in 64 out of 86 (74%) cases. Membrane staining pattern is usually thicker than that seen with thrombomodulin. There were 61 cases in which two or more mesothelioma specific markers were positive. Of these 61 cases, 21 were positive for 4 mesothelioma specific markers, 24 for 3 mesothelioma specific markers and 16 for 2 mesothelioma specific markers. Among the non-specific markers CK7 was positive in 73 out of 84 (87%) cases, CK5 in 25 out of 27 (93%), Vimentin in 63 out of 63 (100%) and MNF116 was positive in 81 out of 84 (96%) cases. Among the epithelial glycoproteins which are typically positive in lung adenocarcinomas, a smaller proportion of mesotheliomas were positive. These include CEA, 3 out of 123 (2%), BerEP4, 10 out of 118 (9%), CD15, 2 out of 26 (8%) and SpA, 0 out of 11 (0%) cases. In the group of 61 cases where two or more mesothelioma specific markers were positive, there were 45 cases negative for CEA and BerEP4. Thirteen cases were negative only for CEA and 3 were negative for Ber EP4. Other markers were positive as follows: EMA, 38 out of 39 (97%) cases, TTF, 0 out of 11 (0%) and p53, 61 out of 74 (82%) cases. Markers used on a much smaller scale included CD44, CD68, CAM5.2, and oestrogen receptor. These markers gave variable results but numbers were too small to derive any conclusion.

Discussion

There is clustering of malignant mesotheliomas in Nottingham and adjoining areas partly related to crocidolite asbestos exposure in a war time gas mask assembly factory.[12] A broad range of antibodies have been in use in our laboratory over the last several years depending upon the preferences of local pathologists and histological features of the tumour. During this time there has been a gradual inclusion of newer markers and a progressive increase in the number of antibodies used in a mesothelioma panel in our laboratory. This increase is evidence based, making the panel more mesothelioma specific in the light of information gained from the literature and our own experience with particular antibodies. Broadly speaking, there are three groups of markers in use: 1) Mesothelioma specific markers which include calretinin, thrombomodulin, CK5/6, HBME-1[8], 2). Non-specific markers include vimentin, EMA, CK7 and MNF116. 3) Epithelial markers such as BerEP4, CEA, EMA, SpA and CD15 are used[7,8,13], where differentiation from adenocarcinoma is required.

The report once again highlights the shift of emphasis from non-specific markers to fairly reliable mesothelium specific antibodies to aid histopathologic diagnosis of malignant mesothelioma.This has been possible due to the introduction of mesothelium associated antibodies. Various cytokeratins have been used to decorate mesothelial cells

Table 1. Mesothelioma specific markers.

Markers	Positive	Negative	Total	% positive
CK5/6	66	11	77/173	86
Calretinin	50	11	61/173	82
Thrombomodulin	86	17	103/173	84
HBME-1	64	22	86/173	74

Table 2. Adenocarcinoma specific markers: positivity in malignant mesothelioma.

Markers	Positive	Negative	Total	% positive
CEA	3	120	123/173	2
BerEP4	10	108	118/173	9
CD15	2	24	26/173	8
SPA	0	11	11/173	0
TTF1	0	25	25/173	0

Table 3. Markers specifically in various histological sub-types malignant mesothelioma.

Marker	Epithelioid	Sarcomatoid	Biphasic	Histology type not specified
CK 5/6	49/49	2/7	9/15	6/6
Calretinin	36/38	2/5	7/12	5/6
Thrombomodulin	54/65	7/9	15/19	10/10
HBME-1	48/55	1/8	13/20	2/3
BerEP4	6/71	1/12	1/23	2/12
Vimentin	31/31	14/14	11/11	7/7
CEA	2/75	00/12	0/22	1/14
CK5	17/17	2/3	4/5	2/2
CD15	1/18	0/2	1/5	0/1
EMA	22/22	3/3	7/8	6/6
CK7	43/50	7/8	12/15	11/11
P53	33/41	12/13	10/12	6/8
MNF116	48/49	11/13	15/15	7/7

the epithelial glycoproteins which are typically positive in lung adenocarcinomas, a smaller proportion of mesotheliomas were positive. These include CEA, 3 out of 123 (2%), BerEP4, 10 out of 118 (9%), CD15, 2 out of 26 (8%) and SpA, 0 out of 11 (0%) cases. In the group of 61 cases where two or more mesothelioma specific markers were positive, there were 45 cases negative for CEA and BerEP4. Thirteen cases were negative only for CEA and 3 were negative for Ber EP4. Other markers were positive as follows: EMA, 38 out of 39 (97%) cases, TTF, 0 out of 11 (0%) and p53, 61 out of 74 (82%) cases. Markers used on a much smaller scale included CD44, CD68, CAM5.2, and oestrogen receptor. These markers gave variable results but numbers were too small to derive any conclusion.

Discussion

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The report once again highlights the shift of emphasis from non-specific markers to fairly reliable mesothelium specific antibodies to aid histopathologic diagnosis of malignant mesothelioma.This has been possible due to the introduction of mesothelium associated antibodies. Various cytokeratins have been used to decorate mesothelial cells but these keratin antibodies also stain epithelial cells making it difficult to distinguish mesothelioma from carcinoma especially adenocarcinoma. Recent introduction of CK5/6 antibody for intermediate-sized basic keratin[14] is a step forward, as this appears to be staining most of the mesotheliomas and absent in pulmonary adenocarcinomas. However, caution is advised as staining has been observed in metastatic non-pulmonary adenocarcinoma.[15] Calretinin, a calcium binding protein is another marker which has been found useful in positive identification of malignant mesothelioma.[16] Thrombomodulin, a surface glycoprotein involved in the regulation of intra-vascular coagulation has a high sensitivity and specificity for mesotheliomas.[17] EMA and HBME-1 show positivity in both adenocarcinomas and mesotheliomas, however, the pattern of staining is different with membranous staining being a characteristic feature in

being a characteristic feature in mesotheliomas and cytoplasmic staining in adenocarcinoma.

In our cases, histopathological features played the most important role in diagnosis. The histological spectrum included epithelial, sarcomatoid, biphasic forms. In a small number of cases histologic types could not be ascertained. In these cases cell morphology, clinicoradiologic features and immunohistochemistry supported a diagnosis of malignant mesothelioma. The epithelial type was commonest and mostly had a tubulo-papillary pattern. The most helpful markers for diagnosis of mesothelioma are CK5/6, thrombomodulin, calretinin and HBME1 (Table 1), Cytokeratin 7 and MNF116 are positive in both, pulmonary adenocarcinomas and mesotheliomas and so are not reliable as discriminatory markers. Whenever there is a question of resolving the differential diagnosis between a mesothelioma and adenocarcinoma, the two most reliable epithelial markers in our laboratory have been CEA and BerEP4. Their positivity favours adenocarcinoma especially when mesothelioma specific markers are negative. In addition EMA membrane positivity versus cytoplasmic positivity is another important feature, which sways the balance in favour of malignant mesothelioma. This feature is enhanced in cytologic preparations such as pleural effusions. It is obvious from Table 2 that in our series, these markers are positive in a small number of cases of malignant mesothelioma corroborating previous studies addressing this issue.[4,7] In mixed or biphasic tumours one should try to pick areas or sections with an epithelial component, as immunohistochemistry is more enhanced and easier to interpret in an epithelial component. Vimentin is positive in all mesotheliomas but not reliable as it is positive in all kinds of tumours and benign mesenchymal tissue. The marker p53 antibody cannot determine the mesothelial origin of a tumour and intense positivity (+++) in most of the tumour nuclei is a strong predictor of malignancy, but this seldom occurs. In frankly malignant mesotheliomas, p53 is usually positive, however, in borderline cases its positivity is variable, hence unreliable. Unfortunately, there will always remain a temptation to include this marker along with a proliferation marker in the panel when faced with a diagnostically difficult situation. It should be used with caution.

Immunohistochemistry is known to be less helpful in the diagnosis of sarcomatoid mesothelioma.[18] Attanoos et al[19] suggest that only one third of cases of sarcomatoid mesothelioma express one of the three markers i.e thrombomodulin, calretinin and CK5/6. In our study we looked into the pattern of marker positivity in the different histological sub-types of mesothelioma. The numbers are too small to draw any firm conclusions, however, it is apparent (Table 3) that in sarcomatoid mesotheliomas among the specific markers in our study, only thrombomodulin is reliable whereas the other three, i.e. CK5/6, calretinin and HBME1 have limited potential. Other markers such as CK7, EMA, vimentin and p53, should be used but caution is advised against over-interpretation of results. Histologic features to consider are that sarcomatoid mesotheliomas can have marked variation ranging from laminar hyaline collagen to frankly sarcomatous areas.[20] Frankly, sarcomatous mesotheliomas are relatively easy to diagnose histologically, whereas if the biopsy is from a Pauci-cellular hyaline collagen area, then none of these markers or histologic features help. Ultimately this requires a re-biopsy or close follow-up in such cases.

Incidence of malignant mesothelioma is rising and is expected to peak in United Kingdom by the year 2020.[21] Pathologists therefore are more likely to come across tissue from suspected cases. When dealing with the possible diagnosis of mesothelioma, it is useful to resort to immunohistochemistry. For positive diagnosis of malignant mesothelioma the panel of markers should include CK5/6, calretinin, thrombomodulin and HBME-1. In epithelial mesotheliomas, where adenocarcinoma is in the differential diagnosis, the panel should include CEA, BerEP4 and EMA. An acceptable pattern of result in an antibody battery along with appropriate clinico-histologic features should include presence of at least 2 mesothelioma specific markers and at least one negative carcinoma marker as an indicator of epithelial or biphasic mesothelioma. Focal positivity for CEA and BerEP4 i.e. less than 10% cells should be ignored. Unfortunately there are occasions where a classic epithelioid mesothelioma is positive for one of the adenocarcinoma markers. In these situations one should resort to electron microscopy, histochemistry or discuss it with clinicians and radiologists. Although p53 is positive in most mesotheliomas, its ability to discriminate benign from malignant mesotheliomas is questionable. One may resort to use of Desmin and EMA in this situation as Desmin appears to be preferentially expressed in reactive mesothelium and EMA in neoplastic mesothelium.[22] Diagnostically, difficult cases should be discussed in multi-disciplinary team meetings with input from clinicians, radiologists and surgeons. Because of medico-legal implications of a diagnosis of malignant mesothelioma, additional samples should be requested by the pathologist if the sample is scanty. It is worth emphasising that a team of Pulmonary Pathologists should deal with tissue from suspected mesotheliomas, as they will be more familiar with the varied histology and immuno-patterns of various markers. In fact, if resources and manpower allow, concept of specialist reporting is the way forward in all areas of histopathology and should therefore be encouraged. Unfortunately, there will always remain a case where conclusive diagnosis is made at autop-

histopathology and should therefore be encouraged. Unfortunately, there will always remain a case where conclusive diagnosis is made at autopsy.

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