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Immunohistochemistry in the diagnosis of malignant mesothelioma

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Summary. The histological diagnosis of malignant mesothelioma of the pleura, especially the distinction from peripheral adenocarcinoma of the lung, may be difficult. The immunohistochemical reports previously published on this subject show diverging results mainly because a variety of antibodies and staining techniques have been used by the different authors. To obtain comparable and reproducible results standard techniques and commercialized antibodies should be applied in routine pathology. In order to investigate the value of immunohistochemistry for the separation of the two entities formalin fixed and paraffin embedded blocks of 47 mesotheliomas and 22 adenocarcinomas were investigated with the PAP technique and commercially available antibodies to carcino-embryonic antigen (CEA), keratin, vimentin, epithelial membrane antigen (EMA), pregnancy specific antigen (SP_1), S-100 protein and monoclonal antibody lu-5 (mAB lu-5). CEA positivity was found in all 22 adenocarcinomas examined, but only 2/47 (4%) of all mesotheliomas showed a positive result. SP_1 was positive in 13/22 (59%) of the adenocarcinomas, whereas only 3/47 (6%) mesotheliomas were positive for this marker. No significant difference in the rate of positive cases in the adenocarcinoma and mesothelioma group could be found with the other above mentioned antigens. The results of our study indicate that especially CEA, but also SP_1 are valuable markers in the diagnosis of malignant mesothelioma.

Key words: Malignant mesothelioma – Immunohistochemistry

Introduction

Many problems may arise in the morphological diagnosis of malignant mesothelioma of the pleura. In the early stage of their evolution these highly malignant tumours may appear well differentiated and have to be distinguished from benign, reactive proliferations of mesothelial cells. Malignant mesothelioma must also be separated from metastasizing carcinomas of any origin and from peripheral adenocarcinoma of the lung, especially bronchioalveolar carcinoma. Careful evaluation of the macroscopic appearance, study of the histological pattern including histochemistry and electron microscopy may fail to reveal the true nature of the tumour. Recently immunohistochemistry has been proposed as an aid in the differentiation of mesothelioma from adenocarcinoma. Various antigens including carcinoembryonic antigen (CEA), keratin, vimentin, epithelial membrane antigen (EMA), pregnancy specific glycoprotein (SP_1) and S-100 protein have been claimed to be present in either mesotheliomas or adenocarcinomas, but the results published are contradictory. This confusing situation has several causes. Many reports stem from laboratories involved in research of immunostaining and antibodies not accessible to other investigators are used. Varying staining techniques (PAP versus avidin-biotin system) and incubation procedures (short versus long incubation) have been applied by different authors. Finally fixation and embedding procedures differ from study to study. It is obvious that the published reports can not be compared with each other and are not reproducible for pathologists involved in routine histological diagnosis. The major purpose of our study was to obtain reproducible results, we therefore used commercially available antisera with standard techniques on formalin fixed, paraffin embedded tissue. We investigated 47 mesotheliomas and 22 adenocarcinomas of the lung with antibodies to CEA, keratin, vimentin, EMA, SP_1, S-100 protein and with an anti-epithelial monoclonal antibody (mAB lu-5).

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Materials and methods

47 mesotheliomas from the Zurich pneumoconiosis research group registered from 1978 to 1983 as malignant pleural me-

sotheliomas were selected for this study. In 10 cases open biopsy material and in 37 other cases autopsy material was at our disposal. All cases met the following criteria: 1. typical gross appearance of mesothelioma; 2. characteristic histological picture with epithelial (24 cases), biphasic (15 cases) or sarcomatoid (8 cases) pattern; 3. absence of periodic acid-Schiff-Diastase-resistant mucosubstances (Kannerstein et al. 1973); 4. exclusion of another primary tumour. Lung dust analysis for asbestos had been done in 44 cases, 41 of them were positive. As controls 22 adenocarcinomas of the lung from the files of the Institute of Pathology of the University of Zurich were investigated.

In all cases formalin fixed, paraffin embedded tissue was available. Immunohistochemical staining with polyclonal antibodies was performed according to a modified peroxidase-antiperoxidase (PAP) method of Sternberger et al. (1970) with some modifications. Monoclonal antibodies were detected by a double sandwich method. Tissue sections were incubated with corresponding monoclonal antibodies for 30 min and subsequently with peroxidase labelled polyclonal goat antibodies to mouse immunoglobulins for 30 min. In a third step peroxidase conjugated swine antibodies to goat immunoglobulins were applied for 30 min. Both peroxidase labelled antisera were diluted 1:20 in PBS with 5% normal human serum (Tago, California USA). Peroxidase was detected by Graham-Karnovsky reagent. Vimentin (clone V9), EMA (clone E29) and mAb lu5 were monoclonal antibodies, all other markers were investigated with polyclonal antisera. Dilutions of primary antibodies were: 1:150 for CEA, 1:100 for keratin, 1:30 for vimentin, 1:30 for EMA, 1:400 for S-100 protein, 1:200 for SP_1 and 1:4 for mAb lu-5. Preincubation with 0.1% trypsin for 8 min at room temperature was carried out for keratin and SP_1. All other sections were not trypsinized prior to the incubation with the antibodies. Positive controls were performed on adequate section of various tissues. For negative controls the specific serum was replaced by normal rabbit serum (1:200) for polyclonal antibodies. For negative controls of the monoclonal antibodies supernatant of a hybridoma line producing IgG_1 kappa antibodies with specificity for human thyroglobulin was used. All specific antisera were obtained from DAKO Immunoglobulin Ltd, Copenhagen, Denmark, except for mAB lu-5, which was kindly provided by C. Stähli, Central Research Division, F. Hoffmann-La Roche & Co. Ltd, 4002 Basle, Switzerland. For all antibodies the reactivity was graded according to the proportion of reactive cells (estimated in percentage) and the staining intensity (weak: +, moderate: ++, strong: +++).

Results

The results of the immunohistochemical reactions are summarized in Tables 1 and 2.

Only two mesotheliomas with an epithelial histological pattern gave a positive staining result for CEA. The positive reaction was weak to moderate and involved about 50% of the tumour cells. In contrast, all adenocarcinomas showed a moderate to strong reaction in 70–100% of the tumour tissue studied (Figs. 1 and 2).

16/47 (34%) mesotheliomas and 5/22 (23%) adenocarcinomas gave a positive staining result for keratin. In both diagnostic groups the staining reaction was weak to moderate and less than 50% of all tumour cells stained. In the mesothelioma group, epithelial tumours (12/24) and the epithelial component of biphasic tumours (4/15) appeared positive, while sarcomatoid areas of biphasic mesotheliomas and all purely sarcomatous mesotheliomas were negative for keratin.

20/47 (43%) mesotheliomas (8/24 epithelial, 7/15 biphasic and 5/8 sarcomatoid) and 3/22 (14%) adenocarcinomas gave a positive staining result for vimentin. Of the 7 vimentin positive biphasic mesotheliomas 3 cases gave a positive reaction in both the epithelial and the sarcomatous part, in 3 cases the sarcomatous areas alone and in one case the epithelial part alone gave a positive staining reaction. Both adenocarcinomas and mesotheliomas stained weakly to moderately for vimentin and the positive reaction usually involved less than 30% of the tumour tissue.

Table 1. Reaction of mesotheliomas with different antibodies

	CEA	Keratin	Vimentin	EMA	mAB lu-5	SP_1	S-100
Mesotheliomas (47)							
Epithelial (24)	2/24	12/24	8/24	18/24	20/24	2/24	0/24
Biphasic (15)	0/15	4/15	7/15	10/15	8/15	1/15	0/15
Sarcomatoid (8)	0/ 8	0/ 8	5/ 8	0/ 8	0/15	0/ 8	0/ 8
	4%	34%	43%	60%	60%	6%	0%

Table 2. Reaction of adenocarcinomas with different antibodies

	CEA	Keratin	Vimentin	EMA	mAB lu-5	SP_1	S-100
Adenocarcinomas (22)	22/22	5/22	3/22	22/22	14/22	13/22	0/22
	100%	23%	14%	100%	64%	59%	0%

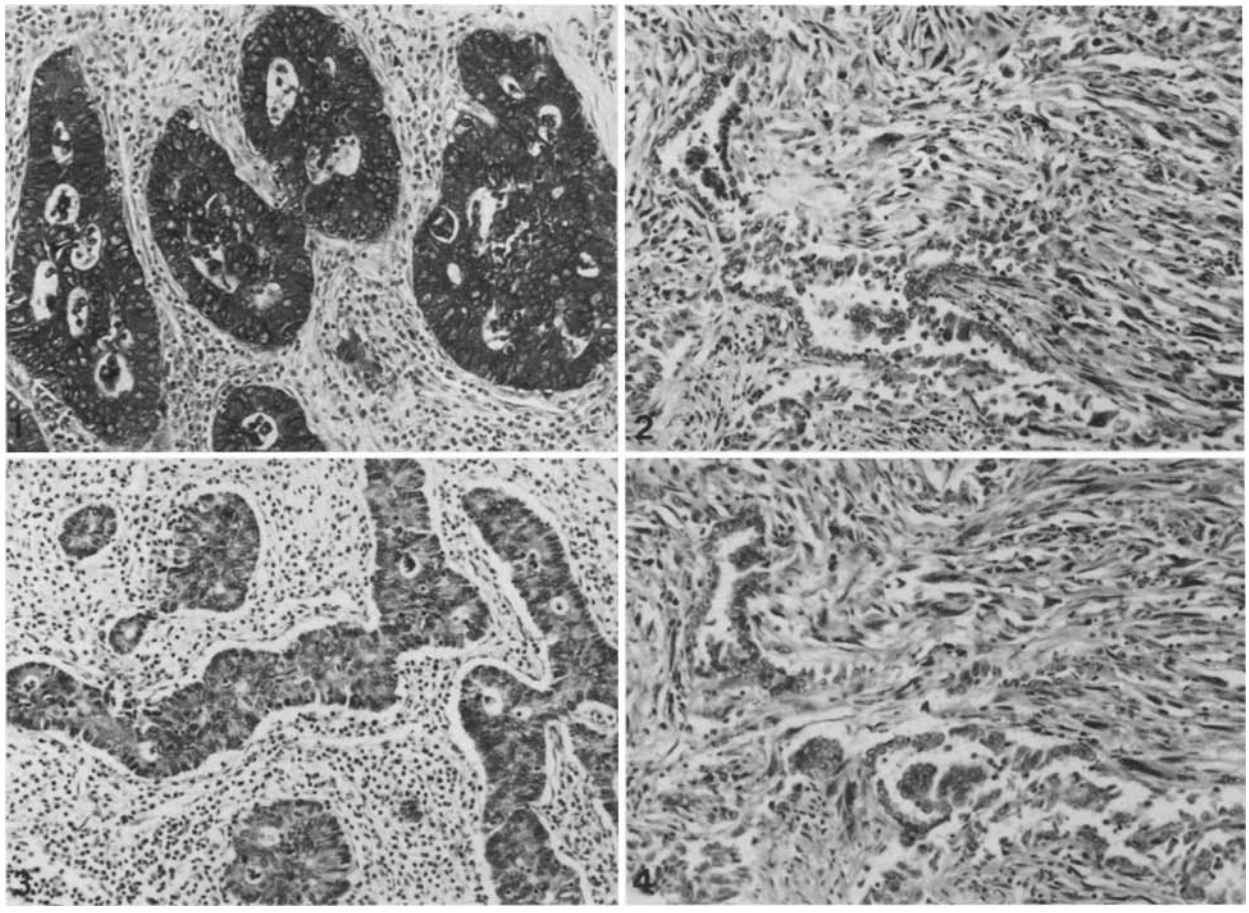

Fig. 1. Adenocarcinoma staining positively for CEA (× 131)

Fig. 2. Biphasic malignant mesothelioma. Incubation with antiserum to CEA shows negative result (× 120)

Fig. 3. Adenocarcinoma staining positively for SP_1 (× 120)

Fig. 4. Biphasic malignant mesothelioma. Incubation with antiserum to SP_1 shows negative result (× 120)

Coexpression of vimentin and keratin was found in one adenocarcinoma and in 6/24 epithelial mesotheliomas.

All 22 adenocarcinomas studied and 28/47 (60%) of the mesotheliomas (18/24 epithelial, 10/15 biphasic) gave a positive staining result for EMA. In only one of the 10 EMA-positive mesotheliomas with a biphasic histological pattern, a positive reaction was found in the spindle cell as well as in the epithelial areas. The other 9 cases were positive in the epithelial part of the tumour only. In mesotheliomas the staining pattern ranged from a weak, but definitely positive reaction in a small proportion of the tumour cells to a strong staining of the whole tumour. All of the 24 adenocarcinomas investigated gave a strongly positive staining reaction involving 60–100% of the tumour tissue.

14/22 (64%) of all adenocarcinomas and 28/47 (60%) of all mesotheliomas stained positively for mAB lu-5. 20/24 epithelial and 8/15 biphasic mesotheliomas gave a positive staining reaction, while all sarcomatous mesotheliomas were negative for mAB lu-5. Of the 8 biphasic mesotheliomas staining positively for mAB lu-5, the epithelial part alone was positive in 7 cases and both epithelial and sarcomatous areas were positive in one case. In the mesothelioma and in the adenocarcinoma group the staining intensity varied from case to case ranging from weak to moderate and involving 30–100% of the tumour tissue.

13/22 (59%) adenocarcinomas stained positive-

ly for SP_1. The staining reaction was usually moderate to strong and involved more than 60% of the tumour tissue (Fig. 3). 2/24 epithelial mesotheliomas and the epithelial part of one biphasic mesothelioma showed a weak and focal positivity for SP_1, involving less than 20% of the tumour tissue. All sarcomatous mesotheliomas were negative for SP_1 (Fig. 4).

All mesotheliomas and all adenocarcinomas were negative for S-100 protein. In many cases peripheral nerve fibres present in the tissue adjoining the tumour gave a strongly positive reaction.

Discussion

The histologic diagnosis of malignant mesothelioma is often difficult. Distinction of epithelial type mesothelioma from adenocarcinoma of the lung is a particular problem. The significance of mucin histochemistry is limited by the low sensitivity of the technique (Kannerstein et al. 1973) and the occurrence of falsely negative results (Kwee et al. 1982; Benjamin and Ritchie 1982).

Recently, much interest has been focussed on immunohistochemistry in the differentiation of adenocarcinoma of the lung and metastases of non-lung adenocarcinomas from mesothelioma. It has been stated that these tumours can be separated by the strongly positive immunohistochemical staining for CEA in adenocarcinomas and the negative reaction for CEA in mesotheliomas (Wang et al. 1979; Kwee et al. 1982; Whitaker et al. 1982; Marshall et al. 1984; Gibbs et al. 1985; Friemann et al. 1986; Bolen et al. 1986). However, studies of Corson and Pinkus (1982), Holden and Churg (1984), Loosli and Hurlimann (1984), Said et al. (1983a), Battifora and Kopinski (1985) have shown a positive reaction of CEA in 15–35% of all mesotheliomas. Most of these CEA-positive mesotheliomas showed only a weak staining reaction which might in part be due to cross reactivity of commercial anti-CEA with nonspecific cross-reacting antigen (NCA) (Nap et al. 1983). In our study only two of the 47 mesotheliomas investigated gave a focal and weakly positive staining reaction for CEA. Interestingly, one of these two cases was positive for SP_1 as well and therefore this tumour is probably not a mesothelioma. The diagnostic value of CEA staining is certainly limited by the fact that an occasional mesothelioma may be CEA positive. Therefore, a positive reaction for CEA does not rule out mesothelioma as proposed by Kwee et al. (1982). Moreover, it seems important to stress that not all adenocarcinomas of the lung stain positively for CEA (Pascal et al. 1977; Goldenberg et al. 1978; Whitaker et al. 1982; Gibbs et al. 1985; Jasani et al. 1985). Nevertheless we feel that a negative reaction for CEA adds considerable support to the diagnosis of mesothelioma.

There is much confusion in the literature concerning the expression of the intermediate filaments keratin and vimentin in mesotheliomas and adenocarcinomas of the lung. Keratin has been described to be present in both adenocarcinomas and mesotheliomas by some authors (Loosli and Hurlimann 1984; Holden and Churg 1984; Kahn et al. 1984; Bejui et al. 1984; Gibbs et al. 1985; Battifora and Kopinski 1985; Blobel et al. 1985; Churg 1985), while other authors (Schlegel et al. 1980; Corson and Pinkus 1982; Said et al. 1983a,b) have demonstrated keratin in mesotheliomas only and describe adenocarcinomas to be negative or only weakly positive for this marker. In our series 16 out of 47 mesotheliomas (33%) and 5 out of 22 adenocarcinomas (23%) were positive for keratin. We have been able to demonstrate keratin in the epithelial part of mesotheliomas only, whereas the sarcomatous areas of biphasic and all purely sarcomatous tumours were negative. Most authors describe both the epithelial and the sarcomatous areas as weakly positive for keratin (Corson and Pinkus 1982; Pileri et al. 1983; Kahn et al. 1984; Gibbs et al. 1985; Battifora and Kopinski 1985; Blobel et al. 1985; Churg 1985), while in other studies no statements are made on this point or the sarcomatous areas are described as keratin negative (Altmannsberger et al. 1982; Bejui et al. 1984). Vimentin has been reported to be positive in mesotheliomas by Blobel et al. (1985), Churg (1985), Jasani et al. (1985) and by Bolen et al. (1986). In our series, 43% of all mesotheliomas were positive for vimentin. Similar to the results of Churg (1985) and Blobel et al. (1985) we found vimentin positivity not only in the sarcomatous but also in the epithelial part of the tumour and in agreement with the findings of Blobel et al. (1985), Bolen et al. (1986) and Churg (1985) vimentin and keratin were coexpressed in six of our mesotheliomas. Coexpression of these two intermediate filaments is not a feature restricted to mesothelial cells (LaRocca and Rheinwald 1984) and mesotheliomas. Malignant tumours including carcinoma of the thyroid (Miettinen et al. 1984), renal cell carcinomas (Holthofer et al. 1983) and various carcinomas growing in body cavity effusions (Remaekers et al. 1983) have been shown to coexpress vimentin and keratin.

Divergent results have been published concerning the expression of vimentin in adenocarcinomas.

Churg (1985) and Blobel et al. (1985) reported adenocarcinomas to be vimentin negative, while Jasani et al. (1985) had 46% vimentin positive cases. In our study 14% of all adenocarcinomas stained positively for vimentin. It seems important to point out that some of our negative results are probably due to formalin fixation and (or) paraffin embedding of the tissue. In fact, Altmannsberger et al. (1981) have shown that formalin fixation and paraffin embedding may destroy or mask antigenic determinants. Interestingly, in one of our vimentin-negative adenocarcinomas and in six vimentin-negative mesotheliomas vascular smooth muscle cells, endothelial cells and fibroblasts also remained unstained, clearly indicating the considerable variation in the preservation of vimentin in formalin fixed material. A second problem is caused by the fact that different types of antisera have been used by different authors and the results of the studies can therefore not be compared. This is demonstrated by the fact that all three authors who described adenocarcinomas to be negative or only weakly positive for keratin used the same antibody (Schlegel et al. 1980; Corson and Pinkus 1982; Said et al. 1983a). The antibody used in our study is a polyclonal antiserum reacting with all epidermal layers and a wide spectrum of other epithelial cells. Based on our results, we feel that keratin and vimentin cannot be used for the discrimination of adenocarcinomas and mesotheliomas when commercially available antibodies are applied to formalin fixed and paraffin embedded tissue. Previous studies with monoclonal antibodies applied to frozen tissue have shown that adenocarcinomas and mesotheliomas can be distinguished from squamous cell carcinomas based on their characteristic keratin components (Moll et al. 1982; Debus et al. 1982). However, so far no cytokeratin antibody exists, which can distinguish adenocarcinoma cells from mesothelial cells (Lang et al. 1986).

Epithelial membrane antigen (EMA), an antiserum raised against human milk fat globule membranes, has been shown to stain a variety of normal and neoplastic human tissues (Sloane and Ormerod 1981). Adenocarcinomas of the lung have been described to be positive for this marker by several authors (Sloane and Ormerod 1981; Loosli and Hurlimann 1984; Marshall et al. 1984; Battifora and Kopinski 1985). Diverging results have been published concerning the expression of EMA in mesotheliomas. Ernst and Brooks (1981), Sloane and Ormerod (1981), Loosli and Hurlimann (1984) and Marshall et al. (1984) have demonstrated the presence of EMA in epithelial, biphasic and sarcomatous mesotheliomas. In contrast, all of the 12 mesotheliomas investigated by Battifora and Kopinski (1985) turned out to be negative for EMA. The authors conclude that EMA is a valuable marker for the distinction of adenocarcinomas and mesotheliomas. Battifora and Kopinski (1985) have used the avidin-biotin complex method, whereas all of the other above mentioned investigators have used the immunoperoxidase technique. Therefore, the negative results of Battifora and Kopinski (1985) are probably the result of the different technique applied. In our series 28 out of 47 mesotheliomas (60%) and all adenocarcinomas investigated stained positively for EMA. In agreement with the findings of Ernst and Brooks (1981) and Marshall et al. (1984) one of our biphasic cases appeared positive not only in the epithelial, but also in the sarcomatous areas. Due to the high proportion of positive cases in both the adenocarcinoma and the mesothelioma group, EMA seems to be of no value for the distinction of these two diagnostic entities.

mAb lu-5 was positive in 60% of all mesotheliomas and in 64% of all carcinomas and therefore does not permit a separation of the two entities either. In tissue sections of 14 adenocarcinomas bronchial mucosa was present, representing a positive control for mAb lu-5. The epithelium reacted positively in 8 cases and was negative in 6 cases. Of these 6 cases the adenocarcinoma was positive once, in the other 5 cases the tumour was negative as well. In these 5 cases the tissue has probably lost its antigenicity due to inadequate fixation. This may explain the lower percentage of positive adenocarcinomas in our series compared to the series of von Overbeck et al. (1985).

SP_1 pregnancy specific glycoprotein, one of the so called trophoblast specific pregnancy proteins, has been shown to be positive in 85% of all adenocarcinomas (Gibbs et al. 1985), while mesotheliomas are reported to be negative for this marker. In our series, three mesotheliomas (6%) and 13 (59%) adenocarcinomas gave a positive staining result. The relatively low rate of positive cases in the adenocarcinoma group indicates that SP_1 should only be used in combination with CEA for the differential diagnosis of mesothelioma and adenocarcinoma.

In our series application of S-100-protein was not useful for the distinction of mesothelioma and adenocarcinoma, in contrast to the findings of Rasmussen and Larson (1985). Our study indicates that especially CEA, but also SP_1 are helpful for the distinction of mesothelioma and adenocarcinoma. A positive reaction for either of these two

markers is a strong evidence against the diagnosis of mesothelioma, whereas a negative result in combination with a typical histology and mucin histochemistry strongly supports the diagnosis of mesothelioma.

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