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Vimentin, carcinoembryonic antigen and keratin in the diagnosis of mesothelioma, adenocarcinoma and reactive pleural lesions

N. Al-Saffar, P.S. Hasleton

Vimentin, carcinoembryonic antigen and keratin in the diagnosis of mesothelioma, adenocarcinoma and reactive pleural lesions. N. Al-Saffar, P.S. Hasleton.

ABSTRACT: An immunohistochemical study of reactive pleural lesions, adenocarcinomas and mesotheliomas using carcinoembryonic antigen (CEA), cytokeratin and vimentin was carried out. All the specimens were obtained at surgery except for 11 mesotheliomas found at necropsy. Vimentin was positive in 23 of 27 mesotheliomas and negative in all the adenocarcinomas and 4 of 17 reactive mesothelial lesions. Conversely, CEA was positive in all the adenocarcinomas but negative in all mesotheliomas. Immunoreactivity for vimentin was seen in only 3 of 11 postmortem mesotheliomas. Vimentin is a useful adjunct to the tissue diagnosis of mesothelioma especially when CEA is negative and cytokeratin positive. Its use appears largely confirmed to well fixed surgically derived tissues. *Eur Respir J., 1990, 3, 997–1001.*

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The diagnosis of malignant pleural mesothelioma is often difficult. It may be confused histologically with a reactive pleurisy or adenocarcinoma. Separation from benign lesions is obviously important. However, to the cynic it may appear less important to distinguish an epithelial mesothelioma from a pleural deposit of adenocarcinoma, both conditions being associated with poor prognosis. However, there are indications [1] that chemotherapy may prolong the life and relieve the suffering of patients with mesothelioma. It is also important for medicolegal reasons to distinguish the two diseases. Until recently a cornerstone of diagnosis of epithelial mesothelioma was the lack of positivity with mucin stains and carcinoembryonic antigen (CEA). Recently CHURG [2] described several cases in which alcohol fixed mesotheliomas stained positively with vimentin, one of the intermediate filament proteins. We report our experience with reactive mesothelial lesions, pleural adenocarcinomas and malignant mesotheliomas using paraffin embedded tissue fixed in conventional formol saline.

Materials and methods

Seventy four specimens were selected for this study. They included 38 malignant mesotheliomas, 19 adenocarcinomas with pleural involvement and 17 cases with a reactive mesothelial proliferation. The "surgically derived" mesotheliomas consisted of 17 of the epithelial type, 6 of the fibrous type and 4 biphasic. Eleven of the 38 mesothelioma cases were necropsy specimens coming mainly from the Medical Boarding Panel (M.B.P.) (Respiratory Diseases). Seven were epithelial, three fibrous and one biphasic.

All biopsy tissues were fixed in neutral buffered formol saline for at least 24 h and routinely processed to paraffin wax in the conventional way. Cases from the M.B.P may have had different formalin fixatives at other hospitals.

Paraffin sections (5 μm) were used for the immunohistochemical study. All the adenocarcinomas showed mucin positivity as demonstrated by diastase/Alcian blue/periodic-acid-Schiff (D/AB/PAS) most having an underlying pulmonary tumour. No tumour was demonstrated in the reactive mesothelial proliferations, most of these cases being derived from pleurectomy specimens operated on for pneumothoraces. Mesothelioma was diagnosed in most cases by lack of mucin and CEA positivity, a tubulo-papillary or sarcomatous growth pattern and the presence of regular open vesicular nuclei. Adenocarcinomas were mucin positive and had hyperchromatic often irregular nuclei. In three cases electron microscopy on epithelial mesotheliomas showed small microvilli and no mucin granules. No computed tomography (CT) scans were available at the time of pathological diagnosis.

A monoclonal anti-vimentin antibody designated RPN-1102 was generously donated by Amersham International. It is an immunoglobulin G (IgG) ascitic fluid mouse monoclonal antibody produced by the hybridoma technique after immunization with porcine eye

lens vimentin. It has previously been shown that it reacted only with vimentin and not with any other intermediate filament proteins [3]. Rabbit anti-shole human keratin antiserum and rabbit anti-CEA were purchased from Dako Corporation, Denmark.

Immunohistochemical staining was performed using the peroxidase anti-peroxidase (PAP) method for CEA and keratin, and the indirect method for vimentin. Sections were deparaffinized with xylene and transferred to alcohol. Endogenous peroxidase activity was blocked by incubation in 0.5% hydrogen peroxide in methanol for 30 min. Sections stained for vimentin were incubated with the monoclonal antibody at a final dilution of 1/2000 for 20 h at 4°C, and then with the rabbit anti-mouse peroxidase conjugate (Dako Corporation) at 1/50 dilution for 1 h at room temperature.

Sections stained for CEA and keratin were pretreated with 0.1% trypsin (Sigma Company) for 15 min at 37°C to unmask antigenic activity. To avoid nonspecific background staining, sections were overlayered with a 1/20 dilution of normal swine serum for 20 min. The primary antiserum was applied to the unwashed sections. The sections were then incubated sequentially with the swine anti-rabbit IgG serum and the peroxidase - rabbit anti-peroxidase complex (Dako Corporation). All the antisera were diluted 1/400 and incubated for 30 min each, at room temperature. The colour was developed with a freshly prepared solution of 3.3' diaminobenzidine tetrahydrochloride with 0.1% hydrogen peroxide. Sections were then washed, counterstained, dehydrated, cleared in xylene and mounted with DPX. Dilution of the antisera and washing of the sections were carried out using tris-HCl buffered saline.

The degree of staining on a section was graded as negative, + = 1–20% positive cells, ++ = 21–50% positive cells and +++ = 51–100% positive cells.

Results

The results of staining for vimentin, CEA and keratin are summarized in table 1. It is clear from table 1 that vimentin was negative in all the adenocarcinomas whereas CEA was positive in all cases of this tumour. Vimentin was positive in the majority (22 of 27, 81%) of surgically derived mesotheliomas. Positivity was seen in all the sarcomatous mesotheliomas (fig. 1), 14 of 17 epithelial type (fig. 2) and 3 of 4 mixed tumours. Vimentin positivity was seen in the mesothelial cells in reactive pleurae (13 of 17, 76%) (fig. 3).

Staining for vimentin was strong in the reactive mesothelium with 10 of 17 cases being 2+ or greater. The sarcomatous and mixed mesotheliomas all stained 2+ or greater. In the epithelial mesotheliomas 9 of 17 cases were 2+ or greater.

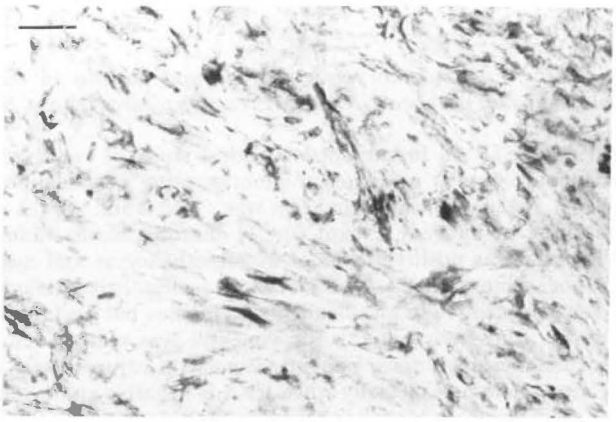

Fig. 1. – Fibroblastic mesothelioma (immunoperoxidase - antivimentin, bar = 50 μ).

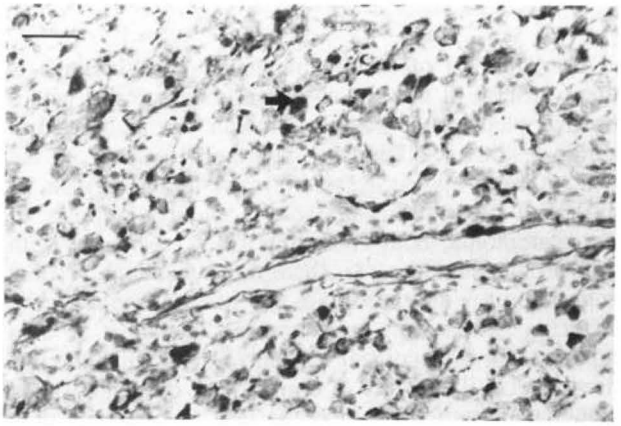

Fig. 2. – Polygonal mesothelioma with some acinar formation (arrow). Blood vessels also stain positively (immunoperoxidase - antivimentin, bar = 50 μ).

Table 1. – The results of staining for vimentin, CEA and keratin in surgical pleural biopsies

			Mesothelioma			Adenocarcinoma
		"Reactive"	Epith	Sarcom	Mixed	
Vimentin	Neg	4/7	3/17	0/6	1/4	19/19
	Pos	13/17	14/17	6/6	3/4	0/19
CEA	Neg	17/17	17/17	6/6	4/4	0/19
	Pos	0/17	0/17	0/6	0/4	19/19
Keratin	Neg	9/17	4/17	4/6	0/4	4/19
	Pos	8/17	13/17	2/6	4/4	15/19

CEA: carcinoembryonic antigen; Epith: epithelial; Sarcom: sarcomatous.

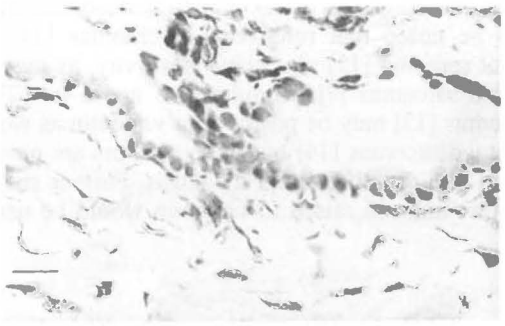

Fig. 3. – Reactive mesothelium with staining of submesothelial tissue (immunoperoxide - antivimentin, bar = 80 μ).

In addition to the mesothelial cells, reactivity for vimentin was observed in other tissue constituents in each section. These included a significant number of fibroblasts, vascular smooth muscle cells, endothelial cells of blood vessels, histiocytes and post-capillary venules present among the lymphoid cell aggregates. Lymphocytes were always negative for vimentin. Positive staining in the other tissue constituents was considered as an internal positive control in each section of reactive pleura, malignant mesotheliomas or adenocarcinomas studied. The staining was strong in all the positive cells, and there was no difference in the intensity of staining between the different cases or between the mesothelial cells and the other positive elements in the sections.

The tumour cells in the 19 adenocarcinomas were negative for vimentin. Positive staining on the other elements in the sections was present in 15 cases.

Keratin was positive in the majority of adenocarcinomas (15 of 19, 79%) and in 10 cases it was 2+ or greater in intensity. Eight of the 17 reactive pleurae were positive with this antiserum (5 were 2+ in intensity, no cases being classed as 3+). Nineteen of the 27 (70%) mesotheliomas were positive with keratin (9 of 17 epithelial were 2+ or greater, 1 sarcomatous showed 2+ positivity and none 3+ and all 4 tumours were 2+ or greater in intensity).

Staining of mesotheliomas from autopsy material

The 11 cases studied comprised seven epithelial tumours, three sarcomatous and one of mixed cell type. Staining for CEA was negative in all cases, but keratin was found in three tumours, (two epithelial and one biphasic). Reactivity for vimentin was present in the two epithelial tumours that stained for keratin. Reactive fibroblasts, vascular smooth muscle cells and endothelial cells of blood vessels in the sections were also positive for vimentin in these two cases. The other nine cases did not show any reactivity when stained with this monoclonal antibody.

Discussion

The present study shows that vimentin will stain most mesothelial cells in biopsy material. It will not distinguish a reactive mesothelial cell population from a malignant one. However, α_1-antichymotrypsin is said to be useful in distinguishing reactive mesothelial cells from malignant ones [4]. More significantly, vimentin will distinguish adenocarcinomas from mesotheliomas. Adenocarcinomas are CEA positive and vimentin negative whereas mesotheliomas are CEA negative and vimentin positive. The ability to use a monoclonal antibody which will positively stain mesotheliomas is an advance since until the present time immunocytochemistry has only been of help in diagnosis in that CEA is negative in this tumour.

Vimentin is one of an intermediate group of filaments which make up the cytoskeleton of mammalian cells. The other intermediate filaments are cytokeratin, desmin, neurofilament and glial fibrillary acid protein. Vimentin stains mesenchymal tissues such as fibroblasts, chondrocytes, vascular smooth muscle, endothelial cells, haemopoeitic tissues and melanocytes.

One of the first studies of vimentin in mesotheliomas was that of CHURG [2] where eight mesotheliomas were studied. Strong staining was seen in the alcohol fixed specimens, the formalin fixed tissues staining less strongly. Three adenocarcinomas and two postmortem pleural derived tissues after alcohol fixation were negative with vimentin. In the present series normal and reactive mesothelial cells stained with vimentin and we have no ready answer as to why in Churg's series the normal pleura did not stain. In our series the reactive pleura was obtained from surgical biopsies and thus had optimal fixation.

JASANI *et al.* [5] studied 44 mesotheliomas and 24 pulmonary adenocarcinomas immunohistochemically. All the tissues were formalin fixed and from postmortems. A different, very sensitive, immunoperoxidase-method was used to that in the present study, a modification of the DNP-labelled antibody technique. 75% of malignant mesotheliomas were positive for vimentin. The figure for adenocarcinomas was 46%. The degree of staining was greater in mesotheliomas than adenocarcinomas. GATTER *et al.* [6] showed that human lung tumours (squamous, adeno, and small cell carcinoma and carcinoid tumours) expressed at least two intermediate filaments in 40% of cases. No mesotheliomas were studied. These two papers would suggest that vimentin is not exclusive to mesotheliomas and has been taken in lung cancers to indicate a tendency for the tumours to express multiple intermediate filament types. However, recently LEADER *et al.* [7] showed that vimentin was a sensitive marker of mesenchymal differentiation in a wide variety of sarcomas. No mesotheliomas were studied by these authors.

Our findings are somewhat at variance with some of the above studies. Vimentin positivity was confined to mesotheliomas, rather than adenocarcinomas. Only 4 of 27 mesotheliomas diagnosed at thoracotomy did not stain. The reason for this may be that most of our PM tissues

were from the M.B.P and had not been optimally fixed. Only two necropsy derived mesotheliomas stained with vimentin. Cytokeratin was not as strongly expressed in necropsy tissues. The monoclonal antibody we used was produced by Amersham International code RPN 1102. It is stated that there is no cross-reactivity with other intermediate filaments including desmin and glial fibrillary protein. In view of the findings of JASANI *et al.* [5] of positive staining in nearly half of the adenocarcinomas it would be interesting to repeat the study with a group of carcinomas using the DNP-labelled antibody technique as well as using different anti-vimentin antibodies.

One of the most detailed recent studies of immunostaining of mesotheliomas is that of BOLEN *et al.* [8]. These authors studied pleura from eight cases where there was either a peritonitis (one case) or an associated carcinoma as well as 9 epithelial mesotheliomas, 7 fibrous mesotheliomas and one biphasic tumour. As in the present study no cases were positive for CEA. In the reactive pleurae most of the staining was confined to subserosal multipotential cells but as these became reactive and acquired cytokeratin, vimentin was lost. A range of cytokeratins demonstrating different molecular weights was positive both in reactive and malignant pleura.

It was in the mesotheliomas that our results are somewhat at variance to those of BOLEN *et al.* [8]. These authors showed no positivity for a vimentin of 58 kdaltons in epithelial mesothelioma though fibrous and biphasic mesotheliomas were positive. All our fibrous mesotheliomas stained positively with vimentin. Fewer epithelial mesotheliomas stained with the antibody but 14 of 17 cases showed positivity. All of the cases of Bolen *et al.* except the mixed mesotheliomas, were surgical specimens. As noted above, necropsy specimens infrequently stain with vimentin.

Mesotheliomas tend to stain strongly with keratin whereas some authors have shown negative or weak staining in adenocarcinomas on frozen section [9]. While we would agree with this for some cases, most of the adenocarcinomas in our series showed strong keratin positivity. WATTS *et al.* [10] suggested that most reactive and malignant mesothelial cells stained positively for keratins with a molecular weight of 63 kdaltons, whereas adenocarcinomas stained with low (45, 46 kdaltons) and intermediate (55 kdaltons) weight keratins. This study was carried out in exfoliated cytology and there were 3 adenocarcinomas staining positively with high molecular weight keratins and conversely 2 mesotheliomas were negative with 63 kdalton keratins. This lack of staining was taken as a lack of differentiation of tumour cells with loss, absence or suppression of this antigen. When one is faced with an individual case this degree of overlap does not help in diagnosis. There is also overlap in staining patterns with mesothelioma and adenocarcinoma.

Vimentin did not stain some epithelial mesotheliomas and one biphasic mesothelioma. All fibrous mesotheliomas were positive. We found it an easy stain to use as there was an inbuilt positive control for endothelial cells, blood vessels and fibroblasts. Thus, while vimentin will, with a negative CEA, give a good guide to the diagnosis of an epithelial mesothelioma, it should be noted that renal cell carcinomas [11] and synovial sarcoma [12] also show positivity, as may unclassified sarcomas [7]. Similarly, B and a few T-cell lymphomas [13] may be positive for vimentin as well as rhabdomyosarcomas [14] but these tumours are unlikely to come into the differential diagnosis. Further staining with other antisera raised to vimentin would be useful.

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Vimentin, antigène carcino-embryonnaire et kératine, dans le diagnostic de mésothéliome, des adéno-carcinomes, et des lésions pleurales réactionnelles. N. Al-Saffar, P.S. Hasleton.
RÉSUMÉ: Nous avons conduit une étude immuno-histochimique dans des lésions pleurales réactionnelles, des adéno-carcinomes et des mésothéliomes, en utilisant l'antigène carcino-embryonnaire CEA, la cytokératine et le vimentin. Tous les specimens ont été obtenus lors d'intervention chirurgicale, sauf 11 mésothéliomes découverts à l'autopsie. Le test au vimentin est positif dans 23 des 27 mésothéliomes, négatif dans tous les adéno-carcinomes ainsi que dans 4 réactions mésothéliales sur 17. A l'inverse, CEA est positif dans tous les adéno-carcinomes, mais négatif dans tous les mésothéliomes. L'immuno-réactivité au vimentin n'a été observée que dans 3 mésothéliomes post-mortem sur 11. Le vimentin peut constituer une aide au diagnostic tissulaire de mésothéliome, particulièrement lorsque le test au CEA est négatif, et celui à la cytokératine positif. Son utilité est largement confirmée lorsque les specimens, bien fixés, sont prélevés chirurgicalement.
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