

Immunohistological staining of reactive mesothelium, mesothelioma, and lung carcinoma with a panel of monoclonal antibodies

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SUMMARY A panel of seven monoclonal antiepithelial antibodies of different specificities, including anticytokeratin, human milk fat globule membrane, Ca, and carcinoembryonic antigen (CEA) were used with the alkaline phosphatase-antialkaline phosphatase (APAAP) immunostaining technique to determine their value in the differentiation between benign and malignant mesothelial cells and lung carcinoma in histological preparations. The anticytokeratin antibody reacted strongly with all cases of reactive mesothelium, mesothelioma, and lung carcinoma. Antibodies to human milk fat globule membrane and the Ca antigen stained mesothelioma and carcinoma and 43% of cases of reactive mesothelium. Staining for carcinoembryonic antigen was not detected in reactive mesothelium or mesothelioma, but was present in most of the lung carcinomas. CEA seemed to be the single most useful marker in distinguishing carcinoma from mesothelioma in that a positive reaction for CEA would indicate carcinoma rather than mesothelioma.

The histological diagnosis of malignant mesothelioma in pleural biopsy specimens is a well recognised problem.¹ Malignant mesothelioma may be indistinguishable from a reactive mesothelial cell proliferation, or from primary, or metastatic carcinoma. The same problem exists in the cytological diagnosis of serous fluids in which malignant mesothelioma cells may be difficult to differentiate from benign mesothelial cells or from metastatic carcinoma by morphological criteria alone.

Recent immunocytochemical studies of serous fluids have shown that monoclonal antibodies are useful in distinguishing benign from malignant cells. Human milk fat globule membrane antigen, carcinoembryonic antigen (CEA), and the Ca antigen have been shown in a wide variety of malignant epithelial cells but have rarely been seen in reactive mesothelial cells.²⁻⁴ A previous study on histological samples of mesothelium reported similar findings with two antibodies against human milk fat globule membrane antigen and an anti-CEA antiserum.⁵

In the present study a panel of seven monoclonal antiepithelial antibodies of different specificities, including anticytokeratin, human milk fat globule

membrane, Ca and carcinoembryonic antigen were evaluated to determine whether they could distinguish between reactive and malignant mesothelial cells and lung carcinoma in histological preparations, using an immunoalkaline phosphatase staining method.

Material and methods

SAMPLES

All tissues were obtained from the surgical pathology files of the histopathology department, John Radcliffe Hospital, Oxford and had been fixed in 10% unbuffered formalin and embedded in paraffin. Cases had been classified according to conventional histological criteria. Samples studied comprised: seven pleural mesotheliomas (most of which were referred to and accepted by the Pneumoconiosis panel); 15 primary lung tumours, which included four squamous cell carcinomas, four adenocarcinomas, five oat cell and two carcinoid tumours; and reactive mesothelial cells from seven cases of recurrent pneumothorax. The lung tumours were selected as representative paraffin embedded specimens from a series of 54 lung tumours previously studied in cryostat sections.⁶ The samples of benign mesothelial cells were selected from

Table 1 Details of monoclonal antibodies used for immunological analysis of mesothelioma, benign mesothelial proliferation, and lung carcinoma

Antibody	Specification	Reference
KL1	Cytokeratin	Viac <i>et al</i> ⁷
E29	Human milk fat globule membrane antigen (EMA)	Cordell <i>et al</i> ⁸
HMFG2		Taylor-Papadimitriou <i>et al</i> ⁹
Ca1	Cancer associated glycoprotein	Gatter <i>et al</i> ¹⁰
Ca2		Ashall <i>et al</i> ¹¹
Ca3		Bramwell <i>et al</i> ¹²
11.285.14	Carcinoembryonic antigen (CEA)	Gatter <i>et al</i> ¹⁰

a large number of cases as clear cut examples of reactive mesothelium. Cases in which any possible confusion might be made with underlying pulmonary tissue were excluded.

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Table 1 gives details of the monoclonal antibodies used in this study. Sections (5 µm) were stained by the APAAP immunoalkaline phosphatase staining method, as described previously.¹³ Prior trypsinisation of sections was not performed.

Results

REACTIVE MESOTHELIUM

Monoclonal antibody KL1 (anticytokeratin) reacted with mesothelium in all seven benign cases. It characteristically stained the single layer of cuboidal mesothelial cells lining the pleural surface with a strong cytoplasmic and surface reaction (figs 1a, 2a). Areas that contained clumps or multilayers of plump reactive mesothelial cells, either on the pleural surface or exfoliated, also reacted strongly with KL1

Antibodies E29 and HMFG2 (antiepithelial membrane antigen) and the three Ca antibodies gave similar staining reactions to each other. Three of the seven benign cases showed positive staining with these antibodies. In these cases the single cuboidal layer of mesothelial cells showed both cytoplasmic and surface staining (figs 1b, c). In areas in which there were multilayers of reactive mesothelial cells these were usually negative (fig 2b), although the occasional exfoliated mesothelial cell was positive with these five antibodies. Two cases had clefts lined by reactive mesothelial cells that were strongly positive (figs 3a, b). Staining for HMFG2 was similar to that seen for E29 and Ca1 in all cases of benign mesothelium.

Anti-CEA did not stain any reactive mesothelial cells.

MESOTHELIOMA

Antibody KL1 (anticytokeratin) stained all seven

cases of mesothelioma examined. Most of the tumour cells were positive in each case, showing strong cytoplasmic staining (fig 4a).

The antiepithelial membrane antigen (anti-EMA) antibodies E29 and HMFG2 showed essentially identical reaction patterns. In four cases the staining was patchy and weak on solid lumps of tumour cells (fig 4b), but where clefts lined by tumour cells were seen these generally gave strong luminal staining. In one case there was only very occasional staining of cells at the periphery of the tumour, and in the other two cases very weak staining of tumour cells was observed.

The three Ca antibodies showed similar staining reactions to the anti-EMA antibodies, although Ca1 was generally weaker. Anti-CEA was negative on these seven cases of mesothelioma.

CARCINOMA

All four cases of adenocarcinoma gave strong positive reactions with the anticytokeratin antibody (KL1) and the anti-EMA antibodies. Staining was usually distributed evenly throughout the tumour. The three Ca antibodies stained all four cases of adenocarcinoma. The staining pattern varied from small foci of positive staining seen in one case to a uniform distribution of positive reaction. Anti-CEA stained three of the four adenocarcinomas, one with a focal staining pattern.

Squamous cell carcinomas showed strong cytoplasmic staining with the anticytokeratin antibody, with a patchy distribution in one case. One anti-EMA antibody (E29) showed positive staining of tumour cells in two cases while the other anti-EMA antibody (HMFG2) stained all four cases, although in two cases only occasional tumour cells were positive. The Ca antibodies showed focal staining of two squamous cell carcinomas. The other two were negative with Ca1 and Ca2, while one showed focal staining with Ca3. Anti-CEA stained three of the squamous cell carcinoma cases in a patchy staining pattern.

Oat cell carcinomas showed positive staining with the anticytokeratin and anti-EMA antibodies in four of five cases. The Ca antibodies were different in their staining reactions. Ca1 stained four cases of oat cell carcinoma, while Ca2 and Ca3 stained only two cases. All showed a focal or patchy staining pattern. Anti-CEA stained three cases of oat cell carcinoma; in two of these cases the staining was weak with a patchy distribution.

Carcinoid carcinomas were positive with the anticytokeratin antibody and negative with anti-EMA, Ca, and anti-CEA antibodies.

Discussion

The aim of this study was to evaluate the reactivity of

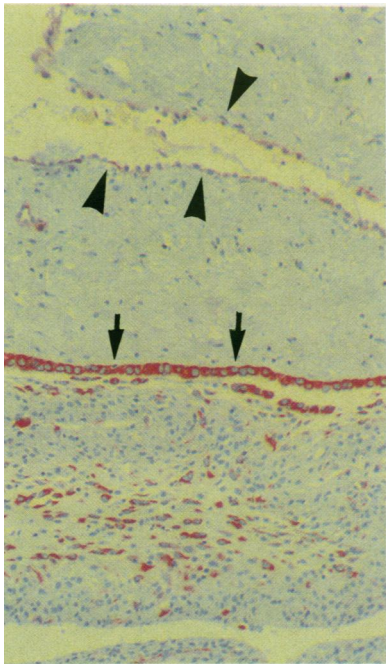


Fig 1a

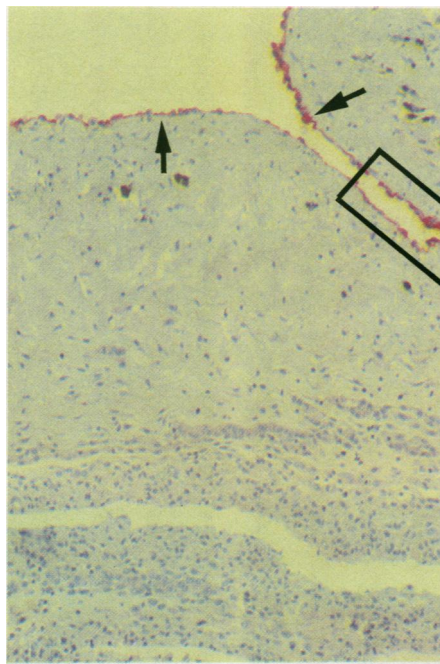


Fig 1b

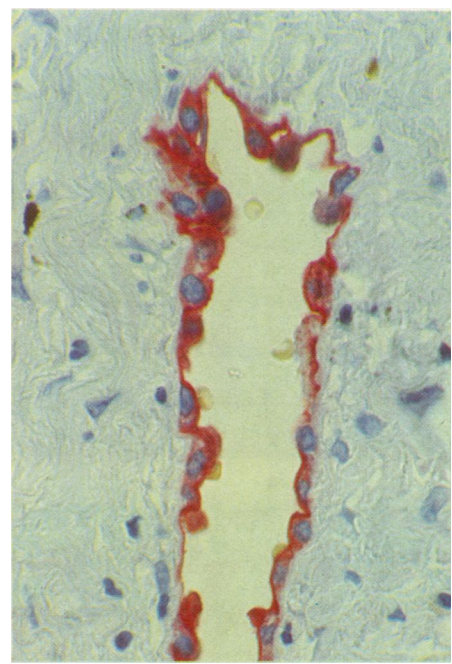


Fig 1c

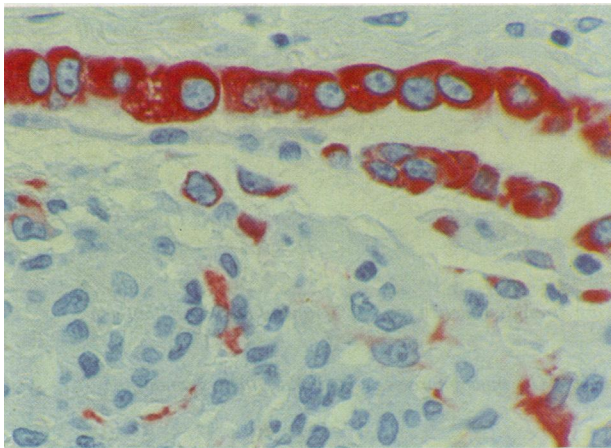


Fig 2a

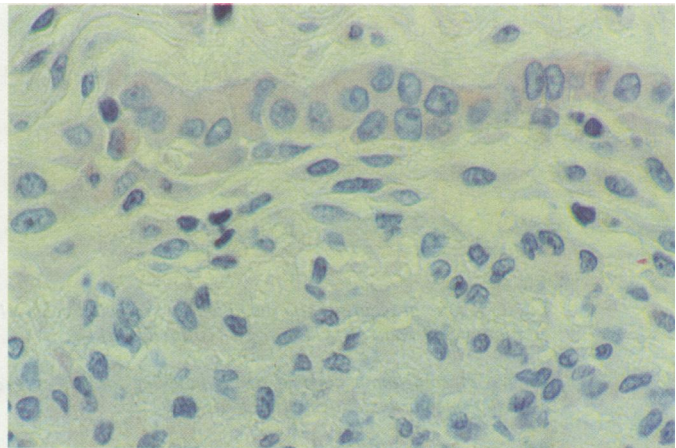


Fig 2b

Fig 1 Section of pleura stained for cytokeratin showing strong staining of plump cuboidal reactive mesothelial cells (open arrows) and scattered exfoliated cells (bottom of picture). Flatter overlying mesothelium (closed arrows) is more weakly stained.

Fig 1b Comparable section from same pleura specimen stained for epithelial membrane antigen (antibody E29). Line of plump mesothelial cells and cells exfoliating from this layer are negative (compare with those in fig 1a), but overlying flattened mesothelium (arrowed) is strongly stained.

Fig 1c High power view of area outlined in fig 1b.

Fig 2 Same biopsy specimen as seen in fig 1. High power view of reactions for cytokeratin (a) and epithelial membrane antigen (b) of plump reactive mesothelial cells seen in figs 1a and b.

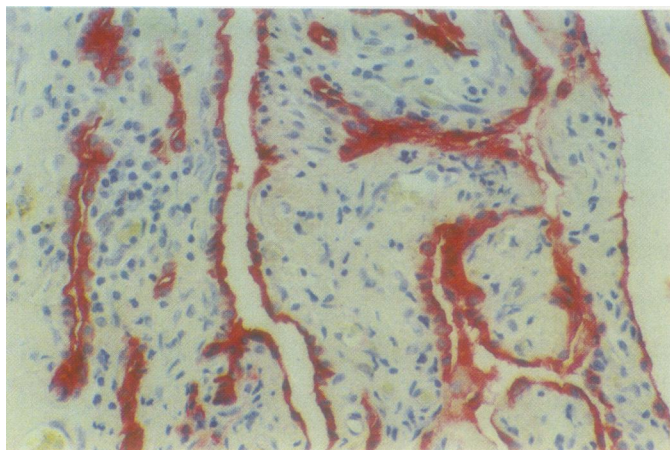


Fig 3a

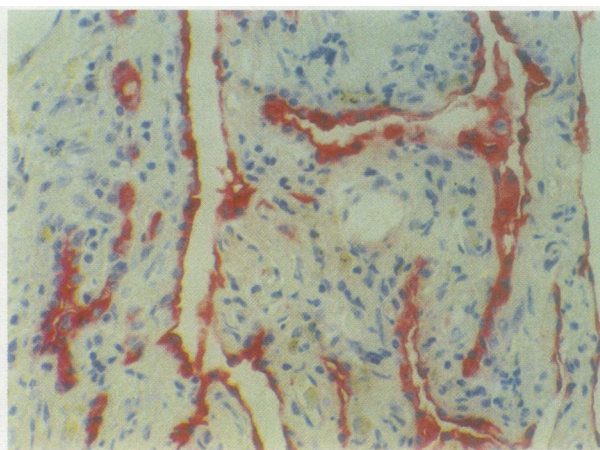


Fig 3b

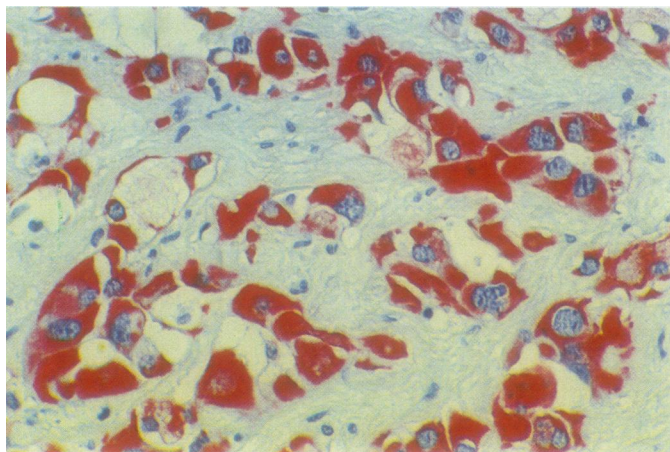


Fig 4a

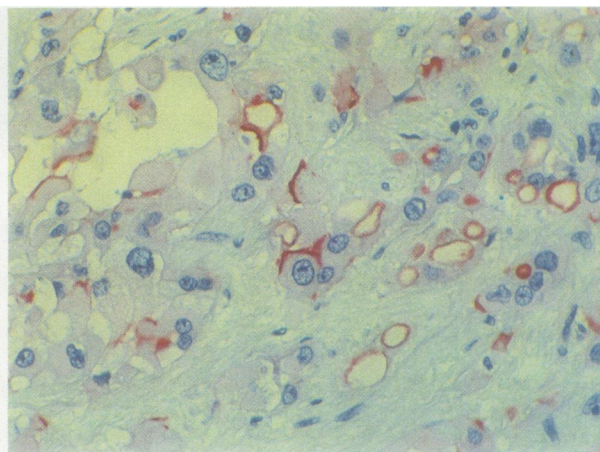


Fig 4b

Fig 3 Clefts in pleural tissue lined with mesothelial cells, which are strongly reactive for (a) epithelial membrane antigen (antibody E29) and (b) Ca antigen (antibody Ca1).

Fig 4 Mesothelioma showing strong labelling for (a) cytokeratin and (b) much more restricted and patchy staining for epithelial membrane antigen (antibody E29).

several different monoclonal antiepithelial antibodies on histological preparations of mesothelioma, benign mesothelial proliferation, and carcinoma and to assess their value in the differential diagnosis of these conditions.

Positive staining with the anticytokeratin antibody KL1 was observed in all cases of benign and malignant mesothelium and carcinoma. These findings are consistent with those of a previous study on exfoliated cells in serous fluids using another monoclonal anticytokeratin antibody of similar specificity.² Kahn *et al*¹⁴ also showed that keratin was present in benign and malignant mesothelial cells and carcinoma cells in

effusions. Previous studies on the diagnosis of these conditions in solid tumours using antikeratin antibodies have differed in their results. Corson and Pinkus,¹⁵ using a polyclonal antikeratin antibody, found that mesotheliomas were strongly positive but that adenocarcinomas were only weakly positive or negative. They suggested that this would be a useful marker to distinguish these two neoplasms. Holden and Churg¹⁶ and Loosli and Hurlimann,¹⁷ however, found keratins expressed in both adenocarcinomas and mesotheliomas.

Other workers have shown varying patterns of keratin expression in bronchial carcinomas.^{18,19} All of

Table 2 Immunohistological results of panel of monoclonal antibodies with benign mesothelium and mesothelioma

	No of cases	KL1	E29	HMFG2	Ca1	Ca2	Ca3	CEA
Benign mesothelium:	4	+	-	-	-	-	-	-
Benign mesothelium:	3	+	+	+	+	+	+	-
Mesothelioma:	7	+	+/-	+/-	+/-	+/-	+/-	-

+ = strong staining; +/- = weak or focal staining; - = no staining.

these studies used polyclonal antikeratin antibodies. As keratins are a group of proteins of different molecular weights the antisera used in these studies almost certainly had different specificities. Gatter *et al*⁶ have recently shown heterogeneous expression of keratins in lung tumours using different monoclonal antikeratin antibodies. The detection of cytokeratins in mesothelioma, benign mesothelium, and carcinomas limits the use of these reagents in their differential diagnosis, particularly in routinely fixed material where the number of suitable anticytokeratin antibodies is so limited.

Immunohistological studies with polyclonal antisera against keratins of different molecular weights have shown that mesotheliomas and benign mesothelium express a 63 kd keratin not detected in most lung carcinomas.²⁰ Such a difference in staining for this 63 kd keratin between mesothelioma and adenocarcinoma may have diagnostic application, and further work, particularly with specific monoclonal antikeratin antibodies, should be undertaken to clarify these results.

Both monoclonal antibodies (HMFG2 and E29) to the human milk fat globule membrane antigen (anti-EMA) reacted with all cases of mesothelioma. Three

of seven cases of reactive mesothelium, however, were strongly positive with these antibodies. Most carcinomas were positive with both antibodies, except two carcinoid tumours and one oat cell carcinoma.

Our previous experience with HMFG2 and E29 on cytological specimens showed strong staining of exfoliated mesothelioma cells. Reactions with exfoliated reactive mesothelial cells were seen in 14 of 22 benign cases stained with HMFG2, and occasionally, with E29, although not all mesothelial cells in any one sample were positive.^{2,8} Epenetos *et al*,⁴ on the other hand, using wet fixed cytological material, did not detect HMFG2 on benign mesothelial cells. Although these differences could be due to different techniques and fixatives used, a study comparing different staining and fixation methods on the same specimens showed that the HMFG2 determinant was occasionally expressed on reactive mesothelial cells.²¹

These previous reports indicated that mesothelial cells could express the HMFG2 determinant. The present study has shown the presence of HMFG2 in reactive mesothelium in three of seven cases. This is in contrast to the findings of Marshall *et al*,⁵ who observed focal staining with HMFG2 in only one of 13 cases of reactive mesothelium. These workers used

Table 3 Immunohistological results of panel of monoclonal antibodies with lung carcinoma

Case No	Cytokeratin	Epithelial membrane antigen		Ca antigen			CEA
		E29	HMFG2	Ca1	Ca2	Ca3	
Squamous cell carcinoma	1	+	+/-	+	+/-	+/-	+/-
	2	+	-	+/-	-	-	-
	3	+	-	+/-	-	-	+/-
	4	+	+	+	+/-	+/-	+/-
Adenocarcinoma	5	+	+	+	+	+	+
	6	+	+	+	+	+	-
	7	+	+	+	+/-	+/-	+/-
	8	+	+	+	+	+/-	+
Oat cell carcinoma	9	+	+	+	+/-	-	+/-
	10	-	-	-	-	-	-
	11	+	+	+	+/-	+/-	NT
	12	+	+	+	+/-	+/-	+
	13	+	+	+	+	-	+/-
Carcinoid	14	+	-	-	-	-	-
	15	+	-	-	-	-	-

+ = strong staining; +/- = weak or focal staining; - = no staining; NT = not tested.

an immunoperoxidase technique and trypsinised their tissue. Marshall *et al*⁵ detected the HMFG2 determinant in mesothelioma and lung carcinoma and suggested that positive staining with this antibody indicates malignancy, while negative staining is more likely to be benign rather than malignant mesothelium. Our findings, however, differ from this and indicate that a positive reaction cannot be used to distinguish a benign from malignant condition.

The three monoclonal antibodies directed against different determinants on the Ca antigen gave similar staining reactions and were positive with three of seven cases of reactive mesothelium, all the mesotheliomas, and most of the carcinomas. Positive reactions of these three antibodies with mesothelioma cells in cytological preparations have been noted. (AK Ghosh, unpublished observations).^{2 3 12} The reactions of the antibodies on benign mesothelial cells in cytological preparations have differed. Ca1 was weakly expressed on mesothelial cells in two of 47 benign effusions (combined data from two series).^{2 3} Ca2 was not detected on reactive mesothelial cells, while Ca3 was detected in benign mesothelial cells in eight of 23 cases.¹² These latter findings suggest that the antigenic determinant recognised by at least two of the Ca antibodies is variably present on mesothelial cells.

A previous immunohistological study of pleural biopsy specimens detected Ca1 in four of eight cases of benign mesothelium, seven of 12 cases of mesothelioma, and three of five cases of metastatic carcinoma.²² Our findings are consistent with these observations and indicate that these antibodies do not distinguish between malignant and benign mesothelium in histological sections.

CEA was absent in all cases of mesothelioma and reactive mesothelium, but was present in most cases of carcinoma, both here and in a previous study of 54 lung tumours, which included cryostat sections from the present cases.⁶ These findings agree with our observations on cytological samples where we found that CEA was absent from benign mesothelial cells, weakly expressed in one case of mesothelioma (subsequent cases of mesothelioma have been negative, AK Ghosh, unpublished observations), and present in 80% of carcinomas.²

Previous immunohistological studies of CEA in mesothelioma have given conflicting results, with some showing no staining^{5 23 24} and others positive staining.^{15 16} Different staining techniques, the use of trypsin, and differing polyclonal antisera could account for these differences. Whether or not the monoclonal or polyclonal antibodies react with non-specific crossreacting antigen may also be important (the monoclonal anti-CEA used here does not react).

There have only been three histological studies

using polyclonal CEA antisera on reactive mesothelium in which no staining was observed.^{5 15 24} Our results are in agreement with these findings.

The reactions of the monoclonal CEA in this study with lung carcinomas were similar to those observed by Gatter *et al*⁶ using the same reagent: most of these tumours showed positive staining. Other workers have detected CEA in lung tumours, particularly in bronchial adenocarcinomas.^{5 16 17 24}

The difference in CEA staining in mesotheliomas and carcinomas makes it a useful marker for differentiating these two neoplasms. A positive reaction with CEA suggests carcinoma and makes a diagnosis of mesothelioma unlikely. A distinction cannot be made (at least with the antibody used in this study), however, between mesothelioma and reactive mesothelium, or between the different histological types of lung carcinoma.

At present there are no reagents that can conclusively distinguish benign from malignant mesothelium. Although monoclonal antibodies to milk fat globule and Ca determinants can help to make this distinction in cytological preparations, our findings in histological preparations are less helpful. The location of positive staining or intensity does not help to make this distinction, as cases of benign mesothelium exhibited strong staining, especially in cleft like areas of cells. This was a feature also observed in mesothelioma. More specific markers for mesothelioma will therefore be required to facilitate their histological diagnosis.

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