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Histologic Distinction Between Malignant Mesothelioma, Benign Pleural Lesion and Carcinoma Metastasis

Evaluation of the Application of Morphometry Combined with Histochemistry and Immunostaining

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Summary. Thirty men and 7 women with malignant mesothelioma seen at the Free University Hospital from 1st January 1960 until 1st July 1981 were reviewed.

The histological, histochemical and morphometrical findings are reported. These findings are compared with 25 cases of pleural metastatic carcinoma and 25 cases of reactive pleural lesions.

Fourty-nine percent of malignant mesotheliomas produced hyaluronic acid, however all cases of pleural metastatic carcinomas failed to produce this substance. All cases of malignant mesothelioma were D-PAS negative while 15 cases of pleural metastatic carcinoma showed reactivity to D-PAS. All cases of malignant mesothelioma and 9 cases of metastases were CEA negative.

To distinguish malignant mesothelioma from metastases it is advisable to perform the D-PAS staining first. If it is negative mesothelioma can be confirmed by showing hyaluronic acid activity. A positive CEA staining rules out mesothelioma. In our study it was shown that with these methods 18 of 37 mesotheliomas could be identified with certainty, and 22 of the 25 carcinoma metastases.

Morphometrically the malignant mesotheliomas could not be distinguished from the metastases, however the reactive pleural lesions had smaller nuclei than the malignant cells with mean values below 30 mu^2. In the malignant cases these values had a range from 36 to 101 mu^2.

In distinguishing between reactive pleural lesions and malignant mesothelioma the production of hyaluronic acid points to the malignant character of the lesion.

Thus histochemistry and immunostaining are important in the distinction of malignant mesothelioma from metastases, while the value

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of morphometry lies mainly in the seperation of reactive lesions from malignant mesothelioma.

Key words: Mesothelioma – Malignant – Morphometry – Histochemistry

Introduction

The incidence of malignant mesothelioma has increased within recent years. This is reflected in the growing amount of related literature. In our institution there were 3 cases from 1960 to 1965, 5 cases from 1965 to 1970, 12 cases from 1970 to 1975, and 14 cases from 1975 to 1980 documented. It indicates that physicians are more frequently confronted with the problems of diagnosis of malignant mesothelioma.

The difficulty in diagnosis of malignant mesothelioma is compounded, above and beyond the usual problem of anaplasia, by its many variations on an underlying theme of dimorphism. The histologic classification of these tumors consists of three categories: epithelioid, fibrous (sarcomatous) and biphasic (mixed) (Enzinger 1969). Other forms are very rare but have been reported e.g. adenosquamous mesothelioma (Kwee et al. 1981). These various expressions are attributable to the multipotentiality of the mesothelial cell (Brandenburg 1952; Klemperer and Rabin 1937).

Another problem in the diagnosis of malignant mesothelioma is its differentiation from benign mesothelial proliferations (Kawai et al. 1981; Klima and Gyorkey, 1977). It was shown that in exfoliative cytology benign cells could be distinguished by their nuclear size and nuclear-cytoplasmic (N/C) ratio (Kwee et al. 1982). Therefore it seems reasonable to expect that morphometry can also be successfull in the histologic differentiation of benign from malignant lesions.

Moreover, in many cases it is difficult to distinguish malignant mesothelioma from carcinoma metastases in histological material (Kannerstein et al. 1977; Wang 1973). The role of histochemical staining in the diagnosis of malignant mesothelioma is known. Both mesotheliomas and metastatic adenocarcinomas are frequently positive on periodic acid-Schiff staining. In contrast to most adenocarcinomas, mesotheliomas become negative after diastase digestion (D-PAS negative) (Griffiths et al. 1980; Kannerstein et al. 1973). Hyaluronic acid production was shown in many cases of mesotheliomas (Griffiths et al. 1980; Harwood et al. 1976; Kannerstein et al. 1973), but is absent in cases of adenocarcinomas.

The purpose of the study reported here was to evaluate the application of morphometry in combination with immuno- and histochemistry in the differentiation of benign pleural lesions from malignant mesotheliomas on the one hand, and from carcinoma metastases on the other.

Materials and Methods

This study is based on an evaluation of autopsy materials obtained from the Free University Amsterdam. From January 1st 1960 to July 1st 1981: 5,261 autopsies were performed. The search of necropsy records revealed a total of 38 cases originally diagnosed as mesothelioma.

Table 1. Pleural metastatic carcinoma

No. of cases	Age at autopsy	Primary site	Diagnosis	Nuclear area mu^2	Standard deviation
1	72	Stomach	Poorly differentiated adenocarcinoma	75.11	29.54
2	52	Stomach	Poorly differentiated adenocarcinoma	64.19	28.74
3	61	Stomach	Tubular adenocarcinoma	85.47	19.72
4	71	Thyroid	Undifferentiated carcinoma, giant cell type	56.36	23.25
5	67	Lung	Bronchogenic adenocarcinoma, acinar type	44.29	12.30
6	81	Endometrium	Moderately differentiated adenocarcinoma	57.77	17.50
7		Endometrium	Moderately differentiated adenocarcinoma	63.97	25.84
8	68	Endometrium	Poorly differentiated adenocarcinoma	46.75	12.29
9	80	Lung	Moderately differentiated bronchogenic squamous cell carcinoma	49.29	19.48
10	58	Ovary	Papillary adenocarcinoma	45.01	16.78
11	58	Colon	Mucinous adenocarcinoma	41.44	14.41
12	58	Lung	Bronchogenic adenocarcinoma, acinar type with mucin	51.88	15.03
13	61	Ovary	Papillary cystadenocarcinoma	42.09	9.23
14	73	Lung	Bronchioloalveolar carcinoma	59.75	18.61
15	50	Lung	Poorly differentiated bronchogenic squamous cell carcinoma	67.84	20.92
16	65	Lung	Poorly differentiated bronchogenic squamous cell carcinoma	36.65	9.39
17	80	Lung	Bronchogenic adenocarcinoma, papillary type without mucin	42.27	11.47
18		Ovary	Papillary cystadeno-carcinoma	37.82	13.05
19	69	Ovary	Moderately differentiated mucinous cyst adenocarcinoma	49.95	19.10
20	65	Lung	Unclassified carcinoma	52.20	18.89
21	71	Lung	Bronchogenic adenocarcinoma, acinar type	57.23	20.68
22	82	Lung	Bronchogenic adenocarcinoma, papillary type	39.56	11.82
23	59	Lung	Bronchogenic adenocarcinoma, acinar type	80.17	25.57
24	52	Lung	Bronchogenic adenocarcinoma, acinar type	92.27	31.26
25	74	Lung	Bronchogenic adenocarcinoma, papillary type	89.85	19.33

Table 2. Twenty-five cases of benign (reactive) mesothelial lesions

No. of cases	Reaction to
4	Pleural adhesions
5	Bronchopneumonia
3	Pleural plaques
3	Metastasis
5	Pulmonary oedema
3	Pulmonary embolism
1	Emphysema
1	Pulmonary haemorrhage

These cases met the criteria for malignant mesothelioma as described by Kannerstein et al. (1977). Two cases originally diagnosed as mesothelioma were subsequently excluded since they were identified as pseudomesotheliomatous carcinomas of the lung (by their D-PAS positive staining etc.) (Harwood et al. 1976). One case was added since it was identified as an adenosquamous mesothelioma of the pleura (Kwee et al. 1981). Thus, a total of 37 cases of malignant mesothelioma were used for this study.

Twenty-five cases of pleural metastatic carcinoma (Table 1) and 25 cases of benign pleural lesions (Table 2) were taken from autopsy cases as controls.

The clinical records of all patients in this study were available for evaluation.

Morphometrical Studies

Formalin fixed (4%) routinely processed 6 μm thick H & E stained sections were used. The nuclear areas of 50 mesothelial cells were measured from each slide with a projection microscope (100×). The slides were projected on a graphic tablet (Bit Pad One, Summagraphics Corp.). In the cases of biphasic mesotheliomas only the nuclei of epithelioid cells were measured. In the group of pleural metastatic carcinomas only nuclei of carcinoma cells were measured. In the cases of benign pleural lesions the nuclei of mesothelial cells were measured.

Statistical Analysis

Statistical analysis of the mean nuclear area and standard deviation of the 50 measured cells was carried out with a part of the program STP on a PDP 11 Dec computer. The descriptive statistics of the parameters were computed for the three groups and Wilcoxon's nonparametric two sample test was used to establish significant differences. As a level of significance $p < 0.005$ (two sides) was adopted.

Histological Studies

In all instances histological preparations were studied by light microscopy. All sections were stained with H & E. Additional stains were performed with periodic acid-Schiff staining methods, both before (PAS) and after diastase digestion (D-PAS) (Culling 1974), and sections were stained for acid mucopolysaccharides with Alcian blue at pH 2.5. In addition, sections were incubated with hyaluronidase from Streptomyces hyalurolyticus (200 turbidity reducing units per 0.5 ml) in 0.02 M acetate buffer (pH 5.0) at 40° C for 2 h, to confirm hyaluronic acid activity (Yamada 1973).

Immunoperoxydase staining for carcinoembryonic antigen (CEA)-like material was performed using the peroxydase anti peroxydase (PAP) method. Deparaffinized sections were incubated sequentially with the appropriate dilution of (1) normal swine serum (2) rabbit anti-CEA serum or normal rabbit serum (control) (3) swine anti-rabbit IgG serum (4) PAP complex (rabbit) (5) 3,3′ diaminobenzidine and hydrogen peroxide solution. All antisera were purchased from DAKO (Denmark). Prior to use, the anti-CEA serum was absorbed with normal human tissue (liver, gastric mucosa) and ultrasonicated leucocytes (to exclude staining of NCA), ac-

cording to Nap et al. (1982). CEA staining was always interpreted by comparing the reaction of the test to the adjacent control section.

In cases with positive CEA staining a further control was obtained by incubation of sections with anti-CEA serum which was absorbed with tissue from a colon carcinoma with proven high content of CEA.

Results

Clinical Records

Thirty-seven cases of malignant mesothelioma were included. The primary tumor was pleural in 32 patients, peritoneal in 4 patients and pericardial in 1. The average age at first presentation was 65 years (range 40–81 years). The peak-age incidence occurred in the 7th decade (Fig. 1). Dyspnea and thoracic pain were the most frequent primary symptoms of pleural malignant mesotheliomas. Radiological changes were relatively nonspecific as described (Solomon 1981) before the era of CT. The median survival time in our series was about 11.5 months after first presentation (range 1–54 months). For the epithelioid mesotheliomas the median survival was 14 months ($n=9$), for the biphasic 11 months ($n=23$) and for the fibrous 7 months ($n=3$). Two cases were incidentally found at autopsy.

Nine of 37 cases of malignant mesothelioma had clinical documentation of asbestos exposure.

The data of 25 cases with benign mesothelial proliferation are shown in Table 2.

Morphometrical Findings

The descriptive statistics and the results of Wilcoxon's non-parametric two sample test were calculated from the morphometrically assessed features of the nuclear area of mesothelioma, metastatic carcinoma and benign mesothelial cells (Table 3). The differences between the mean nuclear areas and standard deviation thereof of the malignant mesothelioma cells and

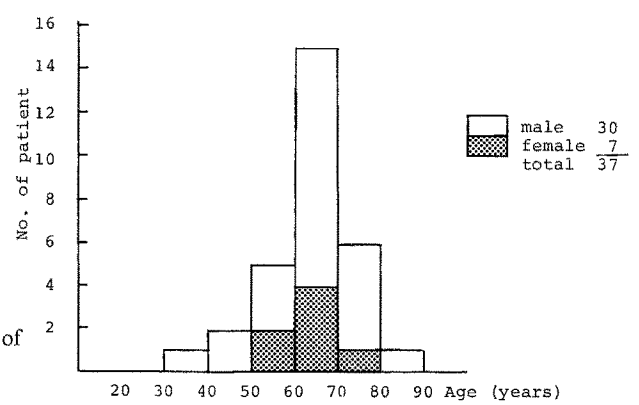

Fig. 1. Age and sex incidence of malignant mesothelioma

Table 3. Descriptive statistics

Diagnosis	Mean nuclear area mu^2	Mean standard deviation of the nuclear area
Malignant mesothelioma	59.3	18.3
Metastatic carcinoma	57.1	18.5
Benign mesothelial lesion	23.7	8.17

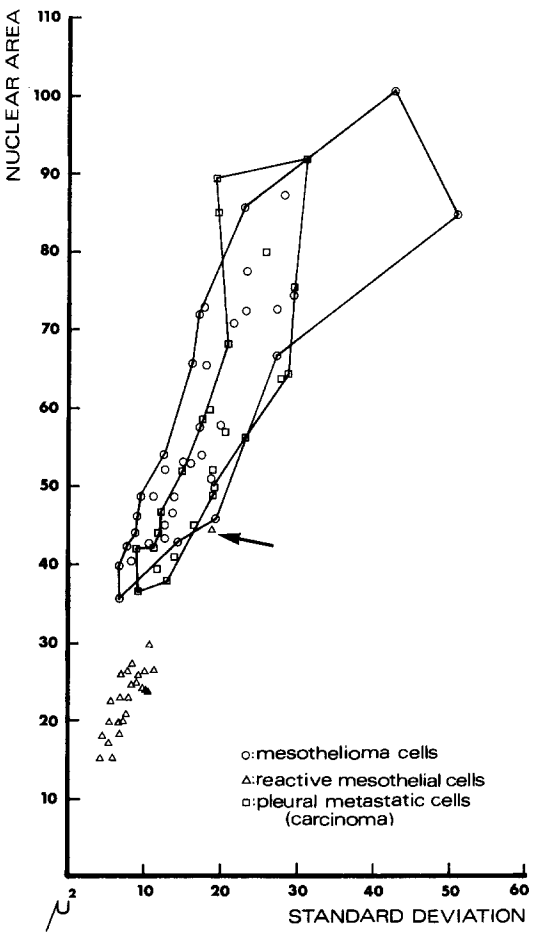

Fig. 2. Scattergram of all cases. A case of early malignant change in a pleural lesion (*arrow*) is in the cluster of malignant groups. (see also Figs. 6 and 7)

the benign mesothelial cells were highly significant ($p \leqq 0.00001$). There were no significant differences between the nuclei of malignant mesothelial cells and carcinoma cells.

All but one of the reactive cases had a mean nuclear area below 30 $m\mu^2$ (range 15.11–29.91 $m\mu^2$). One case had a mean nuclear area of 44.55 $m\mu^2$. The group of reactive mesothelial lesions showed a smaller standard deviation (4.39–18.85) than those of the malignant group (6.93–50.85).

Table 4. Histochemical and CEA findings

Diagnosis	No. of cases	D-PAS		Hyaluronic acid		CEA	
		+	−	+	−	+	−
Malignant mesothelioma	37	0	37	18	19	0	37
Metastatic carcinoma	25	15	10	0	25	16	9
Benign mesothelial lesion	25	0	25	0	25	0	25

The mean nuclear area and standard deviation of each case is plotted in a scattergram (Fig. 2). The one case of "reactive mesothelial proliferation" falling in the cluster of malignant mesotheliomas will be discussed further.

Morphometrically, there were no significant differences between epithelioid, biphasic and fibrous mesotheliomas. However, the peritoneal mesotheliomas ($n=4$) had significantly smaller nuclei, with a range between 42 $m\mu^2$ and 46 $m\mu^2$ ($p=0.03$). There was no correlation between the morphometric findings and the prognosis.

Histological Studies

The histological appearance included the whole range of microscopic patterns. In our series pleural mesotheliomas were classified as mixed type (21), as epithelioid type (8) and fibrous mesothelioma (3 cases). Two of the peritoneal mesotheliomas were of the mixed type and 2 cases were of the epithelioid type. The one pericardial mesothelioma was of the mixed type.

The hyaluronic acid activity was considered positive when the Alcian blue staining was diminished or abolished after hyaluronidase digestion. The results of this staining procedure are listed in Table 4. All pleural metastases and all benign mesothelial lesions were negative while 18 of 37 cases of malignant mesothelioma were positive (Fig. 3).

D-PAS positive cases were considered positive in those cases in which the PAS staining remained positive after diastase digestion. All benign and malignant mesothelial lesions were D-PAS negative, and also 10 of the 25 carcinoma cases (Fig. 4).

The CEA staining was considered positive when brown granular particles were found in the cytoplasm, when the control slide was negative. All benign mesothelial proliferations and malignant mesotheliomas were CEA negative, and 9 of the 25 carcinoma cases (Fig. 5). There were no significant differences in the histochemical- and CEA findings between the fibrous, biphasic and epithelioid mesotheliomas.

From these results it is clear that positive hyaluronic acid confirmation excludes carcinoma. In addition, D-PAS and CEA staining are the important discriminators between mesothelioma and carcinoma, since all mesothe-

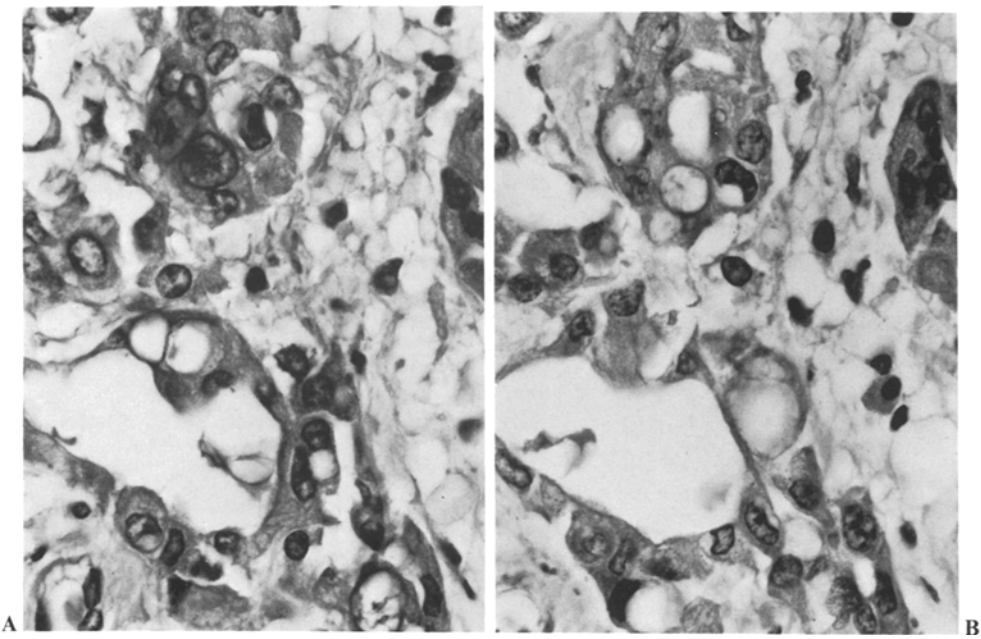

Fig. 3. (A) Alcian blue stained section at pH 2.5. **(B)** Alcian blue stained section at pH 2.5 after incubation with hyaluronidase; the blue color has disappeared as no Alcian blue positive staining can be seen anymore. × 528

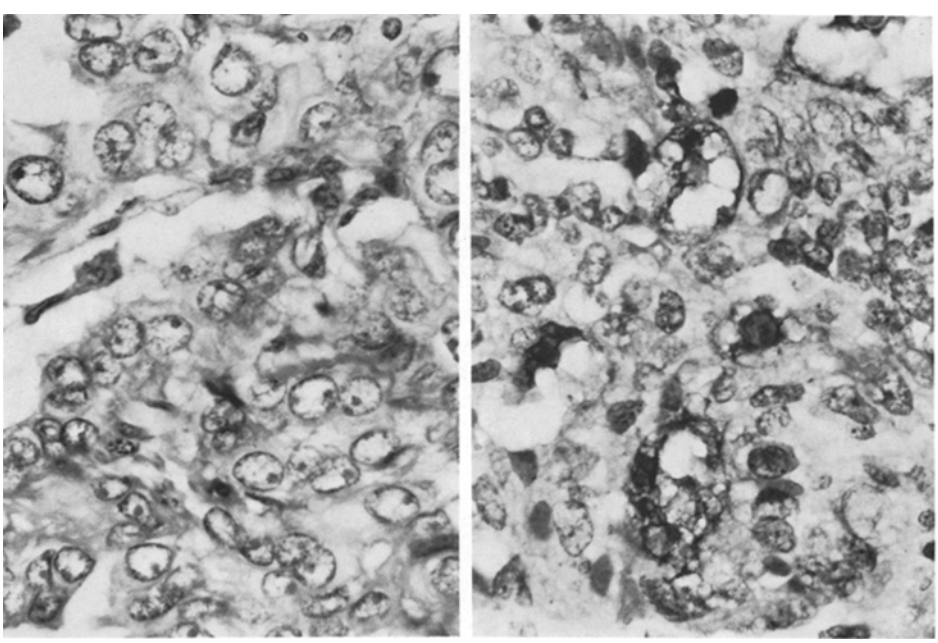

Fig. 4. Malignant mesothelioma with D-PAS negative staining and carcinoma metastasis with D-PAS positive staining. × 528

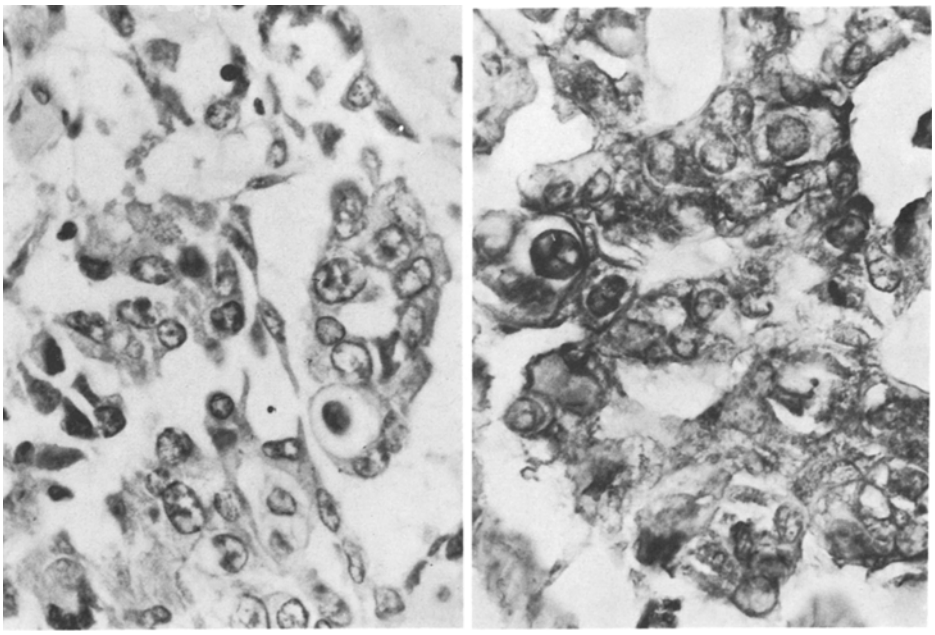

Fig. 5. Malignant mesothelioma: CEA negative. Carcinoma metastasis: Intense staining of the cytoplasm. Immunoperoxydase staining of CEA × 528

liomas are negative with these methods. The CEA positive cases of carcinoma (16 cases) became negative after absorbtion with CEA positive colon carcinoma. In table V are listed those carcinoma cases which were either D-PAS negative or CEA negative. Used in combination, these stains pointed to carcinoma in all but three cases.

Discussion

The histologic diagnosis of mesothelioma is difficult, bigining with the distinction of malignant from benign mesothelial lesions (Kawai et al. 1981; Klima and Gyorkey 1977). Our morphometrical study shows that malignant mesothelioma cells have larger nuclei and a large standard deviation. All but one case of histologically benign proliferations could be separated from the malignant mesotheliomas on the basis of mean nuclear area (Fig. 2). The case of "benign" proliferation falling into the cluster of malignant mesotheliomas is illustrated in Figs. 6 and 7. The patient was an 81 year-old man with asbestosis and unclassified carcinoma of the right bronchus. He was employed for 17 years from the age of 48 in a shipyard. This patient was admitted to the hospital because of dyspnea. Radiologically a left sided pneumothorax and pleural plaque were evident. Examination of lung tissue according to Smith and Naylor (1972) disclosed about 1,800 ferruginous bodies/g wet lung. This case is compatible with the case reported by Lewinsohn (1974), microscopic malignant mesothelioma in pleural plaque due

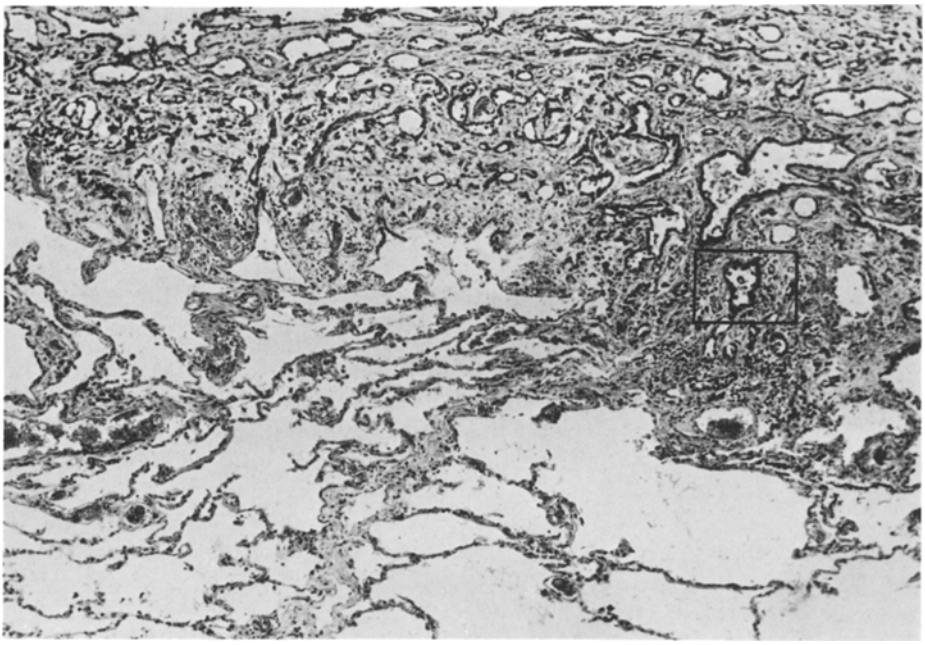

Fig. 6. Pleural lesion with proliferation of mesothelial cells. (*Inset:* Fig. 7). H+E ×52.8

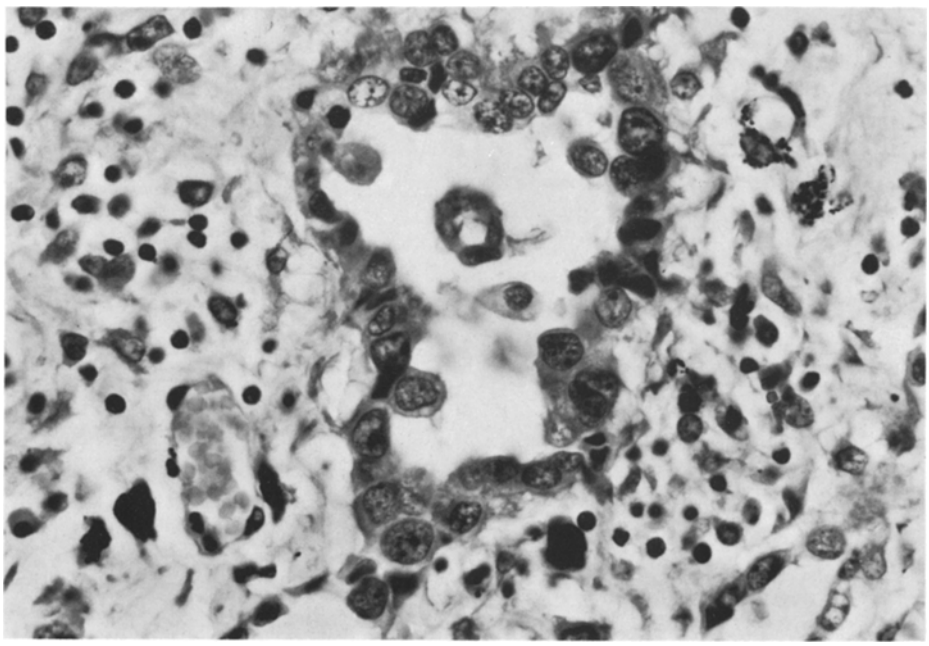

Fig. 7. Higher magnification of Fig. 6 (*Inset*). A microscopic malignant mesothelioma in a pleural lesion. (see Fig. 2 and Discussion). H+E ×528

to asbestos exposure. Another case of simultaneous lung carcinoma and malignant mesothelioma complicating asbestosis is described by Okumura et al. (1980).

Another method to identify malignant mesothelioma is the confirmation of hyaluronic acid in the mesothelial cells (Kannerstein et al. 1973). All the benign mesothelial lesions in our series were negative, whilst 18 of 37 malignant mesotheliomas were positive (Table 3). However, this stain is not helpful in histologic distinction of malignant mesothelioma from synovial sarcoma. Synovial sarcoma shows the same histological picture: spindle-cell, epithelioid, and biphasic with or without formation of hyaluronic acid (Katenkamp and Stiller 1980). However most synovial sarcomas occur in young adults and usually arise from tissues in the vicinity of large joints.

The fibrous type of malignant mesotheliomas does not have specific morphologic features to separate it from fibrosarcoma. Fibrosarcoma is indeed rare in the pleura. A positive result confirming hyaluronic acid production is sometimes conclusive. The ultrastructural features vary. If multiple tissue sections are examined, some typical mesothelial cells can be found from tissue blocks (Kay and Silverberg 1971), also in the material used for ultrastructural studies. In such cases characteristic ultrastructural features of mesothelioma can be distinguished in these epithelioid cells (Davis 1974; Klima and Gyorkey 1977; Stoebner et al. 1979; Suzuki et al. 1976; Wang 1973). The use of anti-mesothelial cell serum in an indirect immunofluorescence assay is described by Singh et al. (1979) and might also help in the establishment of the mesothelial differentiation of the tumor.

There was neither relation between the prognosis of the patient and the morphometrical data of the malignant mesothelioma cells, nor between these and the histologic differentiation. The only striking fact was that the group of peritoneal mesotheliomas ($n=4$) had significantly smaller nuclei ($p=0.03$). These data are in accordance with the cytologic findings of a morphometrical difference between primary peritoneal and pleural mesotheliomas (Boon et al. 1982). Especially in exfoliative cytology, the relatively small nuclei and ample cytoplasm of the malignant mesothelial cells of primary peritoneal mesothelioma can result in a "benign" appearance (Boon et al. 1981) and in delayed diagnosis.

Another problem in the histologic diagnosis of malignant mesothelioma is its distinction from carcinoma metastasis. Morphometry is of no value, because malignant mesotheliomas and carcinomas fall within the same cluster (Fig. 2). All but three carcinomas could be separated from the malignant mesotheliomas (Tables 4 and 5), if D-PAS and CEA staining were performed, in combination with hyaluronic acid confirmation. The diagnostic properties of the D-PAS were of the same magnitude as the CEA staining (respectively 15 and 16 positives in the 25 carcinomas cases, Table 4). The 16 CEA positive cases of carcinoma metastasis became negative after absorbtion with CEA positive colon carcinoma. This implies that CEA of carcinoma metastasis is indeed related to CEA of colon carcinoma. A negative CEA staining result in mesothelioma cases was also observed by Wang

Table 5. Sixteen cases of pleural metastatic carcinoma with either D-PAS or CEA negative staining results

Site of primary carcinoma	D-PAS	CEA
Stomach	−	+
Stomach	−	+
Thyroid	−	+
Endometrium	+	−
Endometrium	−	+
Lung	−	+
Ovary	+	−
Colon	+	−
Ovary	+	−
Lung	−	−[a]
Lung	−	+
Lung	−	−[a]
Ovary	−	−[a]
Ovary	+	−
Lung	−	+
Lung	+	−

[a] Three cases were as well D-PAS as CEA negative (not identified as metastases with these staining methods)

et al. (1979). There were no significant differences in the staining pattern of the fibrous, biphasic and epithelioid malignant mesotheliomas.

In the study presented in this paper it is shown that morphometry and D-PAS, CEA staining and hyaluronic acid confirmation used in combination can be helpful in the histologic diagnosis of malignant mesothelioma.

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References

Boon ME, Posthuma HS, Ruiter DJ, Andel v JG (1981) Secreting Peritoneal Mesothelioma. Virchows Arch [Pathol Anat] 392:33–44

Boon ME, Kwee WS, Alons CL, Morawetz F, Veldhuizen RW (1982) Discrimination between primary pleural and primary peritoneal mesotheliomas by morphometry and analysis of the vacuolization pattern of the exfoliated mesothelial cells. Acta Cytol 26:103–108

Brandenburg W (1952) Die Multipotenz des Mesothels. Gustav Fischer-Verlag VEB, Jena

Culling CFA (1974) Handbook of histopathological and histochemical techniques. Butterworths & Co Ltd, London, p 268

Davis JMG (1974) Ultrastructure of human mesotheliomas. J Natl Cancer Inst 52:1715–1725

Enzinger FM (1969) Histological typing of soft tissue tomours. International Histological Classification of Tumours. No 3. World Health Organization. Geneva

Griffiths MH, Riddell RJ, Xipell JM (1980) Malignant mesothelioma: a review of 35 cases with diagnosis and prognosis. Pathology 12:591–603

Harwood TR, Gracey DR, Yokoo H (1976) Pseudomesotheliomatous carcinoma of the lung. Am J Clin Pathol 65:159–167

Kannerstein M, Churg J, Magner D (1973) Histochemistry in the diagnosis of malignant mesothelioma. Ann Clin Lab Sci 3:207–211

Kannerstein M, McCaughey WTE, Churg J (1977) A critique of the criteria for the diagnosis of diffuse malignant mesothelioma. MT Sinai J Med NY 44:485–494

Katenkamp D, Stiller D (1980) Synovial sarcoma of the abdominal wall. Virchows Arch [Pathol Anat] 388:349–360

Kawai T, Suzuki M, Kageyama K (1981) Reactive Mesothelial Cell and Mesothelioma of the Pleura. Virchows Arch [Pathol Anat] 393:251–263

Kay S, Silverberg SG (1971) Ultrastructural studies of malignant fibrous mesothelioma of the pleura. Arch Pathol 92:449–455

Klemperer P, Rabin CB (1937) Primary neoplasms of the pleura. A report of five cases. Arch Pathol 11:385–412

Klima M, Gyorkey F (1977) Benign pleural lesions and malignant mesothelioma. Virchows Arch [Pathol Anat] 376:181–193

Kwee WS, Veldhuizen RW, Golding RP, Donner R (1981) Primary "Adenosquamous" mesothelioma of the pleura. Virchows Arch A Pathol Anat and Histol 393:353–357

Kwee WS, Veldhuizen RW, Alons CL, Morawetz F, Boon ME (1982) Quantitative and qualitative differences between benign and malignant mesothelial cells in pleural fluid. Acta Cytol 26:401–406

Lewinsohn HC (1974) Early malignant changes in pleural plaques due to asbestos exposure: A case report. Br J Dis Chest 68:121–127

Nap M, Hoor ten K, Fleuren GJ (1982) Cross reactivity with normal antigen in commercial anti-CEA sera used for immunohistology. The need of tissue controls and absorption. Am J Clin Pathol (in press)

Okumura T, Okada M, Tsuji M, Inoue A, Ochiai Y (1980) Mesothelioma with lung cancer complicating asbestosis. Acta Pathol Jpn 30:579–590

Singh G, Whiteside TL, Dekker A (1979) Immunodiagnosis of mesothelioma use of antimesothelial cell serum in an indirect immunofluorescence assay. Cancer 43:2288–2296

Smith MJ, Naylor B (1972) A method for extracting ferruginous bodies from sputum and pulmonary tissue. Am J Clin Pathol 58:250–254

Solomon A (1981) The radiology of asbestos-related diseases with special reference to diffuse mesothelioma. Semin Oncol 8:290–301

Stoebner P, Bernaudin JF, Nebut M, Basset F (1979) Contribution of electron microscopy to the diagnosis of pleural mesothelioma. Ann NY Acad Sci 330:751–760

Suzuki Y, Churg J, Kannerstein M (1976) Ultrastructure of human malignant diffuse mesothelioma. Am J Pathol 85:241–262

Wang NS (1973) Electron microscopy in the diagnosis of pleural mesotheliomas. Cancer 31:1046–1053

Wang NS, Huang SN, Gold P (1979) Absence of carcinoembryonic antigen-like material in mesothelioma. Cancer 44:937–943

Yamada K (1973) The effect of digestion with streptomyces hyaluronidase upon certain histochemical reactions of hyaluronic acid-containing tissue. J Histochem Cytochem 21:794–803

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