

## Invited Review

# Benign multicystic mesothelial proliferation of the peritoneum: Immunohistochemical and electron microscopical study of a case and review of the literature

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**Summary.** We report a case of benign multicystic mesothelial proliferation (the so-called multicystic peritoneal mesothelioma) arising multifocally in the abdomen of a 46-year-old white man. His anamnesis showed an 8-year history of intermittent pain in the right lower abdominal quadrant. Mucin stains, immunohistochemistry, and electron microscopy confirmed the mesothelial origin of the lesion. Review of the available literature allowed us to find another 85 reported cases of benign multicystic mesothelial proliferations of the peritoneum. Out of these cases, eighteen only occurred in men, the majority being reported in middle-aged women mostly with complaints of abdominal pain. Electron microscopy or immunohistochemistry are needed to make a differential diagnosis towards other multicystic lesions, such as peritoneal cystic lymphangioma. Although multicystic mesothelial proliferations of the peritoneum have often been regarded as benign neoplasms, the true nature — neoplastic or hyperplastic — of these lesions still remains greatly elusive. Therefore, we believe that the unbinding term benign multicystic mesothelial proliferation (first used with regard to the unique hitherto reported case arisen in the pleural cavity) should be considered at present more appropriate to indicate even these peritoneal lesions.

**Key words:** Benign multicystic mesothelial proliferations, Peritoneum, Peritoneal neoplasms

### Introduction

Multicystic peritoneal mesothelioma is a very rare and often massive lesion arising from the visceral

and parietal peritoneum of the abdominal cavity and pelvis. It presents unevenly-distributed multicystic masses composed of numerous thin-walled and transparent cavities from a few millimetres up to many centimetres in diameter. Its histological structure is that of one or more variously-sized, rounded or irregularly-shaped cystic spaces separated by varying amounts of connective tissue and lined by a monolayer of cuboidal or flattened mesothelial cells. Although this unusual lesion of the peritoneum has been occasionally described in the past and correctly interpreted as arising from the mesothelium (Plaut, 1928; Lord, 1947; Rhind and Wright, 1949; Krieger et al., 1952; Hinshaw and Phil, 1957; Lascano et al., 1960; Jacobson, 1974), only in recent years has it been clearly defined as a clinicopathological entity and its mesothelial origin confirmed by means of immunohistochemistry and electron microscopy (Mennemayer and Smith, 1979; Moore et al., 1980; Dumke et al., 1983; Schneider et al., 1983; Philip and Reilly, 1984; Toubanc et al., 1985; Miles et al., 1986; Sienkowsky et al., 1986; Alvarez-Fernández et al., 1989). Multicystic peritoneal mesothelioma is usually discovered owing to vague lower abdominal pain or symptoms suggesting partial intestinal obstruction. Some tumors have been, however, disclosed initially as coincidental findings (Hinshaw and Phil, 1957; Lascano et al., 1960; Jacobson, 1974; Dumke et al., 1983). Even if this lesion may develop potentially in all serosal cavities, it has been described in the past in the abdomen only. As a matter of fact, its detection in the pleural is only cavity very recent (Ball et al., 1990). Multicystic peritoneal mesothelioma affects mostly adults, especially middle-aged Caucasian women.

To date, eighteen cases have been described occurring in men, to the best of our knowledge. Because of the rarity of this finding, we report here on

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the clinical, pathological, immunohistochemical, and electron microscopical features of a male patient with benign multicystic mesothelial proliferation observed in our Department of Pathology and Surgery. The lesion involved both visceral and parietal peritoneum with a massive growth which needed to be differentiated from other benign and malignant lesions of the peritoneum. This differential diagnosis may be problematic, at frozen section.

### **Materials and methods**

A 46-year-old white man presented an 8-year history of intermittent pain localized in the right lower quadrant of the abdomen. His medical history included about 20 years smoking of 25 cigarettes a day and a work exposure to silicon dust for 20 years at least. No history of exposure to asbestos was obtained and no family members are known to have had asbestosis or malignant mesothelioma. Physical examination showed a soft, tender, ill-defined mass filling the entire right upper and lower quadrants of the abdomen. Laboratory findings were within normal limits. Ultrasonography and CT scan confirmed the presence in the upper and lower abdominal quadrants of a large well-defined multicystic mass adhering to anterior parietal peritoneum and extending down into the right pelvis above the bladder. The right kidney, the liver and the spleen were normal. At laparotomy, a very large multicystic mass was found occupying the right peritoneal cavity. The mass was attached to parietal peritoneum, cecum and right colon. Other cysts — from a few millimetres up to 4-5 cm in diameter — were found to be isolated or arranged in rows and scattered on loops of small intestine, sigmoid colon, greater omentum and epiploic appendages. No cystic lesions were noted in the liver, spleen, stomach and bladder. The main tumor was excised as completely as possible together with the omentum and a wide sheet of parietal peritoneum. No recurrence was present at two years follow-up. The excised tumor was fixed in 4% formaldehyde solution immediately after receipt from surgery, embedded in paraffin and cut into 3  $\mu$ m to 5  $\mu$ m thick sections. For light microscopical study, standard histological stainings were performed: hematoxylin-eosin, periodic acid-Schiff (with and without diastase predigestion), Masson trichrome, and Alcian-blue stain (with and without hyaluronidase predigestion). Additional sections of the lesion were also examined immunohistochemically by commercially available rabbit polyclonal and mouse monoclonal antibodies to the following antigens: carcinoembryonic antigen (polyclonal, 1:2000, Dakopatts, Denmark) epithelial membrane antigen (monoclonal, 1:40, Dakopatts, Denmark), cytokeratin Cam 5.2 which reacts with cytokeratins of 39, 40 and 50 Kd (monoclonal, 1:10, Becton-Dickinson, California, USA), and alpha-smooth muscle specific actin (monoclonal, 1:200, Sclavo, Italy). All immunohistochemical stains were

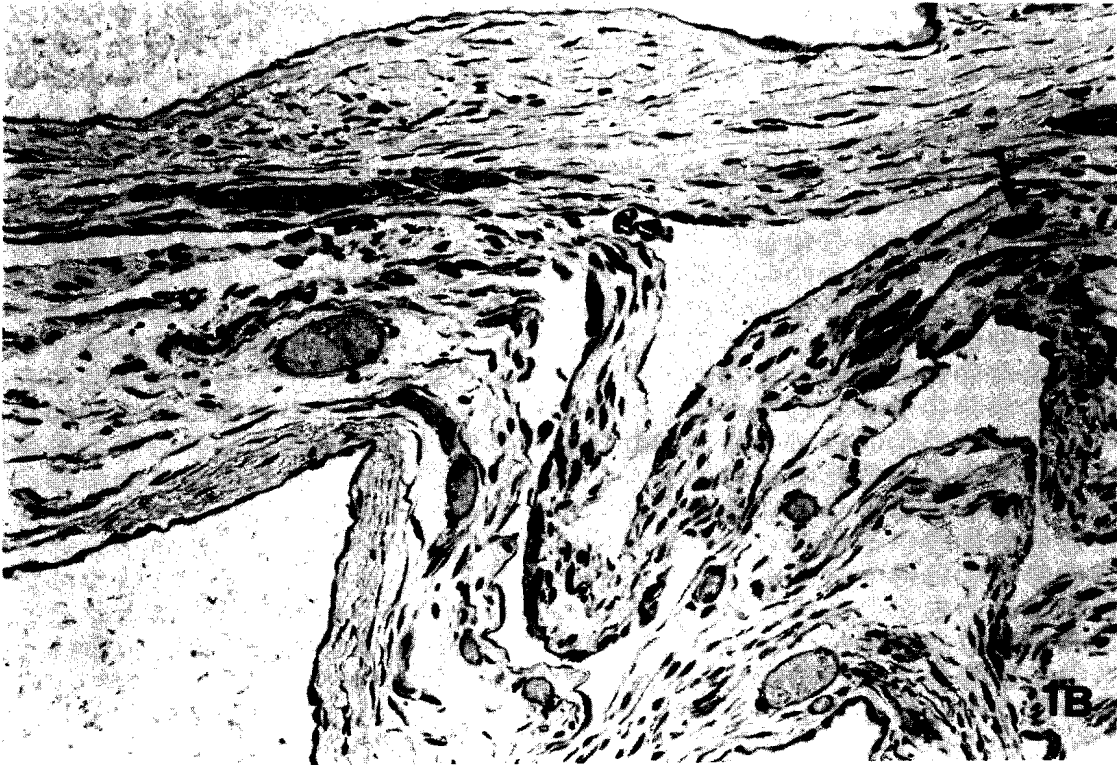
performed by using the three-step avidin-biotin peroxidase method. Tissue for electron microscopy was taken from the formalin-fixed specimens, repeatedly washed in phosphate buffer, fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in epoxy resin (Epon 812 - Araldite 502). Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 10 electron microscope. Three sputum specimens and approximately 1 g of wet formalin-fixed tissue, which included cystic structures and surrounding stroma, were digested in 15 mL of a 1:10 solution of sodium hypochlorite (containing 7% active chlorine) and distilled water, filtered onto cellulose membrane filters and examined by light microscopy for asbestos bodies and by phase-contrast microscopy for asbestos fibres, as elsewhere described (Pelosi et al., 1990). Transmission electron microscopy was also employed for searching in tissue samples for asbestos bodies and fibres.

### **Results**

Grossly, the main tumoral mass measured 30 x 10 x 10 cm and weighed 1350 g. The cut surface exposed multiple fluid-filled cysts containing clear and viscous fluid. The cysts varied in diameter from a few millimetres up to 5 centimetres and were surrounded by scant stroma. Microscopic examination showed numerous, variously-sized, rounded or irregularly-shaped cystic cavities lined by a single layer of uniformly flattened to cuboidal cells. No mitoses were present. Uncommonly, the cells were plump and protruded hobnail-like into the luminal spaces or herniated in the lumen with a papillary pattern (Fig. 1A). Cysts contained faintly granular secreted material in which cells occasionally floated. This material stained with Alcian-blue, but not with periodic acid-Schiff; it was sensible to hyaluronidase. Cysts were separated by varying amounts of loose connective tissue containing capillaries, plump fibroblasts and a scant inflammatory infiltrate composed of lymphocytes, plasma cells and macrophages. Some clusters of mature lymphocytes were present, chiefly in perivascular arrangement. Rounded to spindle-shaped cells resembling those lining the cysts, but supplied with more abundant acidophilic cytoplasm, could be identified in the stroma of the cystic walls. These cells were isolated or arranged in short fascicles and sometimes appeared multinucleate (Fig. 1B). Occasional foci of stromal hyalinization were seen next to cystic cavities. A strong cytoplasmic labelling for low molecular weight cytokeratins was revealed in the cyst epithelium, as well as in the rounded and fusiform cells of the stroma with features of mesothelial elements (Fig. 2A). Immunostainings for CEA and Factor VIII related antigen in the cyst epithelium appeared negative (Fig. 2B). Epithelial membrane antigen labelled the surface of the mesothelial cells coating cystic cavities clearly, but poorly the stromal elements strongly positive for cytokeratins. Some



**Fig. 1A.** Benign multicystic mesothelial proliferation. Numerous cystic cavities are coated with a regular, flattened to cuboidal epithelial layer of mesothelial type, sometimes herniating in the lumen with a papillary pattern (arrow). Cysts contain faintly granular secreted material in which may occasional cells float. H.E.  $\times 100$



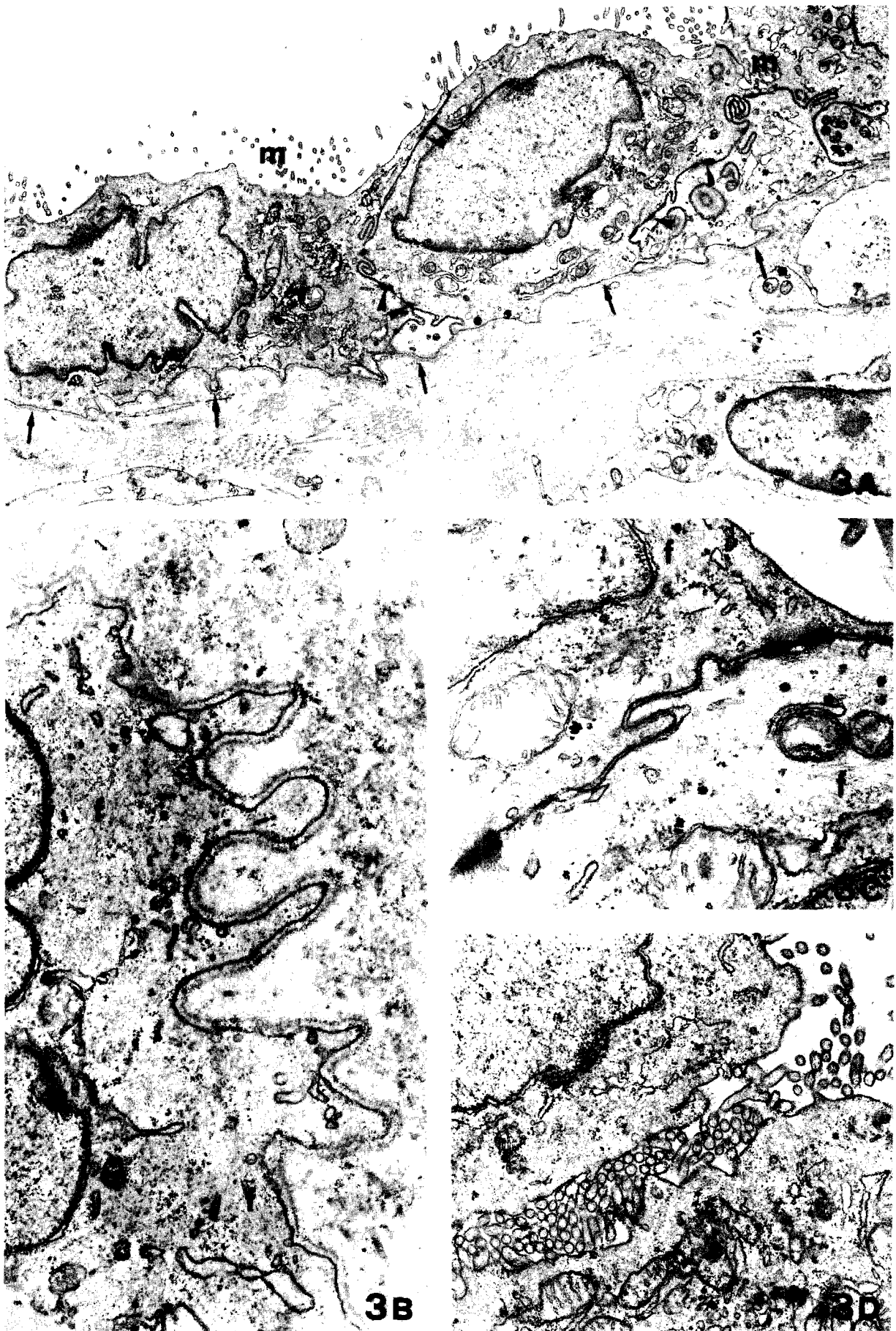
**Fig. 1B.** Benign multicystic mesothelial proliferation. On examination at higher power, the stroma of cyst wall shows vascular channels, fibroblasts and several rounded to spindle-shaped cells with more abundant acidophilic cytoplasm. A multinucleate cell is also seen (arrow). H.E.  $\times 250$

*Multicystic mesothelial proliferation*

**Fig. 2A.** Benign multicystic mesothelial proliferation. Immunoreactivity for low molecular weight cytokeratins (Cam 5.2) labels strongly all the cells lining the cystic cavities and many cells scattered in the connective stroma. ABC method.  $\times 100$



**Fig. 2B.** Benign multicystic mesothelial proliferation. The cyst epithelium shows a negative staining for Factor VIII-related antigen (the inner control of the vascular channels is strongly positive). ABC method.  $\times 100$



**Figs. 3A-D.** Ultrastructural appearance of tumor cells. **A.** The low magnification shows a monolayer of mesothelial cells with numerous slender microvilli (m), prominent intercellular junctions (arrowheads) and a continuous basal lamina (arrows),  $\times 4,500$ . **B, C and D.** High magnifications of basal membrane, desmosomes and microvilli, respectively. Note also scattered granules of glycogen and numerous bundles of microfilaments, some of which are indicated (f).  $\times 23,000$  (**B**),  $\times 27,500$  (**C**),  $\times 23,000$  (**D**)



### *Multicystic mesothelial proliferation*

spindle cells with long cytoplasmic processes and strongly labelled with alpha-smooth muscle specific actin were shown to lie around some cystic spaces. However, the stromal cells supplied with abundant eosinophilic cytoplasm and stained for cytokeratins were reliably unreactive for actin. Electron microscopical examination revealed a monolayer of cells on a well-developed intact basal lamina, with prominent intercellular junctions, numerous, slender microvilli, bundles of microfilaments and scattered granules of glycogen (Figs. 3A-D). Even the above mentioned stromal cells positive for cytokeratins had features of epithelial cells. Some of the spindle-shaped stromal cells exhibited nuclear indentations, microfilaments, dense bodies, pinocytotic vesicles and basal lamina, revealing their smooth-muscle differentiation. Fibroblastic nature of stromal cells was also found. No asbestos bodies or fibres were detected on cellulose membrane filters or seen by transmission electron microscopy in tissue samples.

#### **Discussion**

Benign multicystic mesothelial proliferations of the peritoneum (the so-called multicystic peritoneal mesothelioma) constitute a rare clinicopathological entity. We found in the available pertinent literature another 85 well-documented cases of this type of lesion (Plaut, 1928; Rhind and Wright, 1949; Krieger et al., 1952; Tedeschi and Helpfern, 1953; Hinshaw and Phil, 1957; Case records of the Massachusetts General Hospital, 1960, 1965; Lascano et al., 1960; Lesko et al., 1961; Jacobson, 1974; Loup, 1975; Mennemeyer and Smith, 1979; Moore et al., 1980; Blumberg and Murray, 1981; Carpenter et al., 1982; Dumke et al., 1983; Schneider et al., 1983; Nirodi et al., 1984; Philip and Reilly, 1984; Marhsall et al., 1985; Schneider and Zelnick, 1985; Toublanc et al., 1985; González-Crussi et al., 1986; Miles et al., 1986; Sienkowsky et al., 1986; Cregan et al., 1987; Gandolfi et al., 1987; Pastormerlo et al., 1987; Iversen et al., 1988; Raafat and Egan, 1988; Weiss and Tavassoli, 1988; Alvarez-Fernández et al., 1989; Baddoura and Varma, 1990). Their proportion to peritoneal malignant mesotheliomas is 1.6:10 (Plaus, 1988). Peritoneal multicystic mesothelial proliferations occur chiefly in women (67 cases, 77.9%) rather than in men (19 cases with ours, 22.1%). The average age of the women developing these lesions is 38 years as compared to 47 years for the men. Instances of these lesions have been reported, however, occurring in two children (Raafat and Egan, 1988; Weiss and Tavassoli, 1988) and in a newborn (González-Crussi et al., 1986). Clinically, the most frequent complaint is chronic pain followed by a mass or distention of the abdomen, pelvis or both. Some tumors have been disclosed however, as coincidental findings (Hinshaw and Phil, 1957; Lascano et al., 1960; Jacobson, 1974; Dumke et al., 1983). Characteristic intraoperative findings are those of thin-walled transparent cysts —up to several hundred— ranging in size from a few millimetres up to

many centimetres. Cysts contain watery clear or serous fluid in most patients. In three patients (Nirodi et al., 1984; Philip and Reilly, 1984; Sienkowsky et al., 1986), this fluid was described as jellylike or mucoid. Cysts are attached to serosal and subserosal layers of the parietal and visceral peritoneum without organ infiltration, or are apparently free and floating in the peritoneal cavity. Ascites (Miles et al., 1986), macroscopic haemorrhages and necrosis (Moore et al., 1980; Cregan et al., 1987) have rarely been seen. The reported weight of the lesions ranged from 130 g to 5,800 g.

Peritoneal multicystic mesothelial proliferations are thought to be uncommon, but their rarity could be only seeming because in the past these lesions have been confused clinically and pathologically with other cystic neoplasms of the peritoneum, such as lymphangioma. Therefore, the precise incidence and natural history of these lesions remain largely unknown. Clinical findings are often not specific (Lascano et al., 1960), and preoperative ultrasonography (Gandolfi et al., 1987) and CT (Schneider and Zelnick, 1985) enable one to make only a general diagnosis of benign multicystic disease of peritoneum, especially in instances of circumscribed lesions occurring in young patients. Recently, fine needle aspiration cytology has been employed successfully in identifying these lesions (Baddoura and Varma, 1990). Immunohistochemistry and/or electron microscopy are needed to establish the origin of these lesions, making a reliable differential diagnosis versus the variety of multicystic lesions of the abdomen (Mennemeyer and Smith, 1979; Moore et al., 1980; Foyle et al., 1981; Goepel, 1981; Carpenter et al., 1982; Katsube et al., 1982; Chen and Flam, 1986; McFadden and Clement, 1986; Miles et al., 1986; Dikersin, 1988; Enzinger and Weiss, 1988; Weiss and Tavassoli, 1988; Roismar et al., 1989; Baddoura and Varma, 1990). In the current case, both immunohistochemistry and electron microscopy confirmed a mesothelial origin of the surgically excised lesions. However, we think that these two techniques could be utilized successfully even on incisional biopsies by laparoscopy or cytologic material by fine needle aspiration biopsy. Positive immunoreaction for cytokeratins, as seen by us in some stromal cells, has been reported to be present in both benign multicystic mesothelial proliferations (Enzinger and Weiss, 1988) and in peritoneal inclusion cysts with mural mesothelial proliferation, sometimes mimicking an infiltrative pattern (McFadden and Clement, 1986). However, this latter feature is rather due to the entrapment of proliferating benign mesothelial cells in the fibrous tissue of the cyst septa, and it should not raise suspicion about malignant mesothelioma or metastatic adenocarcinoma. Adenomatoid changes and squamous metaplasia of the mesothelial cells —not disclosed in our case— have not infrequently been described in the lesions (Weiss and Tavassoli, 1988). The main differential diagnosis of the benign

multicystic mesothelial proliferations is lymphangioma of peritoneum (Katsube et al., 1982; Miles et al., 1986; Enzinger and Weiss, 1988). As a matter of fact, some benign multicystic mesothelial proliferations have probably been confused with lymphangiomas on the grounds of the histological details alone, even if detached clumps of redundant mesothelial cells or true papillae occasionally detected within the cystic spaces provide a reliable criterion for distinguishing these lesions from cystic lymphangioma. Distinction between these two benign lesions is of more than academic interest, since multicystic mesothelial proliferations have a greater tendency to recur (30-40% of cases), than lymphangiomas (Mennemeyer and Smith, 1979; Carpenter et al., 1982; Miles et al., 1986). Immunohistochemistry for cytokeratins and EMA enables one to rule out a peritoneal lymphangioma, since this latter is characterized by an endothelial lining reacting with Factor VIII-related antigen, always being unreactive for markers of epithelial differentiation. Even electron microscopy identified correctly these lesions (Mennemeyer and Smith, 1979; Moore et al., 1980; Carpenter et al., 1982; Miles et al., 1986; Roismar et al., 1989). The presence of lymphocyte aggregates and fascicles of smooth muscle cells in a benign multicystic neoplasm of the peritoneum are generally thought to be microscopic hallmarks of lymphangioma. However, this finding should also be considered in the diagnosis of benign multicystic mesothelial proliferations, in our opinion. In fact, we have documented both lymphocytic aggregates in the connective tissue stroma and long, spindle-shaped cells reactive for alpha-smooth muscle specific actin lying around some cystic spaces. Reactivity for alpha-smooth muscle actin is shared from both myofibroblasts and smooth muscle cells, as well as myoepithelial cells (Gugliotta et al., 1988). At present, the distinction of smooth muscle cells from myofibroblasts and myoepithelium relies mainly on ultrastructural criteria rather than immunohistochemical ones (Wargotz et al., 1987). In the current case, in the spindle-shaped cells positive for alpha-smooth muscle actin ultrastructural markers of smooth muscle cell differentiation were identified (Dikersin, 1988). The differential diagnosis of benign multicystic mesothelial proliferation further includes reactive mesothelial proliferations as seen commonly in longstanding peritoneal inflammations (Rosai and Dehner, 1975), peritoneal inclusion cysts with mural mesothelial proliferation (McFadden and Clement, 1986), benign papillary mesotheliomas (Foyle et al., 1981; Goepel, 1981), ovarian and extraovarian papillary neoplasms (Jacobson, 1974; Kannerstein et al., 1977; Foyle et al., 1981; Chen and Flam, 1986), endometriosis, endosalpingiosis and mesonephric remnants (Weiss and Tavassoli, 1988). Malignant mesotheliomas of the peritoneum or metastases of carcinomas rarely needed to be discriminated from benign multicystic mesothelial proliferations. A careful and critical evaluation of the histological,

histochemical, immunohistochemical and electron microscopical results enables one to exclude all these diagnoses in this and in previous cases.

As far as etiology of these lesions is concerned, it still remains unknown. It is well established that many inciting factors such as foreign fibres and dusts, inflammatory mediators, and mechanical injuries may act on mesothelial cells to produce hyperplastic and neoplastic changes. The activation and/or recruitment mechanism of the mesothelial cell proliferation in pathological conditions still remains elusive. Proliferation and inward migration of peripheral mesothelial cells, proliferation and metaplasia of underlying connective tissue cells, and surface attachment and differentiation of free-floating mononuclear cells have all been postulated. It is interesting to stress that identical multicystic and multifocal lesions have recently been disclosed in localities other than the peritoneum, such as the pleural cavity (Ball et al., 1990). Therefore, it is possible that common causative factors are responsible for these lesions of the serous cavities. Theoretically, asbestos might be one of these inciting factors, since it can easily reach both pleural and peritoneal mesothelium. As a matter of fact, an association between asbestos exposure and mesothelial neoplasms is frequently recognized with regard to malignant mesotheliomas (McDonald and McDonald, 1977; McDonald et al., 1989). However, unambiguous evidence of a similar causative association in benign multicystic proliferations has not been unquestionably proven to date (Mennemeyer, 1979; Sienkowsky et al., 1986). Recently, two cases have been reported as having a prior exposure to asbestos 20 or more years before diagnosis, but without recognizable asbestos in tissue samples of the lesion (Kjellevoid et al., 1986; Ball et al., 1990). In our case work history, physical examination, chest roentgenograms and pulmonary function tests enabled us to exclude asbestos exposure. Moreover, analysis of sputum specimens and peritoneal tumor failed to demonstrate presence of asbestos bodies and fibres, as well as of other foreign bodies such as silicon dusts. Unfortunately, lung tissue, which is more relevant for detecting asbestos fibres (McDonald et al., 1989; Pelosi et al., 1990) and silicon dusts, was unavailable for this study.

Multicystic mesothelial proliferations of the peritoneum have often been regarded as benign neoplasms (Mennemeyer and Smith, 1979; Miles et al., 1986) or low-grade malignant neoplasms (Alvarez-Fernández et al., 1989), even if their true nature — neoplastic or hyperplastic — still remains greatly debated. Some authors have also suggested that these lesions may represent developmental dysplasias (Iversen et al., 1988). A reactive hyperplastic nature is suggested by the frequent occurrence of these lesions in abdominal cavity of women with complaints of peritoneal irritation (pelvic inflammatory disease, endometriosis or previous abdominal surgery) in

*Multicystic mesothelial proliferation*

30-40% of cases (Mennemeyer and Smith, 1979; Miles et al., 1986). However, their tendency to locally spread and recur are better in keeping with the assumption that they represent benign mesotheliomas (Weiss and Tavassoli, 1988). The natural history is that of a «per se» benign lesion which may prove, however, to be difficult to eradicate owing to the multifocality of the lesions and their frequently small size which often precludes a complete resection by the surgeon and favours further spread and recurrence (30% of cases) (McFadden and Clement, 1986; Miles et al., 1986). Therefore, we think that a total omentectomy and often a wide excision of the parietal peritoneum should be performed to excise the tumor as completely as possible and minimize the risk of recurrence. To conclude, we think that these lesions should often be regarded as peritoneal inclusion cysts arising from the peritoneal reactive proliferations. The reason for their common recurrence is due to persistence of the original inciting factor(s) and/or the multifocality of the lesions. Whether these lesions represent part of a clinicopathological spectrum including simple localized mesothelial cysts on one hand and a diffuse, multifocal counterpart on the other, still remains to be proven unquestionably. Therefore, we think that the unbinding term benign multicystic mesothelial proliferations may be used to indicate these peculiar lesions, according to Ball et al. (1990).

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