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Multicystic mesothelial proliferation

Immunohistochemical, ultrastructural and DNA analysis of five cases

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Summary. We investigated the clinicopathological findings in five cases of multicystic mesothelial proliferation (MMP). All masses consisted of multiloculated cysts attached to pelvic organs and sometimes growing into the upper abdominal cavity. The cystic spaces were lined by flattened or cuboidal cells. The stroma showed fibrosis, oedema and chronic inflammation. Immunohistochemistry revealed strong positive staining for cytokeratin and epithelial membrane antigen, and focal positivity for vimentin and carcinoembryonic antigen. The endothelial markers were negative. Electron microscopy showed abundant surface microvilli and well-developed basal lamina. DNA analysis identified euploid cell populations in all cases. All but one case had a previous history of abdominal surgery. Despite the worrying appearance the clinical outcome was favourable in all cases; there was one recurrence. Clinical and pathological data support the hypothesis that MMP represent a reactive mesothelial proliferation and not a neoplastic process.

Key words: Multicystic mesothelial proliferation – Immunohistochemistry – Ultrastructure – DNA analysis

Introduction

Multicystic mesothelial proliferation (MMP) is an unusual mesothelial lesion, which has been given many names (Table 1). Whereas there is general agreement on the mesothelial origin of MMP, there is much debate on whether or not this lesion is a true neoplasm, or a reactive process (McFadden and Clement 1986; Weiss and Tavassoli 1988; Ross et al. 1989; Sternberg and Miles 1991).

We report here a histological, immunohistochemical, ultrastructural and DNA analysis carried out in five cases of MMP, in which the clinical course and the pathological findings strongly suggest a reactive nature despite the worrying gross appearance.

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Material and methods

Five cases of multicystic lesion of the peritoneum were selected from the surgical pathology files of the Institute of Pathology, II Faculty of Medicine and Surgery of Naples, Italy, for the period 1984–1990.

Each surgical specimen was fixed in 10% buffered formalin and embedded in paraffin for routine histopathological examination. Tissue sections of 5 μm were stained with haematoxylin and eosin, periodic acid-Schiff (PAS) before and after diastase digestion, alcian blue at pH 2.5 and colloidal iron before and after hyaluronidase, and with mucicarmine. For immunoperoxidase

Table 1: List of alternative names for MMP used in literature

Multicystic peritoneal mesothelioma	Mennemeyer and Smith	1979
	Kjellevold et al.	1986
	Weiss and Tavassoli	1988
	Alvarez-Fernandez et al.	1989
Cystic mesothelioma	Katsube et al.	1982
	Miles et al.	1986
	Sienkowski et al.	1986
Inflammatory cysts of the peritoneum	Lees	1978
Post-operative peritoneal cysts	Monafo and Golfarb	1963
	Gusman et al.	1986
Benign papillary peritoneal cystosis	Jacobson	1974
Infiltrating adenomatoid tumour	Jones and Donovan	1965
Multilocular peritoneal inclusion cysts	McFadden and Clement	1986
	Ross et al.	1989
	Sternberg and Miles	1991
Benign cystic mesothelioma	Moore et al.	1980
	Iversen et al.	1988
	Canty et al.	1990
	Hidvégi et al.	1991

Table 2. List of the primary monoclonal and polyclonal antisera with their corresponding dilutions

Cytokeratin (CAM 5.2)	1:100
Cytokeratin (AE 1.3)	1:100
Epithelial membrane antigen	1:20
Vimentin	1:100
Factor-VIII-related antigen	1:2500
Carcinoembryonic antigen	1:100
Ulex europaeus (UEA 1)	1:2000

study the avidin-biotin-peroxidase complex (ABC) method of Hsu et al. (1981), modified by the use of streptavidin-biotin-peroxidase complex, was applied. The primary monoclonal and polyclonal antisera used are listed in Table 2. Controls for specificity consisted of: (1) incubation of solutions with normal rabbit serum of equivalent dilution instead of the primary antiserum, and (2) sections of known positivity and negativity stained with each batch of slides.

For electron microscopy, formalin-fixed tissues were fixed in 2.5% glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a Zeiss EM 109 electron microscope.

DNA ploidy was evaluted by densitometry. We used sections stained according to the modified Feulgen procedure (Decosse and Aiello 1966) which involves acid hydrolysis in 5 n HCl for 1 h at room temperature.

The measurements were carried out using a Universal Micro Spectrophotometer 30 (UMSP 30, Zeiss) connected to a dual processor system, the IBAS 2000 (Image Basis Analysis System) by Kontron Electronics, Zeiss. We have described the technical procedure elsewhere (Zeppa et al. 1991). For the determination of the 2c value the integrated optical density (IOD) of 20 or 30 lymphocytes was measured in each slide. The IOD of 100–120 cells lining the cystic walls were then measured for each case. To evaluate the DNA ploidy of the lesions the mean IOD of the reference cells modified by a correction factor of 1.19 was used in each case to establish the 2c values. The histograms obtained were evaluated by Auer's classification (Auer et al. 1980).

All but one of the patients were women; the age ranged from 32 to 50 years (mean 39). Previous surgery was documented in four patients (case 1: appendectomy; case 2: appendectomy + colecystectomy; case 3: hysterectomy; case 4: oophorectomy). The most common symptom was intermittent low abdominal pain. Ascites was observed in two patients.

The follow-up time ranged from 14 to 71 months (mean 37). Only one of the five patients had an operatively documented recurrence (case 3, 9 months after surgery); all the patients were alive and well at the time of reporting. The clinical data are summarized in Table 3.

Results

All five cases, showed cystic spaces of different size and shape (Fig. 1) separated by a delicate fibrovascular stroma on light microscopy (Fig. 2). There was some invagination into omental tissue with expansive borders, without signs of true infiltration (Fig. 3a). The cysts were lined by a single layer of flattened endothelial-like (Fig. 3b) and occasionally cuboidal cells (Fig. 3c). Sometimes the cells showed a hobnail appearance (Fig. 3d) and occasionally a papillary-like structure with fibrovascular cores. Focally, in case 2, the epithelium lining the cysts was replaced by a layer of fibrin. In all cases, the cystic spaces were separated by septa containing varying degrees of infammatory infiltrate with a predominance of lymphocytes (Fig. 4), fibrosis, oedema and neovascular formation. Neither adenomatoid change of the mesothelium nor solid nodules of mesothelial cells were present. None of the cases showed mitotic activity. PAS, alcian blue and colloidal iron staining showed granular linear positivity at the cellular apices. Diastase pre-digestion abolished the PAS reaction, whereas hyaluronidase digestion partially removed colloid-iron and alcian blue staining. These findings suggest that the material examined contained hyaluronic acid and sulphated mucosubstances. Mucicarmine staining was negative. Immunoperoxidase staining of the cystic epithelial lining was positive for cytokeratin (CAM 5.2, AE 1.3) (Fig. 5a) and epithelial membrane antigen (EMA) (Fig. 5b). Vimentin and carcinoembryonic antigen were focally positive. All endothelial markers (factor VIII and *Ulex europaeus* lectin) were negative in the cells lining the cysts but positive in the vessels.

Electron microscopic findings in all five cases showed

Table 3. Summary of clinical data of five cases of multicystic mesothelial proliferations

Case	Sex	Age (years)	Location	Ascites	Dimension (cm)	Previous operation	Recurrence	Follow-up
1	M	33	Small bowel mesentery	+	32 × 31	Appendectomy 2 years before	0	AFD (at 71 months)
2	F	42	Pelvis	+	13 × 8	Appendectomy 12 years before Colecystectomy 2 years before	0	AFD (at 48 months)
3	F	50	Pelvis	–	20 × 12	Hysterectomie 4 years before	9 months	AFD (at 37 months)[a]
4	F	38	Pelvis and omentum	–	12 × 10	oophorectomy 3 years before	0	AFD (at 25 months)
5	F	32	Pelvis	–	11 × 8	–	0	AFD (at 14 months)

AFD, Alive, free of disease
[a] After recurrence

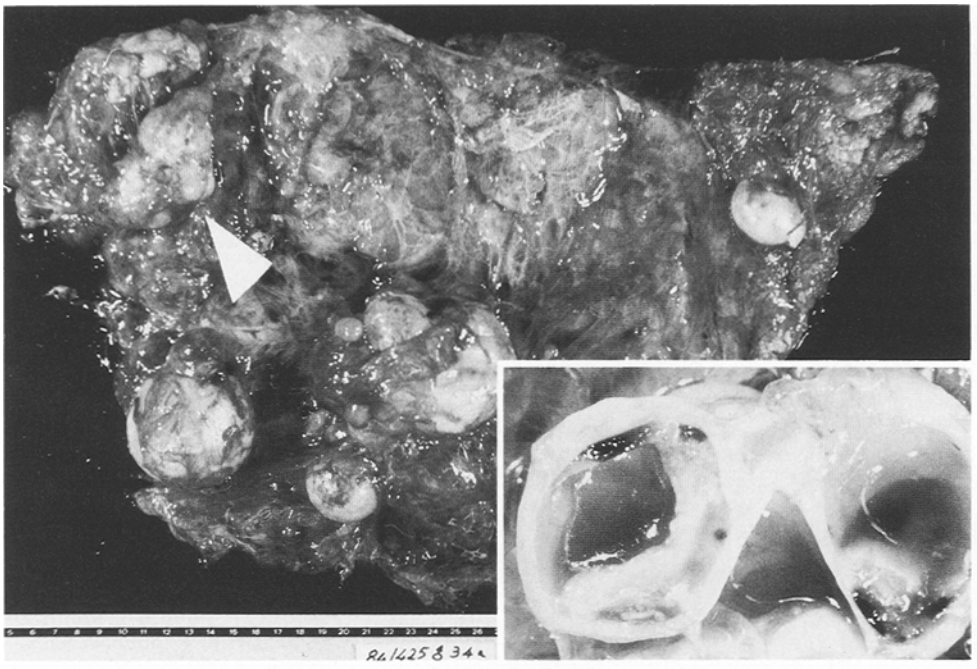

Fig. 1. Resected mesothelial multicystic mesothelioma showing multiple nodules (*arrow*). *Inset*: The cystic spaces are separated by fibrous septa

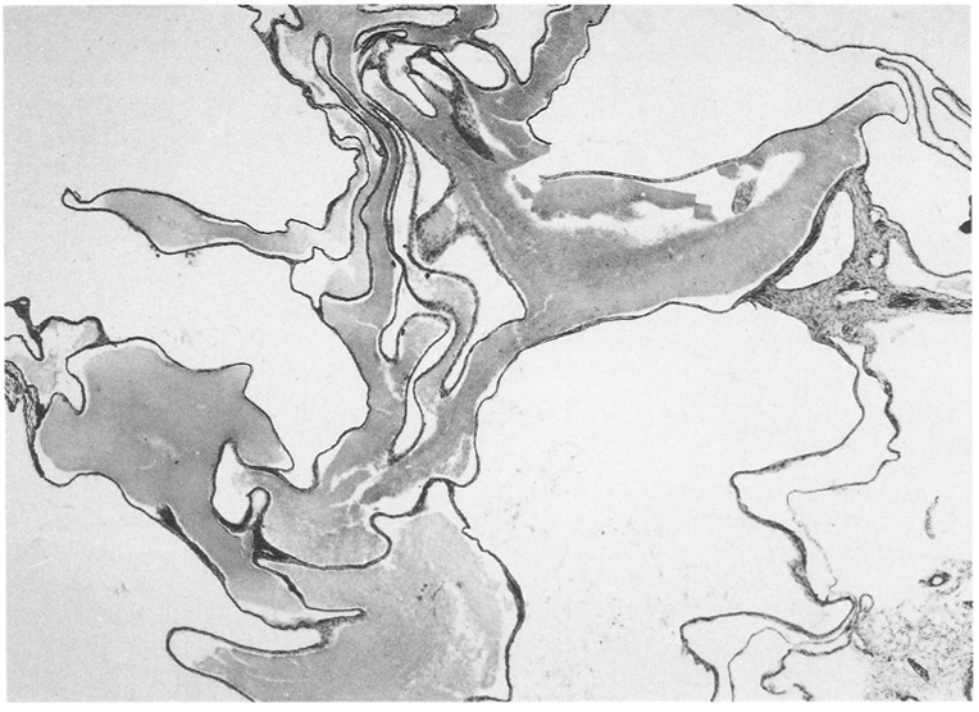

Fig. 2. Multicystic mesothelial proliferation of various sizes and shapes embedded in a delicate fibrovascular stroma. H & E, original magnification $\times 106$

similar features. Both coboidal and flattened cyst-lining cells were lying above a well-developed and intact basement membrane with focal reduplication. The cells showed well developed desmosomal junction complexes and perinuclear bundles of cytoplasmic intermediate filaments. Numerous prominent surface microvilli arranged in tufts were observed at the luminal border (Fig. 6). Abundant organelles such as a well-developed rough endoplasmic reticulum, mitochondria, and free ribosomes were observed. Nuclei were ovoid with a uniform chromatin pattern, and an occasional nucleolous. These findings were consistent with a mesothelial cell origin.

DNA ploidy histograms showed main peaks in the 2c region in all the lesions, small peaks in the 4c region, and a variable amount of cells in the 3c region (Fig. 7a). In two cases (cases 3 and 4) we observed very smalls peaks in the 5c region representing 2 or 3 cells (Fig. 7b). We considered these cells simply the expression of a slightly raised proliferation rate, and the corresponding histograms euploid. Therefore we classified all the cases

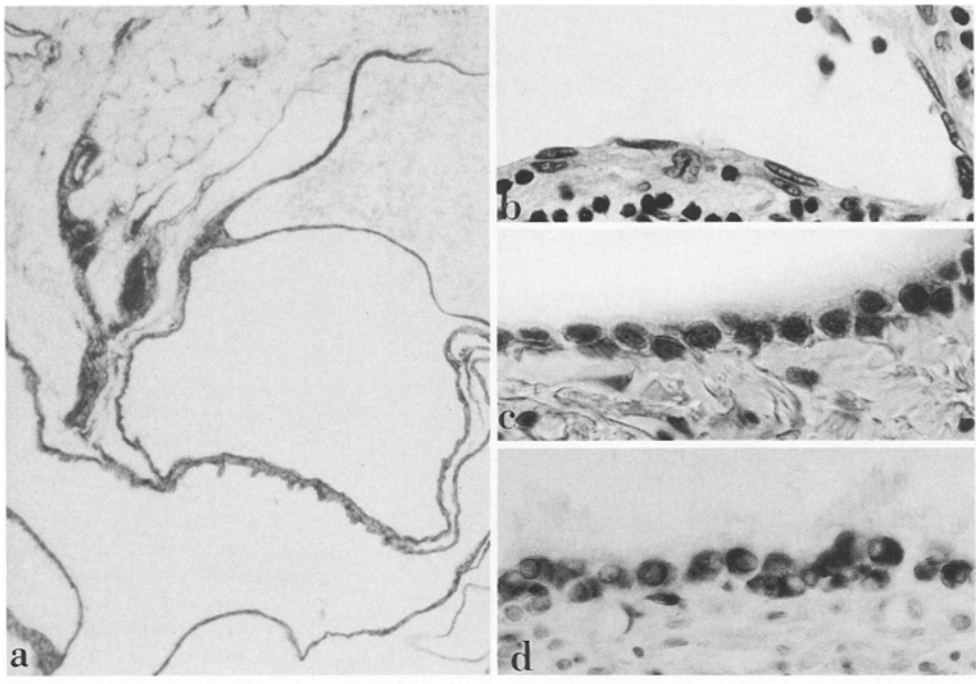

Fig. 3. a Cystic areas invagined into omental tissue. H & E, original magnification ×106. **b** Cysts lined by a single layer of flat, endothelial-like cells. H & E, original magnification ×400. **c** Cuboidal cells lining cystic areas. H & E, original magnification ×400. **d** Hobnail appearance of proliferating cells. H & E, original magnification ×400

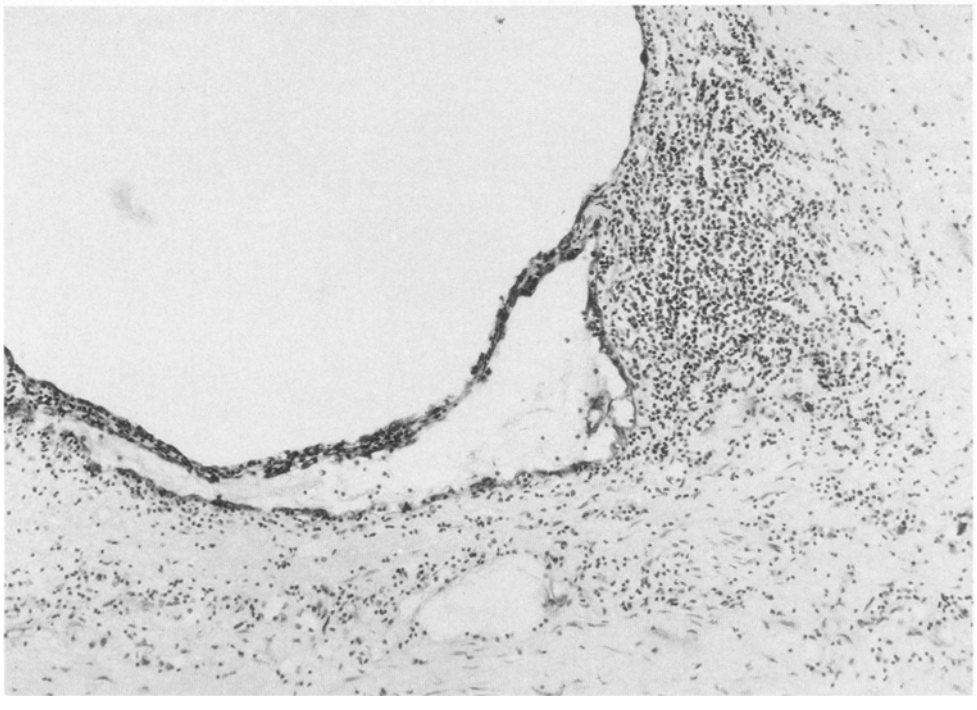

Fig. 4. Locules separated by septa containing varying degrees of inflammatory infiltrate with predominance of lymphocytes. H & E, original magnification ×260

as euploid and the corresponding histograms as Auer's type III (1980).

Discussion

In the peritoneum a large variety of cystic lesions can be found, most of which are lymphatic, while others are mesothelial in origin. To our knowledge Hamdi et al. (1927) and Plaut (1928) were the first authors to hypothesize that some of the peritoneal cysts are histogenetically derived from the mesothelium. These mesothelial cysts may be single or multiple and are subclassified into developmental cysts, unilocular inclusion cysts, free floating cysts and multilocular peritoneal inclusion cysts (Ross et al. 1989). As stated earlier, MMP has been given various names, and very few case series have been described in the literature (Mennemeyer and Smith 1979; Moore et al. 1980; Katsube et al. 1982; Kjellevold et al. 1986; Miles et al. 1986; Sienkowski et al. 1986; Alvarez-

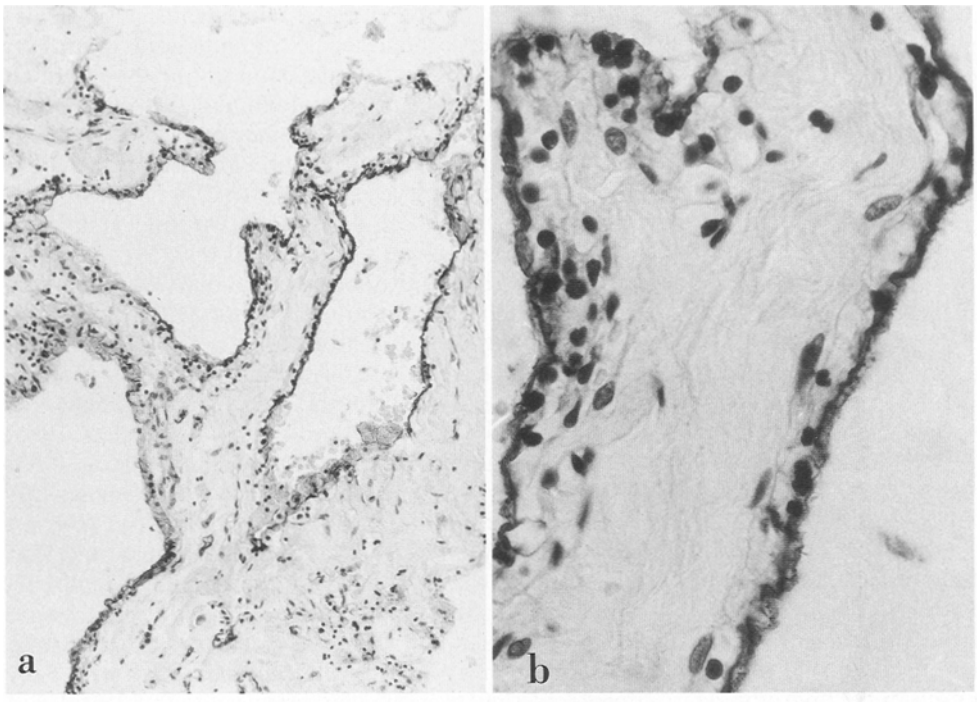

Fig. 5. Immunoperoxidase staining of the cystic epithelial lining was positive to **a** cytokeratin and **b** epithelial membrane antigen

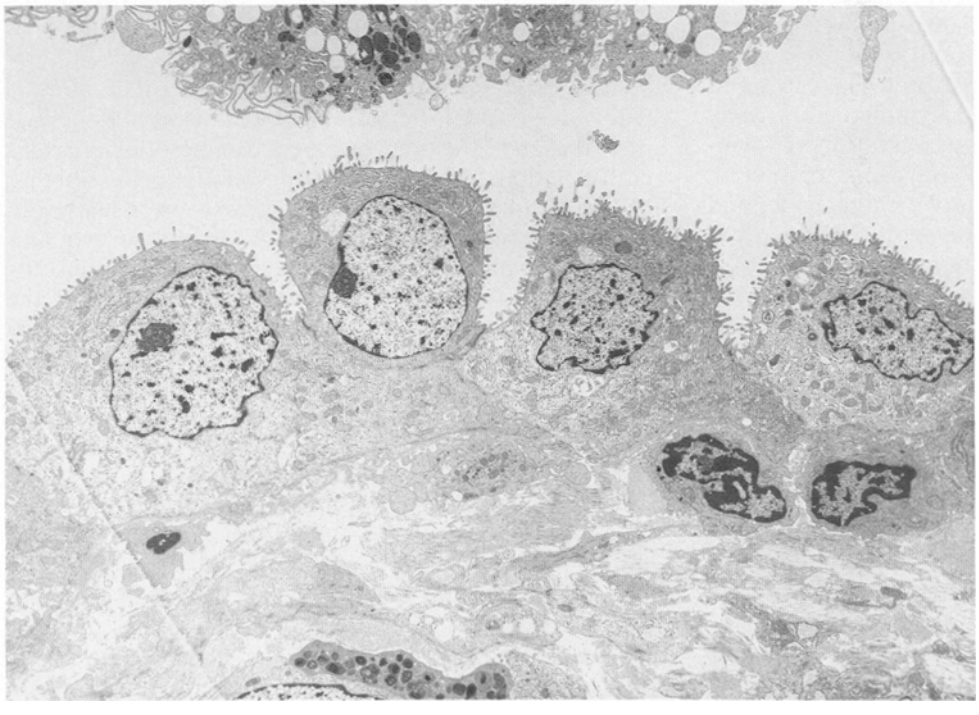

Fig. 6. Ultrastructural features of the cyst lining cells which show numerous microvilli along the luminal surface, desmosomal intercellular junctions, and basal lamina. ×1080

Fernandez et al. 1989; Canty et al. 1990; Hidvégi et al. 1991). Only Weiss and Tavassoli (1988) and Ross et al. (1989) have reported two large series of 37 and 25 cases respectively. It is quite probable that the true incidince of these lesions is underestimated because of the lack of agreement on the terminology and pathogenesis of the lesions.

Our clinical and histopathological findings were similar to those reported in the literature. In our experience and in that of others these lesions occur more frequently in the pelvis in young women (McFadden and Clement 1986; Ross et al. 1989; Sternberg and Miles 1991), and rarely occur in males (Sienkowski et al. 1986; Canty et al. 1990). Similar lesions have been described in the pleural cavity (Ball et al. 1990). MMP present as an abdominal mass, pain and, in two of our cases, with ascites, a finding which is very unusual in those cases reported in literature (Weiss and Tavassoli 1988; Ross et al. 1989). The symptoms are not specific and a laparotomy is always necessary. Multiloculated cystic areas with smooth

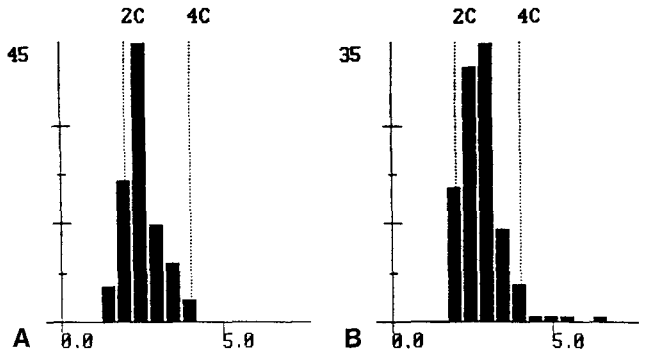

Fig. 7. Histograms of DNA ploidy of cases 2 (**a**) and 3 (**b**) are shown. In **a** a main peak in 2c region and smaller peaks in 3c and 4c regions are shown. In **b** main peaks in 2c, 3c, and 4c regions are shown; smaller peaks (corresponding to 3 cells) are represented in 5c region

surface, filled with clear or gelatinous fluid adherent to adjacent structures are constant features. The cystic areas vary in size from a few millimetres to several centimetres. MMP offer many differential diagnostic problems, and are often confused with multicystic abdominal lymphangioma, which, unlike MMP, occurs predominantly in male children younger than 5 years (Gonzales-Crussi et al. 1986) and are localized in the mesentery of the small intestine, omentum, mesocolon or retroperitoneum, always sparing the pelvis. Lymphangioma may contain chylous material on gross examination and lymphocyte-rich fluid at microscopy (Gonzales-Crussi et al. 1986; Ross et al 1989). Stromal lymphoid aggregates and smooth muscle bundles rarely present in MMP, are a constant feature in multicystic lymphangioma. The cells lining the cysts are similar in both lesions, but in MMP sometimes the cells may show a hobnail appearance, with tufts of proliferative cells and papillae which are never seen in lymphangioma. Immunohistochemical study of cells lining the cystic spaces of the lymphangiomas shows positivity for factor-VIII-related antigen and for *Ulex europaeus* lectin and cytokeratin and EMA are negative, unlike MMP. The ultrastructure of the lymphangiomas shows neither luminal microvilli projections nor desmosomal junctions, the typical features of MMP, and basal lamina is discontinuous (Mennemeyer and Smith 1979). Finally, the lymphangioma rarely recurs after excision. Other conditions that must be considered in the differential diagnosis include adenomatoid tumour, well-differentiated papillary mesothelioma, malignant mesothelioma, and ovarion tumours. The adenomatoid tumour is a benign neoplasm of the mesothelial cells chiefly involving the genital tract in both sexes. It is generally a small, asymptomatic mass, confined mainly to the uterus or the fallopian tubes in females and in the epididymis in males. Rarely does the adenomatoid tumour present a multicystic gross appearance (McCaughey et al. 1985; Sternberg and Miles 1991; De Rosa et al. 1992). There is an overlapping of microscopic features of these lesions, probably due to their common histogenesis. However, the presence of small areas of typical adenomatoid tumour and the gross appearance help in the differential diagnosis. The low-grade monophasic "epithelial" mesothelioma with cystic areas also presents tubulo-papillary areas absent in MMP, and lack the inflammatory and fibrous component common in MMP (McCaughey et al. 1985). Malignant mesothelioma is never entirely cystic on gross examination (Mennemeyer and Smith 1979) and histologically shows a high cellularity and cytological atypia; these latter findings are not observed in MMP. A papillary pattern, higher mitotic activity and an evident nuclear pleomorphism help to differentiate MMP from low-grade serous ovarian carcinomas.

The most controversial aspect of MMP is its biological behavior; different authors have regarded MMP as benign (Mennemeyer and Smith 1979; Miles et al. 1986) or as low-grade neoplasms (Weiss and Tavassoli 1988; Alvarez-Fernandez et al. 1989). Some authors have suggested that these are developmental dysplasias (Iversen et al. 1988). Weiss and Tavassoli (1988) suggested that the lesion is a true neoplasm and not a mesothelial reaction. Their hypothesis is based on the lesion progression seen in one case in their series resulting in death and the mixture of cystic and epithelial-like pattern. They considered that previous surgery was co-incidental and suggested that the lesions are a transitional form between true epithelial mesothelioma and adenomatoid tumour.

Our experience, in accordance with other authors (McFadden and Clement 1986; Ross et al. 1989), suggests that the MMP represent reactive mesothelial proliferative lesions. The inflammatory component, fibrosis, oedema and vascularity of the stroma are usually observed in reactive lesions and DNA analysis further supports this evaluation since all the cases are euploid. Furthermore in four of our five cases and in 60% of the Ross series (1989) there was a history of prior abdominal or pelvic surgery. The more frequent occurrence of these lesions in females suggests that pelvic inflammatory disease, endometriosis and microtrauma due to ovulation may have a role in the pathogenesis of some cases of MMP without a clinical history of previous surgery.

The prognosis in this disease is excellent; in fact, there is no example of disease-related death with the exception of the case reported by Weiss and Tavassoli (1988): a male patient died of local effects of the MMP (massive scrotal extension) after refusing any surgical therapy. The recurrence of MMP often reported in literature must be explained by an incomplete surgical resection due to the large size of the lesions; in fact no malignant degeneration or metastatic potential has ever been demonstrated (Katsube et al. 1982). Ross et al. (1989) have hypothesized that MMP belongs to the wide spectrum of reactive mesothelial proliferative lesions, which includes post-inflammatory mesothelial hyperplasia with adhesions, and those unilocular mesothelial cysts that occur elsewhere in the abdomen.

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