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## Mesothelial Inclusion Cysts (So-Called Benign Cystic Mesothelioma) – a Clinicopathological Analysis of Six Cases

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The report presents six cases of mesothelial inclusion cysts (MIC), detected in five females (22–53 years of age) and one male (47 years old). The lesions were unifocal (four cases) and multifocal (two cases), and were located on the surface of the peritoneum in the *cul de sac*, on the intestines, urinary bladder, uterine adnexa, also involved round ligament within the pelvis and in the inguinal canal (one patient). Additionally, in one female, small cysts, free-floating in the peritoneal cavity were present. In three patients, clinical signs resulted directly from the presence of MIC. One female had been 7 years previously operated on due to endometrioid ovarian cysts. Apart from MIC, three patients presented with concomitant diseases: appendicitis (two cases), peritoneal pseudomyxoma or primary ovarian carcinoma. Gross appearance: the lesions were polycystic, the surgical materials ranging from three fragments measuring 0.5 cm each to seven fragments, with the maximum size of 14×6 cm. The cysts were from microscopic size to 2 cm in diameter, the majority were thin-walled, semitranslucent, filled with clear or yellowish fluid or gelatinous contents. In one case, the cyst walls were thicker and showed intense inflammatory lesions and fibrinous exudate. Microscopically, the majority of cysts were lined with a single layer of flattened or cuboid mesothelial cells (CK+, calretinin+). In two patients, the mesothelium demonstrated diffuse squamous cell metaplasia; in one individual, the cells focally formed small papillae and were vacuolated. No mucus was observed either in the cytoplasm or outside the cells. Immunohistochemical reactions to CEA, ER, PR and MIB-1 were negative. Intramural proliferations and intracystic detached clumps of cells showed both mesothelial cells (without any mi-

totic activity and signs of atypia) and macrophages (CD68+). To date, the follow-up has been 7 years and 3 years in two individuals, and from 1 to 7 months in the remaining three patients – all of them are free from recurrent disease. One female failed to report for follow-up examinations. The report also presents the review of literature.

### Introduction

Mesothelial inclusion cysts (MIC) are quite extensively presented in the literature. In 1928, Plaut published a report entitled "Multiple peritoneal cysts and their histogenesis", which was referred to in the article by Ross [30] and is most likely the first mention of MIC. Yet it was only in 1979 that Mennemeyer confirmed ultrastructurally the mesothelial nature of the layer lining the cysts [19]. Over 70 years, a total of 140 cases of these lesions have been presented, and the terms used to describe MIC have included multicystic (peritoneal) mesothelioma, (benign) cystic mesothelioma, multifocal (peritoneal) (mesothelial) (inclusion) cysts and postoperative peritoneal cysts. The diversified terminology is a reflection of the hitherto unclear histogenesis of MIC, but it emphasizes the property that is constant and common for all MIC cases, i.e. their polycystic and frequently multifocal character. It seems that in recent years, the opinion stressing the reactive, non-neoplastic nature of MIC are predominant.

To avoid ambiguities, it should be emphasized that MIC lesions develop on the surface of serous membranes and differ from isolated mesothelial cysts that float freely in the peritoneal cavity or develop in the parenchyma of various organs (such as the spleen).

## Case Descriptions

The main clinical data are presented in the Table 1.

**Case 1:** a 45-year old male was admitted to hospital in July 1997 with a preliminary diagnosis of appendicitis. For 10 days prior to admission, he had been complaining of a lower abdominal pain and problems of defecation. Abdominal ultrasonography showed a large, multicystic reservoir filled with fluid and extending to the umbilicus. Intraoperatively, a large cystic mass filled with yellow-brownish, clear fluid was noted in the region, along with several smaller but similar cysts that filled the minor pelvis and were accreted to the intestines, urinary bladder and peritoneal wall. Some cysts had thin walls, while others were characterized by thicker, harder walls. The lesions were totally resected.

Macroscopic examination: seven fragments of fibrous tissue, the largest measuring 14x6 cm, on the cross-section showing numerous, small cysts containing clear fluid.

Pathologic diagnosis: peritoneal cystic mesothelioma.

**Case 2:** a 22-year old female admitted to hospital in March 2001 for continued diagnostic and therapeutic management. One month previously, she had been operated on in another hospital due to acute appendicitis. At that time, in the course of an appendectomy, the surgeons noted „mucous masses” in the region of the right uterine adnexa and ascending colon. The masses were referred to histological verification and the diagnosis of the papillary mesothelioma was established. In our center, the patients had abdominal CT that revealed a 10x5 cm tissue mass in the *cul de sac*, the structure of which was not uniform and foamy. Laparoscopy revealed a small volume of fluid in the minor pelvis, with three free-floating gelatinous,

semitranslucent grape-like forms, below 5 mm in diameter, which were completely removed.

Macroscopic examination: several thin-walled cysts, max. 0.5 cm in diameter.

Pathologic diagnosis: cystic mesotheliosis.

**Case 3:** a 47-year old woman was admitted to hospital in May 2004 due to hypogastric pain, painful defecation and increased abdominal circumference persisting for two months previously. Seven years earlier, she had been subjected to a hysterectomy and bilateral adnexectomy due to endometrioid ovarian cysts. Ultrasonography performed in our center showed status post total hysterectomy and, within the *cul de sac*, a cystic-solid structure, 65 mm in diameter, along with space filled with free fluid, 40 mm in diameter. Laparoscopically, numerous, veil-like adhesions with fluid spaces, situated between the sigmoid and the vaginal stump, were detected and then completely resected.

Macroscopic examination: four fragments (of maximal diameter approximately to 5 cm) of loose, whitish, semitranslucent tissue with small collapsed cysts.

Pathologic diagnosis: peritoneal inclusion cysts (the so-called benign cystic mesothelioma).

**Case 4:** a 46-year old female admitted to hospital in May 2004 because of a cyst-like lesion situated in the *cul de sac* that had been detected one month earlier. Ultrasonography revealed an irregularly shaped lesion of a variable echogenicity, measuring 84x64 mm. Additionally, the patient had been on hormonal therapy for several months previously (lynestrenol, estradiol and cyproterone). Laparoscopy revealed a multilocular cyst in the *cul de sac*; the cyst was filled with gelatinous contents. Other findings included acute appendicitis and focal, nonspecific inflammatory le-

**TABLE 1**

Patient No.	Sex/age	Location	Diameter	Previous disease	Coexisting disease
1.	M/45	parietal peritoneum intestines urinary bladder	7 fragments, max. 14x6 cm	–	–
2.	F/22	uterine adnexa ascending colon <i>cul de sac</i>	3 fragments, 0.5 cm	–	appendicitis
3.	F/47	<i>cul de sac</i>	4 fragments, max. 5 cm	endometrioid ovarian cysts	–
4.	F/46	<i>cul de sac</i>	4 fragments, 3-5 cm	–	appendicitis appendiceal adenoma pseudomyxoma
5.	F/53	inguinal canal - round ligament	8x3x2 cm	–	–
6.	F/35	minor pelvis – round ligament	3 fragments, 2 cm	–	ovarian cystadenocarcinoma

sions involving the pelvic peritoneum. A cystectomy and an appendectomy were performed.

Macroscopic examination: the material originating from the *cul de sac* represented four fragments of loose tissue, up to 5 cm in diameter, composed of numerous, small, mostly collapsed, thin-walled cysts.

Pathologic diagnosis: the material collected from the *cul de sac* showed peritoneal inclusion cysts (the so-called benign cystic mesothelioma).

The sections of the appendix and adjacent tissues demonstrated: phlegmonous appendicitis, tubulo-villous adenoma of appendix, peritoneal pseudomyxoma.

**Case 5:** a 53-year old woman was hospitalized in July 2004 due to periodic pain and a sensation of a mass in the right inguinal region. Her preliminary diagnosis was inguinal hernia. Intraoperatively, a cyst involving the round ligament was detected in the inguinal canal and a total cystectomy was performed. In a follow-up examination performed one month postoperatively, no pathologies were noted. The patient failed to report for further follow-up.

Macroscopic examination: a cystic, thin-walled, loose tissue fragment, approximately 8×3×2 cm in size.

Pathologic diagnosis: mesothelial inclusion cysts (the so-called benign cystic mesothelioma).

**Case 6:** a 35-year old woman was operated on in November 2004 due to primary ovarian cancer (serous papillary cystadenocarcinoma). In the course of total hysterectomy, omentectomy and lymphadenectomy, non-specific lesions suspected to be metastatic in origin were noted in the left round ligament.

Macroscopic examination: three fragments of loose, membranous, cystic tissue, up to 2 cm in diameter.

Pathologic diagnosis: mesothelial inclusion cysts.

### Material and methods

The surgical specimens were examined in the Department of Pathomorphology, Collegium Medicum, Jagiellonian University. The tissues were fixed in 10% buffered formalin, routinely processed, embedded in paraffin, and sectioned and stained with hematoxylin and eosin, mucicarmine and alcian blue (pH 2.5). Immunohistochemical staining was performed on paraffin sections using a DAKO Immunostainer (DAKO, Denmark) and primary antibodies NCL-calretinin (1:100, Novocastra), CK (1:50, DAKO), MIB-1 (1:50, DAKO), CD68 (1:50, DAKO), CD34 (1:25, DAKO), CEA (1:50, DAKO), ER (1:50, Novocastra), PR (1:100, Novocastra).

### Microscopic findings

Microscopically, materials from six lesions showed multiple cysts, collapsed or forming grape-like aggregates.

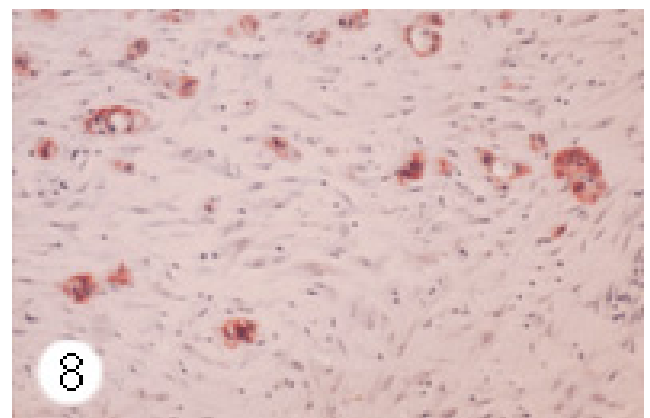
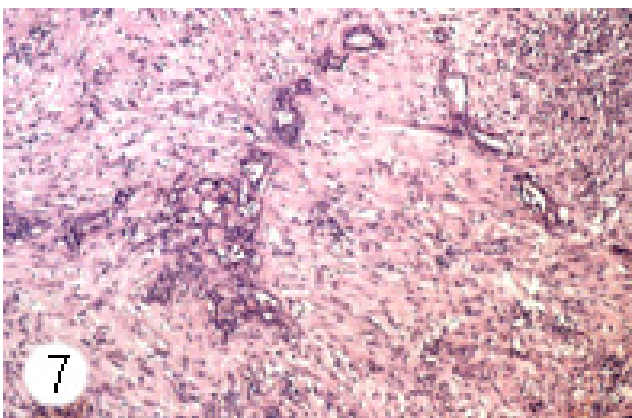
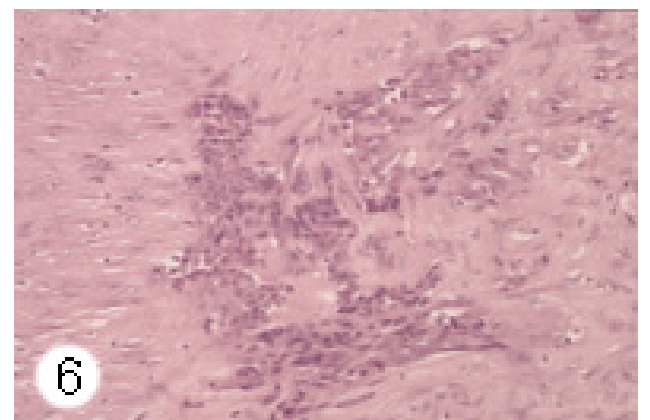
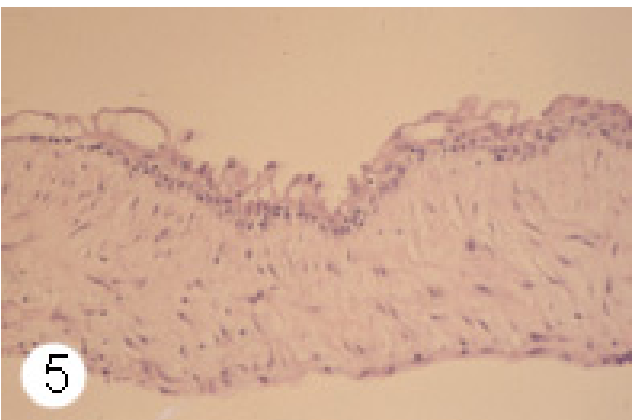
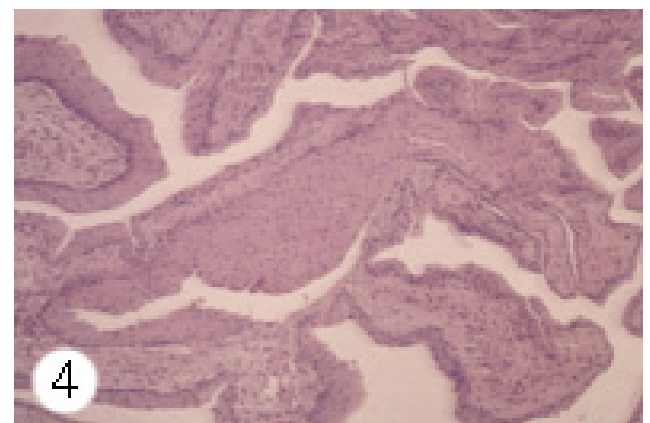
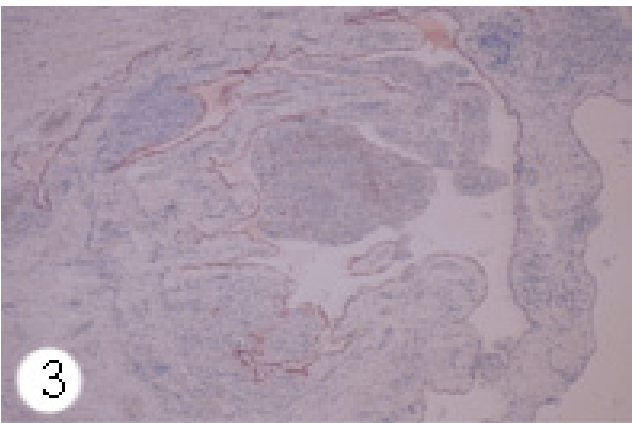
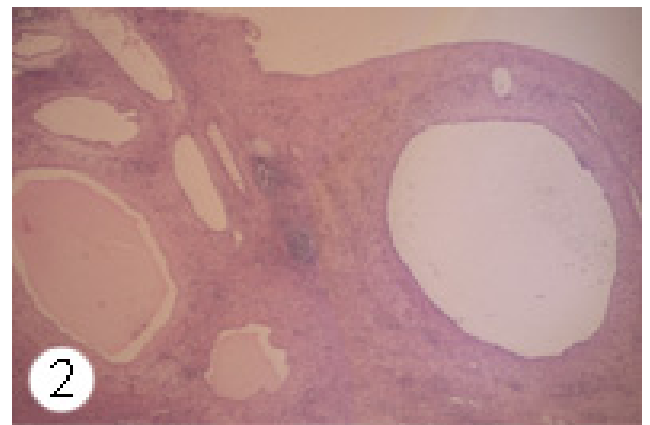
The cyst walls were 0.1 mm or less to 3-4 mm thick and consisted of loose, scanty cellular connective tissue (Fig. 1). The only exception was Case 1, which presented with distinct stromal inflammatory lesions; here, the cyst walls were of the greatest thickness, composed of connective and granulation tissue showing non-specific, chronic inflammatory infiltration (Fig. 2) and hemorrhages. The lining consisted of flattened, endothelial-like cells or alternately of low, cuboid cells that were cytokeratin- and calretinin-positive and corresponded to mesothelial cells (Fig. 3). The mesothelium showed strongly pronounced squamous cell metaplasia (Cases 2 and 3) (Fig. 4). Its cells were also vacuolated and formed small papillae devoid of connective tissue cores (Case 3) (Fig. 5). In lesions with more intense inflammatory involvement, some fragments of the lining were exfoliated and the fibrous exudate adhered to the denuded surface. In Cases 1, 3 and 6 there were intramural mesothelial proliferations. Within the stroma showing inflammatory lesions, mesothelial cells were fairly uniformly mixed with fibroblasts and considerable less numerous macrophages (CD68+). In some fields the mesothelial cells formed focal, small, solid clusters (Fig. 6) or scattered tubular structures (Figs. 7 and 8), which only to a slight degree resembled the arrangement of cells in an adenomatoid tumor. In immunohistochemistry, approximately one half of these tubules proved to be thin-walled vessels with edematous endothelium (CD34+). All the investigated cases were MIB-1-negative in the mesothelial cells, both within the cyst lining and in the focal proliferations. Cell clusters that were freely situated in the cyst lumen partially corresponded to macrophages (CD68+), and partially to exfoliated mesothelium (calretinin +).

Immunohistochemical reactions to CEA, estrogen and progesterone receptors were negative. Histochemical staining (mucicarmine, alcian blue) failed to show mucous substances in any of the six investigated cases.

Microscopically, the six lesions corresponded to the previously described, typical MIC, also when situated extraperitoneally.

### Discussion

Mesothelial inclusion cysts (MIC) develop on serous membranes, mostly on the peritoneum. Isolated cases of extraabdominal location were reported in the spermatic cord [33], peritesticular tissues [25], inguinal region [4], pleura [2], pericardial sac [5] and retroperitoneal space [21, 30]. MIC involve the parietal and visceral layers of the peritoneum, most often at sites where it covers pelvic organs and lines the *cul de sac*, further proceeding to the mesentery and



greater omentum [9, 30, 31, 34]. In rare instances, MIC are situated on the capsule of such organs, as the liver [1, 12], spleen [34] or on the abdominal surface of the diaphragm [8, 23]. MIC form single or multiple foci, more often develop on a wide base, and only in some cases presenting as a pedunculated tumor.

MIC are believed to be associated with endometriosis, as well as present or past abdominal surgery. Such coincidence was observed in 30–66% of patients [30, 31]. By the same token, in at least one third of patients, no such association could be demonstrated. The clinical presentation of MIC was non-specific. The patients usually complained of undefined abdominal pain, painful defecation, nausea, presented urinary symptoms and abdominal mass. Some of these ailments might have directly resulted from the presence of MIC, especially in the case of large tumors and in the absence of other pathologies. In some patients, MIC were detected accidentally, in the course of diagnostic or surgical procedures performed for entirely different reasons. Some patients were totally asymptomatic [21, 31].

From 16 to 23% of MIC were detected in middle-aged or older males [11, 20, 27, 30, 31, 34]. Isolated cases were reported in children [5, 17, 26, 32]. Thus, the majority of MIC (approximately 80%) developed in females, usually between their 20<sup>th</sup> and 50<sup>th</sup> year of life [14, 30, 31, 34], therefore, it is not surprising that the cysts were detected in several pregnant women and in patients in the post-partum period [7, 32]. Yet, MIC does not seem to be a hormone-dependent lesion. In the series of 17 cases investigated by Sawh, only in three, the mesothelial cell nuclei were positive to estrogen and/or progesterone receptors [31].

In all the cases described to date, MIC represented a multicystic structure, consisting of several, over a dozen or a countless number of small cysts. Their diameter ranged from microscopic size to 10 cm [11], and the mean diameter was 0.5–1.5 cm. In the majority of cases, the cysts had thin, smooth walls, 0.1–0.2 mm in thickness, and were semi-translucent. In some patients, the extensive lesions were vividly described as resembling a hydatidiform mole. The cysts contained transparent fluid, usually clear, in more rare instances brownish or golden, and sometimes the contents were gelatinous. The total size of the lesions considerably

differed from patient to patient, starting from several small cysts and reaching a tumor weighing 33 kg [11]. Apart from lesions growing directly on the peritoneum, single patients also manifested a small number of cysts that were floating freely in the peritoneal cavity [13, 34].

MIC show a considerable tendency towards multiple recurrences. The phenomenon is estimated to occur in 50–80% of cases [14, 30]. Recurrent cysts develop several months to many years after primary resection [7, 11, 20, 30, 31, 34]. The longest follow-up period to date has been more than 30 years [13, 34].

Microscopically, the lesions described so far present the same appearance as in our cases. In addition, in some patients, the mesothelium cells showed a hobnail appearance [30], forming small fibrovascular papillae [30, 34], or contained mucus in the cytoplasm [4, 19, 30, 33]. Only in isolated cases did they show mitotic activity and/or atypia [18]. In the stroma of the cysts, foci of pseudoxanthoma cells were seen [9].

MIC should be differentiated from the following conditions:

- epithelial cystic lesions, such as endometriosis (with which they are sometimes associated), endosalpingiosis and müllerian remnants;
- cystic tumors, such as cystic lymphangioma [28, 29];
- malignant mesothelioma [6];
- disseminated adenocarcinoma.

In the latter case, difficulties arise in association with the assessment of intraparietal, epithelioid-like mesothelial cells that are scattered in the stroma of MIC. Some of them may be signet ring-like, and sometimes they form small groups, either solid or interspersed with slits (gland-like structures). An additional occasion for misdiagnosis is afforded by positive staining for mucus in the lumen of the tubules (alcian blue+) or in the apical part of the cytoplasm, as well as a very rare occurrence of mesothelium proliferation in the stroma of adjacent organs, such as the muscular layer of the intestine [4].

At times, these epithelioid-like cells form nodules. Then our primary consideration is the proliferation of macrophages manifested as a nodular hyperplasia [22]. If the mesothelial character of these cells is confirmed, they usually represent reactive proliferation. In such cases, pro-

Fig. 1. A typical for MIC appearance of multiple, collapsed, thin-walled small cysts, lined with a single layer of flattened cells; the stroma composed of loose connective tissue. HE.

Fig. 2. In some cases the stroma of MIC demonstrates inflammatory changes, resulting in wall thickening. HE.

Fig. 3. Positive immunohistochemical reaction to calretinin in the mesothelial cells lining the cysts. Nodular clusters of cells in the MIC wall correspond to granulation tissue.

Fig. 4. Pronounced squamous cell metaplasia of the cyst lining – a phenomenon that is often seen in MIC. HE.

Fig. 5. Slight papillary proliferation and vacuolization of mesothelial cells in the MIC. HE.

Figs. 6 and 7. Intramural proliferation of mesothelium; note solid foci (Fig. 6) and small tubules (Fig. 7) that may suggest a neoplastic infiltration. HE.

Fig. 8. Calretinin decorates mesothelial cells forming small, scattered tubules entrapped within the MIC wall.

liferation is observed just below the lining of the cyst, and it rarely only extends into the cyst lumen. In the stroma, mesothelial cells are interspersed with fibroblasts and endothelial cells and usually do not manifest any mitotic activity. Intraparietal proliferation is apparent in cysts with inflammatory changes and hemorrhages.

In one case, MIC with numerous hyaline globules required differentiation from a cystic form of yolk sac tumor [15].

Gross appearance and histology, the age of the patients (22–52 years of life), the predominance of females (5:1), the location of five lesions within the abdominal cavity, chiefly in the pelvis, as well as the clinical presentation allow for formulating an opinion that our cases represent typical MIC. The location of MIC in the inguinal canal (Case 5) is rare, but, nevertheless, it is fully understandable in view of the peritoneal diverticulum that is present in this area [4]. Cysts that float freely in the peritoneal cavity, as the ones noted in Case 2, are a very uncommon phenomenon, however, they have been described [30]. Some of the previously reported patients demonstrated CEA-positive immunohistochemical reaction, which was unrelated to the presentation of the lesions and the course of the disease [12, 21]. No effect of sex hormones on the development of MIC has been proven. In our six patients, the reactions to estrogen and progesterone receptors were negative, both in the mesothelium (including squamous cell metaplasia), and in the stroma. In addition, a female patient (Case 2) became pregnant following the lesion resection and gave birth to a healthy child. Neither in this patient (Case 2), nor in a 47-year old male (Case 1) was recurrent disease observed (the follow-up duration of 3 and 7 years, respectively). In the remaining three individuals, the follow-up while they are event-free is short (from 1 to 7 months). One female (Case 5) dropped out from follow-up.

The survival prognosis in patients with MIC is good, also in patients with multiple episodes of recurrent disease. The literature reports three cases where the course was fatal [8, 34]. Yet, it does not seem that in these patients, death was indeed directly associated with MIC. In one of the two patients described by Weiss, the tumor was even initially identified as a malignant mesothelioma with a cystic component, while the other patient died with a “giant mass in the abdominal cavity”, but for 12 years previously, he had been steadily refusing any treatment [34]. In the patient reported by Gonzales-Moreno [8], 10 years after the original diagnosis of a recurrent MIC, in its part filling a hernial sac, a 2.5 cm focal lesion was detected, which corresponded to a malignant mesothelioma but showed no junctional component.

The advocates of neoplastic origin of MIC place the lesion between adenomatoid tumor and malignant mesothelioma. To support their opinion, they quote the following observations:

- despite its benign histology, MIC show a strong tendency towards recurrences;
- five cases have been described, where adenomatoid tumors were accompanied by a cystic component that was freely growing on the peritoneal surface and corresponded to MIC [3, 16, 24, 34, 35]; junctional component were also identified [3];
- in relatively numerous cases of MIC, the areas resembling a microcystic form of adenomatoid tumor were found, although they rarely involved a more extensive area.

Differences are also apparent (even if one disregards the obviously different macro- and microscopic appearance):

- as a rule, adenomatoid tumors are single tumors, only isolated multiple cases have been reported, and only in a single patient the lesions simultaneously involved several different organs [10];
- only several MIC had the gross appearance of “tumors” (pedunculated or non-pedunculated).

In addition, in complex tumors (MIC and adenomatoid tumor) one may consider the fact that MIC is a secondary lesion, i.e. it develops in a reaction to the constantly irritating presence of a tumor growing within an organ. Also the junctional component described by Chan [3] that combines the properties of an adenomatoid tumor and MIC may be of a similar origin, since MIC are at times accompanied by mesothelium proliferation and its penetration to the stroma of various organs (the omentum, ovaries) [30], or even to the muscular layer of the intestinal wall [4].

A clear association between MIC and previous surgical procedures and/or inflammatory processes, multifocal character and, on the other hand, frequent proliferation of mesothelium in inflammatory processes, suggest rather a reactive character of the disease. Histologically identified adenomatoid changes within MIC do not favor any opinion, as they are also present in reactive mesothelium proliferations, where no cysts are formed [29].

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