Peritoneal cystic mesothelioma: an electron microscopic and immunohistochemical study of two male patients

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SUMMARY The clinical, pathological, and ultrastructural features of two cases of peritoneal cystic mesothelioma occurring in men were studied. The results of immunohistochemical staining for CAM 5-2, epithelial membrane antigen, carcinoembryonic antigen, and Factor VIII related antigen are reported for the first time and compared with the staining results of two peritoneal cystic lymphangiomas. Although resembling cystic lymphangioma by light microscopy, cystic mesothelioma may have a greater tendency for local recurrence. Staining for CAM 5-2 or epithelial membrane antigen may facilitate the differentiation of these two entities.

Peritoneal cystic mesothelioma is a rare often massive cystic tumour which arises from the abdominal and pelvic peritoneum. Although described as a benign tumour,1 2 cystic mesothelioma has a well recognised tendency to local recurrence,1 3 4 possibly greater than that of peritoneal cystic lymphangioma which it resembles grossly and with which it may be confused by light microscopy.3 5 Since Mennemeyer and Smith's description in 19796 at least 25 cases have been reported. Of these, only four have occurred in men.3 4 6 7

This paper describes the clinical, pathological, and ultrastructural features of two male patients with peritoneal cystic mesothelioma and includes the results of immunohistology not previously applied to this condition.

Case 1

A 35 year old man who was mentally retarded owing to bacterial meningitis in infancy was admitted to hospital in November 1984 with a nine month history of increasing abdominal distension unassociated with other symptoms. No history of exposure to asbestos was obtained.

On examination he looked well without evidence of anaemia or weight loss. The abdomen was moderately distended without evidence of free fluid in the peritoneal cavity. The left testis was undescended but palpable in the left groin. The right testis was normal. Ultrasonography showed the presence of multiple cysts of various sizes in the peritoneal cavity. The liver, kidney, and spleen were reported as being normal.

At laparotomy a very large multicystic mass was found occupying the whole peritoneal cavity. The mass was attached to visceral and parietal peritoneum, greater omentum, and occasional loops of small intestine. No cystic lesions were noted in the appendix, liver, kidney, or spleen. The tumour was excised as completely as possible and weighed 5·8 kg.

The patient made an uneventful recovery and had no recurrence after six months.

Case 2

A 45 year old man was admitted to hospital in December 1981 with a history of suprapubic pain of one week and high incidence of micturition with dysuria of two weeks' duration.

Physical examination showed a globular suprapubic mass extending up to the umbilicus and an apparently separate elongated mass in the left iliac fossa. Haemoglobin concentration, white cell count, and routine biochemical investigations were all within the normal range.

Ultrasonography showed a large well defined multicystic mass extending from above the bladder to the umbilicus and down into the left pelvis, pushing the bladder to the right. The right renal pelvis was mildly

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dilated, suggesting mild obstruction. The left kidney and liver were normal. Intravenous urography showed that both ureters were compressed by a soft tissue mass.

At laparotomy in January 1982 a large multiloculated cystic mass was found adherent to the pelvic wall, the lateral aspect of the sigmoid mesentery, and the surface of the ascending colon. The transverse colon, small intestine, duodenum, retroperitoneum, pancreas, liver, gall bladder, and spleen were normal. Tumour was removed from pelvis, sigmoid, and ascending colon, but excision was incomplete.

At follow up after three years the patient was well with no clinical evidence of recurrence.

Methods

Both specimens were received in 10% formal saline. After fixation the tissue was dehydrated through an alcohol series and xylene for embedding in paraffin wax. Sections (3 µm) were cut, dewaxed, and stained with haematoxylin and eosin, periodic acid Schiff, Van Gieson, and alcian blue (before and after treatment with hyaluronidase). Immunohistological and ultrastructural study of case 2 was carried out retrospectively, tissue being obtained from paraffin blocks made three years before.

Immunohistological staining was performed on 3µm dewaxed sections using an indirect immunoperoxidase procedure. Primary antisera to epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), and prekeratin were raised in rabbits and purified as described previously. The monoclonal antibody to cytokeratin CAM 5.2 was produced by Dr CA Makin, Imperial Cancer Research Fund, and antibodies to Factor VIII related antigen (Factor VIII RA) were obtained from a commercial source (Dako). The two antibodies were used after incubating the sections for 15 minutes with pronase at 37°C. Primary antisera were applied for 90 minutes followed by washing and incubation for a further 90 minutes with alkaline phosphatase conjugated antirabbit or antimouse immunoglobulin (Sigma). The stain was developed in a solution of AS:BI phosphoric acid plus Brentamine fast red and counterstained with Mayer’s haemalum. Appropriate positive and negative control material was included, and sections of two peritoneal cystic lymphangiomas (confirmed by electron microscopy) were also stained for comparison.

Sections for electron microscopy were prepared either from formalin fixed or formalin fixed wax embedded material (case 2). Small pieces of tissue 1 mm3 were transferred into 1% glutaraldehyde in phosphate buffer (pH 7.4) for one hour, then fixed for one hour in 1% osmium tetroxide in phosphate buffer.

Fig. 1 Gross specimen (case 1). Multiple densely packed cysts up to 0.9 cm in diameter.
(pH 7·4), dehydrated, and embedded in epoxy resin. Wax embedded material (case 2) was dewaxed overnight, hydrated, cut into 1 mm cubes, then fixed in 1% osmium tetroxide for one hour, dehydrated, and embedded in epoxy resin. Sections from both cases were cut on a Cambridge Huxley pattern mark II ultramicrotome, mounted on copper grids, stained with 3% alcoholic uranyl acetate and Reynold’s lead citrate, and examined with an Associated Electrical Industries 6B transmission electron microscope at 80 kV.

**PATHOLOGY**
The specimen from case 1 comprised a multicystic mass 32 × 30 × 16 cm that weighed 5·8 kg. The specimen from case 2 consisted of several masses of multicystic tumour measuring 14 × 10 × 8 cm in aggregate. Both tumours were pale brown and consisted of closely packed thin walled cysts 0·1–9·0 cm in diameter, which contained clear or faintly mucoid yellow fluid (Fig. 1). No solid areas, necrosis, or haemorrhage were seen.

**LIGHT MICROSCOPY**
Material from the two tumours was similar and comprised numerous variably sized cysts separated by a loose connective tissue stroma which contained vessels, fibroblasts, and a mild patchy infiltrate of lymphocytes, plasma cells, and occasional eosinophil polymorphs. Bundles of condensed collagen were present at the periphery of a few cysts and distinguished from smooth muscle by Van Gieson's stain. The cysts were lined predominantly by a single layer of spindle cells with elongated nuclei that resembled endothelial cells. A considerable number of cleft like spaces and smaller cysts (in addition to the larger cysts) were almost completely lined by rounded or cuboidal (‘hobnail’) cells with uniform small nuclei; these showed an obvious resemblance to mesothelial cells (Fig. 2). Mild nuclear and cytoplasmic variation was seen, but active pleomorphism, mitoses, and infiltration were all absent in the numerous blocks examined.

Mucin stains combined with hyaluronidase treatment were unhelpful in identifying the mesothelial cells in both cases. The small amounts of intraluminal secretion were faintly and variably positive to alcian blue periodic acid Schiff and periodic acid Schiff diastase, while the cells were negative.

**ELECTRON MICROSCOPY**
In both cases 1 and 2 cells lining the cyst spaces showed ultrastructural features characteristic of mesothelium (Fig. 3) and were of flattened or low cuboidal type resting on a well defined continuous basal lamina. Their luminal surface was microvillous, and the cytoplasm was rich in rough endoplasmic reticulum, free ribosomes, ovoid mitochondria, and bundles of fine filaments (Fig. 3). Cell boundaries were often tightly apposed with prominent desmosome belts, but dilated intercellular spaces were also not uncommon. The adjacent stroma was vascular and contained collagen fibrils.
Electron microscopic and immunohistochemical study of peritoneal cystic mesothelioma

Table 1  Immunohistochemical reactions of cystic mesotheliomas and cystic lymphangiomas

<table>
<thead>
<tr>
<th>Case No</th>
<th>CAM 5-2</th>
<th>Epithelial membrane antigen</th>
<th>Prekeratin</th>
<th>Carcinoembryonic antigen</th>
<th>Factor VIII Related antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>++ +</td>
<td>++ +</td>
<td>±</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2</td>
<td>++ +</td>
<td>++ +</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cystic lymphangiomas</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: + + + Strong positive; + Weak positive; ± equivocal; - negative.

IMMUNOHISTOCHEMISTRY

A similar pattern of staining was seen in both cases. Mesothelial tumour cells stained positively for cytokeratin and epithelial membrane antigen and faintly or equivocally positively for prekeratin. Both cases were negative for carcinoembryonic antigen (Table 1).

Strong CAM 5-2 and epithelial membrane antigen positivity were observed consistently along the luminal surface of both flattened and cuboidal cell types, and in the cytoplasm of the cuboidal cell types, particularly where these were heaped up (Figs. 4 and 5).

Negative staining of mesothelium for Factor VIII related antigen was noted in the presence of strongly positive endothelial cells of adjacent small blood vessels.

Both the cystic lymphangiomas stained negative with all antisera.

Discussion

Of the four cases of cystic mesothelioma previously described in men, three have been confirmed by electron microscopy. Table 2 summarises the main clinical features of these and our two cases. The ages of the male patients at presentation were between 35–54 years and were within the range 20–66 years reported in a predominantly female series.

The most common clinical findings of lower abdominal mass, distension, and pain do not differ from those of their female counterparts. Pain, although an inconstant feature, is more characteristic of cystic mesothelioma than cystic lymphangioma, which usually presents as an asymptomatic mass.

As yet there is no explanation for the predominance of cystic mesothelioma in women. There is no evidence of the causal relationship to asbestos exposure, which, through occupational contact, results in a prevalence of men with malignant mesothelioma.

Cystic mesothelioma can be diagnosed by light microscopy only in some cases. Characteristic features include the presence of multiple cysts lined by flattened and hobnail cells, these cells occasionally bearing discernible microvilli. A picket fence configuration and squamous metaplasia may be present. The cyst walls are formed by fibrous or myxoid connective tissue containing blood vessels,
variable numbers of usually sparse chronic inflammatory cells, and occasional eosinophil polymorphs. Smooth muscle is absent.  

Conditions from which cystic mesothelioma may rarely require differentiation include: reactive mesothelium; endosalpingiosis; malignant mesothelioma with cystic change; pseudomyxoma peritonei; endometriosis; and ovarian carcinoma. The most common differential diagnosis is cystic lymphangioma. Carpenter et al reviewed 25 multilocular peritoneal cysts that had initially been diagnosed as cystic lymphangiomas and reclassified them into two groups. The larger of these comprised cystic lymphangiomas which were more common in adult men and children and which did not recur. The second group consisted of cystic mesotheliomas. These were more common among women and showed a tendency to recur, necessitating frequent surgical intervention more often for diagnosis and treatment. In another series recurrence occurred in 50% (8 of 15 patients) with cystic mesothelioma followed up for more than two years. Among men the behaviour is similar and recurrence has been reported in two cases. In one of these recurrence necessitated four operations over 20 years (Table 2).

On light microscopy cystic lymphangioma consists of endothelial lined cysts, which are separated by connective tissue containing lymphoid cells and follicles with germinal centres. Although sometimes focally cuboidal, the endothelial cells lack microvilli. Smooth muscle is a feature of lymphangiomas and its presence distinguishes the two lesions. On the other hand, it may be scant. The distinction between mesothelium and lymphatic endothelium can readily be made by electron microscopy. The presence of microvilli, numerous desmosomes, intracytoplasmic filaments, and a continuous basal lamina are characteristic of mesothelium, whereas endothelial cells, although containing pinocytotic vesicles, lack microvilli, filaments and

Table 2  Summary of data from six reported cases of cystic mesothelioma and peritoneum in men

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age and sex</th>
<th>Clinical features</th>
<th>Location and size</th>
<th>Histological diagnosis</th>
<th>Treatment</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dumke et al (1983)</td>
<td>46M</td>
<td>Relapse of (R) indirect inguinal hernia</td>
<td>Numerous cysts up to 7 cm in diameter affecting all abdominal organs except liver and parietal peritoneum (2.9 kg)</td>
<td>Benign cystic mesothelioma</td>
<td>Omentectomy and appendectomy; resection of tumour 6 g; intraperitoneal cyclophosphamide</td>
<td>Two and a half years later operation for Lymphangioma inguinal hernia; cysts present in hernia sac</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>Umbilical hernia (recurrence 1) Abdominal pain (recurrence 2)</td>
<td>Cysts in peritoneum at incision site Cysts in parietal and visceral peritoneum</td>
<td>Lymphangioma, possibly mesothelioma</td>
<td>Repair of umbilical hernia Lymphangioma</td>
<td>Excision</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>Acute small bowel obstruction (recurrence 3)</td>
<td>Numerous cysts 0-1-1-5 cm in diameter. Small bowel of pelvis and in peritoneal cavity</td>
<td>Benign cystic mesothelioma</td>
<td>Excision of peritoneum and small bowel mesentry</td>
<td>20 years</td>
</tr>
<tr>
<td>Carpenter et al (1983)</td>
<td>M</td>
<td>Details of single male case not given</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blumberg et al (1981)</td>
<td>44M</td>
<td>Intermittent lower abdominal pain nine months. Irregular tender lower abdominal mass</td>
<td>Cystic mass 4 kg. Cysts 0-5-6-0 cm in lower abdominal cavity and pelvis affecting bladder, seminal vesicles, rectum, sigmoid colon, ascending colon, caecum, small bowel mesentery, pelvic wall</td>
<td>Multicystic peritoneal mesothelioma</td>
<td>Excision (finger dissection, sharp and blunt dissection)</td>
<td>One year (no recurrence)</td>
</tr>
<tr>
<td>Sienkowski et al (this report)</td>
<td>35M</td>
<td>Abdominal distension; no pain</td>
<td>Multicystic mass 5-8 kg in whole peritoneal cavity; attached to greater omentum, visceral, and parietal peritoneum, small intestine</td>
<td>Cystic mesothelioma</td>
<td>Excision</td>
<td>Six months (no recurrence)</td>
</tr>
<tr>
<td></td>
<td>45M</td>
<td>Lower abdominal mass; frequency of micturition and dysuria for two weeks; suprapubic pain for one week</td>
<td>Multicystic mass affecting pelvic wall, sigmoid mesentery, ascending colon</td>
<td>Cystic lymphangioma; cystic mesothelioma (at review after three years)</td>
<td>Excision</td>
<td>Three years (no recurrence)</td>
</tr>
</tbody>
</table>
desmosomal junctions, and rest on a discontinuous basal lamina.

Although applied to only two cases, the results of immunohistochemistry suggest that CAM 5:2 or epithelial membrane antigen positivity may be used to distinguish cystic mesothelioma from cystic lymphangioma. This may include “disease” cases of cystic mesothelioma, in which, by light microscopy, the cyst lining cells appear exclusively endothelial and lack microvilli and in which smooth muscle cannot be definitely excluded, although we have not been able to examine such a case. Immunological methods do not require special fixation and permit wide areas of the tumour to be examined.

The strong positivity of mesothelial cells for CAM 5:2 observed in the cystic mesothelioma conforms with the strong positivity that has been described in normal mesothelium and malignant mesothelioma (4 of 4 pleural and 1 of 1 peritoneal).16

The distribution of epithelial membrane antigen in normal and neoplastic tissues has been described in detail elsewhere.17,18 Peritoneal, pleural, and ovarian mesothelial cells in the resting flattened state show weak inconsistent staining of the surface membrane; rounded reactive cells show some cytoplasmic staining and increased membrane staining. Malignant pleural mesothelioma stains strongly positive. We observed strong surface staining of flattened and cuboidal neoplastic mesothelial cells and additionally focal intracytoplasmic staining of cuboidal neoplastic mesothelial cells.

The faint or equivocal staining for keratin in the cystic mesotheliomas requires further investigation but is consistent with the variable results found for malignant mesothelioma.19,20

The negative staining for carcinoembryonic antigen observed in the cystic mesotheliomas has also been found by other authors in mesothelium21 and in malignant mesothelioma.19,20

Factor VIII related antigen has been found in vascular endothelium22 and inconstantly in malignant mesothelioma.19 The presence of strong positive staining in normal blood vessels in our two cases makes it unlikely that the negative result was caused by loss of antigenicity due to fixation.

In conclusion, the possible difference in rate of recurrence between cystic mesothelioma and cystic lymphangioma makes precise histopathological differentiation important. The diagnosis of cystic mesothelioma may be facilitated by staining for CAM 5:2 or epithelial membrane antigen. This may be of special value in retrospective studies, or when electron microscopy is unavailable.

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References

Requests for reprints to: Dr IK Sienkowski, Department of Histopathology, Mayday Hospital, Thornton Heath, Surrey, England.
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