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Original Paper

Well-differentiated Papillary Mesothelioma of the Peritoneum: A Separate Entity

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Three female patients with a diffuse well-differentiated papillary mesothelioma of the peritoneum are presented. They did not have a history of exposure to asbestos. The peritoneal biopsies were studied extensively, including electron microscopy, nuclear morphometry and DNA ploidy analysis. The results from these more sophisticated investigations confirmed the mesothelial origin and further characterised these lesions. One of the 3 patients has continuing elevated serum CA-125 levels, which increased transiently during and after pregnancy. All 3 patients have done well without therapy, 2 patients being alive and non-symptomatic 6 and 7 years after the initial diagnosis. It is important to distinguish this disorder from the malignant diseases of the peritoneum and the ovary. In view of the indolent course of this subtype of mesothelioma, avoidance of treatment is justified unless there is evidence of progressive disease.

Key words: well-differentiated peritoneal mesothelioma, CA-125
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INTRODUCTION

MESOTHELIOMAS originating from the peritoneal surface of the abdomen are rare neoplasms and account for approximately one third of all mesotheliomas [1, 2]. Most are either solitary and benign or diffuse and malignant [1]. Diffuse malignant peritoneal mesotheliomas occur mostly in men and are associated with asbestos exposure. These are aggressive tumours, with the lifespan from onset of complaints being 3 to 12 months despite intensive therapy [3, 4]. Less than half the peritoneal mesotheliomas have been described as well-differentiated papillary mesothelioma (WDPM) of the peritoneum. This subtype has been recognised as being much less aggressive. It is important to distinguish this tumour from malignant mesotheliomas because of differences in epidemiology, clinical behaviour, treatment and prognosis [5–8]. WDPM is more frequent in women, not associated with asbestos exposure, and may appear as single or multiple lesions. These peritoneal abnormalities are often discovered incidentally during surgery for other reasons and only a minority of patients may have abdominal complaints, which could be associated with this disorder. The macroscopic and microscopic appearance of this tumour may sometimes offer diagnostic problems. We describe 3 patients with WDPM, 1 with persistently elevated serum CA-125 levels, 2 with a long-term follow-up.

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PATIENTS AND METHODS

Patient 1

In 1989, a 44-year old woman was admitted to another hospital with acute abdominal pain. During the appendectomy (on examination: appendicitis), multiple small lesions were seen in the pouch of Douglas and one, located at the mesentery of the colon, was biopsied. The local pathologist diagnosed the material as a well-differentiated papillary proliferation, probably a papillary mesothelioma. A second laparotomy with omentectomy and bilateral oophorectomy followed and a multifocal WDPM, present in all the removed specimens was confirmed. Abdominal fluid also contained WDPM-like material. The patient was referred to our hospital for evaluation and consideration of further treatment. Physical examination, including gynaecological examination and intravaginal ultrasonagraphy, was unremarkable. An additional abdominal CT scan did not show any abnormalities. Serum CA-125, measured before and after the second laparotomy, was normal and remained normal afterwards (<30 U/ml).

The pathology specimens were reviewed and the diagnosis of WDPM was confirmed. After a follow-up of 6 years without therapy, the patient is still without symptoms of disease. All examinations have remained negative.

Patient 2

In 1988, a 30-year old woman, 24 weeks pregnant, was admitted to another hospital with abdominal pain. The results of physical examination were suggestive of appendicitis and appendectomy was carried out. This confirmed the diagnosis. During surgery, abnormal proliferations were noticed on the right ovary and on the surface of the uterus, and were biopsied. The pathologist described the material as "omentum-like tissue, probably of reactive origin". Serum CA-125 was elevated at 1170 U/ml, increasing to a maximum of 1660 U/ml 3 months after the delivery. Postpartum, because of persistently high serum CA-125 values (±1600 U/ml), a laparoscopy was performed and ovarian and peritoneal biopsies were taken. The same lesions were observed as mentioned above and the diagnosis of WDPM was made. This was confirmed by an external panel of expert pathologists. The patient was followed up without therapy. In August 1990, she gave birth to a second child and, after the delivery, an impressive gradual increase of serum CA-125 to a maximum value of 13000 U/ml was observed. Therefore, a new diagnostic laparoscopy followed in January 1991. The diagnosis was again WDPM and she was referred to our hospital for a second opinion. Physical examination was unremarkable as was an abdominal CT scan. The histological diagnosis of WDPM was confirmed on repeat review. Despite the persistent elevation of serum CA-125, no therapy was given. After a follow-up of 7 years, the patient remains well. Serum CA-125 values continue to be elevated (150–400 U/ml), but all other examinations are negative.

Patient 3

In February 1995, a 36-year old woman underwent surgery for a solitary hepatic lesion. She had had abdominal pain in the right upper quadrant for almost 6 months. A CT scan showed a lesion in the left liver lobe. She had used oral contraconceptives for about 12 years. Surgery was done and a 7 cm hepatic tumour was resected, which proved to be focal nodular hyperplasia ("pill adenoma"). During the operation, multiple lesions were seen on the surface of the liver and the diaphragm. These were biopsied. The pathology showed WDPM. All further examinations, including serum CA-125 levels, were negative and have remained so.

Histopathology

All slides were reviewed by the Mesothelioma Panel of The Netherlands. Additional (immuno)histochemistry, electron microscopy, quantitative pathology and DNA flow cytometry were used to establish the definitive diagnosis. Formalin-fixed paraffin embedded tissue specimens were available from all 3 patients. Additional sections were cut for conventional histology and stained for glycogen [periodic acid Schiff (PAS)], mucin (PAS-diastase, mucicarmine, Alcian Blue), and for hyaluronic acid (hyaluronidase/Alcian Blue). For immunoperoxidase staining, 4 μm thick sections were cut and mounted on poly-L-lysine coated glass slides, deparaffinised, rehydrated and treated with 0.5% hydrogen peroxide in methanol to block endogenous peroxidase activity.

The primary antibodies pan-keratin, vimentin V9, epithelial membrane antigen (EMA) (Dakopatts, Denmark), CA-125 (Thamer, The Netherlands) and carcinoembryonic antigen (CEA) (a gift from the National Cancer Institute, The Netherlands) were applied for 1 h at room temperature in moisture chambers. Sections to be labelled with pan-keratin were first digested with trypsin. Labelling with vimentin was preceded by 10 min boiling in a microwave, and pronase was applied for CA-125. These steps were omitted for the other immunostains. Avidin–biotin–peroxidase complex was then added. Sections were developed with diaminobenzidine for 3 min, counterstained with haematoxylin and mounted with depex [9].

Tissue for transmission electron microscopy was obtained by reprocessing selected areas of paraffin-embedded material. This was deparaffinised, washed in 0.1 M cacao-dylate buffer and post-fixed in 2% osmium tetroxide in the same buffer. Representative areas of 1 μm sections, which had been stained in Toluidine Blue, were examined. Mesothelial differentiation was defined by the presence of prominent surface microvilli [6].

The haematoxylin and eosin stained sections were used for morphometry. The mean nuclear area (MNA) was studied with the PRODIT^{R} digitising video overlay system (BMA, The Netherlands) equipped with an automated motorised scanning stage for systematic random sampling. MNA was calculated from measurements of up to 50 nuclei in all cases using a final on screen magnification of 3000×. For pleural mesothelial lesions, a MNA of $\leq 40\ \mu m^2$ correlates with a benign proliferation, and a MNA of $>50\ \mu m^2$ with malignancy (unpublished results).

For DNA flow cytometry 50 μm sections were cut from the paraffin-embedded tissue blocks. Single nuclear suspensions were made as described previously [10]. DNA ploidy was categorised as diploid with a DNA-index (DI) = 1.00, and as aneuploid if $1.00 < DI < 1.90$ or $DI > 2.10$. The DI is the relationship between the G0,1 peak of the aneuploid cell cycle and the G0,1 peak of the diploid cell cycle.

RESULTS

Light microscopy

A uniform papillary pattern was found. The papillae were coarse or branching. Their surface was covered by a single layer of mesothelial cells with only slight cellular atypia (Figure 1). Mitoses were not found. The centres of the papillary

Figure 1. Paraffin section of WDPM composed of coarse, often branching papillae covered by mesothelial cells (HE × 90).

Figure 2. Paraffin section of the surface of WDPM covered by a single layer of mesothelial cells with only slight cellular atypia. No mitoses are present. The centre of loose fibrous tissue contains some lymphocytes, foam cells and fibrosis (HE × 360).

Figure 3. Electron micrograph of the surface of WDPM showing many microvilli and desmosomes, confirming the mesothelial origin of the epithelial lining cells (×22 000).

projections consisted of loose fibrous tissue which contained lymphocytes, some foam cells and fibrosis (Figure 2).

Histochemistry

The PAS-reaction was positive, abrogated with diastase. Alcian blue stained the brush border and the matrix of the papillae; this was reduced by hyaluronidase. The mucicarmine staining was negative (Table 1).

Immunohistochemistry

Pan-keratin showed strong positivity in all cases. Vimentin was also positive. CA-125 and EMA were negative in patient 1 and 2 and positive in patient 3. CEA was negative in all patients (Table 1).

Electron microscopy

In all 3 patients many microvilli and desmosomes were seen (Figure 3).

Table 1. *Results of (immuno)histochemistry, electron microscopy, nuclear morphometry and DNA flow cytometry of WDPM*

	Patient 1	Patient 2	Patient 3
Alcian Blue	+	+	+
with hyaluronidase	−	−	−
PAS	+	+	+
PAS-diastase	−	−	−
Mucicarmine	−	−	−
Keratin	+	+	+
Vimentin	+	+	+
EMA	−	−	+
CA-125	−	−	+
CEA	−	−	−
MAI	0	0	0
EM	microvilli	microvilli	microvilli
MNA	27.4	36.5	n.d.
FC	diploid	n.d.	aneuploid

WDPM, well-differentiated papillary mesothelioma; MAI, mitotic activity index; EM, electron microscopy; MNA, mean nuclear area (μm^2); FC, flow cytometry; n.d., not done.

Nuclear morphometry

Material from patient 1 had a MNA of 27.4 μm^2 (S.D. 5.1) and that from patient 2 a MNA of 36.5 μm^2 (S.D. 8.4). From patient 3 only material after freezing was available, which was not suitable for this type of investigation.

DNA flow cytometry

A diploid DNA pattern was observed in the sample from patient 1 (Figure 4). From patient 2, no adequate material was present. An aneuploid DNA pattern (Figure 5) with a DI of 1.24 was seen in the tumour from patient 3.

DISCUSSION

In this article, we describe 3 patients with a WDPM. This diagnosis must be differentiated from malignant mesotheliomas and ovarian tumours. Until now, the histological appearance of papillar/tubular structures, lined by one layer of uniform cuboidal cells with only slight atypia and a few mitoses

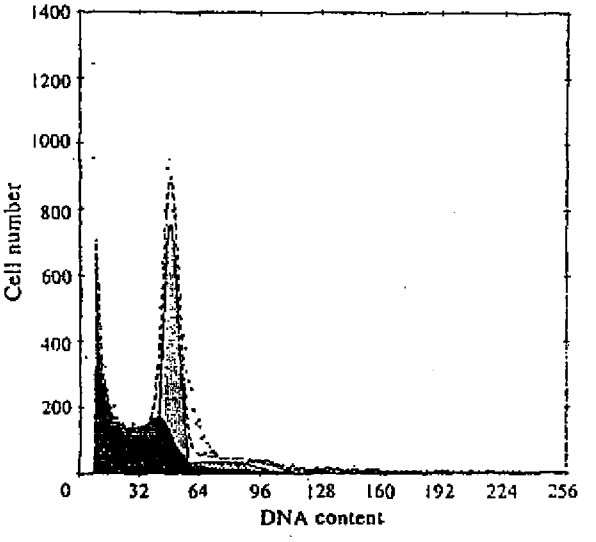

Figure 4. DNA flow cytometry of WDPM material from patient 1 showing a diploid pattern.

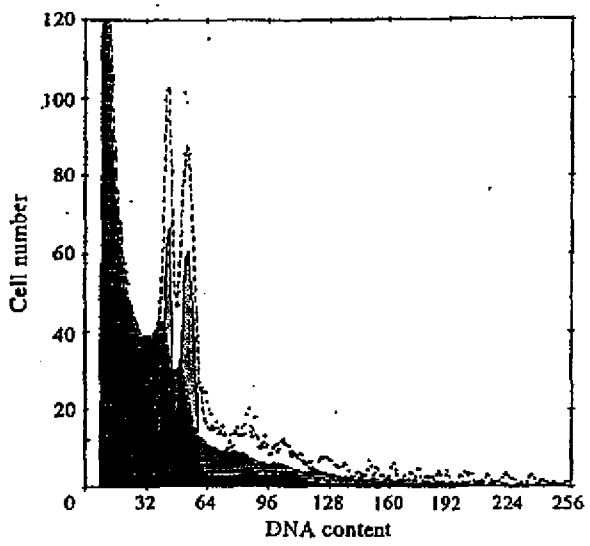

Figure 5. DNA flow cytometry of WDPM material from patient 3 showing aneuploidy with a DNA-index of 1.24.

and without invasive behaviour, has been the cornerstone of the diagnosis of WDPM. In this article, we show that additional morphometric analysis and flow cytometry can be of help in characterising these lesions. In 2 patients, in which this investigation could be done, the MNA was low. This is, in our experience, an indication of benign behaviour. DNA flow cytometry showed a diploid pattern in the first patient but an aneuploid pattern in patient 3. The significance of this finding is not clear, but may indicate the possibility of a less favourable prognosis for patient 3. Ovarian tumours (borderline or malignant) can be distinguished from WDPM by their atypia and number of mitoses. In addition, they are negative for hyaluronic acid and sometimes contain mucin, in contrast with our WDPM samples. When fresh material is available, a panel of specific antibodies can further differentiate ovarian carcinoma from a mesothelioma. In contrast to the findings in patient 1 and 2, a positive reaction with CA-125 and EMA was found in patient 3. CA-125 is a well-known tumour marker for ovarian cancer, but the antigen may also be detected on reactive mesothelial cells as is the case for EMA. The mesothelial origin of all cases was confirmed with electron microscopy, showing many slender microvilli.

The increase of serum CA-125 values observed in 1 of the 3 patients is an interesting finding. Twice, i.e. at the end of the first pregnancy and during the first months after the second pregnancy, patient 2 had astounding serum CA-125 levels (maximum 13 000 U/ml), without symptoms. This finding is even more remarkable considering the fact that the biopsy material from this patient was negative for CA-125. This might be explained by either an artefact of formalin fixation (material from other hospitals) or the rapid release of CA-125 by these cells. Slightly increased serum CA-125 values (up to a maximum of 200 U/ml) are normally seen in the first trimester of pregnancy. High serum levels of CA-125 have been described in patients with malignant peritoneal mesotheliomas [11, 12], but have never been reported in the literature in a case of WDPM. In view of the sharp increase in CA-125 levels during pregnancy, one could speculate about pregnancy-associated hormones and/or growth factors which are able to induce either the proliferation of these peritoneal lesions or their production of CA-125.

WDPM generally behaves as a benign lesion [1–8]. This means that extensive debulking surgery is not indicated and that multiple biopsies may be sufficient. Nevertheless, radiotherapy and chemotherapy have been used in the past to treat these patients [7]. From the scarce data in the literature, one can deduce that morbidity may be induced without clear evidence of a beneficial effect. In our 3 patients, the diagnosis was made by coincidence. The patients were all young women without a history of exposure to asbestos. We decided to withhold treatment and were able to follow up 2 patients for a long period. Despite the generally benign course of WDPM, long-term follow-up is required because of possible sampling errors in the biopsy material and the potency of WDPM to dedifferentiate to true cancer [13].

In conclusion, the diagnosis of WDPM may offer difficulties, both in terms of its pathology and its clinical presentation. However, the multifocal nature of WDPM in our 3 patients, the high serum CA-125 level in patient 2 and the aneuploidy of the material obtained from patient 3, suggest that benign behaviour may not always be guaranteed. Therefore, consultation of other pathologists or pathology panels is highly recommended. Clinicians should be aware of this disease, its clinical features and the indolent course of this disease, in order to refrain from an aggressive therapeutic approach.

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