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Case report

Alveolar soft-part sarcoma of the uterine corpus: histological, immunocytochemical and ultrastructural study of a case

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Summary. A case of alveolar soft-part sarcoma located in the uterine corpus is reported. It was an incidental finding in the hysterectomy specimen of a 40-year-old woman. Light and electron microscopic examination revealed periodic-acid-Schiff-positive, diastase-resistant, membrane-bound cytoplasmic granules and crystalloids. Tumour cells expressed immunoreactivity with vimentin, desmin, cytokeratins, NK1/C3 and HMB-45 antibodies. Four years postoperatively, the patient is still alive without evidence of disease. Differential diagnoses, immunocytochemistry and clinical management of uterine alveolar soft-part sarcoma are discussed and the literature reviewed.

Key words: Alveolar soft-part sarcoma – Uterus – Myometrium – Immunocytochemistry – Electron microscopy

Introduction

First defined in 1952 by Christopherson et al., alveolar soft-part sarcoma (ASPS) is a rare, distinctive, slowly growing neoplasm that most often occurs in children and young adults. Generally located in the deep soft tissues of extremities and head and neck region, it represents between 0.5% and 1% of all soft tissue sarcomas (Enzinger and Weiss 1988). To the best of our knowledge, 15 cases of ASPS have already been described in the female genitalia: 1 in the vulva (Shen et al. 1982), 5 in the vagina (Carinelli et al. 1990; Chapman et al. 1984; Kasai et al. 1980; O'Toole et al. 1985; Tobon et al. 1976), 7 in the uterine cervix (Abeler and Nesland 1989; Flint et al. 1985; Foschini et al. 1989; Gray et al. 1986; Kopolovic et al. 1987; Sahin et al. 1990), 1 in the endometrium (Nolan and Gaffney 1990) and 1 in the myometrium (Gray et al. 1986). The latter was an incidental finding in the hysterectomy specimen of a 43-year-old woman. The tumour presented as an intramural nodule measuring 4 mm in diameter. The patient was still free of disease 9 months after surgery.

The purpose of this paper is to describe the clinical course, histology, immunocytochemistry and ultrastructure of another case of myometrial ASPS – the second reported at this site.

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Case report

A 40-year-old woman (gravida 3, para 3) was admitted because of recurrent abdominal and pelvic pain, intermenstrual bleeding, dysmenorrhoea and dyspareunia. Her past medical history was not significant except for a uterine prolapse treated with a pessary. On gynaecological examination, the uterus was retroverted, painful when moved but not enlarged. The cervix was normal and there were neither parametrial thickening nor abnormal pelvic masses. Cervical smears showed non-specific chronic inflammation only and a chest X-ray was unremarkable. A total abdominal hysterectomy without adnexectomy was performed and uterine ASPS was diagnosed incidentally on pathological examination. The postoperative course was uneventful. Subsequent pelvic echography, abdominal and pelvic CT scan failed to show any residual tumour or enlarged lymph nodes. No further treatment was administered. At last follow-up, 4 years postoperatively, there was no evidence of recurrence or metastatic disease.

Materials and methods

Tissue for light microscopical examination was fixed in 10% phosphate buffered formalin and 4-μm paraffin-embedded sections were stained with haematoxylin and eosin, periodic acid-Schiff (PAS) with and without diastase predigestion, mucicarmine, Fontana-Masson, Grimelius, Masson trichrome, Wilder reticulin and phosphotungstic acid haematoxylin (PTAH).

Additional sections of formalin-fixed, paraffin-embedded material were studied using different monoclonal antibodies: vimentin (Boehringer Mannheim, Rotkreuz, Switzerland; 1 in 400 dilution), desmin (Dakopatts, Copenhagen, Denmark; 1 in 150 dilution), smooth muscle actin (Enzo Biochem., New York; 1 in 3000 dilution), smooth and skeletal muscle actin (Enzo Biochem., 1 in 5000 dilution), panepithelial marker Lu-5 (Von Overbeck et al. 1985)

(Hoffmann-La-Roche, Basel, Switzerland; 1 in 600 dilution), high-molecular-weight cytokeratin, clone 34βE12 (Gown and Vogel 1984) (Enzo Biochem; 1 in 500 dilution), low-molecular weight cytokeratin, clone 35βH11 (Gown and Vogel 1984) (Enzo Biochem.; 1 in 500 dilution), chromogranin (Enzo Biochem.; 1 in 4000 dilution), HMB-45 (Gown et al. 1986) (Enzo Biochem.; 1 in 200 dilution) and NK1/C3 (Gown et al. 1986) (Sanbio, Bioreba, Basel, Switzerland; 1 in 80 dilution). Polyclonal antiserum raised against S100 protein (Dakopatts; 1 in 300 dilution) was also tested.

Enzymatic digestion with protease 27 (Sigma, Basel, Switzerland; 1 mg/2 ml phosphate-buffered saline, 10 min at 37° C) was carried out prior to incubation with antisera directed against keratins and melanoma-associated antigens. The peroxidase-antiperoxidase method was used (Sternberger et al. 1970). Appropriate positive control tissues were processed identically. Endometrium and myometrium with vessels and nerves were also used as internal controls. Negative controls were obtained by replacing the first-step antiserum with normal rabbit or mouse serum or by suppressing the second-step antiserum. Swine anti-rabbit IgG serum (1 in 30 dilution), rabbit anti-mouse IgG serum (1 in 60 dilution), rabbit (1 in 60 dilution) and mouse (1 in 200 dilution) peroxidase-antiperoxidase complexes were obtained from Dakopatts. For electron microscopy study, prefixed tumour tissue samples were washed, post-fixed in buffered 1% osmium tetroxide, dehydrated in graded alcohols and embedded in Epon-Araldite. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined with a Philips 300 electron microscope.

Results

A 3 × 2.5 × 2.5 cm, well-circumscribed firm nodule located deep in the myometrium was found in a 183-g uterus (Fig. 1). The cut surface of the nodule was homogeneously grey without evidence of necrosis or haemorrhage. By light microscopy, the nodule was not encapsulated, had pushing borders and was clearly delineated from the endometrium. A typical alveolar pattern characterized by organoid cellular nests surrounded by delicate fibrovascular stroma was present in about two-thirds of the tumour (Fig. 2). Tumour cells were large with abundant, slightly fibrillary eosinophilic cytoplasm. Nuclei were large, eccentrically located and contained a single prominent nucleolus. Chromatin was frequently marginated against the nuclear membrane giving a central nuclear clearing aspect. Some cells were binucleated or exhibited intranuclear inclusion. PAS-positive, diastase-resistant cytoplasmic granules and crystals were present in about two-thirds of the cells (Fig. 3, inset), especially along the tumour margins. Average mitotic rate was two mitoses/10 high power fields (× 400). Peritumour vascular invasion and a small focus of necrosis were observed. In one-third of the tumour, the characteristic alveolar pattern was replaced by a trabecular arrangement (Fig. 3). In these areas the nuclear pleomorphism was more pronounced and the mitotic rate increased to 4 mitoses/10 high power fields (× 400). Silver and PTAH stains failed to show any melanin pigment, neuroendocrine granules or rhabdomyoblastic differentiation. Mucicarmine stain was also negative. The surrounding myometrium contained foci of adenomyosis and the endometrium was at the secretory phase.

ASPS tumour cells showed uniform and marked cytoplasmic reactivity with NK1/C3, whereas only half reacted with HMB-45. When tested with Lu-5 and low-molecular weight cytokeratin, 10% of the cells showed patchy dot-like cytoplasmic positivity and the surrounding myometrial and vascular wall smooth muscle cells were decorated in the same manner. Most of the cells were clearly positive with vimentin but only weakly positive with desmin. The other antibodies including muscle actins tested yielded negative results.

On electron microscopy tumour cells appeared light or dark depending of the number of intracytoplasmic organelles. Nuclei were large with chromatin condensed at the nuclear membrane and had prominent nucleoli. The cytoplasm contained numerous altered mitochondria, well-developed Golgi complex, abundant rough endoplasmic reticulum and numerous electron-dense,

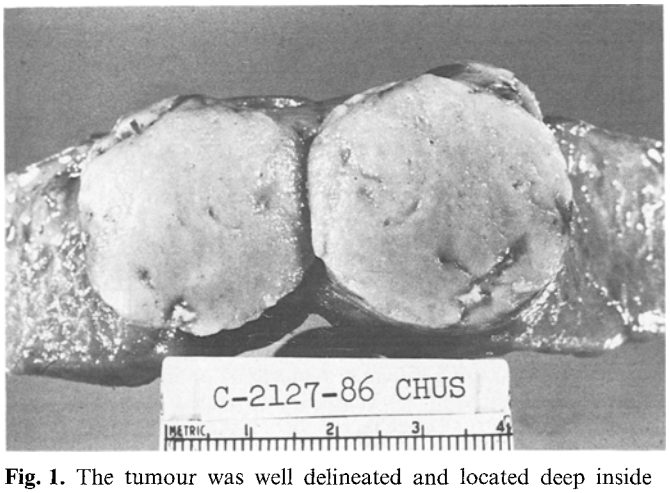

Fig. 1. The tumour was well delineated and located deep inside the myometrium

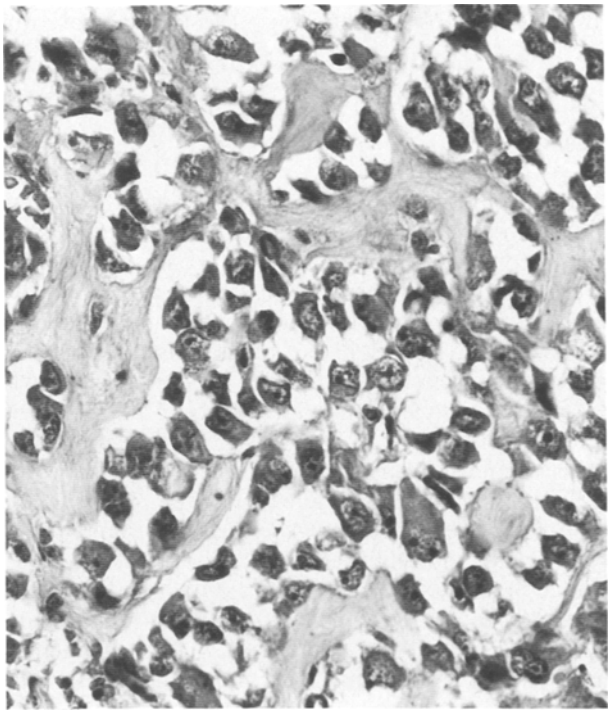

Fig. 2. Tumour area showing typical alveolar pattern. H & E, × 335

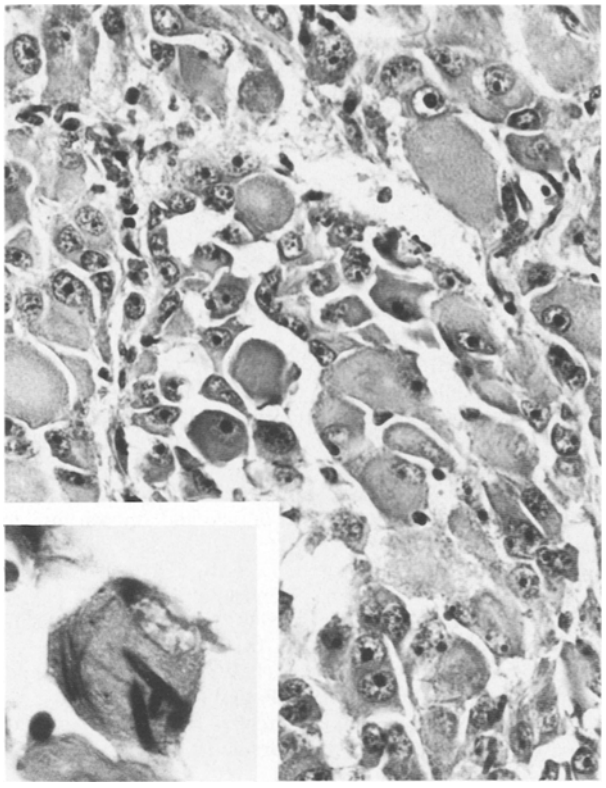

Fig. 3. Tumour area showing trabecular pattern. Cells have abundant cytoplasm, large nuclei and prominent nucleoli. H & E, ×335. *Inset*: PAS-positive, diastase-resistant intracytoplasmic crystals. PAS, ×650

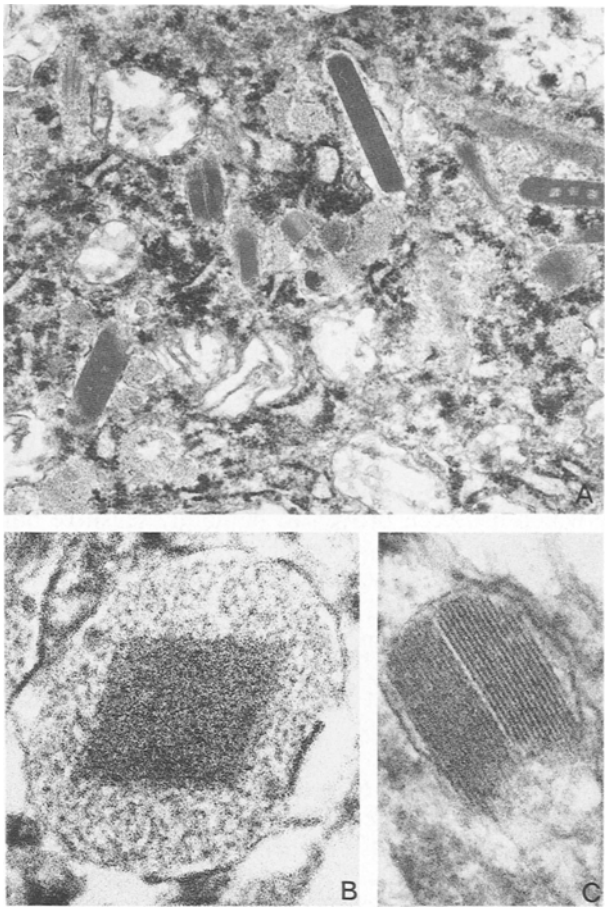

Fig. 4. A Crystals and membrane-bound granules are readily visible in the cytoplasm of some tumour cells. Numerous altered mitochondria and abundant rough endoplasmic reticulum are also present. ×20750. **B** A granule with partial central crystallization. ×103750. **C** A membrane-bound crystal showing unidirectional periodicity. ×78550

membrane-bound granules and crystals (Fig. 4A). Granules with diameters ranging from 150 to 500 nm (average 300 nm) were found. Some of them showed partial central crystallization of their content (Fig. 4B). Crystals were also found lying free in the cytoplasm without any surrounding membrane. They consisted of parallel, 5-nm filamentous structures, each of them being separated by a 3-nm clear space resulting in a 8-nm unidirectional periodicity (Fig. 4C). Some tumour cells were partly surrounded by basal lamina. No cytoplasmic myofilaments, abortive Z bands, myelin figures, melanosomes or premelanosomes were found.

Discussion

When located in unusual sites, ASPS generally prove difficult to classify by light microscopy. In the myometrium, epithelioid leiomyosarcoma, alveolar rhabdomyosarcoma, metastatic malignant melanoma or carcinoma are the main differential diagnoses to consider. Epithelioid leiomyosarcoma is often mixed with foci of the more conventional fusiform leiomyosarcoma. Cells of alveolar rhabdomyosarcoma have less abundant cytoplasm and nuclei are not generally vesicular but are hyperchromatic with numerous mitoses. The multinucleated giant cells typical of this neoplasm were not found in the present case. Carcinomas and melanomas that metastasize to the uterine corpus usually reflect disseminated disease. Carcinomas from breast and gastrointestinal tract are the most common extragenital primaries (Kumar and Hart 1982). Renal cell carcinoma, an ASPS mimicker, metastasizes very rarely to the corpus uteri (Kumar and Hart 1982) and there was no evidence of renal pathology in the present case. In none of these tumours have the PAS-positive, diastase-resistant intracytoplasmic granules and crystals considered characteristic of ASPS been described (Enzinger and Weiss 1988). Other rare tumours which can mimic ASPS have been described in the uterine corpus: malignant granular cell tumour (Mark et al. 1968), paraganglioma (Tavassoli 1986; Young and Thrasher 1982) and rhabdoid tumour (Cho et al. 1989).

With a negative PAS stain and lack of ultrastructural study, the previously published example of malignant granular cell tumour remains a debatable case. The paragangliomas reported in the myometrium by Tavassoli (1986) contained melanotic pigment and were unlikely to be confused with an ASPS.

Although histogenesis and immunocytochemical profile of ASPS are not yet well defined, immunocytochem-

istry may be helpful in differentiating such a lesion from the more conventional uterine tumours. Leiomyosarcomas and rhabdomyosarcomas generally react strongly with desmin and actin antisera, a finding which was not observed in our case. Only rare cases of ASPS have been reported to react with anticytokeratin antibodies (Ben Rhomdhane et al. 1985). The dot-like positivity observed in our tumour is quite similar to that previously reported in smooth muscle neoplasms (Brown et al. 1987; Norton et al. 1987) but clearly different from the one observed in the normal adjacent endometrium and in carcinomas, which can therefore be excluded. The results obtained with both NK1/C3 and HMB-45 (two melanoma-associated antigens) were remarkable and raised the question of a possible malignant melanoma, although no melanosomes or premelanosomes were found at the ultrastructural level. NK1/C3 is an antibody which has been proven to be highly sensitive but non-specific for melanocytic cells; positive results have also been reported in several neuroendocrine tumours and in some breast and prostatic carcinomas (Mackie et al. 1984; Van Duinen et al. 1984; Vennegoor et al. 1985). Recently, Miettinen and Ekfors (1990) studied seven cases of ASPS and all of them showed strong and uniform immunoreactivity with NK1/C3, as did our case.

Subsequently, we have tested several cases of conventional ASPS of the extremities with NK1/C3 and have obtained the same results. We believe that NK1/C3, despite its lack of specificity, may be of practical value in the diagnosis of ASPS in conjunction with electron microscopy. HMB-45, which was initially considered highly specific for cells of the melanocytic lineage (Colombari et al. 1988; Gown et al. 1986), has recently been reported as giving positive results in normal breast and bronchial epithelium as well as in few breast carcinomas and plasmocytomas (Bonetti et al. 1989; Leong and Milios 1989). Since none of the ASPS studied by Miettinen and Ekfors (1990) were positive with HMB-45, the positivity observed in our uterine tumours could be related to cross-reactivity.

In extra-genital sites, ASPS generally displays an indolent clinical course and its prognosis is unpredictable. Complete surgical excision is the treatment of choice. The median survival rate is 7 years, metastases to lungs, bone and brain being the main cause of death (Lieberman et al. 1989). Tumour size less than 5 cm in diameter has also been reported to correlate with a more favourable outcome (Evans 1985). In the present case, referring to previous clinicopathological studies on uterine sarcomas (Marchese et al. 1984; Salazar et al. 1978) the tumour would be staged FIGO IA and best treated by colpohysterectomy with bilateral adnexectomy, removal of .pelvic and paraortic lymph nodes being unnecessay. Despite theoretically incomplete therapy, our patient is still alive, 4 years postoperatively without evidence of disease at the last follow-up. However, owing to the protracted course of ASPS with the possibility of late recurrences and metastases, long-term follow-up is mandatory.

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