Primary alveolar soft part sarcoma of uterine corpus: a case report with immunohistochemical, ultrastructural study and review of literature

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Abstract

Background: Alveolar soft part sarcoma (ASPS) is a rare mesenchymal malignancy. ASPS usually occurs most commonly in the deep soft tissues of the thigh and buttck or the head and neck regions. ASPS that originate from the uterine corpus are even more rare, with only 10 previous cases reported in the English literature.

Case presentation: In our case, the alveolar features were completely lost and the tumour shows a solid, non-alveolar pattern and the nuclei have marked variation in nuclear size, and multinucleation. The correct pathological diagnosis has been made by immuno-histochemical and ultrastructural features, which revealed overexpression of TFE3 and peculiar cytoplasmic crystalline inclusions.

In this paper, an additional case of primary ASPS of uterine corpus is reported with immunohistochemical, ultrastructural study and review of literature in the effort to delineate its clinical and pathological features. In this unusual site, the diagnosis can be problematic because ASPS can mimic other primary or metastatic uterine neoplasms.

Conclusions: Thus, in this unusual presentation an essential diagnostic marker is the nuclear over-expression of TFE3 as well as ultrastructural study, which reveals the presence of peculiar cytoplasmic crystalline inclusions.

Keywords: Alveolar soft part sarcoma, Chromosomal translocation, TFE3 fusion protein

Background

Alveolar soft part sarcoma (ASPS) is a rare mesenchymal malignancy with distinctive histologic and ultrastructural appearance. ASPS was first described in 1952 by Christopherson et al. as a neoplasm with of uncertain histogenesis whose cells were arranged in a pattern mimicking the small air sacks (alveoli) of the lung [1].

In fact, this neoplasm usually shows uniform, organoid nests of polygonal cells, separated by fibrovascular septa and delicate capillary-sized vascular channels. A prominent cellular dyscohesion within the nests results in a distinctive pseudo-alveolar pattern. Sometimes, the alveolar features can be completely lost and the tumour may show a solid, “non-alveolar” pattern [2]. Usually, the nuclei are round-to-polygonal and vesicular, with prominent nucleoli, but cells with marked variation in nuclear size, nuclear-cytoplasmatic inclusions, and multinucleation have been reported [3–5]. The cytoplasmics are abundant granular and may contain periodic acid-Schiff-(PAS) positive, diastase (D)-resistant crystalline structures, rhomboid or rod-like in shape [6]. The first ultrastructural analysis of ASPS, made by Shipkey et al. in 1964 [6] and following studies confirmed the presence of distinctive cytoplasmic crystals which typically were intermingled with dense granules [7–9]. More recently, Ladanyi et al., by a combined ultrastructural and immunohistochemistry study, have demonstrated that these crystals consist of aggregates of the monocarboxylate transporter protein MCT1 and its cellular chaperone CD147 [10].

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Recent cytogenetic studies revealed that ASPSs are characterized by specific chromosomal translocation der(17)t(X;17) (p11;q25) that fuses the transcription factor 3 (TFE3) gene at Xp11 to the ASPL gene at 17q25, producing an ASPL–TFE3 fusion protein [11]. This results in the aberrant and strong nuclear expression of TFE3 which is seen almost exclusively in tumours harbouring the TFE3 gene fusions, such as ASPSs and rare paediatric renal carcinomas [12].

ASPS usually affects adolescents and young adults in the second and third decades with slight female predominance [3]. In adults, this malignancy occurs most commonly in the deep soft tissues of the thigh or buttock, while in children and infants, the head and neck regions are often involved [3]. However, many reports have demonstrated that ASPS can be observed in unusual sites such as the mediastinum, stomach, breast, bone, and urinary bladder [13–19].

This neoplasm has been reported also in the female genital tract [20–26]. As far as we are aware, only 10 cases of ASPS of uterine corpus have been previously reported [4, 5, 27–32]. In these unusual sites, the diagnosis can be problematic because ASPS can mimic other primary or metastatic neoplasms.

In this paper, an additional case of primary ASPS of uterine corpus is reported with immunohistochemical, ultrastructural study and review of literature in the effort to delineate its clinical and pathological features.

Case presentation
A 66-year-old female was hospitalized for atypical vaginal bleeding and anaemia. On gynecologic examination, the uterus was enlarged and the cervix was prolapsed. Transvaginal ultrasound identified a well-circumscribed, intramural nodule measuring 5 cm in diameter, located in the uterine corpus. The patient underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. Intraoperatively, no ascites or adhesions were seen surrounding the uterus, ovaries, and salpinges. No enlarged pelvic lymph nodes were noted neither peritoneal lesions were found.

Gross pathologic examination revealed thickening of the cervical mucosa and an intramural, sharply circumscribed, round, firm, grey-white nodule with trabeculated cut surface located in the endocervix. The uterine corpus was enlarged and deformed because of an intramural nodule, measuring 5 cm in diameter. On cut section, this lesion was well circumscribed, soft, with irregular border, colour varying from yellow, brownish and grey and a large haemorrhagic zone centrally located (Fig. 1). The ovaries and salpinges were macroscopically unremarkable.

Histologically, the cervix showed atrophic epithelium and an intramural leiomyoma. The nodule of the uterine corpus was characterized by neoplastic cells arranged in an organoid pattern (Fig. 2a), with nests surrounded by a delicate fibrovascular septa highlighted by PASD and anti-CD34 stains. Extensive degenerative changes, such as hyalinization, haemorrhage and hemosiderin deposits were observed (Fig. 2b). Most cells had abundant eosinophilic granular cytoplasm, distinct border and vesicular nuclei with prominent nucleolus (Fig. 2a). Other cells were large, with clear vacuolated cytoplasms (Fig. 2c). In some areas, the organoid pattern was lost and the tumour showed solid growth; in these areas, the cells were more spindled and showed nuclear pleomorphism, hyperchromasia and pseudo-inclusions and multinucleation (Fig. 2d). Necrosis and mitoses were absent.

On immunohistochemical analysis, the neoplasm showed focal immunoreactivity to muscle-specific actin, caldesmon and caldesmin. Ki 67 index was very low (Fig. 3a). Strong nuclear positivity to TFE3 was observed in all neoplastic cells (Fig. 3b). Immunostains for CD 34, CD10, microphthalmia transcription factor (MITF), myoglobin, S-100 protein, HMB-45, neuron-specific enolase, synaptophysin, chromogranin, cytokeratin, epithelial membrane antigen (EMA), cyclin D1 and alpha-inhibin were negative. Electron microscopy (EM) examination revealed cytoplasmic crystal inclusions showing periodic pattern of about 10 nm in channels resembling dilated sacs of endoplasmic reticulum (Fig. 4).

Because of immunohistochemical and ultrastructural features, such as focal immunoreactivity to muscle-specific markers (actin, desmin, caldesmin), negativity to other makers, strong and diffuse nuclear positivity to TFE3 and the presence of cytoplasmic crystal inclusions,
the final pathologic diagnosis was primary uterine alveolar soft part sarcoma of uterine corpus. No images suggestive of other primary or metastatic lesions were observed on abdominal ultrasound, chest X-ray, total computed tomography or bone scan.

Seven months after surgery, the patient was free of disease. In fact, when she was readmitted for further examination, no abnormalities were found in both physical examination and imaging studies.

**Discussion**

ASPS is a rare malignant neoplasm, accounting for 0.5–1 % of all soft part sarcomas [33]. ASPS that originate from the uterine corpus is even more rare, with only 10 previous cases reported in the English literature [4, 5, 27–32]. The main clinical and morphological features of 11 cases (including our present case) are summarized in Tables 1 and 2.

The age at diagnosis ranged from 14 to 66 years (median, 43 years). Abnormal uterine bleeding was present in all patients. The size of the neoplasm varied from 0.4 to 7 cm (median, 3 cm) in diameter. In the majority of cases, the neoplasm was an intramural nodule. The diagnosis of ASPS in all cases was supported by histologic and EM examination, which revealed an alveolar pattern and the presence of cytoplasmic crystalline inclusions (Table 2).

Usually, the tumour nuclei were round-to-polygonal and vesicular, with prominent nucleoli, but cells with marked variation in nuclear size, nuclear cytoplasmatic inclusions and multinucleation, with very few mitoses have been observed in our case and in other ASPSs in the uterine corpus [4, 5] and extragenital sites [3].
In our case, the alveolar features were completely lost and the tumour show a solid, non-alveolar pattern and the nuclei have marked variation in nuclear size, nuclear-cytoplasmatic inclusions and multinucleation. The correct pathological diagnosis has been made by immunohistochemical and ultrastructural features, which revealed focal immunoreactivity to muscle-specific markers (actin, desmin, caldesmon), negativity to other makers, strong and diffuse nuclear positivity to TFE3 and the presence of cytoplasmic crystal inclusions.

**Fig. 3** Ki 67 index was very low (a x200). Strong nuclear positivity to TFE3 was observed in all cells (b x200)

**Fig. 4** In an ultrastructural study, neoplastic cells had cytoplasmic crystalline inclusions (arrows) showing unidirectional periodicity
Moreover, the lesion was considered primary uterine alveolar soft part sarcoma because no images suggestive of other primary or metastatic lesions were observed on abdominal ultrasound, chest X-ray, total computed tomography or bone scan.

On the contrary, the review of literature revealed an immunohistochemical profile not very consistent with variable staining using other markers such as vimentin, desmin, myoglobin, muscle-specific actin, S-100 protein, HMB-45, neuron-specific enolase, synaptophysin, chromogranin, cytokeratin, EMA and NK1-C3 (melanoma-specific antibody) (Table 2). Thus, in our case, nuclear over-expression of TFE3 can be considered as an essential diagnostic marker for a correct pathological diagnosis. It has been demonstrated that aberrant and strong nuclear expression of TFE3 is seen exclusively in tumours which contain the TFE3 gene fusions [11], such as ASPS and rare paediatric renal carcinomas [12]. TFE3 immunoreactivity was first tested in the female genital tract in a case of ASPS located in the uterine cervix by Roma et al. in 2005 [26]. TFE3 immunoreactivity in cases of uterine corpus of ASPSs was evaluated only in our case and in the examples reported by Kasashima et al. and by Zhang L et al. [5, 32] (Table 2). The presence of crystalline inclusions on electron microscopy further supports the diagnosis of uterine ASPS.

ASPS of the uterine corpus enters in the differential diagnosis with other neoplasms presenting an admixture of spindle-to-epithelioid cells with eosinophilic-to-clear cytoplasm such as epithelioid smooth muscle tumours, epithelioid endometrial stromal tumours, perivascular epithelioid cell tumours (PECOMAs), uterine rhabdoid tumours, carcinomas, melanoma and malignant paraganglioma. These tumours characteristically do not show expression of TFE3 on immunohistochemical analysis and have other peculiar morphologic and immunohistochemical features.

Epithelioid smooth muscle tumours are composed by mixtures of epithelioid, clear cell. A transition to typical smooth muscle cells in most instances confirms the smooth muscle nature of these tumours [34] and are diffuse immunoreactive for actin and desmin.

Endometrial stromal tumours with a prominent component of epithelioid cells and abundant eosinophilic cytoplasm usually show areas with fusiform cells, characteristic arterioles and strong diffuse cytoplasmic immunoreactivity for vimentin and for CD10 [35].

PECOMAs can arise in the uterus and are characterized by varying amounts of spindle and epithelioid cells with clear to eosinophilic cytoplasm, with immunoreactivity for melanocytic markers, most frequently HMB-45 [36].

Malignant rhabdoid tumour is a highly aggressive tumour in adults and rapidly fatal and was first reported to have been found in the uterus in 1989 [37]. Histologically, this rare neoplasm shows solid sheets of large cells with deep eosinophilic cytoplasm, eosinophilic hyaline cytoplasmic inclusions, eccentric vesicular nuclei and prominent nucleoli. On immunohistochemical analysis, neoplastic cells reveal cytoplasmic staining for vimentin [38] and keratins [38, 39].

Melanoma and carcinoma can be differentiated from ASPS because of greater cytologic atypia, pleomorphism, higher mitotic activity and immunoreactivity, respectively, for epithelial makers and for melanoma-specific antibodies.

Malignant parangangioma is characterized by polygonal to oval cells arranged in distinctive cell balls, called Zellballen, and shows more pronounced cell pleomorphism and immunoreactivity for neuroendocrine markers, such as neuron-specific enolase, protein gene product 9.5, synaptophysin and the presence of neurosecretory granules on EM examination [40].

In extragenital sites, ASPS presents an indolent clinical course with unpredictable prognosis. Metastases to the

| Authors and years | Age | Clinical symptoms | Therapy | Follow-up |
|------------------|-----|-------------------|---------|-----------|
| Gray et al. (1986) [27] | 43 | Menorrhagia | Total hysterectomy | NED 9 Mo |
| Nolan et al. (1990) [28] | 14 | Menorrhagia and expulsion of a necrotic mass per vagina | HBSO | NED 80 Mo |
| Guillou et al. (1991) [4] | 40 | Abdominal pelvic pain, intermenstrual bleeding | Total hysterectomy | NED 48 Mo |
| Burch et al. (1994) [29] | 47 | Intermenstrual bleeding | HBSO | NED 12 Mo |
| Nielsen et al. (1995) [30] | 30 | Menometrorrhagia | Total hysterectomy | NED 66 Mo |
| Radig et al. (1998) [31] | 50 | Abnormal uterine bleeding | HBSO | NED 84 Mo |
| Kasashima et al. (2007) [5] | 50 | Abnormal uterine bleeding | Hysterectomy | NED 8 Mo |
| Zhang et al. (2012) [32] | 57 | Abnormal uterine bleeding | HBSO, lymph chem | NED 1 Mo |
| Present case | 66 | Abnormal uterine bleeding | HBSO | NED 7 Mo |

ASPS alveolar soft part sarcoma, HBSO hysterectomy with bilateral salpingo-oophorectomy, NED not evidence of disease, Mo months
| Authors and years | Macroscopic findings | Microscopic findings | Immunohistochemical findings | Electron microscopy |
|------------------|----------------------|----------------------|-----------------------------|---------------------|
| Gray et al. (1986) [27] | Uncapsulated, circumscribed intramyometrial nodule, 0.4 cm | Large cell, arranged in organoid appearance separated by delicate fibrovascular stroma | ND | Membrane bound crystalline granule inclusions in the cytoplasm |
| Nolan and Gaffney (1990) [28] | Mass of 7 cm, bulging into endometrial cavity | Large cells with granular and vacuolated cytoplasms, organoid arrangement, separated by fibrovascular septae.PASDR crystals | ND | Intracytoplasmic crystalline inclusions |
| Guillou et al. (1991) [4] | 3 × 2.5 × 2.5 cm well-circumscribed intramyometrial nodule | Uncapsulated lesion pushing border delineated by endometrium, large cells with organoid arrangement, separated by fibrovascular septae, granular cytoplasms with crystals. Areas with nuclear pleomorphism | POS Vim, focal POS HMB45, NKJ/C3, patchy dot-like cytokeratin lw POS | ND |
| Burch et al. (1994) [29] | 3-cm endometrial polyp | Large cells with granular cytoplasm, crystals on PAS D | ND | Intracytoplasmic crystalline inclusions |
| Nielsen et al. (1995) [30] 3.5-cm submucosal nodule | Large cell, arranged in organoid appearance separated by delicate fibrovascular stroma crystals on PAS D | ND | ND |
| Radig K et al. (1998) [31] | 3-cm well-circumscribed yellow-whitish, intramural nodule | Pseudoalveolar and trabecular pattern, polygonal and round cells vesicular nuclei, with nucleoli, crystals on PAS D | NEG for S100, Ck, EMA, POS caldes | Intracytoplasmic crystalline inclusions |
| 4.5-cm well-circumscribed, intramural nodule | Pseudoalveolar pattern, granular | Intracytoplasmic crystalline inclusions absent | ND |
| Kasashima S et al. (2007) [5] | 1.9 × 1.9 × 1.0 cm greyish, endometrial exophytic nodule | Large cells with organoid arrangement, separated by fibrovascular septae, granular cytoplasms with crystals | Nuclear POS: TFE3, PGR, ER, CD10 | ND |
| Zhang et al. (2012) [32] | 2.4 × 2.0 × 1.8 cm yellow-whitish exophytic tumour, sub-endometrium of the lower uterine segment | Large cells with organoid arrangement, separated by delicate fibrovascular septa, granular cytoplasms with crystalsM lymph node | NEG: Ae1/AE3, EMA, Sma, Des, S-100, CD10, Synapt, Chrom | ND |
| Present case | 5-cm intramural nodule, well-circumscribed, with irregular border, soft yellow, brownish and grey, with a large hemorrhagic zone centrally located | Solid “non-organoid” pattern, abundant eosinophilic granular cytoplasm, with distinct border, and vesicular nuclei and prominent nucleoli | Focal POS to Sma, desm, Caldes and diffuse and strong nuclear POS to TFE3 | Intracytoplasmic crystalline inclusions |
| | | Areas with spindle elements with nuclear, pleomorphism, hyperchromasia, nuclear cytoplasmic pseudo-inclusions and multinucleations | Diffuse POS nuclear TFE3 | |

**ASPS** alveolar soft part sarcoma, **Caldes** caldesmin, **Chrom** chromogranin, **CK** cytokeratin, **EMA** epithelial membrane antigen, **ER** oestrogen receptor, **caldes** caldesmon, **Immuno** immunohistochemistry, **inh** alpha-inhibin, **Lym** lymph node, **lw** low weight, **MLymph** lymphonodal metastasis, **MTF** microphthalmia transcription factor, **Mo** months, **Myog** myogenin, **Myogl** myoglobin, **ND** not done, **NSE** neuron-specific enolase, **PASDR** periodic acid-Schiff diastase resistant, **PGR** progesterone receptor, **POS** positivity, **Sma** muscle-specific actin, **Synapt** synaptophysin, **TFE3** transcription factor 3, **Vim** vimentin
lungs, bone and brain are the main cause of death [41, 42]. Lesions with size less than 5 cm in diameter seem to be correlated with a more favourable outcome [43]. ASPS in the uterine corpus has a better prognosis than ASPSs in the soft tissues and other cases located in the vagina. All patients with ASPS located in the uterine corpus were alive and well at the time of the last follow-up (Table 1). In contrast, one of six patients with vaginal ASPS died as a result of the tumour. Initially, this patient had a recurrence 4 months after local excision and external radiation therapy and then died of the disease with pulmonary metastases 25 months later [23, 24]. Another patient with vaginal ASPS had a recurrent 1.5-cm mass, 4 months after the initial diagnosis [25].

The more favourable prognosis in ASPS located in the uterine corpus may be due to small tumour size, anatomical location or relative short duration of follow-up. Indeed, in all patients, the ASPS of the uterine corpus, except for the present case and the case reported by Nolan and Gaffney, measured less than 5 cm [28] (Table 2). Only in the case reported by Zhang et al. was the pelvic and para-aortic lymph node metastasis observed at diagnosis, but the duration of follow-up in this case was short (9 months) and it is not possible to establish its outcome [32].

Conclusions
An essential diagnostic marker in this unusual presentation of neoplasms is the nuclear over-expression of TFE3 as well as ultrastructural study, which reveals the presence of peculiar cytoplasmic crystalline inclusions.

Moreover, in our opinion, a larger number of cases of ASPS in the female genital tract with longer follow-up and pathological findings including sizes should help to better define the biological nature of ASPS in the uterine corpus. In addition, although lymph nodes metastasis was observed only in the case of Zhang et al., surgical staging with complete pelvic lymph nodes sampling could be useful to evaluate therapy and prognosis of this rare neoplasm.

Consent
Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Abbreviations
ASPS: alveolar soft part sarcoma; Caldes: caldesmon; Chrom: chromogranin; CK: cytokeratin; EM: electron microscopy; EMA: epithelial membrane antigen; ER: oestrogen receptor; Desm: desmin; HBSCO: hysterecctomy with bilateral salpingo-oophorectomy; Immuno: immunohistochemistry; inh: alpha-inhibin; Lym: lymph node; lw: low weight; MLymph: lymphonodal metastasis; MetF: microphthalmia transcription factor; Mo: months; Myogen: myogenin; Myog: myoglobin; NED: not evidence of disease; ND: not done; NSE: neuron-specific enolase; PASDR: periodic acid-Schiff diastase resistant; PGR: progesterone receptor; POS: positivity; Sma: muscle-specific actin; Synapt: synaptophysin; TFE3: transcription factor 3; Vrm: vimentin.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
GG and ES carried out the study design and writing. TD did the electron microscopy study. EV participated in the literature search. GC performed the operation of the patient. All authors read and approved the final manuscript.

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