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EPITHELIOID TROPHOBLASTIC TUMOR INITIALLY MISINTERPRETED AS UTERINE SMOOTH MUSCLE TUMOR WITH VAGINAL AND SIGMOID COLON METASASES

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ABSTRACT

The epithelioid trophoblastic tumor (ETT) is a rare trophoblastic neoplasm with a wide spectrum of differential diagnoses and unpredictable clinical behaviour.

A 43-year-old woman underwent hysterectomy for an intramural uterine nodule initially misinterpreted as smooth muscle tumor. Subsequently, 91 and 113 months later, two recurrences were discovered: at the vaginal stump and in the sigmoid colon. The histological and immunohistochemical findings of the primary and the metastatic tumors were consistent with the diagnosis of ETT.

Therefore, immunohistochemistry could help in confirming the diagnosis of ETT. In these patients, subsequent to primary surgical treatment, a careful and prolonged follow-up is warranted.

Key words: epithelioid trophoblastic tumor, chorionic-type intermediate trophoblast, metastasis, vagina, sigmoid colon, immunohistochemistry.

INTRODUCTION

Gestational trophoblastic disease constitutes a diverse group of lesions with specific pathogenesis, morphological characteristics, and clinical features [1]. The modified World Health Organization (WHO) classification of gestational trophoblastic diseases includes complete and partial hydatidiform mole, invasive mole, choriocarcinoma, placental site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ETT), exaggerated placental site, and placental site nodule [1]. ETT is an unusual type of trophoblastic neoplasm distinct from PSTT and choriocarcinoma with features resembling a poorly differentiated carcinoma [2].

Originally termed "atypical choriocarcinoma," it was first described in patients with lung metastases that were chemoresistant but amenable to surgical resection [3-5]. Most of these patients had been treated with chemotherapy for choriocarcinoma and hydatidiform mole, some of them with unsatisfactory responses despite prolonged treatment. Therefore, Mazur et al. concluded that the chemotherapy prolonged the course of the disease allowing the atypical pattern to emerge, or that it directly influenced the growth of tumor cells by drug-induced alterations [3]. Similar tumors found in the uteri of 7 patients after the evacuation of hydatidiform moles were reported by Silva et al. [6] as "multiple nodules of intermediate trophoblast."

In 1994, Mazur and Kurman recognized occurrence of similar tumors in the uterus, and proposed the term *epithelioid trophoblastic tumor* (ETT) for this monomorphic variant of gestational choriocarcinoma [7]. Subsequently, in 1998, Shih and Kurman [2] reported 14 cases of ETT, most of which were uterine tumors, describing their clinicopathological and immunohistochemical features. In contrast to lung lesions, uterine ETT developed without a background of prior chemotherapy for choriocarcinoma, suggesting that ETT is a distinct pathologic entity and not simply a treatment-related finding. In 1999, Shih and Kurman suggested that the origin of this disease could be chorionic-type intermediate trophoblastic cells, which are normally seen in the chorion laeve, while placental site trophoblastic tumors originated from the intermediate trophoblastic cells in the implantation site, based on their morphological

and immunohistochemical results [8]. ETT was therefore considered to be a unique tumor originating from intermediate trophoblast, and could be differentiated from other gestational trophoblastic tumors based on its unusual morphological and immunohistochemical characteristics.

Epithelioid trophoblastic tumors are rare. Less than 50 cases have been reported, including tumors termed "atypical choriocarcinoma" and "nodules of intermediate trophoblast" [2-6,9-18]. ETT usually occurs in women of reproductive age [1,2] and shows a wide spectrum of differential diagnoses and clinical behaviour. The diagnosis is usually related to a gestational event, although this may not be a true relationship (up to 25 years later in one case) [17]. Clinically, serum human chorionic gonadotropin (hCG) levels are variable and usually only slightly elevated. Primary ETT has been described in the uterus and the cervix and, in one patient, in the fallopian tube [15], and metastases have been described in the lung, small bowel, and vagina [2,10]. Some cases have presented with extra-uterine disease (lungs, broad ligament) without evidence of a primary tumor [2-5,9,18]. In these cases, the primary (uterine) tumor has presumably regressed as may occur in choriocarcinoma.

The following case describes a patient with ETT which was initially misinterpreted and treated as uterine smooth muscle tumor, in which two recurrences were discovered: one at the vaginal stump 91 months after the hysterectomy, and another 22 months later in the sigmoid colon presenting as stercoral ileus. The clinicopathological features, and immunohistochemical findings of the primary and metastatic tumors, differential diagnoses, and the results of the English-language literature review are presented.

CLINICAL SUMMARY

A 43-year-old woman, gravida 3, para 2, artificial abortion 1, was referred to the Clinic for Gynecology and Obstetrics in March 1995. On her first visit, her uterus was slightly enlarged and a 5 cm mass was detected in the posterior wall by endovaginal ultrasonography, suggesting leiomyoma. Previous reproductive history included two normal term vaginal deliveries in 1983 and 1986, and a first trimester artificial abortion approximately 5 years prior to this event. She had a regular menses at that time and there was no history of chemotherapy or trophoblastic disease. Laboratory data, including complete blood count, blood chemistry and urinalysis, were all well within normal limits. The serum human chorionic gonadotropin (hCG) level was not evaluated before or after the operation.

A total abdominal hysterectomy with bilateral ovarian resection was performed on 23 March, 1995 with a preoperative and presumptive diagnosis of uterine leiomyoma. Intraoperative findings included an enlarged uterus with a clinically suspicious intramural solid/cystic, partly necrotic nodule in the posterior wall, cystic ovaries, normal fallopian tubes, and absence of ascites and lymphadenopathy. She had an uneventful postoperative course and was discharged on the seventh postoperative day. The pathologic examination of the hysterectomy specimen revealed "uterine leiomyosarcoma," and the patient was advised that bilateral salpingo-oophorectomy should be performed with subsequent chemotherapy. The patient insisted on second opinion histopathological examination of the operative specimen in another institution abroad. The second laboratory confirmed that the uterine nodule was a smooth muscle tumor. Nevertheless, according to them it was not a malignant tumor, but benign, "atypical, symplastic leiomyoma". Then the patient was lost to follow-up.

She was hospitalized at the Clinic for Gynecology and Obstetrics for the second time in October 2002, because of sustained vaginal spotting for 10 months. A suspicious ulcerated lesion at the vaginal stump was detected on speculum examination and a punch biopsy was performed. No images suggestive of metastases were observed on computed tomographic (CT) scan of the abdomen and pelvis, abdominal ultrasound, intravenous urography, and chest X-ray. Rectoromanoscopy also revealed normal findings. Serum beta-human chorionic gonadotropin (β hCG) level was found to be 3.59 mIU/ml (normal <6.1 mIU/ml). The histological and immunohistochemical findings of the biopsy material taken from the vaginal lesion, as well as the primary uterine tumor in the operative specimen were consistent with the diagnosis of ETT.

The patient underwent a partial colpectomy and bilateral salpingo-oophorectomy with right inguinal lymph node dissection in another institution. The histopathological analysis of the operative specimen according to the report confirmed the diagnosis of a metastatic ETT at the vaginal stump. According to the anatomical staging system for gestational trophoblastic tumors adopted by FIGO, the disease was in Stage II. The patient was also scored according to the modified WHO scoring system for determining resistance to chemotherapy and was found to have a high-risk of chemotherapy-resistant disease (WHO score = 10). In February 2003 she was commenced on the multiagent combination chemotherapy (methotrexate, actinomycin D, cyclophosphamide and leucovorin) for high-risk gestational trophoblastic disease at the Institute of Radiotherapy and Oncology, following a regime of 3-weekly courses. Nevertheless, after administration of three courses of chemotherapy without significant clinical complications, the patient refused therapy. She remained well until July 2004 when at regular check-up a palpable suprapubical tumor 10 cm in diameter was discovered. Serum β hCG level was 184 mIU/ml.

During the next few weeks her condition deteriorated significantly and she was readmitted at the Clinic for Gynecology and Obstetrics for the third time with symptoms of retrovesical abscess. Abdominal ultrasound revealed a 6.4 x 4.9 x 5.7 cm supravesical mass, suggesting abscess. Two weeks later, in spite of incision and administrated antibiotic therapy, the patient's condition further deteriorated. Native abdominal x-ray confirmed the clinical diagnosis of stercoral ileus and exploratory laparatomy was performed on 3 August 2004. Perforated sigmoid colon infiltrated by a tumor mass with features of stercoral peritonitis was found, and partial sigmoid colon resection was indicated. There was no obvious tumor spread elsewhere in the abdominal cavity. The patient made an uneventful postoperative recovery. Histopathology confirmed metastatic involvement of the sigmoid colon by ETT. The patient's preoperative serum β hCG level 177 mIU/mL decreased to 2.8 mIU/mL one month after surgery. She then received 6 courses of chemotherapy with a regimen of methotrexate, actinomycin D, cyclophosphamide and leucovorin over a 3-month period. There was no clinical evidence of disease at her last follow-up visit in January 2005, almost 10 years after hysterectomy.

MATERIALS AND METHODS

The hysterectomy/bilateral ovarian resection specimen, as well as the biopsy material from the vaginal tumor and the resected sigmoid colon were fixed in 10% neutral-buffered formalin. Representative sections were embedded in paraffin, cut at 5μ, placed on slides, and stained with hematoxylin-eosin. For immunohistochemical staining, additional 5μ thick sections of the primary and the metastatic tumors were deparaffinized, rehydrated, and stained using the streptavidin-biotin peroxidase complex technique (Vectastain ABC kit, Vector Labs, Burlingame, CA). The reaction product was detected with 3,3'-diaminobenzidine chromogen (DAB, Vector Labs). Details about the primary antibodies used, their sources, and working dilutions are listed in Table 1. Antigen retrieval was usually performed by boiling in 10 mM citrate buffer, pH 6.0, for 5-15 minutes, 3 times in a microwave oven, while pretreatment with proteinase K was used for epidermal growth factor receptor (EGFR). Appropriate positive and negative controls were tested along with the tumors. The estimation of the percentage of the immunoreactive cells was determined on the percentage of positive tumor cells in at least 500 randomly selected tumor cells in a single section. The percentage of positive cells was scored semiquantitatively as follows: (-) = no staining, (+/-) = 1-5%, (+) = 6-25%, (++) = 26-50%, (+++) = 51-75%, (++++) = >75% of tumor cells positive.

RESULTS

Gross findings

On gross examination, according to the original histopathological record, the hysterectomy specimen consisted of a uterus, multiple ragged, partly necrotic tumor fragments measuring 0.5 – 2.5 cm, and resected fragments of the right and left ovary. The uterus measured 7 x 8 x 7 cm, and contained a partly solid and partly cystic tumor mass, measuring 6 cm in largest diameter, located entirely within the myometrium of the posterior uterine wall that

was opened intraoperatively. The serosal surface of the uterus was unremarkable, as well as the uterine cavity. However, on cross-section, the myometrium was involved almost entirely through the wall by well-circumscribed, friable, necrotic, and tan to dark-brown nodule. The endometrium was 0.1 cm thick and unremarkable. The cervix measured 3 x 3 x 3cm, and was also unremarkable. The resected right ovarian fragment measuring 2 x 2 x 1 cm contained only few small cortical cysts. The left ovary fragment consisted of a solitary, thin walled cyst with smooth inner surface filled with serous fluid measuring 6 x 5 x 4 cm.

The biopsy material from the ulcerated vaginal lesion consisted of 3 small fragments measuring 0.5 – 0.7 cm in largest diameter.

The resected sigmoid colon segment was 9 cm long, and its serosal surface was covered by purulent exudate. The tumor, a grossly irregular, spongy looking, tanned to dark-brown mass, with small hemorrhagic foci was located at the sigmoid colon wall involving it from the mesocolic fat tissue to the ulcerated mucosal surface. In the middle of the segment there was an irregular perforation with ragged borders, 6.5 cm in largest diameter. A 2 cm long fistulous channel was also present in the involved sigmoid colon wall. The rest of the sigmoid colon wall was unremarkable, as well as the two lymph nodes measuring 0.3 and 0.5cm in diameter, dissected from the mesocolic fat tissue.

Microscopic findings

Microscopically, the uterine tumor had an epithelioid appearance, both in terms of its pattern of growth and the morphology of the neoplastic cells. The tumor was circumscribed, with an expansive pushing border, and was surrounded by a lymphoplasmacytic infiltrate, which extended into the intratumoral stroma. The tumor nests were observed just beneath the endometrium, but some of them reached its basalis layer. The tumor cells grew in anastomosing cords, nests, islands, and sheets. Extensive geographic necrosis accompanied by dystrophic calcification, eosinophilic necrotic debris or hyaline-like material, which resembled keratin material, was seen either focally in the center of massive tumor cell nests or surrounded the nests, coalescing to form large aggregates (Fig. 1a). Typically, a small blood vessel was located within the center of tumor nests. Most tumor cells were mononuclear and had an epithelioid appearance with distinct cell borders, lightly eosinophilic or amphophilic cytoplasm, and nuclei with occasional indistinct nucleoli. The neoplastic cells resembled chorionic-type intermediate trophoblastic cells; they were larger than cytotrophoblastic cells but smaller than implantation site intermediate trophoblastic cells. Occasionally, larger cells were present with convoluted, irregular and slightly hyperchromatic nuclei and moderate amount of eosinophilic cytoplasm, resembling implantation site intermediate trophoblastic cells (Fig. 1b). Mitotic figures of the mononuclear tumor cells varied in different tumor areas from 3 to 9 mitoses (MF, mitotic figures) per 10 high power fields (HPF, x40). Scattered multinucleated cells consistent with syncytiotrophoblastic cells were also present. Other findings included small foci of hemorrhage and the absence of chorionic villi or vascular or lymphatic invasion. Evaluation of the specimen also revealed a proliferative endometrium, focal adenomyosis in the uninvolved myometrium, and chronic cervicitis. The right ovary contained small follicle cysts, while a simple cyst was present in the left ovary.

The metastatic lesions both in the vagina (Fig. 1v) and the sigmoid colon (Fig. 1g) had identical morphology as the primary uterine tumor. The surgical margins of the resected segment of the sigmoid colon as well as two mesocolic lymph nodes were free of tumor.

Immunohistochemical Findings

The results of the immunohistochemistry are summarized in Table 1. Pan-cytokeratin MNF-116 (Fig. 2a), as well as cytokeratin 7, low molecular weight cytokeratin 34βE11, and epidermal growth factor receptor (EGFR) showed diffuse strong reactivity in the tumor cells with a cytoplasmic and membranous pattern of staining. There was also diffuse and less intensive reactivity for epithelial membrane antigen (EMA) and high molecular weight cytokeratin 34βE12. Scattered tumor cells or small clusters also showed positive membranous or cytoplasmic staining for placental alkaline phosphatase (PLAP, Fig. 2b), while reactivity for human chorionic gonadotropin (hCG) was limited to a few of the larger mononuclear and binuclear cells. The tumor cells were also focally positive for CD10 (CALLA), as well as inhibin α (Fig. 2v), and negative for most of the

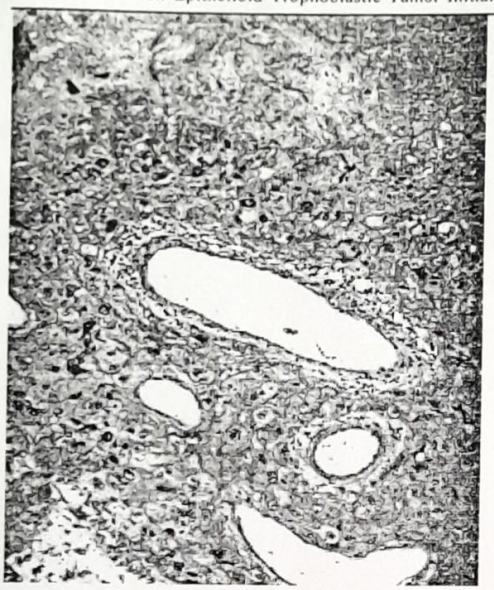

Fig.1a.

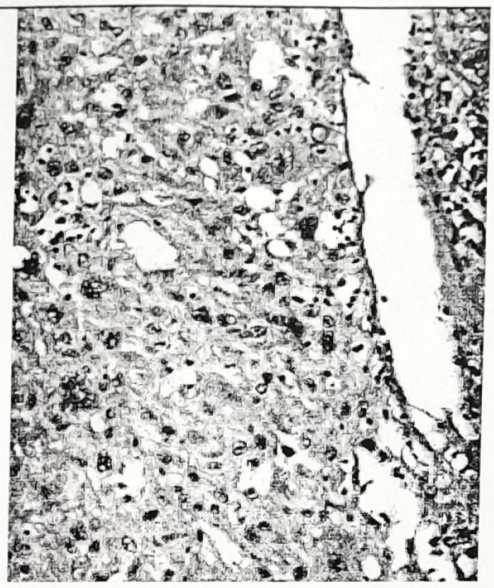

Fig.1b.

Fig.1v.

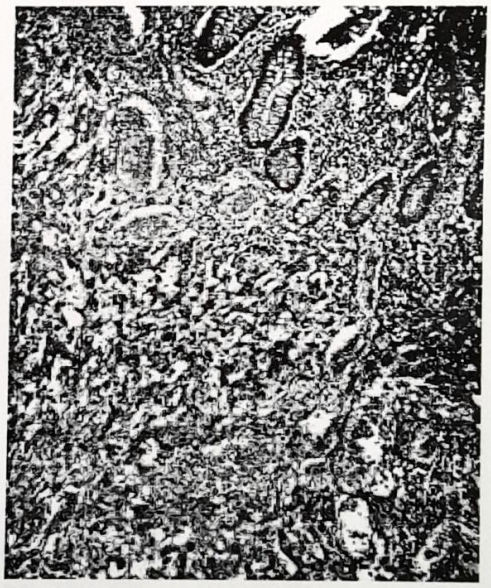

Fig.1g.

Fig. 1. Morphology of the tumor. (**a**) In the hysterectomy specimen, the tumor cells grow in anastomosing cords, nests, islands, and sheets distributed in the hyaline-like material resembling keratin (H&E staining, original magnification, x100). (**b**) These epithelioid islands are composed of cells with abundant eosinophilic cytoplasm with relatively larger and more pleomorphic nuclei, and cells with lightly eosinophilic to amphophilic cytoplasm with smaller and more uniform nuclei. Multinucleated syncytiotrophoblastic giant cells are scattered among the mononuclear cells (H&E staining, original magnification, x200). (v) The vaginal tumor showing a striking epithelioid appearance is present beneath the surface squamous epithelium (H&E, original magnification, x100). (g) The tumor infiltrates the mucosa of the sigmoid colon (H&E, original magnification, x100).

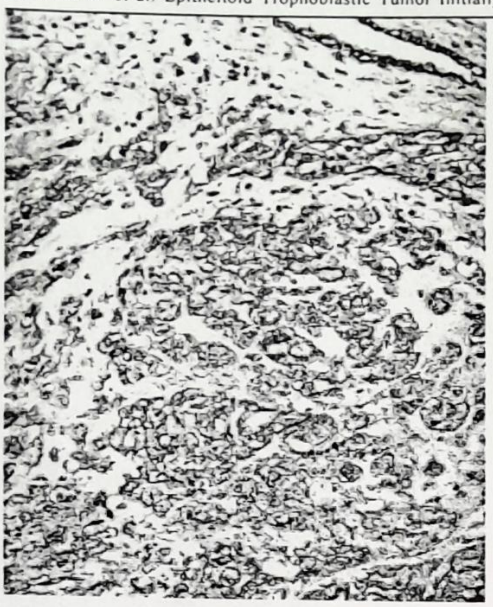

Fig.2a.

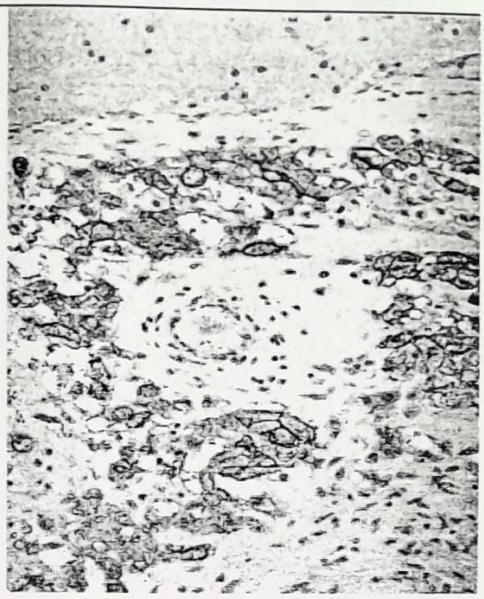

Fig.2b.

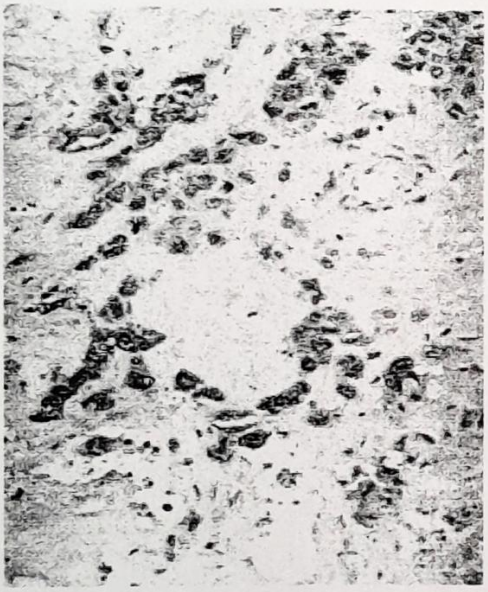

Fig.2v

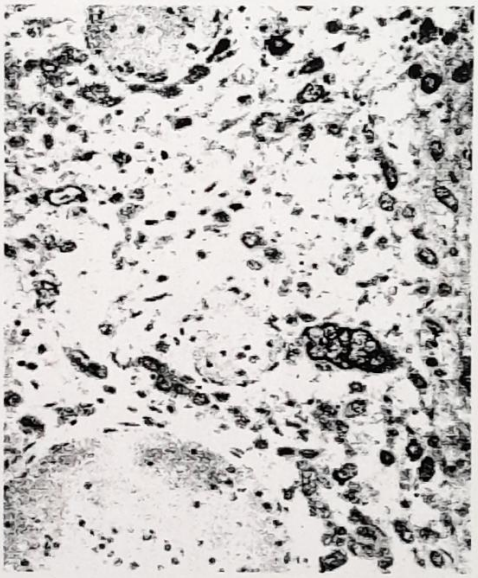

Fig.1g

Fig. 2. Immunohistochemical features of the neoplasm. (a) There is a diffuse strong cytoplasmic and membranous staining for pan-cytokeratin MNF-116 in the primary tumor beneath the basalis layer of endometrium (original magnification, x200). (b) The tumor cells show focal and patchy positive membranous or cytoplasmic staining for placental alkaline phosphatase (original magnification, x200). (v) Inhibin α is focally positive in the cytoplasm of the tumor cells (original magnification, x200). (g) One syncytiotrophoblastic cell as well as a few scattered mononuclear cells show strong cytoplasmic staining for human chorionic gonadotropin in the sigmoid colon tumor (original magnification, x200).

Table 1. Antibodies, their sources, working dilutions, and the results of semiquantitative immunohistochemical scoring of the primary ETT.

Antibody	Clone	Source	Dilution	Results
Pan-cytokeratin	MNF-116	Dako	1:150	++++
Cytokeratin 7	OV-TL 12/30	Dako	1:400	++++
Cytokeratin 20	Ks20.8	Dako	1:500	-
High molecular weight cytokeratin	34²E12	Dako	1:100	+++
Low molecular weight cytokeratin	34²E11	Dako	1:100	++++
CEA (carcinoembryonic antigen)	II-7	Dako	1:400	-
EMA (epithelial membrane antigen)	E29	Dako	1:300	+++
EGFR (epidermal growth factor receptor)	H11	Dako	1:50	++++
Vimentine	3B4	Dako	1:600	-
Inhibin-α	R1	Dako	1:600	++
CD10 (CALLA)	56C6	Vector	1:100	+++
CD117 (c-kit oncoprotein)	T595	Vector	1:40	-
Desmine	D33	Dako	1:200	-
αSMA (α-smooth muscle actin)	1A4	Dako	1:400	-
Caldesmon	h-CD	Dako	1:200	-
CD99	12E7	Dako	1:100	-
CD34	BI-3C5	Dako	1:100	-
Collagen IV	CIV22	Dako	1:100	-
S-100	polyclonal	Dako	1:3000	-
LCA (leukocyte common antigen)	2B11+PD7/26	Dako	1:400	-
hCG (human chorionic gonadotropin)	polyclonal	Dako	1:15000	+/-
PLAP (placental alkaline phosphatase)	8A9	Dako	1:1000	+++
ER (estrogen receptor)	1D5	Dako	1:80	-
PgR (progesterone receptor)	PgR636	Dako	1:350	-
p53 protein	DO-7	Dako	1:500	27%
bcl-2 oncoprotein	124	Dako	1:200	-
Ki-67 antigen	MIB-1	Dako	1:400	23%

Legend: CD, antigen cluster designation; CD10 (CALLA), common acute lymphoblastic leukaemia antigen,; CD99, MIC2 gene products; CD-34, endothelial cell marker; S-100, neuroendocrine antigen; polyclonal, polyclonal antibody; Dako, Dako Cytomation, Glostrup, Danmark; Vector, Vector Labs, Burlingame, CA. Semiquantitative scoring: (-) = no staining, (+/-) = 1-5%, (+) = 6-25%, (++) = 26-50%, (+++) = 51-75%, (++++) = >75%.

remaining antibodies tested. The Ki-67 proliferation index was found to be 23%, while p53 positive immunostaining was present in 27% of the tumor cells. These findings were almost entirely consistent between the uterine tumor and the metastatic tumors, except for significantly higher percentage of inhibin α and hCG positive cells present in the recurrent tumors (Fig. 2g).

Therefore, the clinical, morphologic, and immunohistochemical features of the primary tumor and metastatic lesions were characteristic of ETT, and helped to distinguish it from other trophoblastic tumors, squamous cell carcinoma and smooth muscle tumors.

DISCUSSION

This is the first case report of ETT presenting as a late vaginal metastasis occurring more than seven years following hysterectomy in a patient with an intramural uterine nodule initially misinterpreted as smooth muscle tumor. ETT is a rather new entity of gestational trophoblastic tumor and develops from the neoplastic transformation of chorionic-type intermediate trophoblast, but the overall frequency is difficult to assess because of the paucity of recorded cases [2-6,9-18]. Almost all previously reported cases [2-6,9-18], including those reported under other names but included as epithelioid trophoblastic tumors by Shih and Kurman, occurred in women of reproductive age (range, 15–66 years old, average, 34.7 years). Our patient was 43 year old at the time of hysterectomy. Both in the series of Shih and Kurman [2] and in the other previously published cases [3-6,9-18], there was a history of either a hydatidiform mole or invasive mole that preceded the diagnosis in almost one half of the cases (47.6%). The antecedent gestational

events in the remaining cases included full-term deliveries (31%), and abortions (21.4%) [2-6,9-18]. The interval between the preceding gestational events and the diagnosis of ETT ranges from 1 to 25 years (average, 6.2 years). In our patient the last pregnancy was terminated by artificial abortion 5 years earlier. Contrary to our case, the most common presentation is with abnormal vaginal bleeding, although the presentation in some cases is with lung, vaginal or broad ligament metastases [2-5,9,10,18]. Serum beta-human chorionic gonadotropin (β hCG) levels are always elevated, but generally low (<2500 mIU/mL) at the time of diagnosis [2-5,9,10,18]. In our case slightly elevated serum β hCG levels were detected only when the metastasis in the sigmoid colon emerged. All primary ETTs were reported to be solitary, discrete nodules that deeply invaded the myometrium in 19 cases, the lower uterine segment or endocervix in 8 cases, and the uterine tube in one case [2,6,10-18]. The tumor size was reported to vary from 0.3 to 9 cm [2,6,10-18]. The tumor in our patient is the largest reported uterine ETT, measuring 6 cm in diameter.

Although ETT is an exceedingly rare tumor, the diagnosis can usually be made on morphological grounds. In particular, given the young age of most women with this lesion, especially in the context of pregnancy or elevated serum β hCG, an awareness of this entity in these clinical settings should alert the reporting pathologist. Nevertheless, our case report illustrates the difficulties in diagnosing this rare tumor partly due to the unusual clinical presentation in an asymptomatic woman over 40 years old, who underwent hysterectomy with the diagnosis of a uterine leiomyoma. An additional obstacle in making the correct diagnosis was, most probably, unawareness of the morphology of this tumor and of the entity itself.

Epithelioid trophoblastic tumors, as exemplified by our case, have a distinctive growth pattern and immunohistochemical profile. The differential diagnosis of ETT includes other forms of gestational trophoblastic disease (i.e., placental site nodule, exaggerated placental site reaction, placental site trophoblastic tumor and choriocarcinoma), poorly differentiated carcinoma, particularly keratinizing squamous cell carcinoma of the cervix and epithelioid leiomyosarcoma [1,2,19]. The distinction of an ETT from a placental site nodule (PSN), another trophoblastic lesion originating from chorionic-type intermediate trophoblast that is considered to be the benign counterpart of ETT, is usually made without difficulty. PSNs are microscopic lesions with less cellular and sharp borders, while ETTs are larger and display substantial necrosis. Mitotic counts and Ki-67 proliferative index can assist in the differential diagnosis. Mitotic activity in PSN is low or absent, while the Ki-67 index in ETT (>10%) is significantly higher than in PSN (<10%) [1,8,19]. Exaggerated placental site reaction and placental site trophoblastic tumor are said to show differentiation toward 'implantation-type' intermediate trophoblast and are characterised by an infiltrative growth pattern [1,8,19]. In addition, exaggerated placental site reaction has a benign appearance and almost never produces a tumor mass. Accordingly, mitoses are not seen in exaggerated placental site reaction. Placental site trophoblastic tumor (PSTT) can be difficult to differentiate from ETT as these are both malignant neoplasms of subtypes of intermediate trophoblast. The most characteristic microscopic feature for differential diagnosis between ETT and PSTT might be the manner in which the tumor invades the myometrium and vessels [1,19]. These two tumors of intermediate trophoblast are described as having distinctive immunohistochemical profiles. PSTT is described as being diffusely positive for human placental lactogen (hPL) and focally positive for placental alkaline phosphatase (PLAP), whereas ETT has focal positivity for hPL and diffuse positivity for PLAP. According to Shih and Kurman, immunostaining with Mel-CAM (CD146), a cell-adhesion molecule, could be helpful in differentiating between these tumors because it is highly expressed by PSTT, and only focally positive in ETT [2,8,19]. In addition, HLA-G, a MHC class I antigen, has recently been shown to be a specific marker for normal intermediate trophoblast. HLA-G immunoreactivity was found in all the cells of exaggerated placental sites and PSTTs, and 70-100% of the cells in PSNs and ETTs. Nevertheless, the majority of mononucleotide cells in choriocarcinoma were also HLA-G immunoreactive suggesting that this tumor is related to villous-type intermediate trophoblast [20]. Another, more recent study by Shih and Kurman [21] demonstrated that p63, a

transcription factor belonging to the p53 gene family, could be used in distinction of ETT and PSTT by profiling trophoblastic subpopulations. According to their results, p63 immunoreactivity was found in all ETTs and PSNs. In choriocarcinomas the p63 staining was confined to nuclei of mononucleate cells, while neither PSTTs nor exaggerated placental sites showed detectable p63 immunoreactivity. In the differential diagnosis of a choriocarcinoma, the invasive pattern is very important. ETT forms nodules that invade in an expansive fashion, while choriocarcinoma invades by destroying the myometrium with extensive hemorrhaging. Immunostains for hCG, inhibin-α, cytokeratin, and Ki-67 can be helpful in the diagnosis. The ETT cells immunoreactive for hCG are, like those of PSTT, usually less diffusely distributed than those in choriocarcinoma [1,2,19]. However, in choriocarcinoma, β hCG is diffusely positive, highlighting numerous syncytiotrophoblastic cells, whereas hPL positive cells are less conspicuous [22]. In addition, tumor cells in ETT are positive for cytokeratin and inhibin α [1,19]. Although tumor cells of choriocarcinoma could be positive for cytokeratin, only the syncytiotrophoblastic tumor cells are positive for inhibin α [23]. Mitotic counts can assist in the differential diagnosis, because choriocarcinoma is mitotically active (typically more than 10 MF/10 HPF), while there is usually a lower mitotic rate in ETT and PSTT (mean, 2 MF/10 HPF) [1]. In addition, the Ki-67 nuclear labeling index of ETT has an average of 17.7 ± 4.5% (range, 10%-25%), while that of PSTT is 14 ± 6.9%, and that of choriocarcinoma is much higher at more than 25% (69 ± 20%) [1].

Differentiating keratinizing squamous cell carcinoma of the cervix from ETTs can be challenging, especially in a biopsy or curettage specimen, because of the tendency of ETTs to grow in the lower uterine segment and the cervix [17]. The epithelioid tumor cells of ETT can easily be mistaken for malignant squamous cells, and tumor cells can even replace the surface epithelium and mimic dysplastic squamous epithelium [1,2]. Immunohistochemistry is helpful in the differential diagnosis. β-hCG, hPL and PLAP positive cells are generally not present in squamous cell carcinoma. In addition, most tumor cells of ETTs are positive for inhibin-α and low molecular weight cytokeratins such as cytokeratin 18, whereas squamous cell carcinomas of the uterine cervix are negative for these two markers [1,19]. Furthermore, unlike the relatively low Ki-67 labeling index of ETTs (10–25%), cervical squamous cell carcinomas always have a very high Ki-67 labeling index (>50%) [1,2].

Epithelioid leiomyosarcoma may enter the differential diagnosis, in particular in lesions arising in the uterus. Careful examination of the tumor will usually identify more typical areas of smooth muscle differentiation and should readily distinguish high grade lesions with extensive necrosis from ETT. The immunoprofiles of these two entities are distinctive: epithelioid leiomyosarcoma will express antigens showing smooth muscle differentiation (α-SMA, desmin, HHF35, caldesmon) and not those of trophoblastic disease [1,2,19]. The intramural tumor in our patient had a typical nodular, expansive growth pattern and was initially misinterpreted as smooth muscle tumor. Although the epithelioid nature and atypical constituent cells with eosinophilic or clear cytoplasm and pleomorphic nuclei suggested an epithelioid leiomyosarcoma, the light microscopic features and immunohistochemistry, including cytokeratins and vimentin, desmin, α-SMA and caldesmon highlighted the non-smooth muscle origin of this tumor. In addition, the trophoblastic nature of this neoplasm was confirmed by positive staining for PLAP, hCG, inhibin-α, and CD10 (CALLA). However, we were not able to perform an immunohistochemical study with human placental lactogen (hPL), melanoma cell adhesion molecule (Mel-CAM, CD 146), HLA-G, and p63 since these antibodies were unavailable.

Experience with ETT is limited because less than 50 cases have been reported to date [2-6,9-18]. It is generally accepted that the behaviour of ETT is similar to that of PSTT. Both tend to behave in a benign fashion but metastasis and death occur in approximately 25% and 10% of patients, respectively [1,19]. Similar to choriocarcinoma, lung, liver, abdominal cavity and brain are the sites most frequently involved by metastatic ETTs, as well as PSTTs. Choriocarcinoma and PSTT occasionally metastasize to the vagina, but our search of the English-language literature revealed only one case of ETT with a vaginal metastasis [10]. It is noteworthy that in the present case, the vaginal metastasis occurred more than seven years after

the hysterectomy. Late metastases developing many years after the initial diagnosis of ETT have not been documented to date, although similar behaviour of some PSTTs has already been reported [1]. In majority of the patients in whom ETT recurred the metastases developed in the first 3 years following diagnosis [2-5,14]. Nevertheless, all but five patients with non-recurrent ETTs have been followed-up less than 36 months (range 1-168, mean, 34.5 months) [2,6,11-13,15-17]. In addition, the second recurrence in our patient developed in the sigmoid colon. To date, in only one of the 14 cases of ETT without evidence of prior gestational trophoblastic disease documented by Shih and Kurman [2], the metastasis in the small bowel was reported.

Therefore, due to the small number of cases with ETT that have behaved in aggressive fashion and a relatively short period of follow-up for many cases (mean 3.4 years, range 1 month to 15 years), it was not possible to identify any clinical or morphological feature that predicts outcome [1,2,19]. In general, FIGO stage is the most important prognostic factor for PSTT, although other adverse features such as high mitotic index, with more than 5 MF per 10 HPF and Ki-67 labeling index higher than 50% are more frequently present in malignant examples of PSTT [1]. It is unknown whether a high mitotic index or Ki-67 labeling index are prognostically significant in ETTs, as it is in PSTTs. In our case the mitotic index was from 3 to 9 MF per 10 HPF, which is similar to previously reported in ETT (range, 0-9, average 2 mitoses per 10 HPF) [1,2,19]. The Ki-67 proliferative indices of previously reported ETTs are documented to be around 10% to 25%, although an index as high as 68.6% has been reported [18]. In our case Ki-67 labeling index was 23%. Furthermore, p53 protein was positive in 27% of malignant cells in our case, although it could not be detected in one previously tested ETT [18]. This finding along with bcl-2 negative staining is in concordance with the results reported in a recent study [24] for PSTT, although its prognostic significance needs further elucidation.

Although there are no histological features that reliably correlate with behaviour, it is important to distinguish ETT and PSTT from choriocarcinoma because the recommended treatments differ. According to available data, ETT, like PSTT, may be unresponsive to the chemotherapeutic agents used in the treatment of other types of gestational trophoblastic disease [1,2]. Therefore, the recommended primary treatment is surgical intervention [1,2,19]. Hysterectomy and lung resection have been performed successfully. Long-term survival with persistent and metastatic disease, and also following removal of metastases, has occurred [2-6,9-18]. As for earlier lesions, the effectiveness of curettage and chemotherapy requires more data and further evaluation. Chemotherapy should be considered in ETT if surgical therapy fails. Similarly to PSTT, ETT is relatively resistant to chemotherapy, but there are a few reports of complete response to multiagent chemotherapy [2-6,9-18]. Although serum β hCG levels are variable and generally low in patients with ETT, serum β hCG levels have still successfully been used to monitor treatment.

In summary, we report the unique case of ETT initially interpreted and treated as uterine smooth muscle tumor, which was distinctive by the patient's older age at the time of diagnosis and asymptomatic clinical presentation. Our case exhibited all of the usual gross and light microscopic features of an ETT. The immunohistochemical properties of our case differed slightly from some cases reported in the literature, namely there was a weaker staining for α inhibin, and a weak staining for hCG limited to a few scattered cells in the primary tumor. Furthermore, we could not confirm the results reported by Parker et al. [15] about the CD117 (c-kit oncoprotein) immunoreactivity present in ETT. However, the immunoprofiles of the reported cases are not uniform [2-6,9-18]. In addition, we found positive staining for CD10 (CALLA) which has never previously been reported in ETT. This result is in concordance with the observations reported by Ordi et al. [25] about intensive CD10 positivity present in all types of trophoblastic cells, lesions (hydatidiform moles, PSNs) and neoplasms (PSTT and choriocarcinomas). The ETT in our patient recurred twice in the almost 10 years long follow-up period, developing metastases in the vagina and the sigmoid colon, 91 and 113 months following hysterectomy. Therefore, in patients with ETT, subsequent to primary surgical treatment, a careful and prolonged follow-up is warranted.

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