The value of some immunohistochemical and serological markers for the diagnosis of a peculiar uterine tumor in a postmenopausal woman: case report

Valoarea unor markeri imunohistochimici şi serologici pentru elucidarea unei tumori uterine particulare la femeia în postmenopauză. Prezentare de caz

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Abstract

Introduction: *The epithelioid trophoblastic tumor (ETT) is a rare, unusual trophoblastic proliferation, relatively recently defined as a pathological entity and generally described in women in the reproductive period, far less frequently in the postmenopausal women.*

Case report*: This paper presents the case of a 60 years old patient who was examined by the gynecologist for vaginal discharge and metrorrhagia whose cause could not be established following the examination of the material obtained by uterine currettage. The tumor, placed in the lower uterine segment and the upper part of the uterine cervix, was discovered during surgery with total hysterectomy for persistent metrorrhagia. The diagnosis, originally established in another pathology service, was that of a poorly differentiated squamous cell carcinoma. Further reassessment of the case, by putting together gross, microscopic and immunohistochemical aspects with serological data, established the diagnosis of epithelioid trophoblastic tumor.*

Conclusions*: The case presented herein is the only one diagnosed in the past 17 years in Timişoara, and to our knowledge, the only epithelioid trophoblastic tumor reported in Romania. To be aware of the clinico-morphological particularities of this tumor is particularly important in establishing an accurate diagnosis and in supervised therapy, because the treatment of ETT is different from that of the usual trophoblastic tumors or other uterine tumors that ETT is often mistaken for.*

Keywords: *epithelioid trophoblastic tumor; postmenopause; immunohistochemistry; ßHCG*

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Rezumat

***Introducere**: Tumora trofoblastică epitelioidă (ETT) este o formă rară, neobişnuită de proliferare trofoblastică, relativ recent conturată ca entitate patologică şi descrisă în general la femei în perioada reproductivă, mult mai rar în postmenopauză.*

***Prezentarea cazului**: Lucrarea de faţă descrie cazul unei paciente de 60 de ani care s-a prezentat la medicul ginecolog pentru secreţii vaginale si metroragie a căror etiologie nu a putut fi stabilită în urma examinării materialului obţinut prin raclaj uterin. Tumora, cu localizare cervico-istmică, a fost descoperită intraoperator, în cursul histerectomiei totale efectuate pentru persistenţa metroragiei. Diagnosticul iniţial, stabilit într-un alt serviciu de anatomie patologică, a fost de carcinom scuamos slab diferenţiat. Reevaluarea ulterioară a cazului, prin coroborarea aspectelor macroscopice, microscopice şi imunohistochimice cu datele serologice, au stabilit diagnosticul de tumoră trofoblastică epitelioidă.*

***Concluzii**: Cazul prezentat este unicul diagnosticat în ultimii 17 ani în Timisoara şi, după cunoştinţele noastre, singura tumora trofoblastică epitelioidă raportată în ţara noastră. Cunoaşterea particularităţilor clinico-morfologice ale acestei tumori este deosebit de importantă pentru o încadrare diagnostică exactă şi pentru terapie, deoarece tratamentul este diferit de cel al tumorilor trofoblastice uzuale sau al altor tumori uterine cu care ETT este adesea confundată.*

***Cuvinte cheie:** tumora trofoblastică epitelioidă; postmenopauză; imunohistochimie; ßHCG*

Introduction

The epithelioid trophoblastic tumor (ETT) represents the tumoral subtype, most recently included and characterized, of the trophoblastic tumors group[15]. It is a rare and unusual form of tumor with uterine[2;8;12;14] and rarely extrauterine[5;7] development representing a real challenge in terms of diagnostic and therapy. The tumor is often reported in women in the reproductive period[1;14;15] and much more rarely in the postmenopausal period[2;12].

This paper presents a case of ETT diagnosed in a postmenopausal woman. The diagnosis was suspected after the morphological examination and enhanced by the determination of serologic ßHCG value, being confirmed by immunohistochemistry (IHC) with a wide panel of markers.

Case report

The patient L.E., 60 years old, presented herself at the gynecologist's in January 2007 for vaginal discharge and metrorrhagia that she had been having for 2 months. From her history, we remarked: a normal vaginal delivery at 35 years old, 7 requested abortions, 1 spontaneous abortion at 45 years old, beginning of menopause at 56 years old, diabetes mellitus. The patient said that during her spontaneous abortion, the eliminated tissue fragments presented a grape-like aspect, but there are no data concerning the histopathological aspect of those fragments. Although after the uterine currettage, metrorrhagia persisted for a period of time, in the absence of a hydatidiform mole diagnosis, the patient did not follow any kind of treatment. Following the previously mentioned uterine curettage (January 2007), the tissue fragments examined in our service, did not present any tumoral aspects. The persistence of metrorrhagia imposed total hysterectomy with bilateral salpingo-oophorectomy, performed in a hospital from another town. During the gross examination of the hysterectomy specimen there uterine leiomyomas with dimensions between 4 and 15 mm, endometrium, fallopian tubes and ovaries without notable changes could be observed. At the lower uterine segment and the upper part of the uterine cervix, a nodular tumor was identified, 3/3.5 cm in diameter, distorting the region by bulging out of the

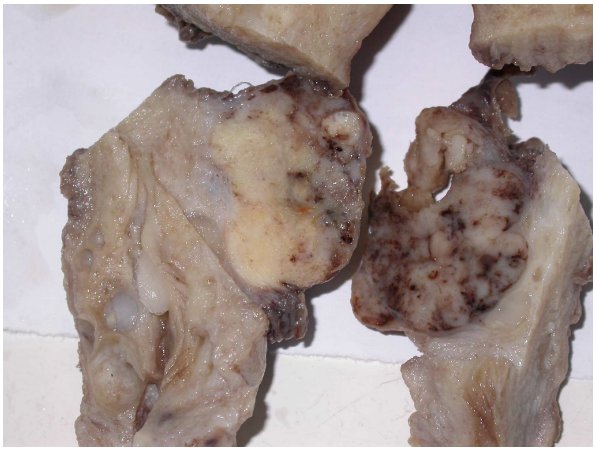

Figure 1. Gross aspect of the tumor which involves the lower uterine segment and the upper cervix.

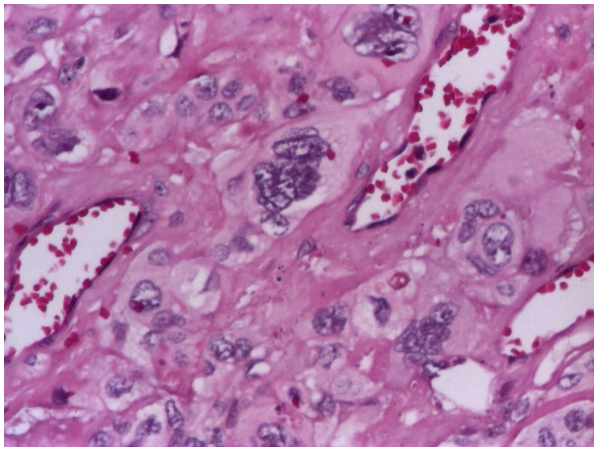

Figure 2. Epithelioid trophoblastic tumor: tumoral cells embedded in an eosinophilic fibrillar hyaline-like material. HE, x400.

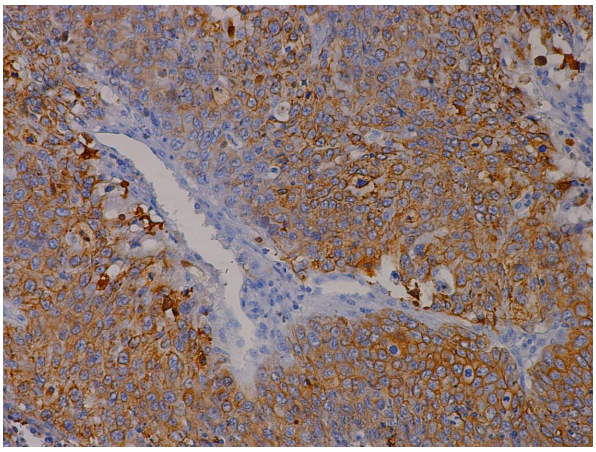

Figure 3. Diffuse expression for CK AE/AE3 in the tumoral cells. Anti-CK AE1-AE3, LSAB2 system, DAB visualization, counterstain with hematoxylin, x200.

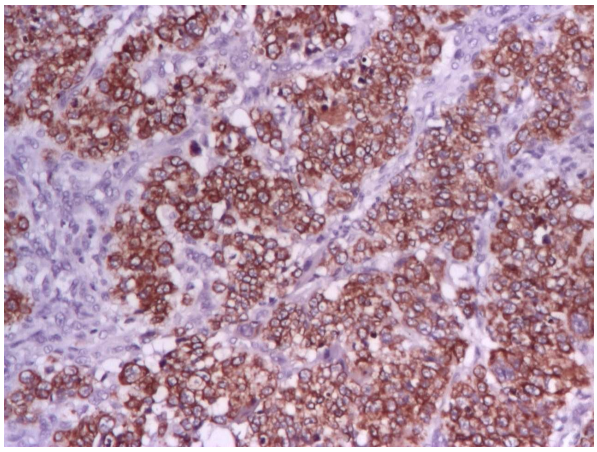

Figure 4. Tumoral cells with diffuse positive reaction for CK 7. Anti-CK7, LSAB2 system, DAB visualization, counterstain with hematoxylin, x100.

organ's outline, apparently circumscribed, with a variegated aspect on cross section: beige-tan-coloured with haemorrhagic spongy areas and dry friable areas of necrosis (*Figure 1*). Seemingly, the tumor did not communicate with the endocervical canal. The tumor discovered during surgery was initially diagnosed as a cervical poorly-differentiated keratinized squamous carcinoma. The histological slides and the rest of the hysterectomy specimen were sent for re-evaluation in our service. Microscopically, on HE stain, we observed a tumoral proliferation with pushing-type margins, with infiltration of the myometrium (95% of its thickness) and of the endocervix, sparing the endocervical glands. The tumor consisted of sheets, islands and cords of epithelial-like cells, polygonal, rounded or elongated, with mostly well-defined cellular limits, eosinophilic, amphophilic or clear cytoplasm, most of them presenting a single pleomorphic, irregular nuclei, some of them vesiculous, with conspicuous nucleoli, others with hyperchromia, bizarre shape and others multilobulated; we noticed areas with high mitotic activ-

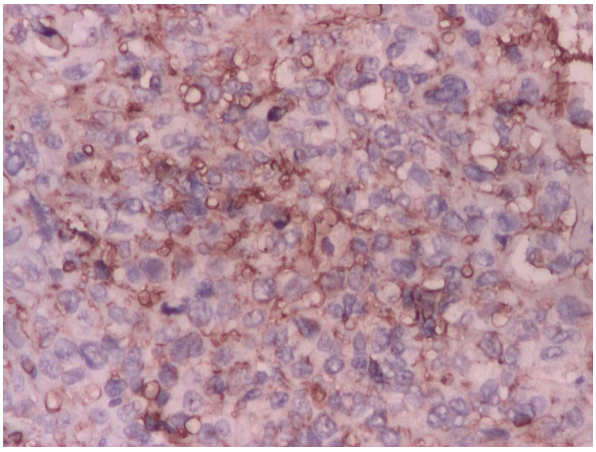

Figure 5. Diffuse positive reaction for CD10 in the tumoral cells. Anti-CD10, LSAB2 system, DAB visualization, counterstain with hematoxylin, x200.

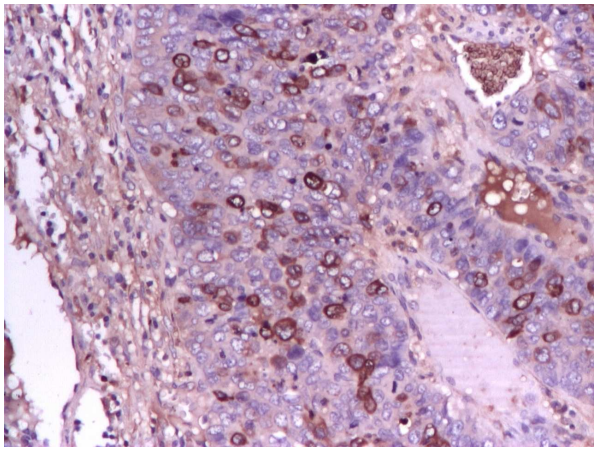

Figure 6. ß HCG expression in the dispersed tumoral cells. Anti-ß HCG, LSAB2 system, DAB visualization, counterstain with hematoxylin, x200.

ity (up to 50 mitoses/10HPF) and frequent apoptoses. The tumor's particularity was given by the predominant perivascular arrangement and less pseudopapillary of tumoral cells around some dilated thin walled vascular spaces, by the large geographical areas of tumor necrosis, by the eosinophilic, fibrillar or hyaline-like zones, which included tumor cells in nests or trabeculae (*Figure 2*); focally we identified dispersed multinucleated syncytiotrophoblastic-type cells. As the histological aspects on routine stain were considered unusual for a conventional uterine tumor, we decided to perform an additional IHC investigation. Additional slides from paraffin blocks were stained with the following antibodies: CK (AE1-AE3), CK7, CD34, smooth muscle actin, vimentin, HMB45, CK20. We noticed an intense positive reaction for CK AE1 - AE3 (*Figure 3*) and CK7 (*Figure 4*) of the tumoral cells and the absence of reactivity for the rest of the analyzed markers. The gross, microscopic and IHC aspects suggested the diagnosis of poorly-differentiated epithelioid trophoblastic tumor, with the recommendation for further IHC investigation. Considering this diagnostic suspicion, the βHCG serum level was determined, which had, 2 weeks after the surgery, a value of 38,68 mUI/ml (reference level <5mUI/ml), with a slow decrease in the next several weeks. Additional IHC investigations, performed at the Diagnostic Center of the National Institute of Research and Development „V. Babes" from Bucharest showed: tumor cells with positive diffuse reaction for CK7, VEGFR2 (*Vascular Endothelial Growth Factor Receptor* 2) and CD10 (*Figure 5*), zonal positivity for VEGFR1 (*Vascular Endothelial Growth Factor Receptor1)*, VEGF *(Vascular Endothelial Growth Factor)*, CerbB2 (++), and dispersed cells with reactivity for βHCG (*Human chorionic gonadotropin*) (*Figure 6*), hPL (*Human Placental Lactogen*) and PLAP (*Placental Alkaline Phosphatase*). We also noted the overexpression of p53 in 35% of the tumoral cells and the lack of reactivity for CK8. Based on the initial diagnosis of cervical squamous cell carcinoma, the patient received pelvic irradiation, concomitant with the administration of chemotherapy with cisplatin. 17 months after the treatment, the patient does not show any signs of relapse or metastasis, being monitored by βHCG values.

Discutions

The trophoblastic gestational diseases represent a heterogeneous group of lesions produced by the proliferation of the trophoblast,

with distinctive histologic, IHC and progressive - therapeutic aspects. ETT is a less common tumor and relatively recently classified as a particular entity within the trophoblastic tumors group[4]. The histological aspects of ETT were described by Mazur in 1989[10], who assigned the term „atypical choriocarcinoma" to the less usual features of some metastases of treated choriocarcinomas. In 1994, Mazur and Kurman[11] proposed the term ETT for the pulmonary metastases discovered in women with choriocarcinoma under chemotherapy, consisting in a monomorphic cellular population, showing no dimorphic/biphasic aspect of choriocarcinoma. Afterwards, similar tumors with uterine localization were reported. In 1998, Shih and Kurman[5] described the clinical, histological and IHC features of ETT, a tumor originating from the intermediate chorionic-type trophoblast, different from placental site trophoblastic tumor (PSTT) and choriocarcinoma. The appearance of tumor in patients with no history of chemotherapy treated gestational trophoblastic disease led to the hypothesis that ETT is a distinct pathological entity and not a therapy-induced lesion[7].

Ninety cases of ETT have been reported up to the present[3], as isolated cases[2;12-14;20] or as small series of cases[3;8;15], most of them in women at the reproductive age[15;16;17] or in their first years of menopause[8]. In the case described herein, the tumor was diagnosed in a 60 years-old woman, 4 years after the beginning of menopause, which is an extremely rare situation mentioned in the literature so far[2;12]. Usually, the tumor is preceded by a gestational event: full-term delivery (68%), spontaneous abortion (16%) or hydatidiform mole (16%)[17], the period of time between these and the appearance of the tumor being extremely variable: from 1 up to 18 years (6.2 years on average)[2;3;15;16;17]. Our patient had in her distant past (15 years ago) a spontaneous abortion, very probably a molar abortion if we take into consideration the description of the eliminated tissue material. The main symptom for the uterine localization of the tumor is the abnormal vaginal bleeding[2;8;11;12], as our data also shows. The serum level of ßHCG is elevated in ETT, but not significantly, usually under 2500 mUI/ml[1;3;8;13;16], thus differing from choriocarcinoma, where the marker is highly elevated. There are reported cases of ETT with normal ßHCG serum values[20]. Due to the absence of a diagnostic suspicion before the surgery or during the initial morphologic evaluation, the determination of ßHCG was performed 2 weeks after the surgery, when the marker still had a slightly elevated value (38,68mUI/ml). The determination of serum ßHCG is very useful in monitoring the disease progression, because concurrently with the appearance of metastases, the marker's level increases significantly. The data concerning the predictive value of serum ßHCG for the disease progression is not clear[8].

Regarding the uterine localization of ETT, the great majority of cases interests the lower uterine segment and the cervix (50%), and the rest, the uterine body (30%)[7;8;15]. Among the extrauterine sites there are mentioned: the ovary[14], the broad ligament[7], the lung[5;20], the small intestine[15], the tonsil[8], being, most likely, tumor extensions or metastatic lesions. There are also reported cases of ETT that coexist with choriocarcinoma, both with uterine and lung localization[8;13;15].

The tumor presents itself as a solitary nodule, relatively well circumscribed, that infiltrates deeply the myometrium and the cervix, usually having 0.5 to 5 cm in diameter, yellow or beige-tan in colour, with areas of hemorrhage and necrosis[1;8;11;13;14], gross features that were also present in our case.

Microscopically, the tumor is characterized by well-circumscribed limits, sometimes with foci of infiltrative growth at the periphery, with a prominent mononuclear cell population and the absence of the biphasic pattern that characterizes choriocarcinoma[1;3]. Even so, some tumoral areas present multinucleated syncy-

tiotrophoblastic-type cells[1;2], as our data also showed. In the case we presented, the localization of the tumor, the growth pattern, the geographical necrosis areas, the presence of eosinophilic fibrillar or hyaline-like material that surrounds the groups and nests of tumoral cells, the perivascular arrangement of the tumoral cells and the haemorrhagic character of the lesion were the first aspects that suggested the exact nature of the tumor.

ETT diffusely expresses epithelial markers (AE1/AE3, CAM5.2, CK7, CK18, EMA), E-cadherin, EGFR, α-inhibin and only focally the „classic" markers of the intermediate trophoblast: hPL, HCG, PLAP and Mel-CAM (*melanoma adhesion molecule*)[2; 8; 15; 20], IHC profile that allows the differentiation of ETT from carcinoma, but cannot discriminate between ETT and PSTT. This last differential diagnosis can be made by evaluating the pattern of tumoral growth: *expansive (with no aspects of diffuse permeation of the myometrium), with a clear limit between the tumor and the surrounding tissues, with tumor cells in nests and cords, with a striking perivascular arrangement,* together with the presence of hyaline-like matrix and positive reaction for p63, all these criteria being present in ETT and absent in PSTT[2;3;5;15]. In addition, the reactivity for hPL is focal in ETT and diffuse in PSTT[3].

The intense and diffuse reactivity for CK AE1 - AE3 and CK7 and the absence of positive reaction for muscle specific markers attested, in the analyzed case, the epithelial nature of the tumor, excluding a non-epithelial uterine tumor with epithelioid aspects like epithelioid leiomyosarcoma or malignant perivascular epithelioid cell tumor („PEComa"). Choriocarcinoma, for which ETT is frequently mistaken, was excluded on the basis of the absence of characteristic biphasic pattern together with only slightly elevated ßHCG serum level. The markers for the intermediate trophoblast (hPL, ßHCG) were only focally positive in the tumoral cells, as literature data also mentioned[8;15].

Recent research has emphasized a series of additional criteria that allow the distinction between ETT and other entities. The expression of cyclin E is more extended in ETT than in the placental site nodule[9]. As in many other reported cases of ETT, in our case, the initial histopathological diagnosis was that of a cervical keratinized squamous cell carcinoma, the main tumor subtype that ETT is mistaken for, because of the more frequent localization in the lower uterine segment and the cervix, the epithelioid aspect of tumor cells and the positive reaction for CK and p63 protein[1;2;6]. In addition, the confusion is due to the hyalinised areas and necrotic debris that are confused with keratin masses, but in ETT there are no true keratin pearls or intercellular bridges characteristic to squamous cell carcinoma. The IHC investigation of p16 expression, absent in ETT, but intensely and diffusely positive in most squamous cell carcinoma, is useful for this differential diagnosis[9]. Moreover, the expression of HLA-G, a specific IHC marker for the trophoblastic cells, is present in ETT and absent in squamous cell carcinoma[18].

Data concerning the treatment and prognostic for ETT aren't yet clear enough, because of the small number of cases correctly classified, reported and monitored. Surgical treatment cures and controls the localized disease.

Chemotherapy is restricted to cases with relapses and metastases[2], although ETT does not respond to the chemotherapy schemes used for other trophoblastic diseases[11]. The EMA-CO (Etoposide, Methotrexate, D-Actinomycin, Ciclophosphamide and Vincristine) scheme is used, but the results are not encouraging. Based on the immunoprofile of the tumor, Liu et al.[8] suggest that chemotherapy with platinum-based schemes could be more efficient. From the therapeutic point of view, it can be appreciated that, when a patient with gestational trophoblastic tumor does not respond to chemotherapy, the possibility of ETT must be

taken into consideration[13].

The prognosis of the tumor is usually good, with a metastatic and death rate of 25% and 10%, respectively[16;17]. At this moment there are no factors with clear predictive value for a bad evolution, but it is supposed that an increased mitotic activity could be associated with an aggressive evolution[1;2;14;15]. Generally, the mitotic rate of ETT is low (between 0-9 mitoses/HPF, with an average of 2 mitoses/HPF), and the Ki-67 index is 10%-25%[15;17]; however, there are reported cases, as the one presented, with an increased mitotic rate, with a Ki-67 index up to 86%[2;3].

The IHC studies brought further arguments for the tumor origin in the chorionic intermediate trophoblast[7;13] and the relationship between the placental site nodule and ETT, advancing also the hypothesis that ETT represents the malignant correspondent of the first one[16]. However, there are groups of researchers who consider that ETT results following some phenotypical alterations in choriocarcinoma under chemotherapy or in a pre-existent hydatidiform mole[17].

In conclusion, ETT represents a rare tumor, especially in postmenopausal women. In fact, the tumor described herein is the single one diagnosed in Timisoara's Pathology Departments and, to our knowledge, the only one reported in the Romanian medical literature. The familiarization of the pathologist with the gross and microscopic aspects of this tumor, the IHC investigations and the information about the ßHCG serum level are especially useful in establishing a correct diagnosis, followed by adequate therapy and follow-up.

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