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Summary: Gestational Trophoblastic Disease can be a challenging area for pathologic evaluation. An update with a focus on pathologic challenges is presented.

Key words: Gestational trophoblastic disease, hydatidiform mole, choriocarcinoma. Trophoblastic neoplasms

Introduction:

Gestational trophoblastic disease(GTD) means different things to the pathologist and the clinician. The pathologist utilizes a classification based on histopathology, while the clinician uses a clinically based classification, and does not always need to submit tissue for histopathology to appropriately manage the patient, particularly with post-molar GTD(table 1). Persistent trophoblastic disease after a mole may be a persistent mole, an invasive mole(fig 1), or much more rarely, choriocarcinoma(fig 2). Management may not require a histopathologic distinction. There are good in-depth reviews of both pathologic and clinical aspects of gestational trophoblastic disease(1-3). This review focuses on updates and challenges for the pathologist. Challenges include distinction of moles from non-molar gestations, recognition of early moles, distinction of complete moles from partial moles, familiarity with lesions of extravillous trophoblast, and recognition of forms of GTD that may be seen by the pathologist after therapy.

## Hydatidiform Mole

Hydatidiform moles are divided into complete and partial hydatidiform moles. These are genetically different lesions, with complete moles usually comprised of a diploid complement, both paternally derived, while partial moles are diandric triploids in most cases. Digynic triploids are not moles. Rarely, complete moles may be biparental, in familial complete moles, associated with mutations of the NLRP7 gene(4). Genetic variations from the usual chromosomal complements seen in molar disease have been reported. Recognizing moles and distinguishing complete from partial moles is important due to differing risk of persistent trophoblastic disease, which is considerably greater after a complete mole(15-20%) than a partial mole(<5%). Recurrence rates of moles in future pregnancies is about the same(1-2%)(2). Histology can be challenging. Below are some specific issues that can arise.

*Early Complete Mole*

Hydatidiform mole was previously diagnosed most often in the second trimester, when marked uterine enlargement, passage of “grapes”, bleeding, and other clinical manifestations became apparent. With the advent of early ultrasounds in pregnancy, most complete moles are detected in the first trimester, before full development of the pronounced histologic features seen in later complete moles, which show the expected edematous avascular villi with marked circumferential trophoblast proliferation. Earlier complete moles have distinct features as well, and familiarity with these features will aid in detection. Features include redundant bulbous terminal villi, hypercellular myxoid stroma, labyrinthine villous stromal capillaries, focal atypical villous and extravillous trophoblast hyperplasia , focal extravillous

trophoblast hyperplasia, hyperchromatic exaggerated implantation site trophoblast, and stromal karyorrhexis and apoptosis(figure 3) (5,6)

*Distinguishing complete from partial moles and moles from non-molar gestations*

Even with subspecialty expertise, diagnosis of moles by evaluation of hematoxylin and eosin stained slides is challenging, with poor interobserver reproducibility(7). Partial moles have less pronounced histopathologic features than complete moles, with less edema and trophoblast proliferation. The presence of fetal tissue, including nucleated red blood cells or fetal membranes, favors a partial mole over a complete mole, but rare complete moles have evidence of fetal tissue, including a complete mole with a twin, or a mosaic complete mole(6). In partial moles, villi tend to be irregular and scalloped, with trophoblast inclusions, which can also be seen in pregnancies with other chromosomal abnormalities, although those pregnancies won't show trophoblast proliferation(figs 4,5). This differs from the more rounded villi of complete moles. Missed abortions can also show edematous avascular villi, although the atypical trophoblastic proliferation of complete moles is absent. In order to further characterize these lesions, a variety of adjunctive techniques have been employed.

*P57 Immunohistochemistry*

P57 immunostaining is helpful, but it is necessary to know how to interpret the results, which are not "positive" and "negative". P57 is a paternally imprinted maternally expressed gene which stains maternal tissues. This allows decidua to serve as an internal positive control. In complete moles, there is no villous staining, although extravillous trophoblast clusters do

stain(fig 6a). The explanation for this staining of extravillous trophoblast clusters is unclear, but has been theorized to be due to incomplete or relaxed imprinting of p57, or less likely due to cross-reactivity(8). In partial moles, there is staining of villous stromal cells and cytotrophoblast(fig 6b).

*Other Adjunctive Techniques*

Ploidy analysis, via flow cytometry, FISH(fluorescent in-situ hybridization) or CISH(chromogenic in-situ hybridization), can be helpful, distinguishing diploidy from triploidy. However, ploidy will not distinguish a diploid missed abortion from a diploid complete mole, or a triploid digynic gestation from a triploid diandric partial mole.

Short tandem repeat(STR) genotyping has emerged as the most useful ancillary technique, not only distinguishing moles from their mimics, and complete mole from partial mole, but also able to distinguish gestational from nongestational trophoblastic disease, as well as recurrent from new molar disease(7,9). STR genotyping can be performed on formalin fixed, paraffin embedded tissue. The limiting factor is the lack of availability of the technique at all centers. As in all techniques, there are rare unusual cases and pitfalls.

*Familial Hydatidiform Mole(biparental complete mole)*

A rare clinical form of complete hydatidiform mole is familial recurrent hydatidiform mole, where the moles are diploid but biparental. This is a recurrent autosomal recessive disorder. Histologically the lesions are similar to sporadic complete moles, and usually are histologically indistinguishable, but the molar features may be less pronounced. P57 immunostaining is the same as for sporadic complete moles(4).

*Mimics of Hydatidiform Mole and GTD*

A variety of lesions may be mistaken for hydatidiform mole or GTD. Features of these are illustrated below:

*Placental mesenchymal dysplasia*

Placental mesenchymal dysplasia is a rare lesion associated with chromosomal abnormalities, Beckwith-Wiedemann syndrome, and stillbirth. It manifests as placentomegaly with cystic dilatation of stem villi which can be mistaken for molar vesicles, as opposed to molar dilatation of terminal villi(10)(figure 7). The cystic dilatation is concentrated near the fetal surface of the placenta, giving it a unique gross appearance.

*Trophoblast inclusions*

Partial moles are characterized by scalloping of terminal villi. These indentations can be seen on histologic sections as trophoblast inclusions if sectioned tangentially. While a rare one is probably of no significance, if increased over 1-2, trophoblast inclusions can also be a manifestation of other chromosomal abnormalities, including triploidy(11) (fig 5), and increased trophoblast inclusions is not a rare finding in spontaneous abortions. These other abnormal gestations will lack the trophoblast proliferation and mix of molar and nonmolar villi of a partial mole.

*Chorangioma with trophoblastic proliferation*

Chorangiomas are fairly frequent in placentas, and represent hemangiomas within the parenchyma. Unless large, they are usually of no clinical significance. Some of these lesions are associated with trophoblast proliferation around the periphery, or tracking along lobules of the lesion(fig 8), and the literature has termed these lesions “chorangiocarcinomas”. This is an unfortunate misnomer, as there have been no reports of persistent trophoblastic disease in mothers or their infants(12)

*Florid trophoblastic proliferation in early pregnancy and tubal ectopic pregnancy*

An area of challenge is the exuberant trophoblast proliferation that can be seen in early intrauterine gestations, as well as in tubal ectopic pregnancy. The trophoblast proliferation in early non-molar gestations has been described as “polar capping”, with the trophoblast at one end of the villus, the side of the direction of implantation. It is possible for a tubal hydatidiform mole to occur, but it is exceptionally rare. P57 Immunostaining may help if this issue arises in the fallopian tube or early intrauterine gestation. If products of conception are examined early enough for there to be no chorionic villi(pre-villous trophoblast), the question of choriocarcinoma may arise. In the absence of availability of short tandem repeat genotyping, the distinction may fall on clinical evaluation, however this is a rare issue.

*Intraplacental Choriocarcinoma*

Intraplacental choriocarcinoma is not seen often, and this may be due to the lack of a grossly recognizable lesion in some cases. It is rare, representing about 0.03% of GTD in one series(13). It is generally found on examination of third trimester placentas. While usually asymptomatic, vaginal bleeding, melena as well as respiratory and neurologic symptoms have been reported(13). Histologically there is marked trophoblast proliferation around one or a cluster of villi , while the rest of the placenta is normal(fig 9). This is an important lesion to recognize and report to the clinicians, as there may be metastatic disease in both mothers and infants(13). Intraplacental choriocarcinoma may be the precursor lesion of gestational choriocarcinoma after a term gestation.

*Pitfalls in following serum beta-hCG:*

Beta-hCG testing can give false positive results unrelated to GTD. This can occur in some tumors(14), end stage renal disease, with some drugs, with heterophile antibody(15), or by misinterpretation of results. A variety of conditions can give false positive urine hcg results(16). False negative results have been reported with GTD(17), and clinical judgment is always important.

A form of hCG, hyperglycosylated hcg(hCG-H) is made by extravillous cytotrophoblasts, as opposed to usual hCG, which is produced by syncytiotrophoblasts. hCG-H is related to normal implantation, and is associated with aggressiveness as well as chemosensitivity of choriocarcinoma, where it tends to be higher(18). It is lower in less aggressive/more chemoresistent GTD.

Quiescent GTD is a presumed inactive form of GTD with low levels of true hCG over several months, with disease undetectable, and the hCG unresponsive to chemotherapy. hCG subanalysis reveals no hyperglycosylated hCG (18) . Long term low b-hCG with no detectible disease may revert to normal at some point, but in one series(19), a large proportion of non-reverters were subsequently discovered to have PSTT or ETT.

*Postevacuation GTD with factors complicating histopathologic diagnosis*

Tissue received by the pathology laboratory for evaluation of persistent GTD after mole can be challenging, and this can be complicated by factors such as insufficient history, or prior therapy. Curettings may only contain scant individual trophoblast cells, sometimes atypical, in blood clot. Theoretically, that finding could represent persistent mole, invasive mole, choriocarcinoma, PSTT/ETT, or a new intrauterine gestation. Lu et al(20) described 4 cases of GTD where masses persisted after chemotherapy, 2 moles, one “GTD” , and one choriocarcinoma. After chemotherapy, the tissue resembled epithelioid trophoblastic tumor(ETT), and the authors suggested that chemotherapy may have eliminated the more primitive choriocarcinoma cells, allowing evolution into a more chemoresistent phenotype of extravillous trophoblast.

A lack of history can also hamper diagnosis. A hysterectomy for GTD was received by the laboratory for a persistent mass(fig 10a). Histologic evaluation showed rare atypical cells within blood clot(fig 10b). The initial history was that the patient had had a mole evacuated somewhere else a few months prior, and there was something "funny about it". The patient's serum hCG was reportedly low. Based on the greater immunostaining with human placental lactogen(HPL) than with beta hCG of the atypical cells from the hysterectomy, the initial impression was of a possible lesion of extravillous trophoblast, such as PSTT. Several weeks later, the prior slides from the molar pregnancy were available for review. They showed a complete mole with a more than usual amount of trophoblast proliferation, with greater atypia. The original diagnosis suggested that this mole may have been evolving into choriocarcinoma, and that the criteria for this were not well established. The reclassified final diagnosis on the hysterectomy was "GTD", no longer thought to represent a lesion of extravillous trophoblast. This case emphasizes the importance of review of prior contributory pathologic material.

**Lesions of Extravillous Trophoblast**

There are 3 types of extravillous trophoblast(EVT), the EVT of the trophoblastic columns, implantation site EVT, and chorionic EVT. These different types of EVT have different immunohistochemical profiles, as do the lesions arising from them(table 2)(21). This category of lesion can be diagnostically challenging, due to overlap of histologic features, as well as lack of familiarity. EVT has also been termed "intermediate trophoblast" in the literature, and some authors continue to utilize this terminology, but this is based more on histopathologic features than on the cells representing an intermediate stage of development.

*Placental Site Nodules/Plaques*

Placental site nodules and plaques are frequently incidental findings in curettings, and it is unclear if they actually contribute to abnormal uterine bleeding. They are thought to be the "graveyards" of prior implantation sites, often remote. The nodules and plaques are of low cellularity, predominantly composed of eosinophilic material(fig 11). A potential pitfall, although rare, are atypical placental site nodules(22). On one series, 3/21 lesions termed atypical placental site nodules(APSN) were associated with GTD on follow-up, developing into either PSTT or ETT. These patients did not have elevated beta hCG. The histologic features of the APSN were intermediate between PSN and PSTT/ETT, with lesions larger than the usual PSN, which rarely exceed 4 mm. APSNs also had increased cellularity, mild atypia, and an increased ki-67 index compared to usual PSN(22).

*Exaggerated Placental Site(EPS)*

Exaggerated implantation sites occur at the time of a gestation, either normal or molar. It is comprised of implantation site trophoblasts infiltrating decidua and between bundles of uterine smooth muscle. When seen in smooth muscle, particularly on curettings and if the chorionic villi have been passed, the concern of PSTT is sometimes raised. Exaggerated placental site(fig 12) has more multinucleation than PSTT, and a ki-67 index of 0, as opposed to PSTT. EPS doesn't form masses like PSTT. In addition, the presence of chorionic villi helps establish the diagnosis.

*Placental Site Trophoblastic Tumor*

Placental site trophoblastic tumor is composed of extravillous trophoblast of implantational type. It is monophasic(fig 13), unlike the biphasic choriocarcinoma, and dissects between bundles of smooth muscle, where it can resemble exaggerated placental site. In addition to the presence of a ki-67 index of about 14%, PSTT is more extensive than EPS, and PSTT is mass forming. PSTT is usually nonaggressive in behavior, but metastatic disease has occurred. PSTT is more chemoresistent than choriocarcinoma

*Epithelioid Trophoblastic tumor*

Similar in behavior to PSTT, epithelioid trophoblast tumor (ETT) is derived from chorionic type extravillous trophoblast, and so has a different immunoprofile than PSTT(table 2). An important characteristic is the presence of geographic necrosis(fig 14). Like PSTT, it tends to be chemoresistent.

*Extrauterine Lesions of Extravillous trophoblasts:*

Lesions of extravillous trophoblast can rarely occur in the fallopian tube, ovary or in paratubal tissue. This must be kept in mind for these rare lesions, and they should be distinguished from

non-gestational choriocarcinoma in the adnexal region. A classification system for these rare lesions has been devised(23).

*Future directions in GTD*

Although still experimental, some feasibility has been established for identifying GTD and distinguishing it from non-gestational hCG-secreting tumors by evaluating circulating cell-free DNA(24).

**Conclusions:**

GTD continues to be a challenge for pathologists. Familiarity with the histology and use of adjunctive techniques for unusual variants and early lesions will assist in the evaluation of these specimens.

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