Gestational Trophoblastic Diseases
Clinical guidelines for diagnosis, treatment, follow-up, and counselling

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Introduction
In 2009, a working committee was established under the Danish Gynecologic Cancer Group (DGCG) with the purpose of implementing registration of gestational trophoblastic diseases in the DGCG database. This guideline represents the Danish Gynecologic Cancer Groups national guidelines anno 2014.

Definition
Gestational trophoblastic diseases is a unifying term for a spectrum of diseases from abnormal proliferation of placental trophoblasts, as seen in hydatidiform mole, to neoplastic trophoblastic diseases.

Limitation of subject
These clinical guidelines deal with the workup, diagnosis, differential diagnostic considerations, treatment, monitoring, and counselling of patients with gestational trophoblastic diseases.

Background
Gestational trophoblastic diseases include hydatidiform mole and non-molar trophoblastic diseases, the latter of which can be divided into neoplastic and non-neoplastic conditions (1).
Hydatidiform mole is divided morphologically into complete and partial hydatidiform mole. Both can develop into invasive hydatidiform mole or persisting trophoblastic disease (PTD). The latter is much more common with complete hydatidiform mole.
Non-molar lesions derived from the placenta include mesenchymal placental dysplasia (MPD), placental site nodule (PSN) and exaggerated placental site, which are non-neoplastic. Neoplastic diseases arising from the placenta include choriocarcinoma (CC), placental site trophoblastic tumour (PSTT) and epithelioid trophoblastic tumour (ETT).

Clinically, distinction is made depending on whether the lesions require treatment such as chemotherapy or surgery or whether only follow-up is needed after primary evacuation.
By far the most common trophoblastic disease is hydatidiform mole. Hydatidiform mole is a histopathologic diagnosis based on the presence in placental tissue of oedematous, abnormal villi plus trophoblastic hyperplasia. Histologically, hydatidiform mole is classified as partial or complete. Hydatidiform mole has a unique genetic constitution because regardless of whether it is diploid or triploid it most often contains two sets of chromosomes from the father.
Hydatidiform mole is seen with a frequency of approximately 1 case per 1000 pregnancies in the Western world (2, 3); in Denmark this corresponds to about 80 to 100 cases a year.
Both the partial and the complete forms of hydatidiform mole are primarily benign but can develop into persistent trophoblastic disease (PTD), invasive hydatidiform mole, choriocarcinoma and PSTT/ETT that require chemotherapy. In Denmark about 10 women a year are treated with chemotherapy after a molar pregnancy and survival is close to 100%.
The frequency of choriocarcinoma is 1 per 20–40,000 pregnancies, whereas PSTT/ETT is even rarer and comprises about 0.2% of the total number of cases of trophoblastic diseases (2). Half of these arise after a normal pregnancy.
Trophoblastic tumours are characterised by the secretion of hCG, and there is a close relation between hCG concentration in the serum and the amount of living trophoblast tissue.

Abbreviations

PSTT	Placental site trophoblastic tumour
ETT	Epithelioid trophoblastic tumour
EPS	Exaggerated placental site
CC	Choriocarcinoma
PSN	Placental site nodule
PMD	Placental mesenchymal dysplasia
hCG	human chorionic gonadotropin
PTD	Persistent trophoblastic disease
MTX	Methotrexate
Act-D	Actinomycin D
BEP	Bleomycin - Etoposide – Paclitaxel
EMA-CO	Etoposide - Methotrexate – Act D - Carboplatin

EPIDEMIOLOGY AND CLINICAL FINDINGS

HYDATIDIFORM MOLE

Hydatidiform mole is known to appear with a frequency of about 1 per 1000 pregnancies in the Western world. I Denmark, during the period 1999 to 2010, 130 to 140 cases were diagnosed yearly, which is a little higher than expected (4). The true distribution of complete and partial hydatidiform mole is not known; many have reported that partial are more commen than complete moles(2). At present, the two types appear to be equally common in Denmark (4).

Risk factors

The two most important risk factors for molar pregnancy are the patient's age and previous molar pregnancy (3). At the extremes, both low and high, in maternal age, there is a significantly increased risk for molar pregnancy and especially for complete/diploid hydatidiform mole (5, 6). The risk increases markedly for women over 40 years of age, where the risk for complete hydatidiform mole is 7.5 times greater than in women between 21 and 35 years of age (3). Previous spontaneous abortions are seen more commonly in women with molar pregnancy (7). According to a British case-control study, the risk for trophoblastic disease is not increased after fertility treatment (8). However, 50% of twin pregnancies with hydatidiform mole and a normal foetus are preceded by fertility treatment. It is not known whether this is because of the stimulation therapy or an underlying impaired fertility in the woman (9). After one molar pregnancy, the risk for a new molar pregnancy increases 1% to 2%, and after two molar pregnancies, the risk for a third is 15% to 20% (10). Molar pregnancies are more common in Asian countries, and here diet is thought to play a role (2).

Symptoms

The four most common gynaecologic symptoms of molar pregnancy are vaginal bleeding (69%–89%), uterus larger than expected for gestational age (with complete hydatidiform mole) (28%–33%), hyperemesis (8 %–22 %) and preeclampsia or hypertension (1 %–3%) (3,11,12,13,14,15). Generally, the symptoms with partial hydatidiform mole are less pronounced than those with complete hydatidiform mole (11,15,16). Complete hydatidiform mole debuts symptomatically about 3 weeks before partial hydatidiform mole. In women with hyperemesis, a sonogram should be taken to rule out molar pregnancy.

Since ultrasound scanning in the first trimester is now routine, up to 40% of molar pregnancies are found today at the 12-week scan, and thus often while the women are still asymptomatic (6,17).

Diagnosis

Ultrasound

The classical sonographic picture of a complete hydatidiform mole is described as "a snow storm", meaning a complex echo pattern with multiple anechoic spaces that fill the uterine cavity. In partial hydatidiform mole, cystic spaces are seen in the placenta together, at times, with a dead foetus – seldom a living foetus. In partial hydatidiform mole, foetal development appears delayed, and the foetus may be malformed (18,19). Thus if, there is a living foetus with biometries corresponding to gestational age, one should suspect a twin pregnancy consisting of a hydatidiform mole and a normal foetus with its own normal appearing placenta.

Today, ultrasound scanning is routinely performed early in pregnancy, which has resulted in the diagnosis of hydatidiform being made earlier than previously (17,20). However, only 40–60% of all molar pregnancies are suspected on routine ultrasound scanning, and the detection rate is better for complete hydatidiform mole (79%) than for partial hydatidiform mole (29%), and best after the 14th gestational week (21). The sensitivity of an "ultrasound suspicion of hydatidiform mole" is 48%, which means that a suspicion of a hydatidiform mole on ultrasound is later confirmed histologically in one of every two cases (22). In order not to overlook a molar pregnancy, evacuated material from pathological pregnancies should be sent for histopathologic and genetic evaluation. (23,24,25).

s-hCG

Human chorionic gonadotropin is a pregnancy hormone secreted by trophoblast cells that is used as a specific marker for trophoblastic diseases. hCG can be measured in urine and blood, and the level of hCG correlates with the volume of trophoblast tissue. In Denmark, quantitative measurement of urine hCG is not done. At diagnosis, serum hCG is often higher in molar pregnancy than in normal pregnancy, and in complete hydatidiform mole, serum hCG is significantly higher than in partial hydatidiform mole. In complete hydatidiform mole, serum hCG is often > 100,000 IU/L (6,11). In partial hydatidiform mole, serum hCG is > 100,000 IU/L in less than 10% of cases (3).

Surgical treatment

Uterine evacuation

Prior to uterine evacuation, serum hCG, haemoglobin, and blood type and crossmatch test, should at minimum be determined. Regular surgical uterine evacuation with suction followed by blunt curettage is recommended (26). Regardless of gestational age and type of hydatidiform mole, a molar pregnancy can often be evacuated with a 12 mm suction catheter. Ultrasonographically guided blunt curettage should be done meticulously but carefully because of the risk of uterine perforation to assure complete emptying of the uterine cavity. Administration of IV syntocinon is recommended with the procedure (3). Medical evacuation is contraindicated because of the increased risk of subsequent need for chemotherapy (RR 1.7) (27). Pretreatment to assure cervical ripening before surgical evacuation does not increase the risk for later chemotherapy (28).

It is important to be aware of the increased risk of bleeding with the surgical treatment of hydatidiform mole; ultrasound with colour Doppler can give indicate hypervascular areas in the myometrium. Patients who are Rh-negative are given immunoglobulins according to normal instructions.

Hysterectomy

As an alternative to uterine evacuation, hysterectomy can be considered in those women who do not wish to retain their fertility. This treatment does not, however, eliminate the risk of PTD (3–5%), and therefore patients after hysterectomy should be follow-up with measurement of hCG according to the same principles as those after uterine evacuation (3).

Follow-up after surgical treatment of hydatidiform mole

Because of the risk of persistent trophoblastic disease (PTD) after a molar pregnancy, serum hCG should be measured weekly after uterine evacuation. It is important that an initial serum hCG value

is measured immediately before (or at a maximum of 24 hours after) evacuation. More than half the patients will have an undetectable serum hCG in the course of 2 months. It is important that the patient uses safe contraception during the whole follow-up period; birth control pills can be recommended and do not increase the risk of PTD (3).
A marked reduction in the length of the follow-up period has taken place during the last few years because the risk of relapse after undetectable serum hCG levels have been attained has been shown to be very low. The latest findings from Charing Cross Hospital, London, show that the risk of relapse after complete hydatidiform mole is 1/400, but only 1/1500 if serum hCG normalises within 56 days (29). The risk of relapse after partial hydatidiform mole is 1/3000.
Given that the patient is informed of this risk, the following follow-up programmes are recommended:

In diploid hydatidiform mole, and in hydatidiform mole (complete and partial) without ploidy determination:
Weekly measurement of serum hCG until serum hCG is undetectable in two consecutive measurements. Hereafter serum hCG is measured once a month. If serum hCG became normal within 56 days after evacuation, the patient can be discharged from follow-up after 4 months. If not, the patient is followed up with measurement of serum hCG once a month for 6 months.

In triploid hydatidiform mole:
Weekly serum hCG measurement until two consecutive undetectable values. Hereafter the patient can be discharged from follow-up.

If "divergent" observations are seen, the length of the follow-up period should be determined at a multi- disciplinary conference with participation of pathologist, gynaecologist, oncologist and geneticist (e.g. if the histopathologic diagnosis is complete hydatidiform mole and genetic studies show triploidy, or if the diagnosis is complete hydatidiform mole and/or diploidy together with positive immunostaining for p57KIP2).

Follow-up after future pregnancies
Patients should be monitored after later pregnancies by measurement of serum hCG 8 weeks post partum. If hCG is not undetectable, the patient should be discussed at a multidisciplinary conference.

PTD (PERSISTENT TROPHOBLASTIC DISEASE)
Up to 20% of patients with a molar pregnancy subsequently develop PTD and are treated with chemotherapy to achieve remission (12,25,30). In the medical literature in English, the expression gestational trophoblastic neoplasia, GTN, is often used.

PTD is diagnosed according to the following criteria (the hCG criteria presuppose that serum hCG levels are measure once a week):
Persistent s-hCG
s-hCG increases during 2 weeks/three measurements
s-hCG decresases less than 10% during 3 weeks/four measurements (plateau)
s-hCG persists with positive measurements for more than 6 months after evacuation

There is a tendency to be less strict regarding the third hCG criterion (elevated serum hCG > 6 months); follow-up can, however, be continued if the serum hCG is low and continues to fall because some patients are slow to excrete hCG. In order not to overlook true PTD, an ultrasound scan of the uterus and possibly a chest x-ray or PET/CT should be performed.

Invasive hydatidiform mole
Invasive hydatidiform mole is a clinical diagnosis based on metastases or invasion of the myometrium. Invasive hydatidiform mole is thus either diagnosed with ultrasound, CT or MR, when pulmonary or hepatic metastases are visualized, or histologically if hydatidiform mole tissue has invaded the myometrium. Invasive hydatidiform mole can give rise to heavy bleeding or pulmonary symptoms. If there is suspicion of invasion into the myometrium, uterine evacuation and biopsy of hydatidiform mole tissue are contraindicated. The diagnosis is confirmed using diagnostic imaging and serum hCG.
Chemotherapy is primary treatment. Some recommend hysterectomy as first choice in patients who do not wish to retain fertility (31, 32). Hysterectomy can also be performed because of uncontrollable vaginal or intraabdominal bleeding and localised chemotherapy-resistant disease (33).

Symptoms of PTD
PTD is usually diagnosed on the basis of an insufficient fall in serum hCG after a molar pregnancy, but in rare cases, molar pregnancy has not been diagnosed upfront. Irregular persistent bleeding after spontaneous abortion or birth can raise suspicion of trophoblastic disease, and one should always measure serum hCG in these patients. The first symptoms of choriocarcinoma and PSTT/ETT can be from metastases, in the form of heavy vaginal bleeding, intestinal bleeding, increased intracranial pressure because of intracranial bleeding or bleeding from other sites. Respiratory symptoms like dyspnoea or chest pain because of pulmonary metastases can also be seen.

Risk factors for PTD
Maternal factors
Patients older than 35–40 years have an increased risk of PTD (3,13,25). Some studies have shown an increased (OR 2.6–4.6) risk for PTD in cases of repeated hydatidiform mole, whereas others have not been able to confirm this (7,14).

Pregnancy-related factors
The majority of studies have found an increased risk for PTD if the initial serum hCG > 100,000 IU/l (13,34).

Morphology
The frequency of PTD after complete hydatidiform mole is reported to be 18–28% (2,35,36). After partial hydatidiform mole, the frequency is 0–5% (2,37,38). In Denmark, PTD was observed in 16% (16/118) of patients with complete hydatidiform mole and in 5 % (7/140) with partial hydatidiform mole (25).

Ploidy
The risk of PTD after diploid hydatidiform mole is reported to be 18–25 % (39,40). In Denmark it is 18% (25).
In four prospective studies in which ploidy was determined using appropriate techniques, no cases of PTD were seen after 258 triploid hydatidiform moles (0%, 95% CI: 0–1.4 %) (41-45). There are, however, case reports of choriocarcinoma after verified triploid hydatidiform moles (46,47,132).

The risk of PTD after tetraploid hydatidiform mole is unknown, but, as the morphologic diagnosis often is a complete hydatidiform mole, the risk of PTD after tetraploid hydatidiform mole should be considered to be the same risk as after a diploid/complete hydatidiform mole (48).

Parental origin of the genome
The risk of PTD after diploid androgenetic hydatidiform mole is approximately 18%. (25,39). In some studies, suspicion of an increased risk of PTD has been raised after androgenetic hydatidiform mole when two different paternal genomes are involved (P1P2) (40,49). Cases of PTD after diploid biparental hydatidiform mole (PM)(50) and after hydatidiform mole with mosaicism between a diploid androgenetic and a diploid biparental cell line (PP/PM) have been observated, see the section on genetics (25,51).

Uterine re-evacuation on suspicion of PTD
Uterine re-evacuation in women with PTD in the hope of reducing subsequent need of chemotherapy is still controversial (2). In a Dutch study, 10% of patients with PTD (diagnosed on the basis of an insufficient fall in hCG) were cured by re-evacuation, and the remaining 90% needed less chemotherapy than the control group to achieve remission (52). In 5% of the re-evacuated patients, there were maternal complications, most commonly perforation and infection. These results were supported by a more recent Dutch retrospective study of 29 patients with PTD, in which the re-evacuated patients had a significantly lesser need for subsequent chemotherapy (53). In a British study, 60% of patients diagnosed with PTD achieved remission after uterine re-evacuation; 78% of the procedures were undertaken due to a dual indication (e.g. bleeding), and maternal complications were not mentioned (54). The success rate for remission was greatest if the serum hCG was < 1500 IU/l. In another study, uterine re-evacuation is advised against because of the low chance of remission and the increased risk for the patient (55). Studies from Charing Cross Hospital, UK, indicate that re-evacuation in patients with serum hCG > 5000 IU/l should be avoided because the subsequent need for chemotherapy is not substantially reduced and the risk of side effects should be considered. (2).
A phase II study under the auspices of the American Gynaecologic Oncology Group is currently underway with the objective of evaluation the value of uterine re-evacuation in patients with PTD (56).
At present, re-evacuation is not recommended as standard procedure.

TWIN PREGNANCY WITH HYDATIDIFORM MOLE AND A NORMAL FOETUS
A twin pregnancy involving a hydatidiform mole and a normal foetus is extremely rare and is seen in 1:20,000–120,000 pregnancies (57,58). On ultrasound scan, a cystic placenta and a normal appearing foetus with a normal placenta are seen (59). Often, but not always, the two placentas appear as two separate entities.
About 50% of these pregnancies take place after previous fertility treatment (9). Twin pregnancies can be difficult to distinguish from partial hydatidiform mole, and thus ploidy and parental origin of the genome should be determined in placental biopsies if the pregnancy is allowed to continue.
A prominent problem in these pregnancies is the evaluation regarding the chance of obtaining a normal child versus the risk of PTD. One large British study found that the chance of obtaining a normal child was 38%, and another with the largest number of cases (90) to date reported that there was a 57% chance of obtaining a normal child (57,58). The median gestational age at birth was 34+1 weeks, and slightly more than half of the children were delivered by caesarean section. Because there is an increased risk of bleeding, preeclampsia and late spontaneous abortion, these pregnancies are an obstetric challenge (58,59,60). Carefully information of the risks involved is mandatory, and the pregnancy must be closely monitored with monthly measurement of serum hCG and ultrasound scans.
In twin pregnancies with diploid hydatidiform mole and a normal foetus, the risk of developing PTD is 26% (58,61). The risk appears to be greatest in those pregnancies that are aborted or cease during the first trimester. There is no indication that the risk of PTD increases in relation to the length of gestation (57,58,62).

REPETITIVE HYDATIDIFORM MOLE
Repetitive hydatidiform mole is seen in 1–2% of those who become pregnant again after a molar pregnancy, corresponding to a 10- to 20-fold increased risk relative to women without previous molar pregnancy (63,64). The empirical risk after two hydatidiform moles is even higher (10–23%). One study has shown that women with repeat hydatidiform mole most often have molar pregnancies of the same histologic subtype (complete/partial) (10).
After two molar pregnancies, between 42% and 67% of women have a normal pregnancy (63,65,66,67).
Women with repetitive hydatidiform mole can be divided into two subgroups (10):
Patients with diploid androgenetic or triploid hydatidiform mole and “normal” reproductive ability
Patients with diploid biparental hydatidiform mole and reduced reproductive ability
Diploid biparental hydatidiform moles have been described mostly in women with repetitive molar pregnancies and/or in families in which a sister has had a hydatidiform mole (50,68,69,70,71). Diploid biparental hydatidiform mole is therefore a predictor for a markedly increased risk of repeat hydatidiform mole. Two genes have been identified that in mutated form give rise in women to an autosomal recessive hereditary disposition to repetitive hydatidiform mole: NLRP7 (72) and KHDC3L (73).
After one molar pregnancy, the woman should be informed about the increased risk of repetitive hydatidiform mole, but also that there is a 98% chance of a normal pregnancy (63). She should be offered early ultrasound investigation in all subsequent pregnancies.
Women with repeat moles should be offered a genetic workup and counselling.

QUIESCENT HYDATIDIFORM MOLE AND FALSELY INCREASED HCG
In a few patients, serum hCG does not fall to an undetectable level but remains at a low value (10–200 IU/l) for months or years after a molar pregnancy. Possible explanations can be false positive values, PTD or “quiescent hydatidiform mole” (74). To rule out false positive hCG values, urine hCG should be analysed, a serum sample sent to a laboratory that uses a different hCG assay, or a series of dilutions be made so that falsely increased hCG values due to binding of heterophile antibodies or LH can be ruled out.
In rare cases, physiologically secretion of a small amount of hCG from the pituitary gland is responsible, which ceases after treatment with birth control pills (75).

If the above-mentioned sources of error can be eliminated and the low serum hCG values are a reality, the reason may be the presence of a quiescent hydatidiform mole. An American centre suggests measurement of hyperglycosylated hCG (H-hCG), which is secreted from invasive trophoblast tissue and can thus be a marker for malignancy. If the concentration of H-hCG is low, the trophoblast tissue is refractory to chemotherapy, and the patient should not be treated. Because 10%–25% of women with quiescent hydatidiform mole at some point will require treatment, these women should be followed up for a long period, even for life, but first treated if hCG or H-hCG begins to raise, a sign of tissue invasion (76). Measurement of H-hCG as a diagnostic measure and as an indication for treatment is, however, controversial and is not done in Europe.

NON-HYDATIDIFORM MOLE TROPHOBLASTIC DISEASES

Choriocarcinoma (CC)

Choriocarcinoma is a malignant trophoblastic disease with a frequency of 1:40,000 pregnancies, corresponding to 1 to 2 patients a year in Denmark. Fifty percent of cases are preceded by a molar pregnancy, whereas 25% are seen after a spontaneous abortion and 25% after a normal pregnancy and birth. The risk factors for CC are high age and previous molar pregnancies (2,3). The tumour is highly vascularised and the first symptom can be heavy vaginal bleeding or bleeding from or in other organs (intestine, nose, throat, lungs, liver, brain). Serum hCG should therefore always be measured in cases of unexplained bleeding. Patients are often acutely ill because of metastases to other organs.
The treatment is primarily chemotherapy and relief of symptoms. Adjuvant hysterectomy and resection of metastases can be necessary (30).

Placental site trophoblastic tumour (PSTT)

Placental site trophoblastic tumour is a very seldomly occurring variant of neoplastic trophoblastic disease that arises from intermediate trophoblastic cells. Its actual incidence has not been established, but PSTT makes up about 0.2% of all cases of trophoblastic diseases, corresponding to one case every 5 years in Denmark (2). The disease is characterised by being able to occur several years after a prior pregnancy. PSTT can develop after both normal and molar pregnancies. It is a slowly growing tumour that metastasises late and often to lymph nodes. The serum hCG level is low in relation to the level seen in the other gestational trophoblastic diseases. In contrast to the other trophoblastic diseases, the serum hCG level in PSTT does not correlate with tumour load or aggressiveness.
PSTT usually presents with irregular vaginal bleeding (80%) or alternatively amenorrhoea. The diagnosis cannot with certainty be made by ultrasound scan, but a scan may show inhomogeneous areas in the myometrium. On suspicion of PSTT, MR or PET/CT scanning can be done, but the sensitivity is not known. About 1% of patients have lymph node metastases at the time of diagnosis (77). PSTT often also metastasises to the lungs.
PSTT is primarily treated surgically with hysterectomy and retention of normal ovaries and in some cases with resection of lymph nodes. (2,78,79). If the woman is young, wedge dissection of the uterus can be considered in order to retain fertility, but one should be aware of the risk of microscopic multifocal disease. Adjuvant chemotherapy is not given with disease in stage 1.
In disseminated disease, multidrug chemotherapy is given (e.g. EMA-EP or EP), but PSTT is less sensitive to chemotherapy than the other trophoblastic tumours.
The best prognostic factor is the time interval from the "responsible" pregnancy (which is not always the latest). In a study of 62 cases, it was shown that all in whom there was a time interval longer than 48 months died of the disease, whereas 98% with a time interval less than 48 months survived (80).
After treatment for PSTT, patients should be followed up with lifelong measurement of serum hCG, see below.

Epithelioid trophoblastic tumour (ETT)

Epithelioid trophoblastic tumour (ETT) is an even more rare variant of neoplastic trophoblastic disease and was described for the first time in 1998 (81). Clinically and histopathologically, ETT is similar to PSTT, and both are derived from the intermediary trophoblast. ETT presents with amenorrhoea or irregular bleeding after a pregnancy and serum hCG is slightly elevated. ETT is often located in the cervix and can be misdiagnosed as planocellular carcinoma. The disease usually occurs before the age of 50, and the clinical picture is one of irregular bleeding and elevated hCG. In the largest and only analysis (78 cases), the median s-hCG was 665 IU/l at the time of diagnosis (82). The antecedent pregnancy was a spontaneous abortion in 50%, hydatidiform mole in 35%, term birth in 10% and ectopic pregnancy in 5%. The only prognostic factor was the FIGO stage. Thus patients with stage 1 disease did much better than patients with stage II to IV disease. In contrast to PSTT, the time interval from the antecedent pregnancy is not a prognostic factor for ETT. There was no increased survival in patients treated with adjuvant chemotherapy. Long-term survival for all stages is reported as being about 40%.
The treatment for ETT is the same as for PSTT.

Follow-up and staging

Follow-up after treatment for PSTT and ETT should be life-long and consist of regular measurement of serum hCG, every month the first year decreasing to once a year after 5 years.
When PSTT or ETT is a coincidental postoperative finding, staging is recommended with use of MRI of the cerebrum and pelvis together with CT of the thorax and upper abdomen.

PATHOLOGIC ANATOMY

The purpose of the pathologic evaluation is to confirm the diagnosis of gestational trophoblastic disease, cf. the WHO classification. The type of tumour is determined in the case of neoplastic lesions, and if hysterectomy is performed, the degree of any local spreading to the myometrial serosa, parametrium and cervix is described.
The pathologic diagnosis of gestational trophoblastic diseases is a challenge and subject to considerable uncertainty (83,84,85). This is due to several factors (see Tabel 1). The extravillous trophoblast has itself invasive characteristics, and it is difficult morphological to determine whether the invasion is controlled, physiological or neoplastic. Suspicion of hydatidiform mole often arises early in pregnancy, at a time when the histologic characteristics are not so well developed and the morphologic overlap is great.
In practice the differential diagnostic problems are among others:
1) Hydatidiform mole vs. abortion with hydropic degeneration.
2) Early complete vs. partial hydatidiform mole.
3) Twin pregnancy with normal conception and (complete) hydatidiform mole vs. partial hydatidiform mole.
4) Other genetic abnormalities with hydropic changes in the placenta (e.g. Beckwith-Wiedemann syndrome and chromosome abnormalities) vs. hydatidiform mole.

5) Normal implantation site and benign trophoblastic lesions vs. trophoblastic tumour.
6) Choriocarcinoma vs. extravillous/intermediary trophoblastic tumour (PSTT and ETT).
7) PSTT vs. ETT.
8) Trophoblastic tumour vs. carcinoma/sarcoma.

PATHOLOGIC DEFINITION AND CLASSIFICATION
(1)
Gestational trophoblastic disease can be divided into three groups:

1. Non-molar, non-neoplastic trophoblastic lesions (only differential diagnostic relevance)
Exaggerated placental site
Placental site nodule
Mesenchymal placental dysplasia

2. Hydatidiform mole: Abnormal products of conception characterised by dysmorphic hydropic chorionic villi and abnormal trophoblast proliferation
Complete hydatidiform mole
Partial hydatidiform mole
Invasive or metastatic hydatidiform mole

3. Trophoblastic tumours: Neoplastic lesions arising from the trophoblast
Choriocarcinoma
Placental site trophoblastic tumour (PSTT)
Epithelioid trophoblastic tumour (ETT)

N.B. Persistent trophoblastic disease (PTD) is not a pathologic diagnosis but solely a clinical diagnosis characterised by static or rising serum hCG values after a molar pregnancy, see section on PTD.

Macroscopic evaluation
Evacuated material: In practice, mainly relevant for early products of conception. The total amount of evacuated material is given in ml. It is investigated macroscopically for the present of spongy placental tissue (chorionic villi), membranes, umbilical cord and foetal parts. Visible cysts are mentioned. If cysts are present, the largest and smallest sizes are noted, and their number or their proportion of the total placental tissue is given. It is noted whether the cystic material is localized or diffusely present. Any suspicion of twin pregnancy is mentioned. If there is clinical, macroscopic or histologic suspicion of hydatidiform mole, representative tissue is embedded in at least five capsules.
Hysterectomy: Uterus removed due to suspicion of invasive hydatidiform mole or trophoblastic tumour is treated according to Danish Gynaecologic Cancer Database's guidelines for corpus uteri cancer.

Histologic evaluation
(1,84-86)

Exaggerated placental site (EPS)
A florid and exaggerated implantation site reaction characterised by extensive infiltration into the endo- and myometrium of extravillous intermediary trophoblast cells, several of which are multinuclear. The cells have abundant eosinophilic cytoplasm and irregular hyperchromatic cell nuclei. Necrosis is not seen. The Ki-67 index is very low (<1 %). Often seen in complete hydatidiform mole. Probably represents a normal physiologic condition and has no clinical relevance if hydatidiform mole is not present.

Placental site nodule (PSN)
Little (1–14 mm) well-defined nodular lesion in the endometrium or cervix made up of extravillous intermediary trophoblast cells in a well-defined eosinophilic matrix (at times multifocal) The cells are seen in a random pattern, singularly, in small groups or strings, sometimes diffusely distributed. Most of the cells have small uniform nuclei with glycogen-containing cytoplasm. A few cells have large irregular hyperchromatic nuclei with abundant eosinophilic cytoplasm. There is no real cell atypia and no mitoses. The Ki67 index is low (<8 %). Often an incidental finding in the uterine corpus or cervix smear that can be seen up to several years after the last pregnancy. Requires no further treatment or follow-up. The term atypical PSN can be used for lesions that are larger, more cellular, with more cell atypia and/or an increased proliferations index, but these findings are not diagnostic for ETT (86).

Complete hydatidiform mole (CHM)
Abnormal product of conception characterised by the following:
Presence of acellular cisterns (vesicles/cysts), often visible macroscopically.
The chorionic villi are plump or polypous, often "cauliflower-like".
No or few trophoblastic inclusions.
Abundant atypical villous trophoblast proliferation, non-polar, focal, multifocal or circumferential (involve the entire periphery of the villus).
The stroma of the villus is myxoid (bluish) with karyorrhexis (nuclear debris), few vessels with no foetal red blood cells and plump stromal cells.
The villi are diffusely involved.
No foetal parts or amnion (except in twin pregnancy with a normal twin and in some cases with mosaicism PP/PM, see genetic section).
Immunostaining: Ki67 index high in the villous stroma and trophoblasts. p57KIP2 often negative (or focal and weak) in the villous stroma and villous cytotrophoblasts (positive control in intermediary cytotrophoblast).

Partial hydatidiform mole (PHM)
Abnormal product of conception characterised by the following:
Presence of a cellular cisterns (vesicles/cysts), often visible macroscopically.
Some of the chorionic villi are large and irregular with clefts, deep invaginations and trophoblastic inclusions in the stroma.
Slight focal trophoblastic proliferation without atypia, at times with fibrinoid degeneration.
The villi are focally involved, i.e. there are two villus populations: a hydropic and a "normal".
Vessels with foetal red blood cells and sometimes foetal parts and/or amnion are present.
Immunostaining: Ki67 index low in the villous stroma and trophoblasts. p57KIP2 often positive in villous stroma and villous cytotrophoblasts as it is in hydropic abortion.

Invasive/metastatic hydatidiform mole
Invasive hydatidiform mole: This diagnosis can usually only be made after hysterectomy. Villi with changes as in hydatidiform mole are seen in the myometrium and/or in the vessels of the myometrium.

Metastatic hydatidiform mole: Pathologic investigation is rare. Extrauterine molar villi are seen in blood vessels or tissue, often vagina or lung.

Choriocarcinoma (CC)
Malignant tumour consisting of layers of biphasic atypical trophoblast predominantly of villous type without chorionic villi. Mixture of atypical syncytiotrophoblasts and cytotrophoblasts as single cells, groups or islands. A varying number of intermediary trophoblast cells are seen. Bleeding is often present (characteristic), necrosis and invasion of vessels. No stroma and no chorionic villi (except in intraplacental CC). Immunostaining shows more hCG expression than hPL expression.
Intraplacental choriocarcinoma: Rare. Biphasic tumour tissue like that mentioned above growing out of the stem villi is seen in the placenta. The tumour is sharply defined histologically, and the surrounding villi are normal.

Placental site trophoblastic tumour (PSTT)
Monophasic tumour composed of extravillous intermediary trophoblasts of implantation site type. Medium to large mononuclear and multinuclear cells with slight to moderate cell atypia, prominent nuclei, eosinophilic or pale cytoplasm, scattered mitoses and intranuclear inclusions. Tumour cells invade the myometrium and grow into the spiral arteries where they induce fibrinoid necrosis (as in normal implantation sites). Cytokeratin and hPL are positive, only focal hCG positivity. Ki67 is positive in > 10 % of cells.

Epithelioid trophoblastic tumour (ETT)
Monophasic tumour composed of extravillous intermediary trophoblasts of chorion laeve type (the free membranes). The tumour is rare and is considered by some to be a subtype of PSTT and by others to be a malignant counterpart of PSN. The tumour cells are monomorphic, smaller than and not as atypical as the tumour cells in PSTT. They grow nodularly, the hyaline matrix is abundant, and sometimes placental site nodules are seen nearby, sometimes with cell atypia. Calcifications are often seen. Ki67 is positive in > 12% of cells.

Immunohistochemistry
All trophoblastic tumours (CC, PSTT and ETT) and the other trophoblastic lesions are positive for low molecular weight cytokeratin (e.g. CK18) and HLA-G. They are focally positive for hPL and hCG (syncytiotrophoblast). hCG, hPL, CD146, P63, Ki67 and cyclin E can be used differential diagnostically (see table 1). (84-87)

Differential diagnostic considerations
1) Hydropic abortion versus hydatidiform mole
In aborted tissue with hydropic degenerative changes, uniform, plump villi with an oedematous hypocellular stroma are often seen. In the stroma, collapsed vessels are also often seen. “True” trophoblastic hyperplasia is not seen, but small trophoblastic “buds” and pseudoinclusions can been seen. The reaction for p57KIP2 is often retained in the villous stroma and cytoplasm.

2) CHM versus PHM

Tabel 1. Characteristics of complete and partial hydatidiform mole

Characteristics	Complete	Partial
Ploidy	Most often diploid	Most often triploid
Foetal parts/amnion	Absent	Usually present
Form of villi	Plump	Clefts and formation of fjords
Stromal apoptosis in villi	Prominent	Limited
Hydropic changes and cisterns	Pronounced, obvious cisterns	Focal, less pronounced cisterns
Trophoblastic proliferation	Circumferential, often marked	Focal and minimal
Trophoblastic atypia	Often	Absent
Implantation site	Exaggerated	Most Often normal
p57KIP2-immunostaining	Most often negative	Most often diffusely positive

p57KIP2 immunostaining: p57KIP2 is a nuclear stain that can help identify cells with only a paternal genome (PP) (seen in section on genetics). In hydropic abortion and triploid hydatidiform moles, the villous stroma and villous cytotrophoblasts are almost always positive. In diploid androgenetic hydatidiform moles, the villous stroma and the cytotrophoblasts are most often negative or only weakly focally positive. A negative p57KIP2 thus strongly suggests complete hydatidiform mole. Extravillous intermediary trophoblasts in trophoblastic columns are a positive control in all cases.
If determination of ploidy has not been undertaken in non-fixed tissue, a FISH analysis or flow cytometry for ploidy on formalin-fixed paraffin-embedded tissue can be considered; the results are, however, less certain than those undertaken in non-fixed tissue.

3) PHM versus twin pregnancy with a molar and a normal product of conception versus mosaicism PP/PM
In twin pregnancy, a sharp macroscopic or histologic division is seen between areas with hydatidiform mole changes in the form of either CMH or PMH and areas with normal tissue. If the hydatidiform mole has only a paternally imprinted genome (which is often the case with CHM), immunostaining with p57KIP2 can clearly differentiate between the different areas. In mosaicism between a diploid androgenetic cell line and a diploid biparental (normal) cell line (PP/PM), areas with hydatidiform mole changes and normal tissue can be seen in localised areas, or the two types of tissue can be seen diffusely scattered amongst each other. Immunostaining of p57KIP2 can give varying results depending on the distribution and type of cells with parental types PP and PM.

4) Other genetic abnormalities causing changes in the product of conception versus hydatidiform mole
In Beckwith-Wiedemann syndrome (BWS) cystic changes (mesenchymal dysplasia) are seen in the placenta, but the changes involve stem villi, which contain foetal stem villous vessels and not distal villi. In chromosome abnormities such as trisomy, hydatidiform mole-like villous changes can be seen, but these are usually

not as prominent and characteristic as those seen in hydatidiform mole. Demonstration of aneuploidy does not rule out the diagnosis of hydatidiform mole, because several examples are known of hydatidiform moles with additional abnormalities, e.g. diploid androgenetic genome (PP) and trisomy (see section on genetics).

5) Normal implantation site and benign trophoblastic lesions vs. trophoblastic tumour
This is a very important differentiation that, like a smear with the presence of atypical trophoblasts, can be very difficult to make. In benign lesions there are no mitoses or necrosis, and immunostaining for Ki-67 shows a low proliferation index (<1 % in EPS, <8 % in PSN). In neoplastic trophoblastic tumours, the Ki-67 index is high (>10 % in PSTT, >12 % in ETT) (86). Always collate with clinical data regarding serum hCG, PTD and time interval from latest pregnancy. The longer the time interval, the greater the risk of trophoblastic tumour.

6) Choriocarcinoma (CC) vs. intermediary trophoblastic tumour (PSTT and ETT)
CC responds well to chemotherapy, whereas PSTT and ETT respond poorly, and thus differentiation has decisive therapeutic importance. CC consists macroscopically of haemorrhagic tissue and is microscopically biphasic of the villous trophoblast type (with a varying number of extravillous intermediary trophoblast cells) with extensive bleeding and necrosis and a high hCG level, whereas PSTT and ETT are monophasic tumours of extravillous intermediary type with less bleeding and necrosis and a lower hCG level. PSTT is similar to implantation site and ETT to chorion laeve. Immunostaining is of limited help (see table 1). The best approach is a thorough investigation of all tissue after staining with haematoxylin & eosin for the presence of syncytiotrophoblasts in CC. In predominantly mononuclear CC, which is seen especially after chemotherapy, the differential diagnosis can be extremely difficult. Mixed tumours with both CC and extravillous intermediary trophoblastic tumour are seen.

7) PSTT vs. ETT
PSTT and ETT are treated in the same way but have different prognoses depending on the time interval from the antecedent pregnancy. Can be reported as extravillous/intermediary trophoblastic tumour. Immunostaining for hPL and p63 can be used, see table 1.

8) Trophoblastic tumour vs. carcinoma or sarcoma
Trophoblastic tumours and benign trophoblastic lesions are strongly positive for cytokeratin. Poorly differentiated carcinomas typically show weaker keratin staining than trophoblastic tumours. Trophoblastic tumours are also strongly positive for HLA-G and may be positive for inhibin, mel-CAM, hPL and hCG (syncytiotrophoblast). They are negative for p16 (cervix cancer), actin and vimentin (sarcoma) (83,88).

In the pathology report of hydatidiform mole, a comprehensive diagnosis is given based on the morphologic picture, immunostaining with p57KIP2 and possible ploidy determination. The results of individual investigation should be evident in the report. The report is subsequently entered into the DGCD database.
If there is lack of agreement between the pathologic and the genetic findings (e.g. p57KIP2 and FISH), the disagreement should be described in the report and be expressed in the conclusion. In such cases the report states: hydatidiform mole, uncertain whether complete or partial.

GENETICS
HYDATIDIFORM MOLE
Approximately half of hydatidiform moles are diploid, half are triploid and <1 % are tetraploid (25,48).

Diploid hydatidiform mole
Diploid hydatidiform moles are most often androgenetic and homozygous. A possible mechanism is that an ovum becomes fertilised by a spermatozoon after which the spermatozoon's chromosomes double and the maternal chromosomes are lost (89,90,91). About 10% of the diploid androgenetic hydatidiform moles are heterozygous (43,92); a possible mechanism is that an ovum is fertilised by two separate spermatozoa and the maternal chromosomes are lost (25,43,93).
Seven percent of hydatidiform moles are (near-) diploid and have a more "complicated" genetic constitution (25,91,94,134). About 3% of cases are mosaics comprising an androgenetic and a diploid biparental cell line (PP/PM). In about 2% of cases, a twin pregnancy exists in which the one conception has a diploid androgenetic genome (hydatidiform mole) and the other has a diploid biparental genome (normal pregnancy) (PP+PM). In about 1% of cases, aneuploidy exists, e.g. diploid androgenesis (PP) and trisomy involving one or more supernumerary chromosomes (e.g. 47,XX,+8). In <1 % of cases all the cells have a diploid biparental genome (PM).
Some women with biparental diploid hydatidiform moles have a recessive hereditary disposition to hydatidiform mole, in that they have inherited a mutation in both alleles1 of the gene NLRP7 (72) or of gene KHDC3L (73). In almost all reported pregnancies in these women, hydatidiform moles have been seen. It is possible that oocytes maturing in women with mutation in both alleles of NLRP7 are abnormally imprinted and that this is the reason for the hydatidiform mole phenotype in their conceptions (95). Normal pregnancies have been described in these women after egg donation (96).
Mutation in NLRP7 and KHDL3C genes does not have phenotypical consequences for the women themselves. Men with a mutation in both alleles have neither affected phenotype nor fertility. It has been suggested that women who are heterozygote for a mutation in these genes could have reduced fertility (97), but to date there is no convincing evidence for this (94,98).

Triploid hydatidiform mole
Triploid hydatidiform moles contain one chromosome set inherited from the mother and two chromosomes sets from the father (PPM). The most likely mechanism is fertilisation of an egg cell with two separate spermatozoa (19,45,99). Triploid conceptions with two sets of maternal chromosomes are not hydatidiform moles (19,100).

Tetraploid hydatidiform mole
Tetraploid hydatidiform moles usually contain a maternal chromosome set and three chromosomes sets inherited from the father (48,92,101,102).

[1] Allele = "copy" of a gene. Women have two alleles of all genes because they have inherited a copy of all genes from their mother and from their father. Men have alleles of genes on chromosomes 1–22 and one allele of genes on the X and Y chromosomes.

Gestational trophoblastic neoplasia (GTN)
Gestational choriocarcinoma, PSTT and ETT can all arise on the basis of a molar pregnancy and a non-molar pregnancy. Even though there are often (always?) somatic genomic aberrations in neoplastic cells, the overall genetic constitution in a GTN is identical with the constitution in the original pregnancy. This means that the genetic constitution in GTN that arises from a diploid androgenetic hydatidiform mole will be diploid androgenetic and that the genetic constitution in a GTN that arises from a non-molar pregnancy will be diploid biparental. In the same way, the overall genetic constitution in a non-gestational trophoblastic tumour in women will be identical with the women's own genetic constitution.
Since treatment and prognosis differs between gestational and non-gestational GTN, and between gestational GTN with biparental and paternal genomes, it is important to genotype these tumors.

GENETIC ANALYSES
Determination of ploidy
Ploidy (number of chromosome sets) can be determined in several ways:
Karyotyping of living cells
Flow cytometry on non-fixed tissue with external control cells
Flow cytometry on fixed tissue
FISH
There are various limitations regarding the use of techniques to determine ploidy on fixed tissue. In flow cytometry on fixed tissue, there is no possibility of including valid control cells. By flow cytometry of fresh contra formalin-fixed, paraffin-embedded specimens of the same tissue, conflicting results were obtained in 4/30 cases (14%) (103). In FISH on fixed tissue, the cell nuclei will be cut through. In DNA purification in fixed tissue, it can be difficult to obtain DNA of adequate quality, and there will often be contamination with maternal DNA

Ploidy is optimally determined when unfixed cells are used, e.g. with karyotyping, flow cytometry (with addition of two different types of control cells with known DNA contents), or FISH on whole cell nuclei. Care should be taken in the use of information regarding ploidy determined on fixed tissue.

Determination of the parental origin of the genome
The parental origin of the genome in a hydatidiform mole or other gestational trophoblastic disease is determined by purifying DNA from the hydatidiform mole/trophoblastic lesion and the mother/woman and comparing the polymorphic DNA markers. Often it is helpful to include DNA from the father/man.
It is often possible to purify DNA from formalin-fixed, paraffin-embedded tissue. But the rate of success is greater and the risk of maternal contamination of tissue from the hydatidiform mole/trophoblastic lesion less if an un-fixed sample is used that can be dissected free of maternal tissue. From the mother/women (and father/man) a blood sample is optimal.
Determination of which pregnancy was the origin of the GTN and study of GTN vs. non-gestational trophoblastic disease
Polymorphic DNA markers in the index GTN are compared with markers in previous pregnancies/children of both the woman and the man. The same technique as for determination of the parental origin of the genome is used.

GENETICS COMPARED WITH OTHER ANALYSES
Genetics and morphology
Molar pregnancies can be classified morphologically or genetically. How great is the agreement between these two classifications?
Most complete hydatidiform moles are diploid androgenetic; partial hydatidiform moles are mostly triploid.
Correspondingly, diploid androgenetic hydatidiform moles are usually complete, triploid hydatidiform moles are usually partial (19,25,89,104-107), whereas most tetraploid hydatidiform moles are described as complete and a few as partial (48). Among these "classical hydatidiform moles", the greatest variability in morphology appears to be among the triploid hydatidiform moles, ranging from complete hydatidiform mole to hydropic degeneration/non-hydatidiform mole (19,25,100,108,109).
Various different morphologies have been described in diploid biparental hydatidiform mole in women with mutations in NLRP7, even among hydatidiform moles in the same woman (72,110,111). Among the few cases of diploid biparental hydatidiform mole in women with mutations in KHDC3L that have been described, the phenotype appears to be mainly complete hydatidiform mole (73,112).
In mosaicism PP/PM, it can be expected that the morphology of the pregnancy varies depending on the prevalence of cells with androgenetic and biparental genomes and dependent of which cell types (e.g. trophoblasts vs. mesenchymal cells) have biparental and androgenetic genomes (91,113) and depending on whether androgenetic cells are present in any foetal tissue. Living children have been born after pregnancies with mosaicism PP/PM (114).

In a twin pregnancy made up of a hydatidiform mole and a normal pregnancy, the mixture of tissues from both conceptions can be confusing. And in both multiple pregnancy and in mosaicism, the correct diagnosis is dependent on whether an adequately large and representative sample is sent for genetic and/or pathologic evaluation.

Genetics and immunochemical staining for p57KIP2
The gene CDKN1C, located on chromosome 11, is parentally imprinted[2] in cytotrophoblasts and villous stromal cells; only the maternal allele is expressed. The gene product of CDKN1C (p57KIP2) can be demonstrated with immunostaining. Cytotrophoblasts and villous stromal cells that are stained with the antibody against p57KIP2 may therefore be considered to contain (at least) only maternally inherited copy of the gene CDKN1C. In most diploid androgenetic hydatidiform moles, the cytotrophoblasts and villous stromal cells do not stain with the antibody against p57KIP2, whereas these cells will be stained in most triploid and tetraploid hydatidiform moles. Some cases in which this prediction has not held true can be explained by the presence of a maternal chromosome 11 in an otherwise diploid androgenetic hydatidiform mole or loss of the maternal chromosome 11 in triploid or tetraploid hydatidiform moles (115). In some biparen-

[2] Parental imprinted genes are genes whose expression ("use") is different from the allele that is inherited from the father and the allele that is inherited from the mother. In some genes, only the allele inherited from the mother is expressed, in other genes, only the allele inherited from the father. Imprinting can, e.g., consist of the presence or absence of a methyl group in certain cytosine bases in or near the gene in question.

tal diploid hydatidiform moles, absence of p57KIP2 has been seen, probably as a consequence of abnormal imprinting (116). Interpretation of the immunostaining of p57KIP2 molar pregnancy with mosaicism is a task for an expert (51,113). The value of immunostaining of p57KIP2 as a predictor for PTD has not been systematically evaluated.

GENETIC EVALUATION/WORKUP AND COUNSELLING
Women/couples with the following history/or family history should be offered evaluation at a clinical genetics unit:
the women/couple have had two hydatidiform moles
the women/couple have had hydatidiform mole and three spontaneous abortions
the woman has had a hydatidiform mole and a family history that suggests autosomal recessive hereditary disposition to hydatidiform mole
the woman has had a hydatidiform mole and has consanguine parents
the women/couple have had a diploid, biparental hydatidiform mole
the woman has a relative with mutation in NLRP7 or KHDC3L.

The workup consists of:
Personal history and family history (pedigree)
Determination of ploidy and the parental origin of the genome in the abnormal pregnancies the women/couple and any affected relatives may have had
If indicated, mutation analysis of NLRP7 and KHDC3L
If indicated, chromosome study of the woman and the partner
If mutations are detected in both alleles of NLRP7 or KHDC3L in the woman:
The probability that a pregnancy of the woman will result in a viable child is most often small, but depends on the type of mutation. Egg donation should be discussed.
There is indication for qualified ultrasound examination of the pregnancy, both in case of egg donation and in case of spontaneous pregnancy. It is unlikely that change in partner or donor insemination will change the risk of recurrence. Prenatal genetic diagnostics are not possible.
The woman's relatives should be offered genetic counselling and genetic testing.
If the history and family history suggests autosomal recessive inheritance, but no mutation in NLRP7 or KHDC3L can be demonstrated in the woman:
The certainty with which a pregnancy in women will result in a living child should be determined based on the woman's history. Egg donation can be discussed, keeping in mind that there is only indirect evidence for its rationality. Qualified ultrasonic monitoring during the pregnancy is indicated, both after egg donation and if spontaneous pregnancy occurs. It is unlikely that change in partner or donor insemination will change the risk of recurrence. Prenatal genetic diagnostics are not possible.
The woman's relatives should be offered genetic counselling.
If a biparental diploid hydatidiform mole is found but there is no information on additional cases of abortion or hydatidiform mole in the women or her family, and no mutation is demonstrated in NLRP7 or KHDC3L:
The morphologic observations regarding the hydatidiform mole should be revised. Is it possible that there is no hydatidiform mole?
The genetic observations regarding the hydatidiform mole should be revised: Is mosaicism possibly present with of two different cell lines? Could there be a structural chromosomal abnormality in the genome of the hydatidiform mole?
If the diagnosis of biparental diploid hydatidiform mole is maintained, the risk of recurrence is unknown, but probably lower than in those women in whom there is evidence of autosomal recessive inheritance.

ONCOLOGIC TREATMENT
In Denmark, approximately 10 patients a year develop gestational trophoblastic disease that requires chemotherapy. The objective of cytostatic treatment is to cure the patient and at the same time retain fertility without risk of secondary malignancy or early menopause.

INDICATIONS FOR TREATMENT
PTD (hCG criterion: increasing, plateau)
Invasive hydatidiform mole/metastatic hydatidiform mole
Choriocarcinoma
Intermediary trophoblastic tumour (PSTT/ETT) (not accessible to surgical therapy)
Persistent heavy vaginal bleeding in a patient with trophoblastic disease

WORKUP BEFORE CHEMOTHERAPY
In accordance with FIGO, the following are required in the workup: chest x-ray, ultrasound scan of abdomen and pelvis, serum hCG, gynaecologic and physical examination, CT is not a requirement but is recommended.
CT can detect pulmonary metastases in up to 40% of the x-ray-negative patients (117) and is therefore recommended. Ultrasound scanning can be used to diagnose disease in and outside the uterus (118). CNS metastases can be detected with CT or MR, and with a spinal tap, hCG can be measured in the cerebrospinal fluid. A ratio of over 1:60 in the concentration of hCG in the spinal fluid: serum is diagnostic for CNS metastasis (35). If a patient has pulmonary metastases, diagnostic workup for brain metastases should be considered (119).

CHEMOTHERAPY
Almost all foreign centres classify patients based on the prognostic parameters in FIGO's scoring system (120) into high- and low-risk groups. Patients in the high-risk groups are treated with highly intensive combination regimes. The treatment of low-risk patients is also more intensive than in Denmark (132, 136). In Denmark, however, a response-adaptive regime is used, in which all patients regardless of risk factors are initially treated with methotrexate (MTX).
By using MTX as initial therapy, a good response is seen in patients (also in high-risk disease), and the risk of sudden life-threatening bleeding, which can be seen at the start of treatment, is reduced to a minimum. The patient can be sustained through the acute phase and afterwards treated more intensely if MTX resistance develops.
This regime has been used in Denmark for more than 30 years, and a recent review of 71 patients treated for post-hydatidiform moles with PTD showed a 100% response, no recurrences and a low incidence of side effects (121). By comparison, the rate of recurrence in the U.K. is 2–3%, and several cases of acute, life-threatening bleeding are seen (2).
Gestational trophoblastic tumours are highly sensitive to chemotherapy, and response-adaptive chemotherapy consists of three lines. Primary therapy (lst line) is oral MTX or I.V. MTX. If resis-

tance develops, I.V. actinomycin D is added as 2nd line therapy, or MTX is replaced with actinomycin D. If the patient does not respond, shift is made to a more intensive regime (3rd line), often BEP or EP.
The response rate to the initial oral MTX therapy is 50%, while 37% are cured with 2nd line therapy. Only 13% of patients have to be treated with high-dose combination therapy to be cured (121). One consolidation treatment is given after therapy has led to undetectable serum hCG values.

Chemotherapeutic regimes
1st line therapy
MTX can be given either orally or intravenously. For oral treatment, 2.5 mg x 4 daily for 5 days/every 3rd week with leucovorin rescue therapy consisting of 1 ml calcium folinate given orally. If I.V. MTX is used, the dose is 250 mg/m2 per week. If there is a high risk of bleeding, giving the first therapy session during hospitalization may be considered. If there are problems with compliance, I.V. or I.M. MTX can be given instead of oral therapy.

2nd line therapy
If kinetic resistance against MTX develops, i.e. serum hCG decreases after each series but increases or stagnates before the next series, act-D 0.5 mg is given daily for 5 days every 3rd week. If MTX intolerance or resistant develops, patients are treated with act-D alone.

3rd line therapy
Combination therapy with bleomycin (30,000 IU I.V. days 2, 9, 16), etoposid (100 mg/m2 I.V. days 1–5) and cisplatin (20 mg/m2 I.V. days 1–5) every third week (BEP), or possibly only etoposid and cisplatin (EP).

Other chemotherapeutic regimes
EMA-CO
EMA-CO (etoposid, MTX, actinomycin D alternating every week with Oncovorin and cyclophosphamide) is used in patients in the high-risk group and patients who relapse while on MTX and/or actinomycin D. Normalisation of hCG is seen in 98% of patients, but the treatment is intensive and toxic (122)
EMA-EP
EMA-EP (etoposid, MTX, actinomycin D, alternating every week with etoposid and cisplatin) is a toxic and resource demanding treatment. In patients with high risk GTN (PSTT, ETT and choriocarcinomas are often refractory to EMA-CO) EMA-EP has been shown to result in a response in 100% and persistent remission in 75% (78).
Taxans
Taxans have been shown to have an effect in previously intensely treated patients, with persistent complete remission in most. This information is, however, based on case reports (137,138).

Acute side effects of chemotherapy
Severe side effects are usually not seen with MTX, but kidney function must be monitored because MTX is excreted renally and can cause uncontrollable toxicity in the presence of renal insufficiency. In addition some patients are poor excretes of MTX. Hepatocellular toxicity is seen in treatment regimes without folinic acid.
Actinomycin D more often than MTX causes hair loss, nausea and vomiting, myelosuppression and stomatitis (123).
Combination regimes like EMA-CO and especially EMA-EP are markedly more toxic, with grades 3 and 4 toxicity in up to 50% (78).
BEP can cause transient myelosuppression, tinnitus, loss of hearing, paraesthesias in fingers and toes as well as skin and lung toxicity. If bleomycin is not included, EP does not cause skin and lung toxicity.

Sequelae to chemotherapy
MTX as single drug does not increase the risk of secondary malignancy, but combination therapy increase the risk of secondary malignancy by a factor of 1.5 and for acute myeloid leukaemia and breast cancer with factors of 16 and 6, respectively (124). After use of combination chemotherapy, menopause appears on average 2 years earlier than after MTX alone (133).

Medical treatment of rare trophoblastic diseases
Choriocarcinoma
Patients with choriocarcinoma are seldom cured with MTX alone, and the primary treatment consists of etoposid and cisplatin possibly also with bleomycin. In order to avoid bleeding complications in these patients, they can initially be treated with MTX alone or in combination with actinomycin D (139). Patients almost always develop resistance to MTX and treatment therefore continues with etoposid, cisplatin and possibly bleomycin (135).

Placental site trophoblastic tumour (PSTT)
PSTT metastasises later than does choriocarcinoma, but tumour spread in the uterus can be extensive. Serum hCG is normally only slightly elevated, whereas serum human placental lactogen hormone (serum hPL) is usually elevated. The tumour is less sensitive to chemotherapy than other trophoblastic diseases, and if possible, surgery should be the first treatment (125).

Epithelioid trophoblastic tumour (ETT)
The course is often benign, but 25% of patients can have metastases and 10% die of the disease. The results of cytostatic therapy are very sparsely described, but as with PSTT, sensitivity to chemotherapy appears to be poor, and for this reason local treatment (such as hysterectomy and resection of pulmonary metastases) should be chosen (81).

Complications
Trophoblastic tumours are highly vascularised, which may cause bleeding after biopsy or start of chemotherapy (35), and massive pulmonary emboli of necrotic tumour tissue may be seen after start of treatment. Biopsy of any metastases is not necessary to make the diagnosis and should be avoided. Pulmonary metastases can cause dyspnoea, cough, haemoptysis and pulmonary hypertension (126-128). In rare cases, intubation or extracorporeal ventilation can be necessary. The prognosis is extremely poor, but curative treatment should be attempted.
The hCG molecule can cross-react with the TSH receptor and cause thyrotoxicosis, which improves when the patient is treated for the malignant disease (127).

Monitoring of the effect of treatment
The effect of treatment is monitored by regular measurement of hCG in serum, and in the case of CNS disease, in the spinal fluid. The serum hCG level is proportionate with the amount of the disease, and regular diagnostic imaging is normally not necessary. The concentration of human placental lactogen hormone (hPL) in the serum can supplement serum hCG in monitoring the effect of

the treatment of PSTT and ETT. Here too, diagnostic imaging is not indicated.

Follow-up after medical treatment

After completion of cytostatic therapy for trophoblastic tumour, the patient should be followed up for 1 year with serum hCG (1–2 times a month for the first 3 months, then every 2ndd to 3rd month). Patients are advised not to become pregnancy during the follow-up period. Gynaecologic examination and diagnostic imaging are not necessary.

At any later pregnancy, serum hCG should be measured 8 weeks after termination of pregnancy.

Treatment of residual disease in patients with normalised hCG

Two studies show that patients treated for metastatic GTN (gestational trophoblastic neoplasia) with residual infiltrates in the lungs after normalisation of hCG can be observed without additional treatment and that survival is the same as in patients without residual infiltrates. Such infiltrates probably consist of non-vital tumour tissue. (129, 130).

Radiotherapy of CNS disease

Local control of the whole CNS is obtained with radiotherapy in 91% with doses of 22–36 GY and in 24% with doses less than 22 GY (131). The downside is transient hair loss and myelosuppression. MTX give intrathecally or in high I.V. doses has the same effect as radiotherapy and can be recommended (124).

SUMMARY

Hydatidiform mole is treated with surgical uterine evacuation with suction and blunt curettage (D).

Medical uterine evacuation should not be used (C).

On clinical suspicion of hydatidiform mole, one representative sample of the evacuated tissue is fixed for histopathologic investigation and one is forwarded unfixed for genetic analysis (D).

Serum hCG is measured on suspicion of hydatidiform mole. At the time of the uterine evacuation, the initial hCG is measured (A).

After a hydatidiform mole that is both triploid and partial, serum hCG is measured weekly until there are two consecutive undetectable values (<1 or <2), after which the patient can be discharged from follow-up (C).

After a diploid hydatidiform mole, a complete mole, or a hydatidiform mole without valid ploidy determination, serum hCG is measured weekly until the value is undetectable (<1 or <2). If serum hCG is undetectable within 56 days after evacuation, the patient can be discharged from follow-up after an additional 4 monthly measurements. If serum hCG is first normalised after 56 days, the patient is follow-up with monthly serum hCG measurement for 6 months.

Safe contraception should be used during the follow-up period (A).

If hCG stagnates (less than 10% fall over three measurements), increases, or if hCG can be demonstrated for longer than 6 months, the patient by definition has persistent trophoblastic disease (PTD). A chest x-ray should be taken and a gynaecologic ultrasound scanning performed. The patient is referred to oncologic treatment (A).

Uterine re-evacuation as a treatment for PTD can, in general, not be recommended because the rate of remission is low, and there is the risk of perforation of the uterus (C).

In all following pregnancies, the woman is offered an early ultrasound scan, e.g. in gestational week 8 (D).

8 weeks after termination of all future pregnancies, serum hCG is measured (D).

In PTD and invasive hydatidiform mole, the primary treatment is MTX, either orally every 3rd week or I.V. every week (B).

In MTX-resistant PTD, I.V. act D is added (or replaces the MTX) (B).

3rd line chemotherapy is BEP or EP, alternatively EMA-CO (B).

Choriocarcinoma is primarily treated with chemotherapy. Hysterectomy and/or resection of metastases are possible treatments (A).

Placental site trophoblastic tumour (PSTT) and epithelioid trophoblastic tumour (ETT) are primarily treated with hysterectomy. In the case of disseminated disease, chemotherapy is considered(A).

The risk of reoccurrence after trophoblastic disease treated with chemotherapy is approximately 3%. Most reoccurrences are seen within 12 months, and for this reason monitoring of hCG is recommended for 1 year, the first 3 months once or twice a month, thereafter every 2nd to 3rd month.

Patients with PSTT and ETT are monitored with measurement of hCG throughout their lifetimes (C).

In genetically verified twin pregnancy with hydatidiform mole and a living foetus, the pregnancy can continue if serum hCG is monitored and ultrasound scans regularly preformed, and possible obstetric complications dealt with (C).

In the case of recurrent hydatidiform mole and/or familial hydatidiform mole, patients should be referred to genetic workup and counselling (C).

Women with a hereditary disposition to hydatidiform mole because of a mutation in NLRP7 should be informed of the possibility of becoming pregnant via egg donation (C).

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APPENDIX

EVIDENCE

The prevalence of PTD after diploid hydatidiform mole is 18%, after triploid hydatidiform mole 0% (95% confidence interval: 0–1.4%) (Evidence IIa)

Classification of hydatidiform mole according to ploidy (diploid/triploid) compared to rmorphology (complete/partial) gives a better discrimination between hydatidiform moles with high risk and hydatidiform moles with low risk of PTD (Evidence IIa)

Patients over 40 years with a molar pregnancy have an increased risk of PTD (Evidence IIa)

If the initial serum hCG is greater than 100,000 IU/L, the risk of PTD is increased (Evidence III)

10% of hydatidiform mole patients with an insufficient fall in serum hCG can expect remission after uterine re-evacuation alone (Evidence III)

The incidence of twin pregnancies with hydatidiform mole and normal foetus is 1:20,000–120,000 pregnancies, corresponding to 0.5–3% of registered trophoblastic diseases (Evidence IIa)

The risk of PTD after a twin pregnancy with hydatidiform mole and a normal foetus is approximately 25% and does not deviate significantly from the risk associated with a singleton hydatidiform mole (Evidence III)

About 60% of women with a twin pregnancy with hydatidiform mole will produce a living baby: median length of gestation is 34 weeks (Evidence III)

The frequency of hydatidiform mole among women who have previously had one hydatidiform mole and later become pregnant is 1–2% (Evidence IIa)

The empirical risk of a new molar pregnancy after two hydatidiform moles is 10–23%. (Evidence IIa)

The group of women with two or more hydatidiform moles is heterogeneous. Some probably have a low risk of recurrence; others have a hereditary very high risk of recurrence (Evidence III)

Women with mutation in both alleles of NLRP7 or both alleles of KHDC3L have an autosomal recessive hereditary disposition to hydatidiform mole with a high penetrance (Evidence III)

Women with mutation in both alleles of NLRP7 can achieve a normal pregnancy using egg donation (Evidence III)

50% of patients with PTD can be cured with MTX alone (Evidence IIa)

In 75% of patients with MTX resistance, hCG normalises on actinomycin D therapy (Evidence IIa)

Actinomycin D causes more often than MTX hair loss, nausea and vomiting, myelosuppression and stomatitis (Evidence IIa)

Menopause occurs 2 years earlier in women given combination chemotherapy compared with those that receive MTX alone (Evidence grade IIa)

Combination regimes (EMA-CO and especially EMA-EP) cause grades 3 and 4 toxicity in up to 50% (Evidence IIa)

There is not complete agreement between the results of ploidy determination with flow cytometry on fixed and on unfixed tissue (Evidence III)

There is not complete agreement between the histopathologic and the genetic classification of hydatidiform mole (Evidence IIa)

TABLE OF IMMUNOHISTOCHEMICAL CHARACTERISTICS OF GESTATIONAL TROPHOBLASTIC DISEASES
(Modified from Trophogram, Shih IM, chap. 20, Blaustein's Pathology of the Female Genital Tract, Sixth edition)

Tumour	Cell type	CK18	HLA-G	Ki-67 index	hCG	P63	hPL	CD 146	Cyclin E
EPS	Intermediary trophoblast	++	++	<1 %	-*	-	++	++	++
PSTT	Intermediary trophoblast	++	++	>10 %	-*	-	++	++	++
PSN	Intermediary trophoblast	++	++	<8 %	-/-	++	-/+	-/+	-/+
ETT	Intermediary trophoblast	++	++	>12 %	-/+*	++	-/+	-/+	++
CC	Syncytiotrophoblast and cytotrophoblast	++	++	>40 %	++	-/+	-/+	-/+	

The table can be used as an algorithm. First, confirmation of suspicion of trophoblastic cells with staining for CK18 and HLA-G. hCG-positive syncytiotrophoblast cells suggest choriocarcinoma. If choriocarcinoma is ruled out, P63 and hPL are used to differentiate between tumours consisting of intermediary trophoblast of chorion type or implantation type. Ki-67 can in addition differentiate between EPS/PSTT and PSN/ETT. Because the range of the Ki-67 index in PSN/ETT is very narrow, cyclin E can be used for additional discrimination.
*Positive in multinuclear intermediary trophoblast cells.

FLOWCHART IN A GYNECOLOGICAL SETTING