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HYDATIDIFORM MOLE –

PREVALENCE AND OUTCOME

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Hydatidiform mole – prevalence and outcome

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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Keep your eyes on the stars and your feet on the ground.

- Theodore Roosevelt

To my family, for love and support.
ABSTRACT

Background: Hydatidiform mole (HM) is a genetically abnormal pregnancy with malignant potential, which exists in two forms, complete (CHM) and partial hydatidiform mole (PHM). The incidence rates (IR) of HM show wide geographic variations, due to differences in registration practices and methods of estimating rates. With increasingly easy access to medical care, and introduction of better diagnostic techniques, pathological pregnancies are detected at an earlier gestational age (GA). As a result of earlier detection, the presenting symptoms and clinical features originally described for second trimester molar pregnancies, are becoming less frequent and the diagnostic criteria have changed. Women with a diagnosis of HM are of childbearing age, and many are eager for a new pregnancy. The aims of this thesis were to investigate the current epidemiology and diagnostic accuracy of HM, and to evaluate pregnancy outcomes subsequent to a HM.

Methods: The study population in studies I, III and IV were all women with HM in Stockholm County 1991-2010 or subgroups thereof, and the cohort in study II was all births in Sweden 1973-2009. Study I compared the main presenting symptoms and clinical features of CHM and PHM, as well as of current CHM compared to a historic cohort. In study II, subsequent pregnancy outcomes of women with a history of HM were evaluated and compared to women with no history of HM. Incidence rates of HM, in relation to age groups and time periods, were investigated in study III. Finally, study IV examined the accuracy and the inter-rater agreement in the diagnosis of HM, by re-evaluating histological slides, and applying ancillary methods, including immunohistochemistry (IHC) and image cytometry.

Results: The overall IR of HM was 2.08/1000 deliveries and 1.48/1000 viable conceptions. A temporal increase in the IR of HM was seen, while the proportion of malignant progression remained constant. The registered number and proportion of PHM increased, and a significant overdiagnosis of PHM was demonstrated. Women with HM were diagnosed 1-2 gestational weeks earlier than previously described in Stockholm County. Vaginal bleeding was the main presenting symptom for women with CHM (57%), but less common compared to a historic cohort (84%, p<0.01). The majority (53%) of women with PHM were asymptomatic at the time of diagnosis. A maternal history of HM was associated with a lower risk of preeclampsia (PE) (OR 0.75) and a slightly increased risk of preterm birth, stillbirth and large for gestational age birth (LGA), although the risk increases were inconsistent when stratified by the relationship between the molar exposure and the rankorder of subsequent births.

Conclusion: The results indicate, that while the IR of HM is increasing, the rate of malignant progression remains constant. Women with HM are diagnosed at successively earlier gestational ages, with symptoms not differing from those of a normal miscarriage. The earlier detection also makes the histological diagnosis difficult, and ancillary diagnostic methods should be applied to avoid a misdiagnosis of HM, and potentially an undetected malignancy. Women with a history of HM can expect normal future reproductive outcomes.
LIST OF SCIENTIFIC PAPERS

I. Joneborg U, Marions L.
   
   **Current clinical features of complete and partial hydatidiform mole in Sweden.**
   

II. Joneborg U, Eloranta S, Johansson AL, Marions L, Weibull CE, Lambe M.

   **Hydatidiform mole and subsequent pregnancy outcome: a population-based cohort study.**


III. Joneborg U, Folkvaljon Y, Papadogiannakis N, Lambe M, Marions L.

   **Temporal trends in incidence and outcome of hydatidiform mole: a retrospective cohort study.**

   Submitted

IV. Joneborg U, Marions L, Sirotkina M, Wessman S, Carlson J, Papadogiannakis N.

   **Overdiagnosis of partial hydatidiform moles – a consequence of underutilization of ancillary diagnostic techniques?**

   Submitted
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>Act-D</td>
<td>Actinomycin-D, chemotherapeutic drug commonly used in treatment of GTN</td>
</tr>
<tr>
<td>AVM</td>
<td>arteriovenous malformation</td>
</tr>
<tr>
<td>CHM</td>
<td>complete hydatidiform mole</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CR</td>
<td>complete remission</td>
</tr>
<tr>
<td>EMA/CO</td>
<td>chemotherapeutic regimen</td>
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<tr>
<td>EP/EMA</td>
<td>chemotherapeutic regimen</td>
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<tr>
<td>ETT</td>
<td>epithelioid trophoblastic tumor</td>
</tr>
<tr>
<td>FIGO</td>
<td>Fédération Internationale de Gynécologie et d’Obstétrique</td>
</tr>
<tr>
<td>FSH</td>
<td>follicle-stimulating hormone</td>
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<tr>
<td>GTD</td>
<td>gestational trophoblastic disease</td>
</tr>
<tr>
<td>GTN</td>
<td>gestational trophoblastic neoplasia</td>
</tr>
<tr>
<td>hCG</td>
<td>human chorionic gonadotropine</td>
</tr>
<tr>
<td>hCG-H</td>
<td>hyperglycosylated hCG</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>hematoxylin and eosin stain</td>
</tr>
<tr>
<td>HM</td>
<td>hydatidiform mole</td>
</tr>
<tr>
<td>ICD-O/2</td>
<td>international classification of diseases for oncology version 2</td>
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<tr>
<td>ICD-O/3</td>
<td>international classification of diseases for oncology version 3</td>
</tr>
<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
</tr>
<tr>
<td>IR</td>
<td>incidence rate</td>
</tr>
<tr>
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<td>International Society for the Study of Trophoblastic Diseases</td>
</tr>
<tr>
<td>LGA</td>
<td>large for gestational age</td>
</tr>
<tr>
<td>LH</td>
<td>luteinizing hormone</td>
</tr>
<tr>
<td>MBR</td>
<td>Medical Birth Register</td>
</tr>
<tr>
<td>MGR</td>
<td>Multi-Generation Register</td>
</tr>
<tr>
<td>MTX</td>
<td>Methotrexate, chemotherapeutic drug commonly used in treatment of GTN</td>
</tr>
<tr>
<td>NM</td>
<td>non-molar gestation</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>PE</td>
<td>preeclampsia</td>
</tr>
<tr>
<td>PHM</td>
<td>partial hydatidiform mole</td>
</tr>
<tr>
<td>PROM</td>
<td>premature rupture of membranes</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
</tr>
<tr>
<td>PSTT</td>
<td>placental site trophoblastic tumor</td>
</tr>
<tr>
<td>RCR</td>
<td>Regional Cancer Register</td>
</tr>
<tr>
<td>SCR</td>
<td>Swedish Cancer Register</td>
</tr>
<tr>
<td>SGA</td>
<td>small for gestational age</td>
</tr>
<tr>
<td>SNOMED</td>
<td>systematized nomenclature of medicine</td>
</tr>
<tr>
<td>SymPathy</td>
<td>regional pathology database</td>
</tr>
<tr>
<td>TE/TP</td>
<td>chemotherapeutic regimen</td>
</tr>
<tr>
<td>TSH</td>
<td>thyroid-stimulating hormone</td>
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1 INTRODUCTION

GESTATIONAL TROPHOBLASTIC DISEASE

The term gestational trophoblastic disease (GTD) encompasses a broad spectrum of disorders, all originating from the placental tissue. GTD mostly affects young women of childbearing age, and are classified as premalignant or malignant lesions. The premalignant hydatidiform mole (HM) is a genetically abnormal pregnancy, which exists in two distinct forms, complete (CHM) and partial hydatidiform mole (PHM). The malignant forms of GTD are invasive mole, choriocarcinoma, placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT). A HM can progress into any of the malignant forms, while any non-molar pregnancy can develop into all of the malignant tumors except for invasive mole. The common name for the malignant forms of GTD is gestational trophoblastic neoplasia (GTN) (1). The relationship between different types of antecedent pregnancies and GTN is illustrated in Figure 1.

Figure 1. Schematic illustration of Gestational Trophoblastic Disease – GTD

Since the introduction of chemotherapy for the treatment of GTN in the 1950’s (2), women with this type of neoplasia have had an excellent prognosis, especially when treated in early stages of disease. Early detection of GTN is thus of great importance. Over the years, the management of pathological pregnancies has changed, mainly due to introduction of new diagnostic techniques, like high-resolution pelvic ultrasound, and new treatment methods, such as prostaglandins and anti-progesterone for the treatment of incomplete miscarriages and early pregnancy terminations. This has had an impact on the diagnostics of molar pregnancies, which are now diagnosed at an earlier gestational age, with the implication that both the clinical presenting symptoms and histological changes are less pronounced. Also, with medical termination of early pregnancies, the products of conception are not sent for
histological analysis to the same extent as when pregnancies were surgically terminated. All of this could potentially lead to molar gestations going unnoticed, with a risk of late presentation of GTN. It is of utmost importance to have an organizational structure, that promotes accurate detection of molar pregnancies and careful post-molar surveillance, to ensure early detection, and thus early treatment, of women with GTN. The rationale for this thesis was to investigate the current epidemiology, diagnostic accuracy and outcome of women with a diagnosis of hydatidiform mole, and to identify possible areas of improvement.
2 BACKGROUND

2.1 DEVELOPMENT OF TROPHOBLASTIC CELLS AND THE PLACENTA

The fertilized ovum is transported through the fallopian tube and enters the uterine cavity after three to four days. At that time, after a series of mitotic divisions, it has developed into a blastocyst, consisting of an inner cell mass and an outer cell layer. The inner cell mass will develop into the embryo, and the outer layer consists of trophoblastic cells, which provide the embryo with nutrients and give rise to the embryonic part of the placenta. The trophoblastic cell layer differentiates into two distinct cell populations; an inner proliferating layer of mononucleated undifferentiated cytotrophoblasts and an outer layer of multinucleated differentiated syncytiotrophoblasts, forming villous structures. The primary villi consist of a cytotrophoblastic core covered by a syncytiotrophoblastic cell layer. Syncytiotrophoblasts invade the endometrial stroma with implantation of the blastocyst (Figure 2), and is the cell type that produces human chorionic gonadotropin (hCG). The cytotrophoblasts continually differentiate into syncytiotrophoblasts, thus acting as syncytiotrophoblastic stem cells.

During further development, secondary and tertiary, or definitive, placental villi are formed, when the core of the villus is penetrated by mesodermal cells, which differentiate into villous capillaries. Meanwhile, the intermediate trophoblasts, which also develop from the cytotrophoblasts, progressively penetrate into the overlying syncytiotum until they reach the maternal endometrium, where they infiltrate the implantation site, and invade and replace the spiral arteries. The uteroplacental circulation is thus established. The intermediate trophoblasts also anchor the placenta to the uterus. The mechanism of trophoblastic invasion is similar to tumor invasion (3-6). Molar gestations and gestational trophoblastic neoplasms all originate from the placental trophoblast, and all three types of trophoblastic cells may result in GTD.

Figure 2. Implantation of a blastocyst

Drawing by Elias Joneborg
2.2 HISTORY OF GESTATIONAL TROPHOBLASTIC DISEASE

There are records of GTD since antiquity. In 400 BC, Hippocrates first described hydatidiform mole as “dropsy of the uterus”. The term hydatid, meaning droplike, was first used by Aetius of Armida, a physician at a Byzantine emperor’s court, in 600 BC, describing a uterus filled with bladder like objects. The next record of a mole is the anecdote of Margaret, Countess of Henneberg, who in 1276 delivered 365 “children”. After that, other anecdotal accounts appear in the medical literature, but it was not until 1827 that a Parisian midwife, Marie Anne Victoire Boivin, in her Nouvelles Recherches de la Mole Visiculaire hypothesized that a hydatidiform mole was of chorionic origin. The first true examples of choriocarcinoma were three cases reported in 1877 by Hans Chiari, later professor of pathology in Vienna. Chiari recognized their association with recent pregnancy, but did not recognize their trophoblastic origin. Choriocarcinoma was long believed to be a sarcoma originating from the placental site. The modern history of choriocarcinoma begins in 1895 with the work of Felix Marchand, who demonstrated that this malignancy was an epithelial tumor derived exclusively from trophoblastic tissue. He called it ”chorionepithelioma”. In 1903, this theory was also accepted by the British Obstetrical Society. Chorionepithelioma was known as a highly aggressive tumor related to pregnancy, with rapid hematogenous dissemination and almost uniformly fatal results. Cases of spontaneous regression were reported, but the mortality rate was over 90% (7-11).

2.3 HUMAN CHORIONIC GONADOTROPIN

The first pregnancy tests were complicated. In 1927 Aschheim and Zondek demonstrated that urine and blood from a pregnant woman had a gonad-stimulating effect. By injecting these substances into immature female mice, they were able to induce follicular maturation and luteinization. The early pregnancy tests, usually referred to as the Friedman test, used urine from pregnant women to induce ovulation in mice and rabbits. In 1960, Wide and Gemzell in Sweden, developed an immunological hCG assay using hemagglutination inhibition reactions for quantitative determination of hCG in human urine, which could be used as a simple and rapid pregnancy test. Brody and Carlström continued the work on pregnancy tests using complement fixation. In 1964, the radioimmunoassay for hCG was invented, which revolutionized pregnancy testing. It was now possible to measure very low concentrations of hCG, and readily available pregnancy tests spread to laboratories across the world (12-17).

2.3.1 Subtypes of hCG

hCG refers to five different molecules with an identical amino acid sequence, but all with different structure and biological function (18, 19).
2.3.1.1 The pregnancy hormone hCG

The pregnancy hormone hCG is a glycoprotein made up of an α-subunit and a β-subunit. It is produced in the placental syncytiotrophoblastic cells. The α-subunit is identical to the α-subunits of the other glycoprotein hormones; luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH), while the β-subunit is unique to hCG. hCG functions as a hormone, promoting production of progesterone. From the time of implantation, hCG will take over the role of LH in promoting progesterone production, by acting on the hCG/LH receptors in the corpus luteal cells of the ovary. After the first weeks of pregnancy, the syncytiotrophoblastic cells of the placenta take over the production of progesterone. It is also believed that hCG plays a role in the differentiation of cytotrophoblasts to syncytiotrophoblasts, as well as in promoting angiogenesis of the uterine vasculature. In early pregnancy, hCG is proportional to the increasing mass of syncytiotrophoblasts, to reach a peak concentration at about ten weeks of pregnancy. After that, the proportion of terminally differentiated syncytiotrophoblasts, which are poor producers of hCG, increase, and the level of hCG is reduced (20).

hCG is an ideal tumor marker for GTD. Trophoblastic tumors containing as little as 10 000 cells cause elevated serum concentrations of hCG, which makes it the most sensitive of known tumor markers (19, 21).

2.3.1.2 Hyperglycosylated hCG (hCG-H)

hCG-H is produced by the cytotrophoblasts of the placenta and has an autocrine function in promoting invasion of the uterus at implantation and growth of the placental tissue during pregnancy. It also promotes growth of trophoblastic malignancies, such as choriocarcinoma and germ cell tumors. Because of its invasive properties, hCG-H has also been called invasive trophoblast antigen (ITA) (22).

2.3.1.3 Sulfated hCG

Sulfated hCG is produced by the gonadotropic cells in the pituitary gland. The pituitary hCG-production follows LH-production in both women and men, at about 1/50 the serum concentration of LH. It is believed that sulfated hCG supplement the function of LH in promoting androstendione and progesterone production and ovulation.

2.3.1.4 hCGβ-subunit (hCGβ) and hyperglycosylated hCGβ

hCGβ is produced by almost all malignancies, even non-gestational, in advanced stages. This subunit lacks hCG activity, but promotes cancer growth by preventing apoptosis Expression of hCGβ in a tumor is associated with an adverse prognosis (18, 23).
2.3.2 Non-pregnancy hCG-increase

A positive pregnancy test in a woman where pregnancy cannot be confirmed, always causes some concern. There are several possible reasons for this finding.

2.3.2.1 False positive hCG

A false positive hCG in serum is often caused by antibodies to animal immunoglobulins in the serum, most commonly heterophilic antibodies, which bind to monoclonal antibodies used in common hCG-assays, giving a false positive result. Since immunoglobulins are not excreted into urine, a negative hCG in urine is a reliable sign of a false positive hCG in serum (19).

2.3.2.2 Pituitary hCG

In cases of gonadal suppression, with the absence of estrogen and progesterone feedback to the hypothalamus, gonadotropin releasing hormone (GnRH) pulses become maximal, resulting in increased levels of LH, hCG and FSH by the gonadotropic cells. The serum concentration of hCG will under these circumstances be slightly increased. This is the case in menopause or gonadal suppression induced by chemotherapy. Pituitary hCG can be confirmed by the measurement of LH and FSH, which should be at postmenopausal levels, or administration of an oral contraceptive or estrogen replacement, which suppresses this iatrogenic elevation of hCG (18).

2.3.2.3 Gestational Trophoblastic Disease (GTD)

Virtually all trophoblastic tumors produce hCG, and investigation for GTD should be undertaken in cases of unexplained increased serum concentrations of hCG. Monitoring after evacuation or chemotherapy is also based on the determination of hCG in serum. An over-expression of hCGβ is common in the more malignant forms, as well as an increased hCG-H/hCG ratio (22, 23).

2.3.2.4 Non-trophoblastic malignancies

Expression of hCGβ can also be seen in non-trophoblastic cancers, especially germ-cell tumors, and is associated with adverse prognosis due to the growth promoting effect exerted by this subunit (23).

2.3.2.5 Quiescent hCG

There are women with stable low levels of hCG persisting for months or years, but without clinical or radiological evidence of disease. This condition is called quiescent hCG. It is believed that hCG is produced by inactive trophoblastic cells, with the potential for malignant transformation. Most, but not all, occur after a history of GTD. The hCG levels will not be affected by surgery or chemotherapy. It is proposed that hCG-H could be a marker for malignant transformation. These women need to be under careful longtime surveillance to ensure that a GTN could be diagnosed at a treatable stage (24-26).
2.3.2.6 Familial hCG

Familial hCG syndrome is a very rare genetic condition, entailing slightly increased serum concentrations of hCG. It affects both men and women, and only a few families in the world have been diagnosed with this syndrome (27).

There are several commercial hCG assays for the analysis of hCG. It is important for the clinician to know which specific assay is used, its advantages and pitfalls. Preferably, the analyses of hCG should be centralized to laboratories with knowledge of GTD. Serum from a patient should always be analyzed with the same method at the same laboratory for reliable comparison (28, 29).

2.4 HYDATIDIFORM MOLE

2.4.1 Epidemiology

Reported incidence rates (IR) of HM show a wide geographic variation, with rates ranging from 1-12/1000 pregnancies. Asia has reported higher rates than Europe and North America, but reliable comparisons are difficult (30, 31). The differences can partly be explained by discrepancies in the use of hospital-based and population-based data, and the use of different control groups. Since pregnancy is a pre-requisite for the development of a HM, the ideal denominator for calculating IR would be all conceptions. However, since it is not possible to gather information on conceptions, IR have been based on estimates of clinically recognized pregnancies, viable conceptions, total births or live births, which adds to the variation in reported rates. For women in the upper and lower extremes of reproductive age, comparison with the number of births will produce an over-estimation of the real risk of having a molar conception because of the high proportion of pregnancies leading to terminations in these groups. Also, lack of central pathology review and registers in many countries add to the difficulties (32, 33). More recent reports indicate a decreasing trend in the Asian population, attributed to improved medical care and to social, economic and educational changes (34-36), while some European countries demonstrate an increasing rate over time (37, 38).

Swedish IR of HM have been estimated in three previous reports, with rates ranging from 0.64/1000 medically registered pregnancies (births and terminations) in 1970 to 1.46/1000 deliveries and 0.9/1000 pregnancies (births, terminations, miscarriages and ectopic pregnancies) in 1992 and 1.20/1000 deliveries in 2011 (39-41). These three reports have used different national or regional registers to retrieve the molar conceptions and different denominators, which make them difficult to compare. Furthermore, an under-registration of around 20% of all cases of HM in the Swedish Cancer Register (SCR) has been demonstrated, which further adds to the difficulty of correct IR (41, 42). Reporting of GTD to the SCR is mandatory, and reports are made separately by both the clinician and pathologist. The SCR does not differentiate between CHM and PHM, treatment data and recurrences are not registered, and there are no records of post-molar GTN. Since the establishment of the
SCR in 1958, on average 108 cases of HM and 4-5 cases of choriocarcinoma are reported annually (Table 1). Data extracted from the last 15 years show an increase to almost 140 cases of HM reported annually, while the number of choriocarcinomas remains the same.

Table 1. Cases of GTD in the Swedish Cancer Register 1958-2014

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>C24*</th>
<th>ICD-O/2*</th>
<th>ICD-O/3*</th>
<th>C24*</th>
<th>ICD-O/2*</th>
<th>ICD-O/3*</th>
<th>Total (n)</th>
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<td>Complete hydatidiform mole or Partial hydatidiform mole</td>
<td>801</td>
<td></td>
<td></td>
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<td>3360</td>
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<tr>
<td>Partial hydatidiform mole</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Complete hydatidiform mole</td>
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<td>M9100/0</td>
<td>1248</td>
<td>1308</td>
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<td>2556</td>
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<td>Placental site trophoblastic tumor</td>
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<td>M9104/1</td>
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<td>1</td>
<td></td>
<td>16</td>
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<tr>
<td>Placental site trophoblastic tumor or Epithelioid trophoblastic tumor</td>
<td></td>
<td>M9104/3</td>
<td></td>
<td></td>
<td></td>
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<td>Epithelioid trophoblastic tumor</td>
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<td>M9105/3</td>
<td>M9105/3</td>
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<td></td>
<td></td>
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<tr>
<td>Malignant hydatidiform mole</td>
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<td>805</td>
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<td></td>
<td></td>
<td>42</td>
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<td>Invasive mole or Choriocarcinoma or Epithelioid trophoblastic tumor</td>
<td></td>
<td>806</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>171</td>
</tr>
<tr>
<td>Invasive mole</td>
<td></td>
<td>M9102/1</td>
<td>M9102/1</td>
<td>13</td>
<td>2</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td></td>
<td>M9103/0</td>
<td>M9103/0</td>
<td>30</td>
<td>19</td>
<td></td>
<td>49</td>
</tr>
</tbody>
</table>


**SCR: /b Suspected invasiveness

2.4.2 Risk factors

Several potential risk factors for the development of a HM have been evaluated. The association between maternal age and HM is well established, with a correlation between very young or advanced maternal age and higher rates of HM, especially CHM (43). In Sweden, there is a growing trend towards delaying childbirth to later in life, something which is reflected in many of the western societies worldwide (44). The mean maternal age at first child in Sweden has increased from 26.3 to 28.9 years between 1990 and 2010. Women in Stockholm follow the same trend, but are on average two years older than Swedish first-time mothers in general, with a mean age at first child of 28.3 years in 1990 and 31.2 years in 2010 (45).

Another well known risk factor for a HM is a prior molar pregnancy. The risk of a repeat mole is 1-2% after one previous HM and 15-20% after two previous molar gestations (46-48). Some of the repeat moles are due to familial clusters of bi-parental HM, which is
associated with mutations in the NLRP7 or KHDC3L genes, and almost no chance of a normal pregnancy (49, 50).

Ethnicity is believed to be another possible risk factor for HM, with higher risks for women of Asian origin. Although the high incidence rates of HM reported from some areas of Asia seem to be decreasing, Asian women have been reported to have an almost twofold increased risk of developing molar pregnancies compared to non-Asians (30, 35, 51).

Additionally, an increased risk of molar pregnancy has been associated with dietary factors, such as reduced intake of dietary carotene and animal fat, and a history of spontaneous abortions and fertility problems (52-54)

2.4.3 Genetics
HM is a genetically abnormal conception, characterized by an over-expression of paternally derived genes. The subtypes CHM and PHM are genetically distinct entities. A CHM is almost always diploid, with endoreplication following monospermic or dispermic fertilization of an ovum, where the maternal chromosome is lost. In a PHM, dispermic fertilization of an ovum leads to a triploid conception (55-58). A consequence of the dominance of paternal genomes in both subtypes of HM seems to be trophoblastic hyperplasia, which is more pronounced in CHM. The presence of a maternal genome in PHM leads to more fetal development and less trophoblastic hyperplasia. A rare subgroup exists, which has the typical morphological appearance of a CHM, and is genetically diploid, but bi-parental. The bi-parental familial recurrent HM is associated with mutations in the NLRP7 or KHDC3L genes, with an autosomal recessive inheritance pattern, which predisposes affected women for recurrent pregnancy loss, mainly CHM (49, 50, 59) (Figure 3).

2.4.4 Pathology
HM arise from the villous trophoblasts of the placenta, and exhibit distinctive morphological characteristics, mainly related to abnormal trophoblast proliferation. These architectural changes of the placenta are originally described in the second trimester of gestation, and are less pronounced in the first trimester, since the morphologic features depend on the developmental stages of the villous tissues. The diagnosis of a HM is based on histopathological examination of the products of conception, with well established diagnostic criteria. These are, however, less reliable when pathological pregnancies are diagnosed at an earlier gestational age, something which is common today, with the frequent use of obstetric ultrasound. Also, non-molar gestations with hydropic degeneration can sometimes mimic an early HM (59-62)
2.4.4.1  Complete Hydatidiform Mole (CHM)

The classical morphologic features of CHM are characterized by enlarged hydropic villi with marked to extensive circumferential trophoblastic hyperplasia, central villous cistern formation, trophoblastic nuclear pleomorphism and trophoblastic pseudo-inclusions. No fetal parts or villous blood vessels are present. In the first trimester, however, other diagnostic features, including stromal karyorrhectic debris, irregular budding architecture of the villi and empty collapsed villous blood vessels, may be more important. Trophoblast hyperplasia is always present (59) (Figure 4).
2.4.4.2 Partial Hydatidiform Mole (PHM)

Morphologic features of PHM are characterized by the presence of two villous populations, some enlarged with hydropic changes and some small, fibrotic and normal-appearing, mild trophoblastic hyperplasia, and evidence of fetal tissue. The first trimester features may include villous stromal fibrosis, irregular dentate villi, numerous villous blood vessels and fetal nucleated red blood cells. Trophoblast hyperplasia is mild to moderate, but always present (59) (Figure 5).

Figure 4. Images of CHM (photos by Nikos Papadogiannakis).

A. Macro photo of CHM. B. H&E slide of CHM. C. CHM with p57-negative staining

Figure 5. Images of PHM and non-molar gestation (NM) (photos by Nikos Papadogiannakis).

A. H&E slide of PHM. B. PHM with p57-positive staining. C. H&E slide of NM. D. NM with p57-positive staining.
2.4.5 Ancillary diagnostic techniques

The use of ancillary techniques is described to increase the diagnostic accuracy of HM. The genetic profiles and over-expression of paternal genomes, which characterize a HM, is the basis for the established complimentary diagnostic techniques.

2.4.5.1 Immunohistochemistry (IHC)

p57\textsuperscript{KIP2} is a paternally imprinted gene, which is expressed predominantly from the maternal alleles in most tissues. It functions as a cell cycle inhibitor, and is strongly expressed in the cytotrophoblasts and villous mesenchyme in the normal placenta throughout gestation. There is no expression in the syncyiotrophoblasts. In CHM, which lack maternal nuclear DNA, p57\textsuperscript{KIP2} is significantly under-expressed, or not expressed at all. In PHM and non-molar gestations (NM), which have at least one set of maternally derived chromosomes, the p57\textsuperscript{KIP2} expression is normal. Immunohistochemical staining for p57\textsuperscript{KIP2} can reliably differentiate CHM from PHM and NM. Since maternal decidua and extra-villous trophoblasts always stain positive, they will function as an internal control (63-65).

2.4.5.2 Genetic evaluation

Ploidy analysis of the products of conception is helpful in distinguishing a triploid PHM from a diploid CHM or NM. There are different methods for determining ploidy status in a specimen.

*Flow cytometry* has the benefit of being able to analyze the ploidy status of a large number of cells quickly. However, it includes all cells in the analyzed tissue section, including maternal cells if present. A large proportion of contaminating maternal cells in a specimen will reduce the proportion of triploid cells, making it difficult to interpret the histogram as triploid. Also, actively dividing diploid cells may have an increased DNA content and can imitate a triploid histogram pattern.

The use of *image cytometry* has the advantage of visualization of the analyzed cells, allowing for selection of trophoblastic cells for ploidy analysis. This will reduce the risk of false diploid results. A limitation is that actively dividing diploid trophoblastic cells might falsely show up in the non-diploid area on the histogram, without a clearly defined triploid peak, which could lead to misinterpretation of ploidy status.

One advantage of *fluorescence in situ hybridization - FISH* is that it can detect chromosomal abnormalities in individual trophoblastic cells, and thereby accurately determine ploidy status, however it cannot distinguish between paternal and maternal genetic material (66).

In recent years, *molecular genotyping* has been shown to further add to the diagnostic accuracy of HM. This technique enables identification of the parental source of the genome, which differentiates between PHM and non-molar digynic triploid miscarriages. It can also confirm the gestational origin of a trophoblastic tumor (67-70).
2.4.6 Presenting symptoms and diagnostic tools

The clinical presentation of second trimester CHM has been well described in the medical literature, including symptoms such as vaginal bleeding, anemia, hyperemesis, hyperthyreosis and early onset preeclampsia. The excessive trophoblastic proliferation of a CHM causes markedly elevated levels of hCG, and features including excessive uterine size and theca lutein cysts are frequently seen in late second trimester presentation (71). PHM have been described to present with similar, but less pronounced symptoms (72). Because of the hydropic placental villi, second trimester CHMs demonstrate a characteristic vesicular ultrasonographic picture, called a ”snowstorm pattern” (Figure 6). It has classically been associated with ovarian theca lutein cysts and no fetal development (73). These features are, however, not present in the first trimester of pregnancy. With the increasing use of high resolution pelvic ultrasound in the management of early pregnancy complications, HMs are today predominantly diagnosed in the first trimester. This makes ultrasound a less reliable diagnostic tool, especially for PHM, which can resemble a missed abortion (74-78). To achieve a correct diagnosis, histological examination is necessary. With earlier detection of HMs, the presenting symptoms have changed and become less pronounced. Vaginal bleeding is now the most common symptom of a molar pregnancy, but many women are asymptomatic at the time of diagnosis (79-81). With the introduction of medical abortion and medical treatment of incomplete spontaneous abortions in Sweden (82, 83), the products of conception are not sent for histological examination to the same extent, which increases the risk of missing a HM. The symptoms of a HM today resemble those of a normal miscarriage, and with ultrasound being less reliable in the first trimester, it can be difficult for the clinician to decide when to proceed with a histological examination.

Ultrasound may also detect arteriovenous malformations (AVM), which can be associated with GTD, especially in its malignant forms (Figure 6) (84). In GTD, angiogenesis is enhanced by the trophoblastic proliferation, and the production of high levels of hCG can predispose to the formation of AVM with high blood flows (85). AVMs usually resolve with the resolution of the molar pregnancy or with treatment of GTN.

**Figure 6.** Ultrasonographic images of GTD (*photos by Elisabeth Epstein*)
2.5 GESTATIONAL TROPHOBLASTIC NEOPLASIA (GTN)

2.5.1 Definition

After a diagnosis of a molar pregnancy, women are enrolled in an hCG-surveillance program to enable early detection of malignant transformation of the HM. hCG, produced by the trophoblastic cells, is considered to be a disease-specific marker for GTD. It is easily measured quantitatively in both serum and urine, and the levels of hCG have been shown to correlate with disease burden. A plateaued or rising hCG concentration signifies malignant change. Studies show that approximately 15% of CHM and 1% of PHM undergo malignant transformation (86-88). Before the development of effective medical treatment of GTN, hysterectomy was the standard procedure in cases of malignant transformation. After introduction of chemotherapy as a curative treatment for choriocarcinoma, however, surgical resection has become an unusual practice, precluding histological assessment and diagnosis. The term post-molar GTN is used for the malignant progression of HM, independent of the histological subtype of GTN, and treatment is started upon clinical diagnosis.

The current internationally established criteria for the diagnosis of post-molar GTN were adopted by the FIGO Committee on Gynecologic Oncology in 2000 (89), although several centers include additional criteria, or use their own.

*FIGO criteria for the diagnosis of post-molar GTN*

1. GTN may be diagnosed when the plateau of hCG lasts for 4 measurements over a period of 3 weeks or longer (days 1, 7, 14, 21).

2. GTN may be diagnosed when there is a rise of hCG on three consecutive weekly measurements, over a period of two weeks or longer (days 1, 7, 14).

3. GTN is diagnosed when the hCG level remains elevated for 6 months or more. *

4. GTN is diagnosed if there is a histologic diagnosis of choriocarcinoma.

*Omission of this criterion proposed (90, 91)*

The diagnosis of GTN after any non-molar pregnancy is also rarely based on histology, but rather on clinical symptoms, radiological findings, hCG-levels and likelihood (young women with a relatively short interval to an antecedent pregnancy). Biopsies for histological confirmation is not recommended because of the extremely hemorrhagic trophoblastic tissue, which might cause life-threatening hemorrhages (92).
2.5.2 Histological subtypes of GTN

2.5.2.1 Invasive mole

Invasive mole is the most common form of GTN, and consists of molar tissue which has invaded the myometrium, with a risk of uterine perforation and intra-abdominal hemorrhage. It has the potential to set extra-uterine metastatic lesions, particularly to the vagina and lungs. When histological confirmation is possible, it is characterized by enlarged molar villi that have penetrated deeply into the myometrium, and it can be distinguished from choriocarcinoma by the maintained villous structures (30, 93, 94) (Figure 7). Differential diagnoses could be placenta increta or percreta.

2.5.2.2 Choriocarcinoma

Gestational choriocarcinoma is an invasive neoplasm of the placental trophoblastic epithelium, which affects approximately 1 in 40,000 pregnancies in Europe and North America. It can originate from any type of pregnancy, although it is approximately 1000 times more common after a HM than after a normal conception. It is estimated that 25% of gestational choriocarcinomas occur after parturition, 25% after an abortion or miscarriage and 50% after a HM, most likely a CHM. Choriocarcinoma is a highly malignant hCG-producing tumor with rapid growth and a tendency for early metastasis. The lesions are extremely hemorrhagic, and the presenting symptoms are usually vaginal bleeding or bleeding from the metastatic sites. Biopsies are generally not recommended, but when tissue can be safely obtained, the histological picture is characterized by hemorrhagic lesions with central necrosis, prominent vascular invasion and an avillous biphasic/bilaminar architecture of syncytiotrophoblasts and cytotrophoblasts (Figure 7). The histological picture is identical to that of non-gestational choriocarcinoma, which is a rare carcinoma usually originating from the ovaries. Genotyping is necessary to differentiate these two entities, reflecting the causative pregnancy in cases of gestational choriocarcinoma. While gestational choriocarcinoma is highly sensitive to chemotherapy and has a good prognosis even in metastatic disease, non-gestational choriocarcinoma has a worse prognosis (30, 32, 33, 94, 95).

2.5.2.3 Placental Site Trophoblastic Tumor (PSTT)

PSTT is a rare form of GTN occurring in approximately 0.2% of all cases of GTD (96). Like choriocarcinoma, it can also originate from any type of pregnancy and most patients present with either vaginal bleeding or amenorrhea. PSTT is a slow-growing tumor, which infiltrates the endometrium and myometrium. Compared to choriocarcinoma, it produces less hCG and metastasizes later, but more commonly involves regional lymph nodes. Histological verification is needed for the diagnosis of PSTT, which is a neoplastic proliferation of the extravillous placental implantation site intermediate trophoblasts. Typically, the malignant cells infiltrate between muscle fibers and bundles without extensive hemorrhage and necrosis, and as in the normal placental site, the tumor displays a characteristic form of vascular invasion in which the walls of blood vessels are replaced by trophoblastic cells (Figure 7).
The tumor cells stain positive for human placental lactogen (hPL), but only focally positive for hCG. Unlike other forms of GTD, the serum levels of hCG are generally low, and can in some cases be absent. Management of PSTT differs from that of choriocarcinoma and invasive mole since the tumor is more resistant to chemotherapy (94, 97, 98).

2.5.2.4 Epithelioid Trophoblastic Tumor (ETT)

ETT is a recently described, extremely rare type of malignant trophoblastic tumor which resembles a carcinoma. It is composed of chorionic-type intermediate trophoblastic cells, but is distinct from PSTT, with a different immunohistochemical staining pattern. ETT often occurs in the lower uterine segment or in the cervix, where the stratified neoplastic cells, which replace the cervical glandular epithelium, can simulate a keratinizing squamous cell carcinoma. IHC will be helpful in these cases. Similar to PSTT, ETT presents with low serum levels of hCG, and responds poorly to conventional chemotherapy, why hysterectomy is the treatment of choice (94, 97, 99).

Figure 7. Images of GTN (photos by Joseph Carlson)

A. H&E slide of invasive mole, x20. B. H&E slide of choriocarcinoma, x 200. C. H&E slide of PSTT, x400.

2.5.3 Staging and risk scoring of GTN

FIGO anatomical staging is not sufficient to predict the outcome and prognosis of women with GTN. Throughout time, different countries and societies have used a different mix of anatomical and clinical factors in staging of GTN. The most established clinical scoring system was developed by Bagshawe in the UK, comprising ten factors, including age, type of antecedent pregnancy, AB0 blood groups, size and number of metastatic lesions (100). In 1983, WHO adopted nine of these factors in a scoring system, which, over the years, has been modified and revised. After several meetings at the international societies involved in the study and treatment of women with GTD, a consensus on staging of GTN was reached and recommended to the FIGO Committee on Gynecologic Oncology and adopted in 2000 (Table 2) (101-104).
Table 2. FIGO 2000 staging and classification of GTN

**FIGO Anatomical staging of GTN**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Disease confined to the uterus</td>
</tr>
<tr>
<td>Stage II</td>
<td>GTN extends outside the uterus but is limited to the genital structures (adnexa, vagina, broad ligament)</td>
</tr>
<tr>
<td>Stage III</td>
<td>GTN extends to the lungs with or without genital tract involvement</td>
</tr>
<tr>
<td>Stage IV</td>
<td>All other metastatic sites</td>
</tr>
</tbody>
</table>

**Modified WHO prognostic scoring system as adapted by FIGO**

<table>
<thead>
<tr>
<th>FIGO scoring</th>
<th>0</th>
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<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt;40</td>
<td>≥40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Antecedent pregnancy HM</td>
<td>Abortion</td>
<td>Term</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Interval from index pregnancy (months)</td>
<td>&lt;4</td>
<td>4-&lt;7</td>
<td>7-&lt;13</td>
<td>≥13</td>
</tr>
<tr>
<td>Pre-treatment SHCG (IU/L)</td>
<td>&lt;10³</td>
<td>10³-&lt;10⁴</td>
<td>10⁴-&lt;10⁵</td>
<td>≥10⁵</td>
</tr>
<tr>
<td>Largest tumor size (cm)</td>
<td>&lt;3</td>
<td>3-&lt;5</td>
<td>≥5</td>
<td>-</td>
</tr>
<tr>
<td>Site of metastasis Lung</td>
<td>Spleen, kidney</td>
<td>Gastro-intestinal</td>
<td>Liver, brain</td>
<td></td>
</tr>
<tr>
<td>Number of metastases -</td>
<td>1-4</td>
<td>5-8</td>
<td>&gt;8</td>
<td></td>
</tr>
<tr>
<td>Previous failed chemotherapy -</td>
<td>-</td>
<td>Single drug</td>
<td>2 or more drugs</td>
<td></td>
</tr>
</tbody>
</table>

The prognostic score predicts the risk of developing resistance to single-drug chemotherapy, and is calculated by adding the scores of the individual variables. A risk score of 0–6 indicates a low risk of resistance, while women with a risk score of ≥7 are at high risk of developing drug resistance, and require treatment with multi-agent chemotherapy (105). In recent years, the term ultra high-risk has been used for women with very advanced disease and a risk score of ≥12. In this group, the risk of bleeding complications at onset of treatment is high, and a modified induction therapy is recommended (106).
2.5.4 Treatment

2.5.4.1 Development of chemotherapy as a treatment of GTN

It is known that fetal development is dependent on folinic acid. It was therefore suggested that gestational trophoblastic tumors might be sensitive to anti-folinic acid therapy. The first report of women with choriocarcinoma successfully treated with Methotrexate (MTX), a folinic acid antagonist originally used for treatment of leukemia, came from the US in 1956 (2, 107). This was followed by reports from the UK, where combination chemotherapy was successfully used, hypothesizing that drug resistance would develop less readily if a combination of drugs was used (108, 109). Over the years, the development of effective treatment of GTN has progressed, and today there are established first line regimens for women in the low-risk, high-risk and ultra high-risk groups respectively, as well as salvage therapies.

2.5.4.2 Treatment of low-risk GTN (score 0-6)

As a consequence of hCG surveillance, approximately 95% of patients with post-molar GTN are in the low risk group. The recommended treatment in this group is single-agent chemotherapy with MTX and folinic acid or Actinomycin D (Act-D). Several studies have tried to compare the efficacy of regimens including MTX with Act-D regimens, but have failed to determine which is the preferred first line treatment. It seems as Act-D might induce complete remission (CR) to a higher extent, but has more severe side effects. Studies indicate a CR rate of 50-90% after first line single-agent chemotherapy, and an overall remission rate and survival of approximately 100%. In cases of drug resistance, second and occasionally third line therapies will usually salvage the patients. Treatment is recommended to continue for 6 weeks after normalization of the tumor marker hCG to minimize the risk of relapse. Since survival is so high, it is considered adequate to start with the least toxic therapy. For women who have completed child bearing, hysterectomy might hasten the course of treatment, but will not necessarily eliminate the need of chemotherapy. The role of a second dilatation and curettage is under debate, but does not seem to affect the need of chemotherapy other than in certain circumstances, why it is not generally recommended (110-115).

2.5.4.3 Treatment of high-risk GTN (score ≥7)

Most women in the high-risk group have not been registered for hCG follow-up and present with widespread disease months or years after the causative pregnancy, with varying symptoms from different disease locations. These women are very unlikely to be cured with single-agent chemotherapy. Several multi-agent chemotherapy combinations have been used as first line treatment of high risk GTN. The most widely adopted regimen today is EMA/CO, consisting of etoposide, MTX and Act-D alternating with cyclophosphamide and vincristine, which has proven to be efficient with acceptable and manageable side effects. Overall survival has been reported to be 75-90%, with 86% reported from the UK with central registration of all cases of GTD. Bad prognostic factors for survival are the presence of liver and brain metastases, more than 2.8 years since the antecedent pregnancy and prior use of
chemotherapy (116-119). To reduce early deaths in patients with very advanced disease, so-called ultra-high risk patients, induction therapy with low dose etoposide and cisplatinum (EP) was introduced, resulting in reduced mortality in early bleeding complications. This management, together with exclusion of patients with non-gestational choriocarcinoma by genetic analysis, has increased the overall survival to 94% (106, 120). For women with chemo-refractory disease, platinum-based regimens, such as EP/EMA (etoposide and cisplatinum alternating with EMA) or TE/TP (paclitaxel and etoposide alternating with paclitaxel and cisplatinum) are commonly used, with a salvage rate of approximately 70% (121-124). Surgery can be considered for well resectable unifocal lesions, but delays in treatment should be avoided. In very selected cases, high-dose chemotherapy with autologous stem cell support has been used with varying success (125, 126).

2.5.4.4 Treatment of PSTT and ETT

The management of PSTT differs from all other forms of GTN, since this neoplasm is more resistant to chemotherapy. Hysterectomy with regional lymphadenectomy/lymph node sampling is the recommended treatment in tumors confined to the uterus and platinum-based multi-agent chemotherapy, such as EP/EMA or TE/TP, is used in metastatic disease (127). The risk factor most strongly associated with outcome is time from the antecedent pregnancy, where diagnosis more than four years after the previous pregnancy seems to result in adverse outcomes (96, 128). For young women with a strong desire to preserve fertility, wedge resections of the uterine tumors have been tried, but the risk of residual microscopic disease in the remaining part of the uterus may endanger oncological safety, and this practice should be preceded by careful counseling (129-131). There is little clinical data on ETT, which is thought to have a behavior similar to PSTT. Since PSTT is such a rare form of GTD, the International Society for the Study of Trophoblastic Diseases (ISTS) registers all reported cases in an international database.

2.5.5 Long-time outcome

2.5.5.1 Relapse

The overall risk of relapse after treatment for GTN is approximately 3%, with a 2% risk for women in the low risk group, and an 8% risk for women in the high risk group. Most relapses occur within the first year after completion of chemotherapy, and during this time women are recommended to undergo hCG monitoring and to refrain from new pregnancies. Almost all relapses occur within the first five years. A majority of women, who have relapsed after primary treatment, can be salvaged by further chemotherapy (132).

2.5.5.2 Late sequelae

Late sequelae after treatment for GTN are quite rare, even though there are reports of second malignancies. More recent studies show that there is no increased risk of second cancers in the whole cohort of women treated for GTN. However, in the subgroup of women treated with combination therapy, there is an increased risk of especially leukemia. The risk is
highest after more than six months of combination treatment including etoposide and alkylating agents. MTX alone does not seem to increase the risk of second tumors (133, 134). Combination chemotherapy for GTN has also been shown to induce menopause approximately three years earlier than average menopausal age (134, 135).

2.5.5.3 Subsequent pregnancy outcome

Treatment of GTN does not seem to affect future fertility and women who wish to conceive after chemotherapy for GTN can expect a more than 80% chance of birth of a healthy child (136). Women who conceive within six months of completion of chemotherapy, however, seem to have a higher risk of spontaneous abortion, although early conception does not affect the risk of relapse (137-139). After treatment of GTN, the risk of stillbirth in subsequent pregnancies is around 1.5%, which is slightly higher than for the normal population. There seems to be no increased risk of adverse pregnancy outcomes after a HM with spontaneous regression of hCG, except for the previously documented risk of a repeat molar pregnancy (46, 47, 140, 141). There are, however, very few studies on the risk of adverse maternal outcomes in subsequent pregnancies after a diagnosis of GTD.

2.6 ORGANIZATION OF GTD IN SWEDEN

Sweden has a population of almost 10 million. Women with HM are diagnosed and followed up at all approximately 50 gynecological departments in the country. It is mandatory for both the pathologist and clinician to report cases of HM to the Swedish Cancer Register (SCR), although reports have shown a considerable under-registration of GTD in the SCR (41, 42). hCG-surveillance is undertaken at the gynecological department, which has diagnosed the HM. The hCG follow-up has varied in length between hospitals, and there is no central register for GTD or hCG regression curves. Sweden is divided into six regions with tertiary care university hospitals. In cases of GTN, women are referred for treatment to one of five tertiary referral centers for GTD. It has been estimated that approximately 50% of all Swedish cases of GTN are referred to and treated at Karolinska University Hospital in Stockholm (142). National guidelines for the management of GTD have recently been completed, in order to secure that all GTD patients receive equal treatment and care. A future goal of the national working group on GTD is to start a national register for GTD, and to centralize pathology and hCG-measurements.
3 AIMS OF THE THESIS

The overall aim of this thesis was to increase knowledge of the prevalence, management and outcome of gestational trophoblastic disease in Sweden.

The specific research questions of the thesis were:

- What are the current presenting symptoms in women diagnosed with hydatidiform moles, and can they be used as indication of the need of further investigation of the products of conception?

- Is there a risk of subsequent adverse maternal and offspring pregnancy outcomes after a maternal history of hydatidiform mole?

- What is the current incidence of hydatidiform mole and post-molar gestational trophoblastic neoplasia in Stockholm County, and has it changed over time?

- What is the accuracy of histopathological diagnosis of hydatidiform mole? Is there a significant misdiagnosis of hydatidiform mole? To what extent are ancillary diagnostic methods used, and how much do they increase diagnostic accuracy?
4 PATIENTS AND METHODS

4.1 STUDY POPULATIONS

Two separate populations were studied in this thesis; one regional cohort of women with a diagnosis of HM, and one national cohort encompassing all births in Sweden and related to a maternal history of HM.

4.1.1 Populations in studies I, III and IV

The basis for studies I, III and IV is a cohort of 956 women diagnosed with HM in Stockholm County 1991-2010. Subpopulations of this cohort, represented by women diagnosed and treated at Karolinska University Hospital in Stockholm, were studied in papers I and IV, while the whole cohort of women was studied in paper III (Table 3).

Table 3. Characteristics of the study populations in studies I, III and IV (medians (range)).

<table>
<thead>
<tr>
<th></th>
<th>Population study I (n=331)</th>
<th>Population study III (n=956)</th>
<th>Population study IV (n=328)</th>
</tr>
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<tbody>
<tr>
<td>Maternal age years</td>
<td>31 (14.6-57.8)</td>
<td>31 (14.1-57.8)</td>
<td>-</td>
</tr>
<tr>
<td>Gestational age completed weeks</td>
<td>12 (4-21)</td>
<td>-</td>
<td>9 (5-19)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1 (0-8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Parity</td>
<td>2 (1-12)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-molar gestation</td>
<td>-</td>
<td>-</td>
<td>219b</td>
</tr>
<tr>
<td>CHM</td>
<td>164</td>
<td>533</td>
<td>36b</td>
</tr>
<tr>
<td>PHM</td>
<td>167</td>
<td>422</td>
<td>72b</td>
</tr>
</tbody>
</table>

a HM impossible to subtype, included in total HM
b The number of each type of conception in the original diagnosis.

4.1.2 Population in study II

Study II is based on 3,622,412 births in Sweden 1973-2009, and related to an antecedent diagnosis of HM in 3071 of the mothers from 1958 and onwards (Figure 8).
**Figure 8.** Study population in study II.

- **All singleton births in the Swedish MBR**
  
  \( n=3,730,789 \)

- **Exclusion of multiple births**
  
  \( n=90,128 \)

- **Exclusion of children with missing data on maternal country of origin**
  
  \( n=169 \)

- **Exclusion of children born to a woman with childbirth and HM registered the same date**
  
  \( n=17 \)

- **Exclusion of children with missing data on SGA or LGA**
  
  \( n=18,063 \)

- **Merged with other register data**
  
  \( n=3,622,412 \)

- **3,622,412 singleton children and 1,878,917 mothers left for final analysis**

- **No exposure**
  
  3,617,226 children

- **Exposure to a prior HM**
  
  5186 children

  - **Exposure of HM prior to index pregnancy**
    
    2867 children

  - **At least one birth between the HM exposure and the index pregnancy**
    
    2319 children
4.2 METHODS

The thesis includes methods from epidemiology (papers I, II and III) and laboratory medicine (paper IV).

4.2.1 Data sources in studies I-IV.

Data were obtained from four Swedish nationwide population-based registers and two regional registers. Record linkage between different registers is made possible by the Swedish personal identity number, which is an individually unique national registration number assigned to all Swedish citizens at birth or first permanent residency (143).

4.2.1.1 The Swedish Board of Health and Welfare

The Swedish National Board of Health and Welfare holds descriptive statistics on all pregnancy terminations, including information on women’s age, gestational age and method of termination. No personal identification is possible, precluding recode linkage to other national registers. Regional descriptive data on calendar year of pregnancy termination and maternal age was collected for paper III.

4.2.1.2 The Swedish Cancer Register (SCR)

The SCR was established in 1958, and since then it is mandatory for both clinicians and pathologists to report all new cancer cases and some pre-malignant conditions, including HM. The SCR does not differentiate between CHM and PHM, nor does it register cases of post-molar GTN, treatment data or recurrences. The overall completeness of the SCR is high (144), but there is a documented under-registration of HM of approximately 20% (41, 42). For the purpose of papers I, II and III, information on women with a diagnosis of HM was collected from the SCR.

4.2.1.3 The Medical Birth Register (MBR)

The Swedish MBR was established in 1973 and holds records of more than 98% of all births in Sweden. Information on maternal characteristics, reproductive history and complications during pregnancy, delivery and the neonatal period are recorded (145). Since 2008, births are recorded from gestational week 22, and before this year, from gestational week 28. Information on births was collected from the MBR for papers II and III.

4.2.1.4 The Multi-Generation Register (MGR)

The MGR encompasses all individuals in Sweden born in 1932 or later, and who resided in Sweden at some point after 1961. It holds information on reproductive history and allows for identification of family structures. For paper II, information on births before 1973, not encompassed in the MBR, was retrieved from the MGR.
4.2.1.5 The Hospital Discharge Registers

Upon discharge from hospitals in Sweden, all patients are given a primary discharge code, many times also secondary and tertiary codes, as well as codes for any procedures which have taken place. The codes are linked to the patient through the personal identity number. The Karolinska University Hospital in Stockholm is the referral center for all women with GTN in Stockholm County and neighboring regions. Since cases of post-molar GTN are neither registered in the SCR nor in the pathology database, the Discharge Register of Karolinska University Hospital was used to retrieve information on women treated for post-molar GTN for paper III, and to find any additional women with HM for paper I.

4.2.1.6 The regional pathology database SymPathy

Sweden does not have a central registration for pathology. In Stockholm, the pathology database SymPathy (Tieto AB, Malmö, Sweden) contains information on results reported by pathology departments at the five regional and university hospitals with gynecological in-patient and emergency wards. The SymPathy database distinguishes between CHM and PHM, but does not contain information on cases of post-molar GTN, since this diagnosis is based on criteria rather than histopathology. Information from the SymPathy database was retrieved to identify women with a diagnosis of CHM and PHM for papers I and III.

4.2.2 Laboratory work in study IV

4.2.2.1 Re-evaluation of histological slides

Histological hematoxylin and eosin (H&E) stained slides of HM and miscarriages were blindly and separately re-evaluated by two placental pathologists. In cases of missing slides, but available blocks, new slides were prepared. Established criteria for the histologic diagnosis of CHM and PHM were used in the evaluation.

4.2.2.2 Immunohistochemistry (IHC)

Immunohistochemical stains were performed on representative formalin-fixed paraffin-embedded, 3µm thick tissue sections, with a mouse anti-human monoclonal antibody against the paternally imprinted but maternally expressed p57 protein (clone KP10), using the BenchMark Ultra automated staining system (Ventana Medical Systems Inc.). Slides were counterstained with hematoxylin. Immunoreactivity was assessed in the cytotrophoblasts, syncytiotrophoblasts, intervillous trophoblasts and villous mesenchyme. Only distinct nuclear staining was scored as positive, and positive staining of the intervillous trophoblast and villous mesenchyme served as background control.

4.2.2.3 Image cytometry

Ploidy status of the tissue was measured by image cytometry, using the AHRENS ICM Cytometry System (Meßtechnische Beratung, Bargteheide/Hamburg, Germany). Single cell
nuclei were defined in feulgen-stained sections from formalin-fixed, paraffin embedded blocks, and measured by means of their absorption of transmitted light. The integrated optical density (IOD) values were calculated by transforming the absorption values into extinction values and correcting for background absorption. From this, the DNA value that entered the histogram was calculated from the extinction integral over the nuclear area, using a calibration factor determined through measurement of diploid reference cells. A cluster analysis detected the different peaks within the histogram, giving a numerical ploidy number. The histogram, the numerical value and the number of analyzed cells were then weighed together to give an adequate interpretation of the ploidy of the specimen.

4.3 STUDY DESIGNS

4.3.1 An overview of all studies

Table 4. Overview of all studies, designs and statistical methods

<table>
<thead>
<tr>
<th>Study design</th>
<th>Inclusion criteria</th>
<th>Cases</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>Retrospective cohort</td>
<td>HM treated at Karolinska University Hospital 1991-2010</td>
<td>331</td>
</tr>
<tr>
<td>Study 2</td>
<td>Retrospective population-based cohort</td>
<td>Singleton births in Sweden 1973-2009</td>
<td>3,622,412</td>
</tr>
<tr>
<td>Study 3</td>
<td>Retrospective observational</td>
<td>HM diagnosed in Stockholm County 1991-2010</td>
<td>956</td>
</tr>
<tr>
<td>Study 4</td>
<td>Retrospective reliability</td>
<td>HM diagnosed at Karolinska University Hospital 2004-2008</td>
<td>328</td>
</tr>
</tbody>
</table>

4.3.2 Design and analysis in study I

Study population

In paper I, women with a diagnosis of HM were retrieved from the regional cancer register (RCR), the pathology database SymPathy and the Karolinska University Discharge register. A total of 341 medical journals of women diagnosed and treated at Karolinska University
Hospital, Solna and Huddinge, 1991-2010 were analyzed, and 331 were included in the final analysis. Demographics, including maternal age, gravidity, parity, gestational age (GA) and subtype of HM, were recorded.

**Outcome measures**

Outcome measures were the documented presenting symptoms and clinical features, and analyzed separately for CHM and PHM.

- Presenting symptoms included vaginal bleeding, hyperemesis, abdominal pain and preeclampsia.
- Clinical features included uterine size, serum level of hCG and ultrasonographic pattern.

Temporal trends for the presenting symptoms and features of CHM were analyzed by comparison to a historical cohort of CHM from 1988-1993 at The New England Trophoblastic Disease Center, Boston, USA

**Statistics**

For comparison of mean and medians, Student’s unpaired t-test and Mann-Whitney U-test were used. Chi square test was used for analysis of differences in proportions, regarding categories of clinical presenting symptoms and features, for the subtypes of HM, and for temporal trends of the proportion of the clinical presenting symptoms in our cohort of CHM compared to the historic cohort. A confidence level of 99% was used for statistical significance.

**4.3.3 Design and analysis in study II**

**Study population**

A total of 3,730,789 births were identified in the MBR between 1973 and 2009. After exclusion of multiple births, missing data on maternal country of origin, children born to a woman with a childbirth and a HM registered the same date, and children with missing data on large for gestational age (LGA) and small for gestational age (SGA), 622,412 children and 1,878,917 mothers were left for the final analysis (Figure 8).
Exposure variable

Maternal history of HM prior to childbirth.

Information on exposure was extracted from the SCR, and yielded 4,940 cases of HM. By record linkage between the MBR and the SCR, 3071 women with a HM prior to at least one of their childbirths and 5186 exposed births were included in the final analysis. The study population was further stratified into maternal history of HM prior to the index pregnancy (n=2867) and maternal history of HM and at least one birth between the HM and the index pregnancy (n=2,319). Information on births occurring prior to 1973 was retrieved from the MGR (Figure 9).

Outcome measures

Adverse maternal and offspring pregnancy outcomes.

- Adverse maternal outcomes included preeclampsia (PE), pregnancy hypertension, placental abruption and premature rupture of membranes (PROM).

- Adverse offspring outcomes included congenital malformations, preterm birth (delivery <37 gestational weeks), stillbirth, neonatal mortality (child died <28 days post partum), SGA and LGA.

Statistics

Unconditional logistic regression analysis was used to estimate the association between maternal history of HM and different adverse maternal and offspring outcomes, and adjusted for maternal age at delivery (<20 years, 20-29 years, 30-39 years and 40 years or older) and region of birth (Europe, Africa, Asia and America). Odds ratios (OR) with 95% confidence intervals (CI) were calculated and potential confounding variables (maternal age at index birth, country of origin, education, smoking at first visit to antenatal care and body mass index) and HM exposure prior to the index birth was assessed using multinomial regression. p<0.05 was considered statistically significant.
4.3.4 Design and analysis in study III

Study population

The Stockholm RCR, SymPathy database and Karolinska University Hospital Discharge Register yielded 956 women with HM and 77 women with post-molar GTN from Stockholm County 1991-2010. Information on deliveries was retrieved from the MBR and information on the number of pregnancy terminations from the Swedish National Board of Health and Welfare. The incidence of HM was calculated by the number of deliveries as well as viable conceptions (births and pregnancy terminations), and reported per 1000 births and per 1000 viable conceptions. The cohort of women with a diagnosis of HM was stratified into seven age groups (<20, 20-24, 25-29, 30-34, 35-39, 40-44 and ≥45), and into the subgroups CHM and PHM for additional information. For assessment of temporal trends, the study period was stratified into four 5-year intervals, 1991-1995, 1996-2000, 2001-2005 and 2006-2010. The incidence of post-molar GTN was defined as the number of post-molar GTN divided by the number of HM at risk during each period.
Outcome measures

- Temporal change in the incidence rate of HM per 1000 deliveries and per 1000 viable conceptions.
- Temporal change in the proportion of women with a diagnosis of post-molar GTN.

Statistics

Student’s unpaired t-test was used for comparison of mean age and Fisher’s exact test was used to analyze the proportion of women progressing into post-molar GTN. Poisson regression models were used to compare age-specific incidence rates, and stratified by time period. OR of GTN after CHM was analyzed by logistic regression. The two-sided significance level was set to \( \alpha = 0.05 \) in all analyses.

4.3.5 Design and analysis in study IV

Study population

All 116 cases of HM diagnosed 2004-2008 at the pathology department at Karolinska University Hospital were retrieved from the pathology database SymPathy, and 232 cases of NM were retrieved as controls. After exclusion of cases with missing or insufficient material, the final analysis encompassed 328 histological slides, 109 with an original diagnosis of HM and 219 with an original diagnosis of NM.

Re-assessment of histological slides

- A blinded re-evaluation of all histological slides was performed by two independent placental reference pathologists. The macroscopic diagnoses were coded as 0= NM, 1=suspicion of mole/mole of uncertain subtype, 2=CHM, 3=PHM, 4=not assessable. All cases of HM, and all cases where there was a diagnostic disagreement between the three evaluations, were further analyzed with complimentary diagnostic methods.
- IHC for p57\textsuperscript{KIP2} was performed for all cases of HM, and all cases where there was a diagnostic disagreement between the three separate evaluations.
- Image cytometry for analysis of DNA ploidy status was also performed for all cases of HM and all cases where there was a diagnostic disagreement between the three separate evaluations.
Statistics

Sensitivity, specificity and positive predictive value (PPV) of the original diagnoses were calculated for each diagnostic category (NM, CHM and PHM). Chi square analysis was used for comparison of the proportions of HM and NM in the original and the final diagnosis. The level of significance was set to p<0.05. Inter- and intra-rater agreement was calculated with non-weighted Fleiss and Cohen’s kappa statistics. The kappa coefficient was interpreted according to Landis & Koch, where κ<0.20 is poor, κ=0.21-0.40 is fair, κ=0.41-0.60 is moderate, κ=0.61-0.80 is good and κ=0.81-1.0 is excellent agreement.

4.4 GENERAL NOTE ON STATISTICS

Student’s two-tailed t-test was used for comparison of continuous variables with an estimated normal distribution, and Mann-Whitney U test for the comparison of medians. Differences in proportions between groups were analyzed with Chi square test or Fisher’s exact test. Logistic regression was used to calculate OR and to control for confounders. Fleiss and Cohen’s kappa statistics were used for inter-rater agreement. Statistical significance was set at a 95% confidence level, with a p-value<0.05. Statistical analyses were performed using Stata version 12.1 (StataCorp 2011. StataStatistical Software: Release 12. College Station, Tx: StataCorp LP), R, version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) and Statistica for Windows, version 12.0, (Statsoft Inc., Tulsa, OK, USA).

4.5 ETHICAL PERMISSION

All studies were approved by the Research Ethics Committee of Karolinska Institutet, Stockholm, Sweden.
5 RESULTS

5.1 STUDY I - CURRENT CLINICAL FEATURES OF COMPLETE AND PARTIAL HYDATIDIFORM MOLE IN SWEDEN

5.1.1 Main findings

The main presenting symptom for women with CHM was vaginal bleeding, while the majority of women with PHM were asymptomatic at the time of diagnosis. A temporal change in clinical features at presentation for CHM was seen, compared to a historic group from the New England Trophoblastic Disease Center in Boston, USA (NETDC). The results are presented in tables 5 and 6.

Table 5. Presenting symptoms of CHM and PHM.

<table>
<thead>
<tr>
<th>Presenting symptom*</th>
<th>CHM (n=162)</th>
<th>PHM (n=164)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>93 (57.4)</td>
<td>67 (40.9)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Hyperemesis</td>
<td>9 (5.6)</td>
<td>2 (1.2)</td>
<td>n.s</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>7 (4.3)</td>
<td>8 (4.9)</td>
<td>n.s</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>1 (0.6)</td>
<td>0</td>
<td>n.s</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>52 (32.1)</td>
<td>87 (53.0)</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

*Information was not available for 5 women.

5.1.2 Additional results

5.1.2.1 Demographics

A total of 164 CHM and 167 PHM were studied. No difference in demographics regarding median age, gravidity and parity was seen. There was a higher proportion of women ≥40 in the group of CHM (23% vs 7%, p<0.01).

The mean and median GA was 11.8 and 11.4 weeks for CHM and 13.3 and 13.7 weeks for PHM (p<0.01).
Table 6. Presenting symptoms of current CHM compared to a historic cohort from NETDC*

<table>
<thead>
<tr>
<th>Presenting symptom</th>
<th>1991-2010 (n=162)**</th>
<th>1988-1993* (n=74)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>93 (57)</td>
<td>62 (84)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Hyperemesis</td>
<td>9 (6)</td>
<td>6 (8)</td>
<td>n.s</td>
</tr>
<tr>
<td>Uterine size greater than date***</td>
<td>19 (13)</td>
<td>21 (28)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>1 (0.6)</td>
<td>1 (1.3)</td>
<td>n.s</td>
</tr>
</tbody>
</table>

*Soto-Wright, 1995, New England Trophoblastic Disease Center (NETDC)

**Information not available for 2 women

***n=146

5.1.2.2 Uterine size

Excessive uterine size (≥4 gestational weeks) was reported in 13% and 3% of women with CHM and PHM respectively (p<0.01). On the contrary, uterine size small for gestational age (≤4 weeks) was found in 48% of women with PHM.

5.1.2.3 Ultrasound

The ultrasonographic picture was suggestive of a HM in 73% of CHM and 35% of PHM (p<0.01). 55% of the cases of PHM had an ultrasonographic appearance of missed abortion. 9/39 women, who subsequently developed post-molar GTN, had a primary ultrasound examination that was not suggestive of a HM.

5.1.2.4 hCG-level

The median hCG level was 172 000 (1830-3520 000) IU/L for CHM and 35 000 (1761-597000) IU/L for PHM (p<0.01) (Figure 10).
5.2 STUDY II - HYDATIDIFORM MOLE AND SUBSEQUENT PREGNANCY OUTCOME: A POPULATION-BASED COHORT STUDY

5.2.1 Main findings

We found no evidence that women with a history of HM were at increased risk of adverse maternal pregnancy outcomes in subsequent pregnancies. On the contrary, they had a 25% lower risk of PE compared to the reference group. For women exposed to a HM just prior to the index delivery, the risk of preterm birth was increased by almost 25% (OR 1.23), and women with at least one birth between the HM and the index birth were at increased risk of large for gestational age (LGA) birth (OR 1.35) and stillbirth (OR 1.82). The absolute risk of stillbirth in this group was still very low, 0.69%, compared to 0.37% in the reference group (Tables 7 and 8).
Table 7. OR and 95% CI for the association between a maternal history of HM and adverse maternal pregnancy outcomes in deliveries between 1973 and 2009 in Sweden.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Model 1*</th>
<th>Model 2*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No history of HM</td>
<td>Previous history of HM (ever)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00 (reference)</td>
<td>0.75 (0.59-0.96)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.023</td>
</tr>
<tr>
<td>Number of events</td>
<td>69,262</td>
<td>77</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>3,617,226</td>
<td>5,186</td>
</tr>
</tbody>
</table>

*Both models were adjusted for maternal age at birth, maternal country of origin and the correlation between siblings using a robust estimator of the standard error

5.2.2 Additional results

5.2.2.1 HM exposure

A total of 0.14% of all 3,622,412 births included in the analysis had been exposed to a prior maternal molar pregnancy. There was an increase in the exposure of HM prior to childbirth with increasing maternal age (p<0.001), increasing level of maternal education (p<0.001) and increasing maternal body mass index (BMI) (p=0.0155). Maternal country of origin did not affect the exposure of HM.

5.2.2.2 More on subsequent pregnancy outcomes

Women with a history of HM were not only at no increased risk of adverse maternal outcomes and at a lower risk of PE in subsequent pregnancies, they also were at no increased risk of small for gestational age birth (SGA), congenital malformations or neonatal mortality. The occurrence of a repeat molar pregnancy was 0.4%.
Table 8. OR and 95% CI for the association between maternal history of HM and adverse offspring pregnancy outcomes in deliveries between 1973 and 2009 in Sweden.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Model 1*</th>
<th>Model 2*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No history of HM</td>
<td>Previous history of HM (ever)</td>
</tr>
<tr>
<td>Large for gestational age</td>
<td>1.00 (reference)</td>
<td>1.19 (1.02-1.39)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00 (reference)</td>
<td>1.19 (1.02-1.39)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.025</td>
<td>0.019</td>
</tr>
<tr>
<td>Number of events</td>
<td>118,328</td>
<td>215</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>1.00 (reference)</td>
<td>1.13 (1.00-1.27)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00 (reference)</td>
<td>1.13 (1.00-1.27)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.059</td>
<td>0.025</td>
</tr>
<tr>
<td>Number of events</td>
<td>184,171</td>
<td>296</td>
</tr>
<tr>
<td>Still birth</td>
<td>1.00 (reference)</td>
<td>1.13 (0.74-1.71)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00 (reference)</td>
<td>1.13 (0.74-1.71)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.569</td>
<td>0.021</td>
</tr>
<tr>
<td>Number of events</td>
<td>13,283</td>
<td>22</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>3,617,226</td>
<td>5,186</td>
</tr>
</tbody>
</table>

*Both models were adjusted for maternal age at birth, maternal country of origin and the correlation between siblings using a robust estimator of the standard error.

5.3 STUDY III - TEMPORAL TRENDS IN INCIDENCE AND OUTCOME OF HYDATIDIFORM MOLE: A RETROSPECTIVE COHORT STUDY

5.3.1 Main findings

The overall incidence rate of HM 1991-2010 was 2.08/1000 deliveries and 1.48/1000 viable conceptions. There was a significant temporal increase in the incidence rate of HM from 1.66/1000 deliveries and 1.21/1000 viable conceptions in 1991-1995 to 2.31/1000 deliveries and 1.66/1000 viable conceptions in 2006-2010 (Tables 9a+b). During the period under study, a significant temporal increase in the total number and proportion of PHM was also demonstrated (Figure 11). Among 956 women with HM, 77 (8%) progressed into post-molar GTN. There was evidence of a slight, but non-significant, increase in the risk of malignancy in the two last 5-year periods under study.
### Table 9a. Incidence rates (IR) of HM per 1000 deliveries with 95% CI

<table>
<thead>
<tr>
<th>Period</th>
<th>IR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991-1995</td>
<td>1.66</td>
<td>(1.44-1.91)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>2.09</td>
<td>(1.81-2.40)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>2.22</td>
<td>(1.96-2.51)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>2.31</td>
<td>(2.06-2.59)</td>
</tr>
</tbody>
</table>

### Table 9b. Incidence rates (IR) of HM per 1000 viable conceptions with 95% CI

<table>
<thead>
<tr>
<th>Period</th>
<th>IR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991-1995</td>
<td>1.21</td>
<td>(1.04-1.39)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>1.44</td>
<td>(1.25-1.65)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>1.58</td>
<td>(1.39-1.79)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>1.66</td>
<td>(1.48-1.86)</td>
</tr>
</tbody>
</table>

**Figure 11.** Temporal changes in the proportion of CHM and PHM.
5.3.2 Additional results

5.3.2.1 Maternal age

During the period under study, the mean age of women diagnosed with a HM increased from 30.3 to 33.5 years (p<0.01). The highest incidence of HM was observed in women above the age of 40. Women of 45 years and above demonstrated a considerably higher risk of a molar conception, 1 in 17 deliveries and 1 in 43 viable conceptions, compared to younger women.

5.3.2.2 Registration in the SCR

The proportion of HM reported to the SCR increased over time, from 55% (109/197) in 1991-1995 to 84% (246/306) in 2006-2010.

5.4 STUDY IV – OVERDIAGNOSIS OF PARTIAL HYDATIDIFORM MOLES – A CONSEQUENCE OF UNDERUTILIZATION OF ANCILLARY DIAGNOSTIC TECHNIQUES?

5.4.1 Main findings

The final pathological diagnosis differed from the original diagnosis in 32/328 (9.8%) cases. Compared to the final diagnosis, there was a minor overdiagnosis of HM (108/328 (33%) vs 87/328 (27%), p=0.07) and a significant overdiagnosis of the subtype PHM (72/328 (22%) vs 47/328 (14%), p=0.015) in the original diagnosis, while the diagnostics of CHM did not markedly differ (Figure 12).

The inter-rater agreement was moderate to good overall, and good to excellent for the reference pathologists. The inter-rater agreement was best for NM and CHM. The intra-rater agreement was moderate to good, and also best for NM and CHM.

Ancillary diagnostic methods were used in the original diagnosis of the 328 products of conception in 74 cases (23 %), mainly in the diagnosis of CHM. When ancillary techniques were applied in the diagnosis of PHM, IHC only was the most commonly applied method.

5.4.2 Additional results

5.4.2.1 Gestational age

Data on GA was available in 290 gestations (88%), 152 (52%) of which were based on early ultrasound, and 138 (48%) on menstrual data. After exclusion of three third trimester pregnancies suspicious of twin molar pregnancies or PHM, the mean and median GA of the conceptions were 9.6 weeks and 9 weeks respectively, with a range of 5-19 weeks. For CHM, GA was only based on menstrual data, with a median of 10 weeks. For PHM, the median GA was 8 weeks based on ultrasound, and 14 weeks based on menstrual data. For NM the median GA was 8 weeks based on ultrasound and 10 weeks based on menstrual data.
**Figure 12.** Study population, original and final diagnosis in Study IV.

348 original slides

- 116 HM
  - 1 slide excluded due to incorrect
  - 4 blocks not possible to retrieve

- 232 NM
  - 7 blocks not possible to retrieve

**RE-EVALUATION**

- 110 HM
  - 1 tetraploid p57+ conception

- 226 controls
  - 7 slides with insufficient material excluded

328 slides for final diagnosis

**FINAL DIAGNOSIS**

- 87 HM
  - 40 CHM
  - 47 PHM

- 241 NM
5.4.2.2 Sensitivity, specificity, PPV and change of diagnosis

The sensitivity, specificity and PPV was 90%, 100% and 100% respectively for CHM, 94%, 90% and 61% for PHM and 90%, 97% and 99% for NM in the original compared to the final diagnosis.

The final diagnosis matched the original diagnosis in 296/328 (90.2%) of all cases. Ancillary methods changed the diagnosis in all of the remaining 32 cases. In 19/32 cases, a final diagnosis could not be easily assigned due to discrepant results between one or more of the ancillary studies. These cases were resolved after consensus discussion. Of these, 8/8 slides with an original diagnosis of CHM were assessed to be CHM, and of 11 slides with a primary diagnosis of PHM, were 7 assessed to be PHM and 4 were assessed to be NM.
6 DISCUSSION

6.1 MAIN FINDINGS

Women with HM presented with symptoms resembling those of a normal miscarriage. For women with CHM, the main presenting symptom was vaginal bleeding. Compared to a historic group of women with CHM, there was a significant change in clinical features at presentation, with a reduction of vaginal bleeding from 84% to 57% and of uterine size large for date from 28% to 13%. A majority of women with PHM were asymptomatic at the time of diagnosis, and a pathological pregnancy was only detected by ultrasonography.

Women with a history of HM were at no increased risk of adverse maternal outcomes in subsequent pregnancies compared to women with no history of HM. On the contrary, they had a lower risk of preeclampsia in their pregnancies following a HM. A maternal history of HM was a risk factor for premature birth in the first birth following the molar pregnancy, while women with at least one birth between the HM and the index birth were at increased risk of LGA birth and stillbirth. The absolute risk of stillbirth in this group was still very low, 0.69%, compared to 0.37% in the reference group. The risk of repeat HM was 0.4%.

The overall incidence rate of HM in Stockholm County 1991-2010 was 2.08/1000 deliveries and 1.48/1000 viable conceptions. A significant temporal increase in both the incidence rate of HM and the total number and proportion of PHM was demonstrated. Among the 956 women with a diagnosis of HM during the period under study, 77 (8%) progressed into post-molar GTN. There was evidence of a slight, but non-significant, increase in the risk of malignancy in the two last 5-year periods under study.

After re-evaluation and assessment of pathological slides of HM and miscarriages during a 5-year period, a non-significant overdiagnosis of HM and a significant overdiagnosis of the subtype PHM was found. The inter-rater agreement for pathologists of mixed experience in placental pathology compared to reference pathologists was moderate to good overall, and between reference pathologists good to excellent. The intra-rater agreement was moderate to good. The best agreement was found for NM and CHM, while the diagnosis of PHM demonstrated the lowest grade of agreement. Ancillary diagnostic methods had been applied in 23% of all original diagnoses, mainly in the diagnosis of CHM.
6.2 INTERPRETATION OF THE RESULTS IN CONTEXT

6.2.1 Early detection and diagnosis of HM

HM is a rare condition, which most general gynecologists and pathologists will rarely encounter. The classical symptoms and diagnostic features associated with a molar pregnancy are described for second trimester gestations, and are rarely seen today. With the introduction and frequent use of early obstetric ultrasound, pathological pregnancies are diagnosed and terminated in early pregnancy, before the development of both pronounced symptoms and a typical ultrasonographic appearance. In the cohort of women with HM in study I, 43% were asymptomatic at the time of presentation, and a pathological pregnancy was only detected by ultrasound. In the stratified cohorts, we found that vaginal bleeding was still the main presenting symptom for women with CHM, although occurring significantly less frequently than described in most historic cohorts (71, 79). The lack of both symptoms and clinical features in this study are, however, similar to what is described in more contemporary studies (80, 81). The majority of women with PHM in our cohort were asymptomatic at the time of presentation, and ultrasound predicted a HM in only one third of these women. Overall, a molar diagnosis was suspected at ultrasound examination in only slightly more than half of all cases.

Compared to a previous Swedish study on a similar population (78), the molar pregnancies in study I were diagnosed on average one gestational week earlier. In study IV, the median GA of CHM in the same population was one additional week lower, which implies that the difficulties in the clinical diagnosis of HM remain, or may increase. The earlier diagnosis of pathological pregnancies also has implications on the histopathological diagnosis. The histological diagnostic criteria for HM are, in the same way as the clinical symptoms, originally described for second trimester gestations. In the first trimester, the villous changes are less pronounced, which makes it more difficult to distinguish a molar from a non-molar specimen. In Sweden, surgical evacuation of products of conception is becoming less frequent due to the widespread use of medical treatment of both pregnancy terminations and incomplete and missed miscarriages. Medically treated pathological pregnancies are less likely to be sent for histopathological examination than surgically evacuated, which may increase the risk of missing a HM. It has been described that women who go through terminations of pregnancy for non-medical reasons, where no pathological examination of the products of conception has been made, are at increased risk of life-threatening complications from GTN (146). It can be hypothesized that medical termination of pathological pregnancies, such as incomplete or missed miscarriages, will lead to an even higher risk of undetected GTN. Since the clinical presentation rarely indicates a molar gestation today, and ultrasonography is becoming less reliable, it is difficult to properly guide the clinician in discriminating which products of conception to send for histopathological examination.

In studies I and III, as has been described in numerous articles (43, 147, 148), we found that CHM was more common in women above the age of 40, and that a majority of women with CHM presented with a level of hCG $\geq 100 \, 000$ IU/L. Even though it is not possible to decide
on a cut-off level of age or hCG when to further analyze the products of conception, it could be recommended that the clinician should bare the diagnosis of HM in mind in women above the age of 40, with a sonographic picture of a pathological pregnancy and high levels of hCG. It is important to remember, however, that even if the proportion of HM compared to normal pregnancies is higher in older women, the highest absolute number of molar pregnancies will be found in women of normal childbearing age. When no pathological examination of the products of conception has been performed, women should be recommended hCG-testing a few weeks after their miscarriage.

6.2.2 More on diagnostics of HM

With the detection and diagnosis of HM in earlier GA, the architectural changes of the molar villi are less pronounced than originally described for second trimester HM. New diagnostic criteria for first trimester HM have been established (59, 149), but the distinction between molar and non-molar specimens, as well as between the subtypes of HM, can still be difficult when the diagnosis is based on morphology alone. Misdiagnosis of PHM and hydropic abortions, and also early CHM, is not uncommon, and a reliable diagnosis will depend on the use of complimentary diagnostic methods. The literature has demonstrated that even experienced pathologists show a marked variability in the diagnosis of HM (150, 151). In study IV we demonstrated a significant overdiagnosis of PHM, which was mainly due to misdiagnosis of normal miscarriages and a lack of usage of available ancillary diagnostic techniques. The median GA of these conceptions based on menstrual data was two weeks higher than based on ultrasound, suggesting that they were mainly missed miscarriages. Non-viable products of conception are likely to develop hydropic changes before evacuation, resulting in a more difficult morphological diagnosis. There was also a small non-significant underdiagnosis of HM, which could potentially have been harmful to the individual woman.

Despite available complimentary diagnostic methods, they were only used in approximately half of all cases with an original diagnosis of PHM, and in many times only IHC was applied, to distinguish the specimen from a CHM, but not ploidy analysis for the distinction from a non-molar specimen.

Corroborating results from other reports, the inter-rater agreement of both molar and non-molar gestations studied in study IV, was better for reference pathologists than a group of pathologists with mixed experience in placental pathology and GTD (152). The highest level of agreement was found for non-molar gestations and CHM. Since HM is a rare and difficult diagnosis, for the detection of which pathology plays a crucial role, it seems reasonable to have a central pathology review for molar specimens and also, possibly as part of national guidelines, a flow chart for the usage of ancillary diagnostic techniques.

Based on the results in study IV, the following suggestion for a flow chart has been made (Figure 13). For the individual woman, a correct diagnosis is of utmost importance, to either ensure that she will be under surveillance for an adequate period of time, or to make certain
that she will not be subjected to unnecessary hCG controls, during which she has to refrain from a new pregnancy.

**Figure 13.** Flow chart pathology
6.2.3 Incidence rate of HM

True incidence rates of HM are difficult to evaluate due to lack of complete data on both HM and pregnancies. Many countries chose to present incidence rates based on the total number of deliveries, a parameter which is usually registered. Deliveries will, however, overestimate the incidence rate in the extremes of maternal age, when pregnancies are terminated to a higher extent. Viable conceptions (births and pregnancy terminations) has been introduced as a denominator in the estimation of incidence rates of HM, and will give a more accurate estimate of the risk of HM for women of all age groups. Another pitfall in determining correct incidence rates, is many times the lack of central registration of GTD, making it difficult to find all molar pregnancies. The overall incidence rate of 2.08 HM/1000 deliveries and 1.48 HM/1000 viable conceptions in Stockholm, demonstrated in study III, is higher than has been previously described in Sweden and also in neighboring countries, such as Denmark, Finland and Holland, with similar populations (37, 40, 41, 153, 154). To ensure the detection of as many HM as possible, we used the regional pathology database, in addition to the regional and national registers, which hold records on HM. This probably accounts for at least some of the increase in the documented incidence rate of HM in our study compared to previous reports. Unfortunately, this evaluation has not been possible to do on a national level, because of the lack of a central pathology diagnostic register. Interestingly, the Stockholm incidence rate of HM is similar to that described in England, with well established central registration of GTD (38).

Study III also demonstrated a continuous temporal increase in the incidence rate of HM in Stockholm. During the period under study, there was a significant increase in the mean age of women diagnosed with a HM, which may have contributed to the higher incidence of molar pregnancies, but cannot fully explain it. A temporal increase in the incidence of HM has also been reported from England and Holland, both countries with national registers of GTD (37, 38). Again, Denmark and Finland, have a stable incidence rate of HM, which is similar to what has been described in earlier Swedish studies based on similar national cancer- and in-patient registers (40, 41, 153, 154).

The increase in both the absolute number and proportion of PHM in study III probably also accounts for some of the increase in the incidence rate. It is difficult to extrapolate the overdiagnosis of PHM found in study IV to the incidence study, since the re-evaluation and re-assessment of the histopathological diagnosis was only performed at Karolinska University Hospital, and only encompassed five years, but if we assume that the overdiagnosis of PHM was true for the whole Stockholm cohort 2001-2010, the proportion of PHM in the cohort of total HM would have decreased by approximately 10%, but would still be 15-20% higher than in the cohort 1991-2000.

Records extracted from the SCR demonstrate a temporal increase in the absolute number of HM registered. This is in line with the increasing proportion of HM registered in the SCR, which was shown in study III. However, there still seems to be a considerable under-registration of HM in the SCR. In 1991-2010, 2368 cases of HM were reported to the SCR.
During the same period, 956 women with a diagnosis of HM were included in the Stockholm cohort of HM. Since approximately 25% of all deliveries in Sweden take place in Stockholm, it could be estimated that approximately 3800 HM should have been reported to the SCR during this time period. As long as there is no national register for GTD, the importance of reporting all cases of GTD to the SCR should be emphasized, to enable a correct evaluation of the incidence rate of HM on a national level.

6.2.4 Post-molar GTN

HM is classified as a premalignant condition, with a risk of progression into post-molar GTN. GTN is a malignancy, which responds extremely well to chemotherapy, and in the low-risk group usually following a HM, the expected cure rate is approximately 100%. On the contrary, an undetected GTN can progress rapidly with a risk of life-threatening complications and a considerably worse prognosis. Study III demonstrated that the risk of post-molar GTN after a CHM was 13% and after a PHM 2%. This result is comparable to results from other European studies (148, 155), but is lower than in reports from the USA and South America, something which is usually attributed to different treatment indications rather than a true difference in the rate of post-molar GTN (156, 157). The risk of malignant progression is significantly higher for CHM, which is reflected in the duration of the recommended hCG surveillance. In study I, ultrasound was not indicative of a molar gestation in 23% of all HM which later progressed into GTN, suggesting that these women could have been lost to follow-up had the clinician not chosen to send the products of conception for further analysis. A correct histopathological diagnosis, differentiating between the subtypes of HM, is crucial for enabling the clinician to enroll women in adequate hCG surveillance programs. Underdiagnosis of HM may lead to an undetected, potentially life-threatening, GTN, while overdiagnosis of HM will force women of child-bearing age to postpone a new pregnancy during the surveillance period.

6.2.5 Subsequent pregnancy outcome

Women with a diagnosis of HM are in their fertile years, and many wish for a new pregnancy as soon as possible. It has been shown, however, that many women suffer from anxiety and future fertility fears after a HM (158, 159). Few previous studies have reported on the maternal risks in subsequent pregnancies after a diagnosis of GTD, while many reports have described an increased risk of a repeat molar pregnancy of 1-2% after a previous HM (46, 47, 141), and an increased risk of miscarriage for women who become pregnant within six months after treatment for GTN (137, 138). Some reports also indicate a slightly increased risk of stillbirth of 1-2% after chemotherapy for GTN (47, 136, 160). Study II is the largest study published on pregnancy outcome subsequent to a HM to date. However, it has not been possible to distinguish between women with HM with spontaneous regression and women treated for post-molar GTN, which, according to data from study III should be
approximately 8%. For this reason, it cannot be excluded that differences in adverse obstetric outcomes reflect chemotherapy related effects, rather than the molar diagnosis. In study II, the risk of stillbirth for women with a history of HM was 0.69% compared to 0.37% in the reference group, which is lower than in previous studies on pregnancy outcomes after GTD. Since only women treated for GTN seem to be at risk of stillbirth in subsequent pregnancies, the relatively low risk in our cohort of women with GTD was expected. Also, the increased risk of stillbirth was only true for women with at least one birth between the molar exposure and the index birth, indicating that stillbirth in our cohort may be influenced by unknown confounding factors, rather than the previous HM.

The increased risk of LGA birth in the same subgroup of women is also difficult to correlate to the molar exposure, and might also be related to other confounding factors, such as an increase in body mass index (BMI) with each pregnancy. Both LGA birth and stillbirth are more common in women with higher BMI (161, 162).

Study II also demonstrated an increased risk of preterm birth, which was restricted to women with a first pregnancy after a molar diagnosis. This finding is difficult to correlate to the previous HM itself, and may instead be related to other associated factors. Women with a diagnosis of HM have been reported to suffer from a higher level of anxiety and fear of an adverse pregnancy outcome than women in general, and high levels of perceived stress is associated with preterm birth (163, 164). The increased risk of preterm birth only in the pregnancy following the HM, may indicate that women, who already have given birth to at least one child after the molar exposure, experience lower levels of anxiety and fear of an adverse pregnancy outcome. Studies designed to examine perceived stress levels in subsequent pregnancies, and pregnancy outcomes following a molar pregnancy, need to be performed to verify this assumption. A history of pregnancy termination, particularly following surgical management, has also been associated with preterm birth (165). It can be assumed that the absolute majority of women with a history of HM have undergone surgical uterine evacuation, which also may be associated with their risk increase of preterm birth.

All observed risk increases discussed above were, although statistically significant, small and inconsistent across exposed groups when stratified by the relation between the molar exposure and the rank order of subsequent births. This may indicate that the elevated risks of adverse outcomes reflect confounding factors, rather than the HM itself.

The results from study II are reassuring in that there is no increased risk of adverse maternal outcomes in pregnancies subsequent to a maternal history of HM. On the contrary, we demonstrated a decreased risk of PE in women previously exposed to a HM. Since GTD, especially in its malignant forms, to some extent can be seen as defective placentation, it could be assumed that the risk of PE, which is also a defective placentation disorder, would be increased. The finding of a decreased risk was therefore surprising. Since PE is more common in late pregnancy (166), this outcome may partly be explained by the increased risk of preterm birth in women exposed to a HM, thus reducing the time they were exposed to the risk of developing PE.
The occurrence of repeat mole of 0.4% in study II was low compared to reported estimates of 1-2% in previous studies. This may reflect not only under-reporting of HM, but also confusion as to whether a new HM represents a new event or a recurrence, since the SCR does not register disease recurrences. Since there is a familial disposition for recurrent HM, in many cases based on specific mutations, the low occurrence of repeat mole may also indicate that familial recurrent HM is not highly represented in the Swedish population.

In conclusion, based on the results in study II, women can be safely assured that a new pregnancy after a previous HM is associated with very low risks of adverse outcomes, including a low risk of repeat mole.

6.3 METHODOLOGICAL CONSIDERATIONS AND LIMITATIONS

In the study of a rare disease like HM, the retrospective cohort design allows for the collection of sufficient data of the rare exposure, but with limited control over the already existing data, which may be incomplete, inaccurate or inconsistently measured. When studying rare diseases, prospective studies are difficult to perform, since they would necessitate very large cohorts to address the relationship between exposure and outcome. All four studies included all registered cases of HM within the context of each separate study design, using all available regional or national registers. A disadvantage with register studies is, however, that they suffer from a risk of misclassification. A differential misclassification may lead to both type I and type II errors, while a non-differential misclassification may lead to an under-estimation of the association between the exposure and outcome.

6.3.1 Systematic error

6.3.1.1 Selection bias

In study I, all available medical charts of women with a diagnosis of HM at a large urban university hospital were collected. It was, however, not possible to retrieve all charts, especially from the earliest period under study, since these medical charts were archived differently, and some were simply missing. From 1996, it was possible to retrieve many charts from a computerized archive. Charts from women with post-molar GTN were, however, often recorded in the computerized archive even from earlier dates. There is a possibility of selection bias of women with malignant progression in the retrieved charts, which may affect the presenting symptoms and clinical features, making them not representative of the population under study. The higher rate of post-molar GTN in study I compared with study III, supports the hypothesis of a selection of women in the treatment group in study I.

In study IV, the selection of the miscarriages used as controls, may also be subjected to selection bias. During the study period, the clinical routines for the treatment of miscarriages
changed from surgical evacuation to mostly medical treatment. Since all miscarriages selected as controls had undergone surgical treatment, there might have been a selection of cases not representative of the whole population of miscarriages, leading to a risk of over- or under-estimation of the misclassification of HM in this population.

6.3.1.2 Information bias and confounding

In all four studies, cases of HM were retrieved from register data. Based on the results of study IV, where we found a significant overdiagnosis of PHM, there might be a risk of misclassification of HM in general in the regional pathology database. Opposite to that, there is a documented under-registration of HM in the SCR.

In study I, a misclassification of HM might have under-estimated the documented clinical presenting symptoms and features (type II error). Since the study only encompassed cases of HM, there was no risk of over-estimation of symptoms by case selection in this cohort. The individual medical charts were assessed by the same researcher, and one main presenting symptom was assigned to each woman. In addition, clinical features such as uterine size, sonographic findings and serum levels of hCG were documented. Since the same researcher assessed all of the medical charts, the rates of the studied variables are likely to be reliable for the cases encompassed in the study population, however, it cannot be excluded that there was a differential misclassification of one of more of the variables, over-or under-estimating the symptoms of the whole study population.

In study II, a major strength was the nationwide population-based design and the large size of the study population. Data extracted from the MBR is considered highly reliable, and allowed assessment of rare pregnancy outcomes, such as PE, PROM, gestational hypertension, placental abruption, stillbirth, neonatal mortality, SGA and LGA, as well as adjustment for potential confounders, such as maternal age and ethnicity. The under-registration of HM in the SCR, though, might have under-estimated the risk of adverse outcomes in the exposed group, while the absence of data on post-molar GTN in the SCR and the inclusion of women treated for GTN, might have over-estimated the risk, by measuring chemotherapy related effects rather than the molar exposure.

Study III suffers from the same risk of misclassification of HM as studies I and II. Since we included all cases of HM during a 20-year period, it was not possible to re-evaluate the pathological slides to confirm the diagnosis. Also, there is a risk that the data on post-molar GTN was not complete, or that the treatment criteria, i.e. the diagnosis of post-molar GTN, differed during the study period. This is, however, not likely, considering that all women with post-molar GTN were treated at the same department, with few changes in the staff, implying that the same treatment criteria probably were applied. The data on births from the MBR and on pregnancy terminations from the Swedish National Board of Health and Welfare were highly reliable. The stratification of age groups and time intervals in study II ensured that the incidence data was correlated to age and time period, and thus more specific.
Advantages of study IV were the relatively large number of slides re-evaluated, and that the pathologists performing the re-evaluations, as well as the IHC and interpretation of the image cytometry, were blinded to the original diagnosis. For a correct interpretation of a possible underdiagnosis of HM, the control group would ideally have included all miscarriages during the period under study. Since evaluation of histological slides and performing complimentary diagnostic techniques, as well as interpreting the results, is very time-consuming and resource intense, it was not possible to include more than two cases per HM. This will obviously decrease the reliability of the interpretation of underdiagnosis of HM.

6.3.2 External validity

Studies I and III represent the early pregnancy population at large urban hospitals, with easy access to prenatal and medical care. The demographics, access to gynecologists and routines regarding examination of products of conception may vary across the country, mainly between urban and rural areas, but the results from these studies are probably applicable to the Swedish population as a whole. Since the diagnosis of post-molar GTN is based on internationally established criteria, there should be no national differences in the diagnosis and proportion of post-molar GTN. Study II is a population-based study, encompassing all births and cases of HM in Sweden during the period under study, which should largely eliminate the sampling bias on a national level. Although all three study populations reflect the Swedish pregnancy and obstetric population, the results may be applicable to other high resource populations similar in background and age. Study IV was also conducted at a large urban university hospital with expertise in the field of GTD and access to complimentary diagnostic techniques, which might have influenced the results, and therefore might not be representative of all areas of Sweden. This study was a qualitative assessment of the local routines and results, and is not automatically generalizable to other hospitals or areas in Sweden or in other countries.
7 CONCLUSIONS

• Vaginal bleeding is the main presenting symptom for women with CHM, and the majority of women with PHM are asymptomatic at the time of diagnosis. The presenting symptoms and clinical features of women with a diagnosis of HM do not differ from those of a normal miscarriage, and are not helpful in the consideration of a molar diagnosis. A combination of maternal age, hCG-level, sonographic appearance and the clinical picture will in each case have to guide the clinician how to further manage the individual woman. If no histopathological analysis of the products of conception is performed, women should be recommended hCG-testing a few weeks after a miscarriage.

• Women with a history of HM are at no increased risk of adverse maternal pregnancy outcomes in subsequent pregnancies, and even have a lower risk of PE than the reference population. After a previous HM, women have an increased risk of preterm birth in their first pregnancy following the molar exposure, and an increased risk of LGA birth and stillbirth in pregnancies with at least one childbirth between the HM and the index birth. The risk of adverse offspring outcomes after a maternal history of HM are, however, slight and inconsistent, and barely differs from that of the general population. Women treated for a HM should be reassured that they can expect normal future reproductive outcomes.

• The overall incidence rate of HM in Stockholm County 1991-2010 was 2.08/1000 deliveries and 1.48/1000 viable conceptions. A significant temporal increase in the incidence rate during the period under study was demonstrated, which may partly be explained by increasing maternal age and an increase in the absolute number and proportion of PHM. The overall proportion of post-molar GTN was 8%, with a non-significant increasing trend.

• After re-assessment of all cases of HM and twice as many NM during a 5-year period, a non-significant overdiagnosis of HM and a significant overdiagnosis of PHM was demonstrated. The misclassification of PHM largely depended on underutilization of available ancillary diagnostic techniques. The inter-rater agreement in the diagnosis of HM and NM was better for reference pathologists than pathologists with mixed experience in placental pathology and GTD, and the best agreement was found for NM and CHM. Based on the results of this study, we suggest standardized protocols for the evaluation of products of conception, with clear indications for when ancillary diagnostic methods should be applied.
8 FUTURE PERSPECTIVES

This thesis was to a large extent an investigation of the prevalence, management and outcome of GTD in Sweden. The work with this thesis, as well as my daily work with women with GTD, has raised many questions, which would be interesting to explore in future research and of benefit to these women. To collect enough cases for any prospective studies, most of these possible future projects would demand cooperation across country borders, something which may be possible within the existing international organisations for GTD.

For the direct benefit of the GTD patients, certain areas of the field of GTD need to be further explored:

- Can we find a marker indicative of malignant progression of HM?

Most studies have focused on hCG as a tumor marker, and hCG regression nomograms as indicators for malignant development, but this has not been able to differentiate between women who will develop and who will not develop post-molar GTN at an early stage. Perhaps genetic studies, based on the genes predicting repetitive HM, could improve not only understanding of possible causative genes for HM, but also for GTN.

Invasive moles could be seen as a placentation disorder, with too deep placentation. Perhaps a defective uterine lining could predispose for GTN, and studies on the mechanisms involved in placentation could lead further.

- Is it possible to develop a targeted drug for GTN?

GTN is most often treated successfully with chemotherapy, but in cases of chemo resistance, women have a much worse prognosis. Since the tumor cells produce hCG, which is a promoter of cancer growth, it would be ideal to target hCG. There have been studies on hCG vaccines, which up to date have not fulfilled expectations. Perhaps there is more to do in this area, or in the study of the signaling pathways associated with tissue remodeling, angiogenesis and the immunological alterations associated with pregnancy.

- Can we predict recurrent GTN?

GTN will often present with uterine vascular anomalies, which regress and disappear on treatment and normalization of hCG. Is it possible to predict uterine recurrences by the existence of persisting or re-appearing AV malformations?
The Swedish national registers, based on the national personal identity number, constitute a solid basis for epidemiological studies. Establishment of a national register for GTD, with documentation of several variables of interest, would make it possible to monitor GTD on a national level, as well as to perform national epidemiological studies. Several possible associations between HM or GTN and other medical conditions or causes of death could easily be explored. For a small treatment center, as well as a small country like Sweden, easily available patient data is of great importance, to ensure that patient outcomes are comparable to those of larger centers and to standard. During the work with this dissertation thesis, a group working on national guidelines for GTD has been formed and guidelines worked out. The establishment of a national register for GTD is currently one of the aims of this group.

Besides improving the understanding of GTD with scientific research, there is much to do in improving care for women with GTD, by adjustments in the organization of the management of GTD, both regarding diagnosis and treatment. Since GTD is a rare disease, and GTN is even more seldomly encountered, it seems reasonable to centralize the care of at least the high risk or ultra-high risk cases of GTN, and to establish a central pathology review for GTD.
9 SAMMANFATTNING PÅ SVENSKA

Mola hydatidosa (HM), på svenska kallad druvbörd, är en genetiskt avvikande graviditet med ett överskott av kromosomer från fadern. Den finns i två olika former, komplet (CHM) och partiell mola (PHM), och kännetecknas av en ökad tillväxt av moderkaksvävna. En molagraviditet kan utvecklas till en malignitet, och kvinnor med diagnosen HM följs därför med kontroller av graviditetshormonet hCG, som är en idealisk tumörmarkör.

Incidensen av HM i världen är svår att uppskatta, då olika länder skiljer sig åt beträffande registrering och hur incidensen beräknas. I västvärlden brukar man ange en incidens på 1-3 HM/1000 graviditeter.

Med ökad tillgänglighet till medicinsk vård och bättre diagnostiska metoder, upptäcks molagraviditeter allt tidigare. Det gör att symptomen och de kliniska fynd som ursprungligen beskrivits vid HM är mindre uttalade idag, liksom de förändringar i graviditetsvävnaden som ligger till grund för diagnostiken. Det kan därför vara svårt att skilja HM från vanliga missfall om man inte använder tilläggsanalyser som stöd för diagnosen.

HM drabbar kvinnor i barnafödande ålder, som i många fall är angelägna om en ny graviditet. Syftet med den här avhandlingen var att undersöka förekomsten av såväl HM som proportionen av HM med malign utveckling, den aktuella symptombilden och diagnostiska säkerheten vid HM, samt eventuella risker för moder eller barn vid graviditeter efter en tidigare HM.


Sammanfattningsvis fann vi en ökande incidens av HM i Stockholm, med upptäckt vid allt kortare graviditetslängder och med symptom som ej nämnvårt skiljer sig från normala missfall. Diagnostiken av framförallt PHM är svår och tilläggsanalyser bör användas. Kvinnor med en tidigare HM kan förvänta sig normala framtida graviditeter.
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11 REFERENCES


