Improving management of gestational trophoblastic neoplasia

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Publication of this thesis was generously sponsored by
ChipSoft
Bayer HealthCare Pharmaceuticals
Medical Dynamics

ISBN/EAN 978-90-9027082-1

Cover, design and layout: In Zicht Grafisch Ontwerp, Arnhem
Print: Ipskamp Drukkers, Enschede

Improving management of gestational trophoblastic neoplasia

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op donderdag 24 januari 2013
om 15.30 uur precies

door

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geboren op 8 juni 1984
te Doetinchem

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1

General introduction

Gestational trophoblastic disease

Gestational trophoblastic disease (GTD) comprises a spectrum of diseases arising from abnormal proliferation of trophoblastic tissue of the placenta. The modified World Health Organization classification of gestational trophoblastic disease broadly divides GTD into molar lesions and non-molar lesions.[1] The molar lesions include partial and complete hydatidiform moles and invasive moles. The nonmolar lesions include choriocarcinoma, placental site trophoblastic tumour (PSTT), epithelioid trophoblastic tumour (ETT), and miscellaneous trophoblastic lesions including exaggerated placental site and placental site nodule. However, this does not provide a distinction between malignant or non-malignant disease. Clinically, GTD can be divided on the one hand into benign trophoblastic lesions including exaggerated placental site, placental site nodule and hydatidiform moles and trophoblastic neoplasia on the other.

Hydatidiform moles (HMs) are abnormally developing pregnancies characterized by hydropic swelling of the villi and trophoblastic hyperplasia with or without development of a foetus. They are usually benign, but may acquire malignant potential. HMs are classified as complete hydatidiform mole (CHM) or partial hydatidiform mole (PHM). PHMs are usually triploid, having two paternal and one maternal set of chromosomes, whereas CHMs are diploid and androgenetic, having two paternal chromosome sets.[2] In most patients, HM regresses spontaneously after suction curettage of the molar tissue from the uterus. However, in 15% of CHM and 0.5-6.6% of PHM trophoblastic tissue remains active after evacuation of the mole as shown by persistent high or even rising human chorionic gonadotrophin (hCG) concentrations in blood.[3-5]

According to the criteria of the International Federation of Gynecology and Obstetrics (FIGO), post-hydatidiform mole trophoblastic neoplasia (GTN) is defined as 1) a plateau in weekly serum hCG concentrations for four consecutive measurements, 2) an increase in serum hCG levels in three weekly consecutive measurements, 3) persistence of detectable hCG levels for more than 6 months after evacuation, and 4) the presence of a histologic diagnosis of choriocarcinoma.[6] In the Netherlands, an additional criterion was added to this definition in 1993. At least one of the values should exceed the 95th percentile of the hCG regression corridor of uneventful decline as constructed by Yedema *et al.* [7]

Gestational trophoblastic neoplasia (GTN)

Gestational trophoblastic neoplasia (GTN) represents a unique group of tumours that include invasive moles, choriocarcinoma, PSTT and the rare ETT.[8] Invasive moles occupy a special position within the spectrum of GTN. Invasive moles are clinically

considered as gestational trophoblastic neoplasia because they can locally invade and distantly metastasise, but biologically they represent abnormally formed placental tissues rather than true neoplasia.[8] An invasive mole is a hydatidiform mole in which hydropic villi invade the myometrium or blood vessels or, more rarely, are deported to extra uterine sites.[1] Invasion can range from superficial penetration to extension through the wall, with perforation or involvement of the broad ligament.[1] Extra uterine molar disease is characterised by the presence of molar villi within blood vessels without invasion of adjacent tissue. Therefore this process is called deportation rather than metastasis.[9] Invasive moles may regress spontaneously, and the risk of progression of invasive mole to choriocarcinoma appears to be no greater than that of progression from a non-invasive HM.[9]

Invasive mole must distinguished from choriocarcinoma, the latter being a highly malignant tumour. Approximately 50 percent of all cases of gestational choriocarcinoma develop following a hydatidiform mole, 25 percent are following a spontaneous abortion or ectopic pregnancy, and 25 percent are following a term pregnancy.[10] Both invasive mole and choriocarcinoma after a HM are manifested by a plateau or elevation in the serum hCG concentration. Consequently, clinically it is often not possible to distinguish between these lesions. However, histopathologically, choriocarcinoma should not be diagnosed in the presence of chorionic villi.[1] PSTT arises from intermediate trophoblast, unlike choriocarcinoma, which arises from villous trophoblast. PSTT grow more slowly, metastasise later, more commonly involve lymph-nodes and produce less hCG than choriocarcinomas.[11] PSTT should be suspected if hCG concentrations are low for the volume of disease present on imaging and hCG free β-subunit values are high.[11;12] The term ETT was introduced to describe an unusual type of trophoblastic tumour that is distinct from PSTT and choriocarcinoma with features resembling a carcinoma.[1;13] The frequent location of ETT in the low uterine segment and endocervix, as well as the epithelioid appearance of ETT, can lead to confusion with squamous cell carcinoma.[14] GTN can occur after any type of antecedent pregnancy, including HM, non-molar abortions, ectopic pregnancies and term live births.[15] The most frequent antecedent pregnancy is a hydatidiform mole. It is usually unknown whether GTN after a HM represents an invasive hydatidiform mole, choriocarcinoma or PSTT, since histological confirmation at the time of rising hCG is only occasionally obtained as the disease is usually eliminated with chemotherapy without surgery.[8;16] A biopsy is discouraged since it can cause life-threatening haemorrhage, and a second curettage is usually not recommended, since it is only curative in a limited number of patients and bears the risk of uterine perforation, although it might be beneficial in some patients with low-risk GTN and reduce the number of courses of chemotherapy required for serum hCG normalisation.[16-19] A prospective randomised controlled trial would be required to investigate the true curative effect of a second curettage.

Management of GTN

When the diagnosis of GTN is established, thorough assessment of the extent of disease as well as an evaluation for risk factors should be performed. A chest x-ray is essential, since pulmonary metastases are common. Chest CT is not needed when the chest x-ray is normal, since discovery of micrometastases does not affect outcome.[20] However, recent studies showed that lung involvement on CT imaging (but not on chest x-ray) may predict the need to switch to second-line chemotherapy.[21] Nevertheless it is chest x-rays that are used for counting the number of lung metastases to evaluate the risk score.[6] In patients with vaginal or lung metastases or with pathologic diagnosis of choriocarcinoma, a CT scan of the abdomen including the liver and MRI of the brain is indicated.[11;22]

Patients with GTN are classified as having low-risk or high-risk disease using the modified WHO prognostic scoring system as adapted by FIGO (Table 1).[6] Risk factors that are taken into account include age, antecedent pregnancy, interval from index pregnancy in months, pre-(chemotherapeutic)treatment serum hCG concentration in IU/L, largest tumour size including the uterus, site and number of metastases, and previous failed chemotherapy. Each risk factor is given a score of 0, 1, 2 or 4. Patients with a combined prognostic score under 7 are considered low-risk and can usually be treated using single-agent chemotherapy. A total prognostic score of 7 or more is considered a high-risk score. These patients are more likely to develop resistance to methotrexate (MTX), and are therefore treated with multi-agent chemotherapy. In the Netherlands, since 1983, a clinical classification system is used proposed by the

Table 1 Modified WHO prognostic scoring system for GTN as adapted by FIGO

Scores	0	1	2	4
Age (years)	<40	≥40	–	–
Antecedent pregnancy	mole	abortion	term	–
Interval months from index pregnancy	<4	4–6	7–12	>12
Pre-treatment serum hCG (IU/L)	$<10^3$	10^3–10^4	10^4–10^5	$>10^5$
Largest tumour size (including uterus)	–	3–4 cm	≥5 cm	–
Site of metastases	lung	spleen, kidney	gastro-intestinal	liver, brain
Number of metastases	–	1–4	5–8	>8
Previous failed chemotherapy	–	–	single drug	2 or more drugs

Dutch Working Party on Trophoblastic Disease (Table 2).[23] This system defines high-risk disease as presence of one or more of the following features: insufficient response to single-agent chemotherapy; metastases in more than one organ; metastasis in liver, spleen, kidneys, gastro-intestinal tract, bones or brain; antecedent term pregnancy; and an interval of more than 12 months between the end of the antecedent pregnancy and the start of treatment. Absence of all of these features is defined as low-risk disease.

Table 2 Risk classification of GTN by the Dutch Working Party on Trophoblastic Disease

Low-risk GTN	Demands all of the following conditions: 1. Antecedent pregnancy: hydatidiform mole or abortion* 2. No metastases or metastases in vagina or lung 3. No previous chemotherapy 4. Interval between evacuation and start of chemotherapy less than 12 months
High-risk GTN	Demands one or more of the following conditions: 1. Failure of previous (mono-) chemotherapy 2. Metastases in more than one site (outside the uterus) 3. Metastases in one or more of the following organs: liver, spleen, kidney, gastrointestinal tract, bones or brain 4. Antecedent pregnancy: term 5. Interval between end of antecedent pregnancy and start of chemotherapy more than 12 months

* There is no consensus on classification of non-molar abortion as low- or high-risk disease

In the Netherlands, patients with GTN are treated in different referral hospitals with the Dutch Working Party on Trophoblastic Disease, founded in 1971, having a registering and advisory function. Treatment of low-risk disease consists of single-agent chemotherapy. The most widely used chemotherapy drugs are MTX and actinomycin D. In general, the 5-day MTX or actinomycin D protocols (intramuscular or intravenous (iv) push daily for 5 days every other week) and the 8-day MTX-folinic acid regimen are most effective.[24] In the Netherlands, low-risk patients receive intramuscular MTX (1 mg/kg) on days 1, 3, 5 and 7 alternating with oral folinic acid (FA) 15 mg on days 2, 4, 6, and 8, repeated every two weeks. Low-risk GTN is extremely responsive to chemotherapy, and short-term toxicity of MTX is lower than that of actinomycin D.[23;25;26] Treatment continues until the hCG level is normal and then for a further consolidation period. In patients who develop

resistance to MTX or relapse after completing MTX therapy, the level of hCG indicates the mode of further treatment. Patients with a low serum hCG level and no signs of metastatic disease can be treated with actinomycin D, whereas patients with high hCG levels should be switched to multi-agent chemotherapy in the form of EMA/CO (etoposide, MTX and actinomycin D, alternating with cyclophosphamide and vincristine).[26] The current Dutch guideline for GTN formulated by the Working Group Oncologic Gynaecology states that treatment with actinomycin D can be considered if the serum hCG is lower than 100 IU/L.[23] However, a cut-off for the serum hCG concentration in ng/ml (measured with the Nijmegen radioimmunoassay) has not been formulated. Initially in the UK, in patients who developed MTX resistance or toxicity with a serum hCG concentration of <100 IU/L, therapy was changed to single-agent actinomycin D (0.5 mg intravenously daily for five days, repeated every two weeks). For patients whose hCG was greater than 100 IU/L at the time of developing MTX resistance or toxicity, EMA/CO treatment was used.[26] Nowadays, the UK gestational trophoblastic disease service has increased the hCG concentration at which multi-agent chemotherapy is started to > 300 IU/L, to reduce the number of women being exposed to greater toxicity.[11]

Patients with high-risk disease are primarily treated with EMA/CO. This regimen is repeated every two weeks until disease remission and then continued for an additional three cycles. Management of PSTT differs from that of other forms of GTN. A hysterectomy with sampling of pelvic lymph nodes is the primary mode of treatment in patients with non-metastatic disease.[27] Patients with metastatic PSTT require more aggressive treatment with an additional platinum-containing chemotherapy regimen such as EMA/EP (etoposide, MTX and actinomycin D, alternated with etoposide and cisplatin) until 8 weeks of normal hCG concentrations are recorded.[11;28] The EMA/EP regimen is different as compared to EMA/CO as etoposide and actinomycin D are administered for one day rather than two, and cyclophosphamide and vincristine are replaced by etoposide and cisplatin. EMA/EP is the most commonly used regimen for patients resistant to EMA/CO or with disease relapse after initially achieving disease remission with EMA/CO.[29] A hysterectomy should also be considered in women with chemotherapy-resistant disease.

Disease incidence

Before attempting to contribute in optimising the management of GTN, we would like to gain insight into how many patients are affected by GTD in the Netherlands. In North America and Europe, rates of HM are about 0.5 to 1 per 1000 pregnancies. Higher frequencies have been reported for some areas of Asia and the Middle East, with rates ranging from 1 to 12 per 1000 pregnancies.[30-32] Incidence rates for chorio-

carcinoma range from 2-7 per 100,000 pregnancies in Europe and North America, to 5-200 per 100,000 pregnancies in Asia.[33;34]

The variation in worldwide incidence rates results in part from discrepancies between population-based and hospital-based data. Many older studies used hospital-based data, which tend to show a higher incidence of GTD than those reported in population-based studies. This may be due to underestimation of the population at risk (e.g. the number of pregnancies, deliveries or live births) in hospital-based studies. In the Netherlands, patients with GTD are registered at the Dutch Central Registry for Hydatidiform Moles (DCRHM) residing at the Radboud University Nijmegen Medical Centre (RUNMC). This voluntary registry serves as an epidemiological database and provides a national hCG assay service to gynaecologists, and currently contains 4,190 records of patients with GTD. This corresponds to an average of 120 GTD patients registered per year. Since registration is voluntary, registration bias may occur and uncertainty exists on the completeness of the registration. Currently, no population-based data on the incidence of GTD are available from the Netherlands.

Prediction of GTN

HCG follow-up after evacuation of hydatidiform moles is essential to identify patients requiring chemotherapeutic treatment for post-molar GTN. However, post-molar GTN seems to be pre-programmed, since delayed evacuation at an increased gestational age does not increase the risk of requiring chemotherapeutic treatment.[35] Therefore many investigators have attempted to predict at an early stage which patients will go into disease remission and which patients will develop malignant disease. Most of this research has focused on hCG in serum [36-38], but also investigators have studied whether urine hCG could be reliably used to predict disease remission.[39] Some authors attempted to find absolute serum hCG levels predictive of outcome.[40] Others attempted to predict post-molar GTN using ratios of two hCG values in serum.[41-43] Also, normograms were developed for normal hCG regression after evacuation by plotting serum hCG values against time for patients that showed spontaneous disease remission and patients that developed GTN.[7;44-46] Although these methods aid in earlier diagnosis of GTN, still no generally accepted method is available for prediction of GTN prior to the clinical detection of GTN by FIGO 2000 criteria.

Treatment of low-risk GTN

As mentioned before, low-risk GTN patients are treated with MTX until the hCG level is normal and then for a further consolidation period. The number of courses of MTX administered after normalisation of serum hCG levels may influence the risk of disease relapse.[47;48] Previous studies showed that relapse rates in low-risk GTN patients range from 1.8 to 8.1%.[26;49-52] However, studies on relapse rates often combined relapse rates of low- and high-risk disease or included relapse after subsequent second-line chemotherapy because of resistance to MTX.[48;49;53] In 74 low-risk GTN patients treated with MTX/FA in the Netherlands a relapse rate of 8.1% was found.[50] McNeish *et al.* studied 324 low-risk GTN patients treated with MTX/FA in the UK and found a relapse rate of only 3.4%.[26] Definitions of relapse differed between these studies, and a comparative study on the influence of different numbers of consolidation courses on relapse rates has not been carried out.

Treatment of high-risk GTN

Although survival of patients with low-risk disease is almost 100% [26;51], patients still die from high-risk disease, with reported survival rates of 87 to 91% after treatment with EMA/CO and adjuvant cisplatin-based chemotherapy, surgery and radiotherapy.[54-57] Although the group of patients who died from GTN provides a unique insight in what can be learnt for future management of GTN, literature on fatal cases of GTN is limited and mostly dated.[58-60] Lurain *et al.* studied 48 patients who died from invasive mole or choriocarcinoma from 1962 to 1979 at the Trophoblastic Disease Centre of Northwestern University.[58] Mazur *et al.* studied the 31 autopsied cases within this group.[59] Five patients presented with a fulminant clinical course and died before any chemotherapy could be given. Overall, tumour haemorrhage and pulmonary insufficiency were the most common causes of death. Moodley *et al.* described more recently a profile of mortality among women with GTD infected with the human immunodeficiency virus (HIV).[60] Of the 15 deaths, 5 patients were admitted in very poor general condition precluding the administration of chemotherapy. Five HIV-infected patients and 5 non-HIV infected patients received chemotherapy. The causes of death in the 10 patients included widespread disease, multi-organ failure and toxicity due to chemotherapy. An overview of patients who died from GTN despite more up-to-date treatment standards would provide further information on potential areas to further improve current practice.

Today, the most widely accepted initial treatment for high-risk trophoblastic tumour is EMA/CO chemotherapy introduced in 1979 by Newlands and Bagshawe.[61] EMA/CO (day 1: etoposide 100 mg/m^2, actinomycin D 0.5 mg iv bolus MTX 300 mg/

m^2; day 2: etoposide 100 mg/m^2, actinomycin D 0.5 mg/m^2, folinic acid rescue 15 mg every 12 hours for four doses; day 8: etoposide 100 mg/m^2, vincristine 1 mg/m^2, cyclophosphamide 600 mg/m^2) is a relatively well-tolerated regimen. In a series of 45 patients treated with EMA/CO, alopecia was universal, but there was no life-threatening toxicity.[55] However, EMA/CO is associated with long-term toxicity in the form of secondary malignancy and an earlier start of menopause.[62;63] Triple therapy with MAC (MTX, actinomycin D plus either chlorambucil (original regimen) or cyclophosphamide (modified regimen)) was once the preferred first-line treatment for high-risk patients, but this passed out of use because of low remission rates of 51 to 73%.[64;65] When the combination of cyclophosphamide, hydroxyurea, actinomycin D, MTX with folinic acid, vincristine, and doxorubicin (CHAMOCA) was compared to MAC in a randomized trial, the remission rate was lower and toxicity was higher with CHAMOCA compared to MAC.[65] A few years earlier the successful use of cisplatin was reported in patients with disseminated testicular carcinoma, including testicular choriocarcinoma, and the first preliminary studies on the use of cisplatin is gestational choriocarcinoma were reported.[66-68] In 1982, a multi-agent chemotherapy schedule (EMACP, day 1: etoposide 100 mg/m^2, MTX 100 mg/m^2 iv bolus; 200 mg/m^2 cyclophosphamide 600 mg/m^2; day 2: etoposide 100 mg/m^2, actinomycin D 0.6 mg/m^2; day 3: etoposide 100 mg/m^2; day 4: etoposide 100 mg/m^2, cisplatin 60 mg/m^2; day 5: etoposide 100 mg/m^2) was designed by the Dutch Working Party on Trophoblastic Disease, aiming to compose a schedule that could be repeated frequently with a short interval between two courses, causing less myelosuppression than CHAMOCA and containing the new agents etoposide and cisplatin. However, due to the toxicity associated with cisplatin, on an international level preference was given to an initial treatment regimen without cisplatin for high-risk patients. Although multi-agent chemotherapy in the form of EMACP was applied in the Netherlands for an extensive period of time, it has not been compared to EMA/CO chemotherapy in the treatment of patients with high-risk GTN.

Prediction of treatment failure in high-risk GTN patients

Not all patients are cured with initial multi-agent chemotherapy in the form of EMA/CO or EMACP. Some patients develop resistance to multi-agent chemotherapy during treatment, whereas others achieve complete remission but develop disease relapse afterwards. Patients with chemo resistant GTN have a worse outcome compared with patients with relapsed GTN.[69] For these patients, treatment options are limited. Salvage chemotherapy in the form of EMA/EP alone or combined with salvage surgery is reported to achieve remission in 70 to 88% of patients resistant to EMA/CO.[29;54;70] Early identification of patients resistant to multi-agent chemotherapy

is highly important, since these patients are at risk for a fatal outcome. Previous studies determined predictive factors of a poor outcome.[71-73] Factors determined to significantly influence the chance of resistance to initial chemotherapeutic treatment (requiring additional treatment) were presence of metastatic disease, metastases to other sites than the lung or vagina, prior unsuccessful chemotherapy and duration of disease for more than 4 months, although these factors were analysed for initial single-agent chemotherapy for low-risk disease and initial multi-agent chemotherapy for patients with metastatic high-risk disease combined.[72] Kim *et al*. found that a tumour age of more than 12 months, metastases to more than two organs and incomplete previous treatment were independent risk factors for poor prognosis.[71] In patients with two and three of these factors, the death rates were 17.7 and 56.6%, respectively. Previously, van Trommel *et al*. developed a normogram for the regression of hCG in the treatment of low-risk GTN with single-agent MTX to identify resistance to MTX at an early stage. This normogram allows the identification of 50% of patients needing alternative therapy with a specificity of 97.5% before the fourth course of MTX.[74] However, a common definition for resistance to multi-agent chemotherapy is still lacking. More importantly, no method is available to predict resistance to multi-agent chemotherapy.

Outline of the thesis

The main aim of the present thesis was to further optimise the management of patients with GTN by attempting to predict post-molar GTN and resistance to multi-agent chemotherapy. In **Chapter 2** we determine the incidence and time trends of GTD in the Netherlands using population-based data in order to evaluate the extent to which women in the Netherlands are affected by this disease. In **Chapter 3** we propose a model based on the slope of the linear regression line of three consecutive serum hCG measurements in the first weeks after evacuation for the prediction of post-molar GTN. Early prediction of GTN allows start of treatment at a less advanced stage of the disease and thus contributes to a favourable outcome. However, despite the start of adequate treatment, some patients will develop resistance to chemotherapy or relapsed disease after achieving disease remission. In **Chapter 4** we compare in a retrospective setting the relapse rates of women completing MTX therapy for GTN in the Netherlands and the UK. Disease relapse requires treatment with multi-agent chemotherapy, which, in contrast to MTX, is associated with an increased incidence of secondary malignancies and with the risk of an early menopause. Therefore an additional course of MTX would be preferred if this decreases the chance of developing relapsed disease. **Chapter 5** reviews all patients who died from GTN over the last four decades in the Netherlands and aims

to evaluate whether treatment was given according to the protocol or guideline at the time of diagnosis and to reveal possible implications for future management. Previously, cisplatin has been identified as an active agent for achieving complete remission in high-risk GTN. However, introduction of cisplatin to the treatment regimen may cause a higher toxicity, resulting in dose delay or dose reduction. For this reason in **Chapter 6** we evaluate the efficiency, survival rate and complications of cisplatin-based combination chemotherapy (EMACP) as compared to the EMA/CO schedule for the treatment of high-risk GTN. For patients who will not be cured with EMA/CO or EMACP, respectively, an early switch to EMA-EP chemotherapy of salvage surgery is highly important. Therefore in **Chapter 7** we aim to construct a normogram for the hCG regression in patients with high-risk GTN successfully treated with EMA/CO chemotherapy in order to predict patients developing resistance to EMA/CO. The results presented in these chapters and possible implications for future management are generally discussed in **Chapter 8** and summarised in **Chapter 9.**

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Increase in the incidence of gestational trophoblastic disease in the Netherlands

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Gynecologic Oncology 2011; 121: 334-338

Abstract

Background We determined the incidence and time trends of gestational trophoblastic disease (GTD) in the Netherlands using population-based data.

Methods Data on patients with a pathologically confirmed diagnosis of GTD from 1995 to 2008 were obtained from PALGA, a national archive containing all histopathology reports in the Netherlands. Data on number of deliveries were obtained from the Database of Statistics Netherlands.

Results During the study period, 4249 GTD patients were registered. Overall incidence rates of hydatidiform mole (HM), choriocarcinoma and placental site trophoblastic tumour (PSTT) were 1.34 per 1000 deliveries, 3.1 per 100,000 deliveries, and 1.0 per 100,000 deliveries, respectively. Incidence rates of HM increased from 1.02 per 1000 deliveries in 1995 to 1.56 per 1000 in 2001, an increase of 0.091 per year (95%CI 0.081-0.101). After 2001 incidence rates remained constant (increase per year -0.010, 95%CI -0.045-0.024). Maternal age and ethnicity are known to influence the risk of HM. Highest incidences were observed in women under 20 and over 40 years of age. The proportion of deliveries accounted for by women over 40 years of age increased from 1.5% to 2.9%, whereas women under 20 accounted for 1.5% of deliveries. The proportion of live births of Asian descent increased from 2.6% to 3.7%.

Conclusion The incidence of GTD in the Netherlands increased significantly from 1995 to 2008. This can partially be explained by increased maternal age and increased proportion of live births of Asian descent. Part of the increase might result from improved diagnostic techniques. However, these factors do not seem to account for the total observed increase and part of the increase therefore remains unexplained.

Introduction

Gestational trophoblastic disease (GTD) consists of a heterogeneous group of disorders that are characterized by an abnormal proliferation of trophoblastic tissue. The modified World Health Organization (WHO) classification of GTD includes exaggerated placental site, placental site nodule, complete and partial hydatidiform mole, invasive mole, choriocarcinoma, placental site trophoblastic tumour (PSTT), and epithelioid trophoblastic tumour (ETT).[1;2] Hydatidiform mole (HM) is the most common form of GTD. The reported incidence varies widely in different regions of the world. In North America and Europe, rates of HM are about 0.5 to 1 per 1000 pregnancies. Higher frequencies have been reported for some areas of Asia and the Middle East, with rates ranging from 1 to 12 per 1000 pregnancies.[3-6] As has been reported for HM, incidence rates for choriocarcinoma also differ markedly throughout the world. In Europe and North America, rates of 2 to 7 per 100,000 pregnancies have been reported, whereas in Asia rates have been noted as high as 5 to 200 per 100.000 pregnancies.[3-5;7;8] PSTT is a rare form of GTD, with fewer than 250 cases reported in the literature.[9]

Comparisons of GTD incidence rates among different regions in the world are limited by the various methods used to determine rates. The variation in worldwide incidence rates results in part from discrepancies between population-based and hospital-based data.[10-12] In addition, incidence rates may be based on the total number of pregnancies, deliveries, or live births.[3;12] Furthermore, under-registration of GTD might occur, as was demonstrated by Flam *et al.*[13] Their study showed that 25% of patients with a diagnosis of HM were not included in the Swedish Cancer Registry. This further underlines the importance of an accurate, centralized registration of this disease. In the Netherlands, a unique nationwide network and archive was established in 1971 under the name of PALGA ('Pathologisch Anatomisch Landelijk Geautomatiseerd Archief'; Pathological Anatomy National Automated Archive), in order to facilitate the optimal use of histopathology and cytopathology data for research and quality control. Since 1991, this registration has encompassed all sixty-four pathology laboratories in the Netherlands and has complete coverage: every histopathological and cytopathological diagnosis made in the Netherlands is entered into this registration.[14] The aim of the present study is to determine incidence rates and time trends of GTD in the Netherlands with the use of a population-based database from 1995 to 2008.

Methods

Histopathology database

The PALGA database is a national archive containing abstracts of all histopathology and cytopathology reports generated in the Netherlands, with a nationwide coverage since 1991. Abstracts of all pathology reports are generated automatically at the participating laboratories and transferred to the central data bank. These abstracts contain encrypted patient identification data (including date of birth, gender and the first eight characters of the patient's family name), a summary of the pathology report, and a so-called PALGA diagnosis, a coded diagnosis line based upon standard pathology terminology. This code consists of a term indicating the anatomical location, type of sample and a morphological term describing the finding.[14] The present study includes data recorded in the PALGA database between 1995 and 2008. For each report, first initial, age, date of pathology report, summary text and diagnostic codes were made available.

Patient selection

A selection of the central database was obtained containing records of patients diagnosed with GTD. Selection was based on the following items: 'placental site nodule', 'exaggerated placental site reaction', 'mole', 'complete mole', 'partial mole', 'invasive mole', 'persistent trophoblastic disease', 'trophoblastic proliferation', 'choriocarcinoma', 'metastasis choriocarcinoma', 'intratubular choriocarcinoma', 'placental site trophoblastic tumour' and 'trophoblastic pseudotumour'. Epithelioid trophoblastic tumour is not yet recorded as a PALGA thesaurus diagnosis. These search terms selected 4494 abstracts. All patients with a histologically confirmed diagnosis of GTD were identified in the database. Revisions from abroad were excluded (n=5). The remaining reports were reviewed and categorized according to the WHO classification of GTD: exaggerated placental site or placental site nodule, complete mole, partial mole, invasive mole, choriocarcinoma, placental site trophoblastic tumour, and epithelioid trophoblastic tumour. Two categories were added to this classification: a category of 'unspecified HM' was added for moles that were clearly HM, but a further classification into partial or complete was not made. In addition, a category of 'abortion or HM' was added, in which those patients were categorized of whom the pathologist was not sure whether the specimen concerned a hydatidiform mole. Inconclusive reports were reviewed and classified by an expert pathologist, specialized in gynaecological pathology. Patients that were eventually not diagnosed with GTD were excluded from further analysis (n=227). Thirteen patients were diagnosed as having persistent trophoblastic disease (PTD). Since PTD is a clinical diagnosis and not a pathological diagnosis, these patients were also excluded from further analysis. A total of 4249 patients were included in the study.

The study was carried out in the Netherlands in accordance with the applicable rules concerning the review of research ethics committees and informed consent.

Statistical Analyses

Incidence rates of HM, choriocarcinoma and PSTT were calculated as the number of patients diagnosed in the PALGA database divided by the total number of deliveries. Data on the number of deliveries were obtained from Statistics Netherlands and included live births and stillbirths from 24 weeks gestation and onwards.[15] Data on the descent of live births, according to the country of origin of the parents, was also obtained from Statistics Netherlands. Incidence rates of HM were calculated per 1000 deliveries. Incidence rates of choriocarcinoma and PSTT were calculated per 100,000 deliveries. Trends in the incidences over time were calculated using linear regression. Regression coefficients were calculated per 1000 deliveries from 1995 to 2008. Statistical analysis was performed using SPSS software (version 16.0).

Results

Gestational trophoblastic disease

Between 1995 and 2008, a total of 4249 cases of gestational trophoblastic disease were registered in the Netherlands. The age of the patients ranged from 13 to 73 years with a median age of 30.0 years. Evaluation of the pathological reports revealed complete and partial HM in 30.2% and 44.5% of patients, respectively. Patients diagnosed with a mole not further specified into a partial or complete mole comprised 11.6% of all GTD patients, and 8.6% were categorized as having an 'abortion or HM' (Table 1). A significant trend to an increase in the overall incidence of GTD could be observed (increase per year 0.032, 95% CI 0.012-0.052). The frequency and incidence of GTD registered in the Netherlands over fourteen years is shown in Table 2. The annual number of GTD averaged 303 cases per year. The overall incidence was 1.56 per 1000 deliveries per year.

Hydatidiform mole

A total of 3,668 HMs were registered during the 14-year study period. Median maternal age was 30.0 years (range 13-59 years). In order to assess a possible time trend in the incidence of hydatidiform mole, patients were analyzed according to the year of diagnosis. Figure 1 shows trends in the annual incidence rates for hydatidiform mole over the years. The overall incidence rate in total HM (complete, partial and unspecified hydatidiform mole) was 1.34 per 1000 deliveries (262 cases per year). Incidence rates of HM increased from 1.02 per 1000 deliveries in 1995 to 1.56 per 1000 deliveries in 2001, an increase of 0.091 per year (95% CI 0.081-0.101). Given the

Table 1 Frequency and incidence rate of types of gestational trophoblastic disease (GTD) in the Netherlands, 1995-2008

Type of GTD	N	Percentage	Incidence rate*
EPS / placental site nodule	88	2.1	0.032
Complete HM	1285	30.2	0.470
Partial HM	1892	44.5	0.692
Invasive mole	9	0.2	0.003
Unspecified mole	491	11.6	0.180
Abortion or HM	366	8.6	0.134
Choriocarcinoma	87	2.0	0.032
PSTT	30	0.7	0.011
ETT	1	.0	0.0004
Total	**4249**	**100**	**1.56**

*per 1000 deliveries

approximately 200,000 deliveries each year in the Netherlands, this corresponds to an increase of 18 molar pregnancies per year. After 2001 the incidence rate remained constant (increase per year -0.010, 95% CI -0.045-0.024). Regression was calculated for several windows around 2001 and results proved to be very similar.

The incidence of both complete and partial HM increased across the study period. In complete moles, a gradual increase was observed, from 0.34 per 1000 deliveries in 1995 to 0.63 per 1000 deliveries in 2008 (0.015 per year, 95% CI 0.007-0.022). In partial moles, a strong increase was initially observed from 0.44 per 1000 deliveries in 1995 to 0.82 per 1000 deliveries in 2002 (increase per year 0.061, 95% CI 0.048-0.074), however, after this, the incidence rate in partial moles did not significantly change (-0.020 per year, 95% CI -0.047-0.007). In contrast, the incidence rate of unspecified moles gradually decreased during the study period, from 0.23 per 1000 to 0.10 per 1000 deliveries, respectively (-0.012 per year, 95% CI -0.015 - -0.008). The incidence of 'abortion or HM', a category where uncertainty existed concerning the diagnosis, remained constant throughout the years (increase 0.002 per year, 95% CI -0.007-0.010). Overall, in this category an incidence of 0.13 per 1000 deliveries was observed.

Choriocarcinoma and PSTT

During the study period, 91 cases of gestational choriocarcinoma were reported to the PALGA database, giving an overall incidence of 3.1 per 100,000 deliveries. Thirty

Table 2 Frequency and incidence rates of gestational trophoblastic disease (GTD) in the Netherlands by year, 1995-2008

Year	Number of GTD^	Deliveries	Incidence rate*
1995	229	191735	1.19
1996	243	190742	1.27
1997	266	193722	1.37
1998	293	200679	1.46
1999	326	201708	1.62
2000	352	207872	1.69
2001	355	203861	1.74
2002	322	203268	1.58
2003	331	201421	1.64
2004	289	195020	1.48
2005	279	188893	1.48
2006	338	185913	1.81
2007	319	182117	1.75
2008	307	185408	1.66
Total	**4249**	**2732359**	**1.56**

*per 1000 deliveries
^ including 'abortion or HM'

patients were diagnosed with PSTT, resulting in an overall incidence of 1.0 per 100,000 deliveries.

Maternal age in hydatidiform mole

Figure 2 shows incidence rates of HM for different maternal age categories as a function of time. The highest incidences were observed in patients under 20 years of age and in patients over 40 years of age. An overview of all deliveries in the Netherlands in different age categories is given in Figure 3. The number of women delivering after the age of 40, as is shown on the right y-axis, increased 85%, from 2950 in 1995 to 5469 in 2008, respectively. However, this accounts for an increase from 1.5% to 2.9% of the total number of deliveries. The number of deliveries in women younger than 20 increased from 1995 to 2001, after which the number returned to the same level as observed in 1995, and accounts for 1.5% of the total number of deliveries. Most deliveries occurred in the group of women between 30

and 35 years old. Calculating the contribution of each age category to the total incidence of HM shows that no age category solely caused the increase of HM incidence.

Figure 1 Incidence rate per 1000 deliveries for hydatidiform mole (HM) patients over time (1995-2008) in the Netherlands. Total HM includes complete, partial and unspecified HM.

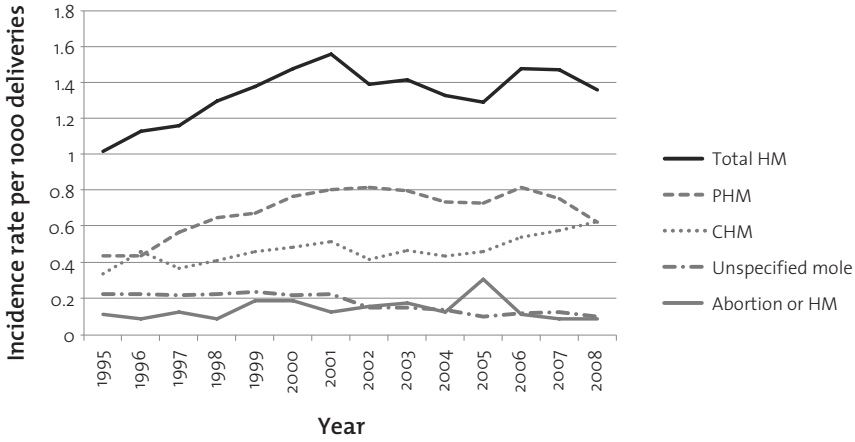


Figure 2 Age-adjusted incidence rate per 1000 deliveries in hydatidiform mole patients. Incidence rates of HM for different maternal age categories are shown as a function of time.

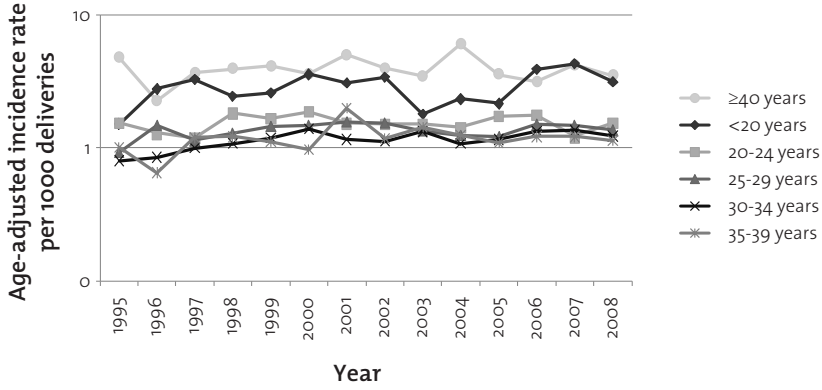
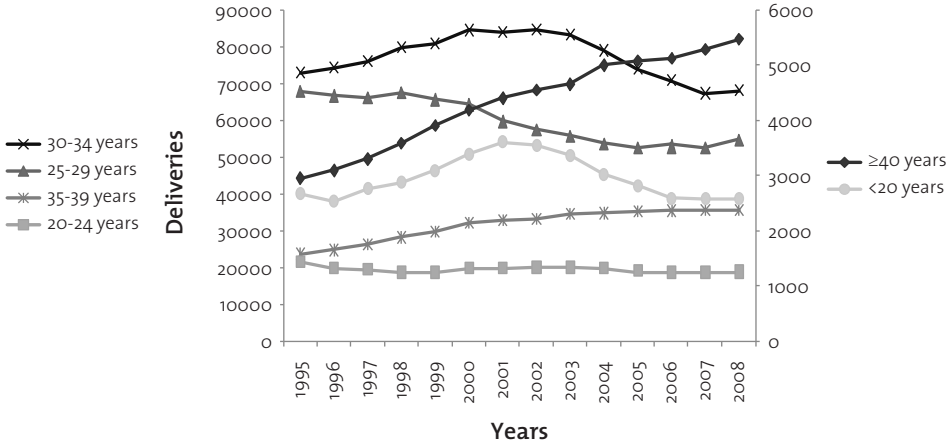


Figure 3 The total number of deliveries in different age categories over time (1995-2008) in the Netherlands.



Ethnicity in hydatidiform mole

Since women of Asian descent are thought to have a higher risk of developing a HM, the influence of the number of live births from Asian descent on the total number of live births was analyzed. In the Netherlands, the number of live births with at least one parent of Asian descent increased more than 40%, from 4886 in 1995 to 6853 in 2008. This accounts for an increase from 2.6% to 3.7% of the total number of live births.

Discussion

The present study provides long-term nation-wide data on the incidence of GTD in the Netherlands. Studies presenting incidence rates after the early 1990s are limited. An overview of more recent population-based studies on the incidence of HM and choriocarcinoma is given in Table 3. Many older studies used hospital-based data, which tend to show a higher incidence of GTD than those reported in population-based studies. This may be due to underestimation of the population at risk in hospital-based studies, especially in under-developed countries, since many births take place at home, and high-risk pregnancies or cancer cases are more likely to receive hospital care than are uncomplicated deliveries.[12;16;17]

Differences in reported incidence rates can also be accounted for by inaccurate ascertainment of the number of patients as a function of the number of gestational events. Different denominators, which represent the population at risk, are used in

published studies. The preferred denominator for women at risk of GTD is 'all women who have conceived'. Since this number is unknown, ratios for GTD are presented in relation to the number of pregnancies, deliveries, or live births. The item 'pregnancies' usually includes live-births, stillbirths, spontaneous and induced abortions, and ectopic pregnancies, and represents the closest approximation to the population at risk. 'Deliveries' does not take into account the number of conceptions that terminate because of induced or spontaneous abortion, and in addition lacks a smaller number of ectopic pregnancies. When the denominator 'live births' is used, even more conceptions at risk are being excluded.[5;12] Because these denominators underestimate the size of the population at risk, the estimates of the incidence of GTD are likely to be too high.[12] However, since the total number of deliveries is inevitably collected more accurately than the number of pregnancies, we used 'deliveries' as the denominator to calculate incidence rates in the present study.

In our study, an overall incidence rate of HM of 1.34 per 1000 deliveries was found. This is consistent with rates found in previous population-based studies in Scandinavia (1.1 per 1000 pregnancies and 1.46 per 1000 deliveries in Denmark and Sweden, respectively) and the United States (1.19 per 1000 pregnancies).[16;18;19] Higher incidences have been found in Ireland[17] and Japan[20], with rates of 2.2 per 1000 deliveries and 2.23 per 1000 live births, respectively. However, incidence rates in Japan declined substantially, hereby approaching incidence rates found in Europe and the United States.[20;21] Flam *et al.* showed that there were no significant differences over time in the incidence of HM in Sweden.[16] Interestingly, in the present study, incidence rates increased, from 1.02 per 1000 deliveries in 1995 to 1.56 per 1000 deliveries in 2001, respectively, after which the incidence rate has remained constant. Similarly, an increasing trend in the incidence of GTD was found in Northern England, from 1.26 in 1991 to 1.63 per 1000 live births in 1999, respectively.[22]

Concerning choriocarcinoma, an incidence of 3.1 per 100,000 deliveries was found in the present study. This is in accordance with data from other population-based studies conducted in Western countries. The reported incidence was between 2.4 and 4.0 per 100,000 deliveries in Sweden[16], Denmark[18], New Mexico[8] and Finland[7]. In the present study, an overall incidence of PSTT of 1.0 per 100,000 deliveries was demonstrated. The epidemiology of PSTT is hardly described. We found no other studies presenting incidence rates on PSTT.

The influence of maternal age on the risk of HM has been studied extensively.[16;18;23-29] An increase in maternal age has been proposed as a possible explanation for an increasing incidence of HM. Analyses of the maternal age in HM patients in our study revealed that the highest incidences were observed in patients under the age of twenty and in patients older than forty, confirming previous literature.[3;22;23;28] However, both age categories accounted for only a small percentage of the total number of deliveries. The increased numbers of deliveries in these age categories can,

therefore, only partially explain the observed 30% increase in the incidence of HM in the total group.

Another explanation of the increasing incidence of HM might be found in an increase in women of Asian descent. Many studies have demonstrated the relation between ethnicity and risk of molar pregnancy.[19;22;25;30] Women of Asian descent are thought to have two- or threefold higher rates of HM compared to Caucasian women.[6;22] However, since the proportion of live births of Asian descent increased only 1.1%, this can only for a small part explain the observed increase in the incidence of HM.

To what extent epidemiological trends are a result of the improvement of diagnostic techniques for GTD is unknown.[12] During the years, diagnostic options in the field of GTD significantly advanced.[31] Flow cytometric DNA ploidy analysis can distinguish diploid complete mole or hydropic abortuses from triploid partial moles by analyzing a large number of random nuclei. Flow cytometry was first described in the PALGA records in 1994, and has since then been widely used as a rapid and easy test for ploidy. The introduction of flow cytometry might explain the observed increase of partial moles. Partial moles that were previously erroneously diagnosed as hydropic abortion, could since then be proven to be triploid using flow cytometry. Since partial moles account for an important part of the total amount of HMs, this might explain the increase of the incidence of total HM. With the introduction of p57kip2 immunohistochemistry, first mentioned in our PALGA records in 2002, a better differentiation between complete and partial mole has become possible. The p57kip2 gene is paternally imprinted and expressed from the maternal allele. Since complete moles lack a maternal genome, p57kip2 immunostaining is absent, whereas hydropic abortuses and partial moles show positive staining.[32] In the present study, the incidence of unspecified moles significantly decreased during the study period, mostly after p57kip2 IHC was introduced. Simultaneously, an increase in the incidence of complete moles was observed, suggesting that these unspecified moles were mainly categorized as complete moles. However, this will not have influenced the overall incidence of HM since unspecified moles were also considered in the calculation of the overall incidence of HM. The category 'abortion or HM' remained constant throughout the years. The fact that this category of disease did not diminish with the introduction of new diagnostic techniques might suggest that these are in fact mostly non-molar pregnancies.

Since part of the observed increase of GTD seems to remain unexplained, questions may arise concerning the influence of possible shortcomings of the registration system used. However, the PALGA system was founded in 1971 and reached complete national coverage in 1991. Since our data were obtained from 1995 onwards, this to our opinion should ensure good reporting.

Strengths of our study are the large series of patients with GTD that were analyzed. Additionally, collection of patients by means of the PALGA registry ensured

non-selected data. Limitations of our study are the retrospective analysis of the pathological abstracts and the fact that no histopathological review of the cases was performed.

In conclusion, the overall incidence of GTD is low, but significantly increased over the last fourteen years. This can only partially be explained by increased maternal age and an increased portion of live births of Asian descent, since their part of the total number of deliveries is small. In addition, part of the documented increase might also result from improved diagnostic techniques. However, these factors do not seem to account for the total observed increase and part of the increase therefore remains unexplained. Rates found in HM and choriocarcinoma are consistent with rates found in previous population-based studies in Western countries. To provide useful information, population-based studies should be performed, preferably through a centralized registry to ensure unbiased and non-selected data.

Table 3 Incidence rate of HM and choriocarcinoma reported in population-based studies since 1990

		Hydatidiform mole Incidence per 1000			Choriocarcinoma Incidence per 100.000		
Region	**Authors** (Year)[Ref]	**Pregnancies**	**Deliveries**	**Live births**	**Pregnancies**	**Deliveries**	**Live births**
Asia							
Japan	Matsui *et al.* (2003)[33]			1.61			
Japan	Hando *et al.* (1998)[20]			2.23			
Okinawa Islands (Japan)	Sakumoto *et al.* (1999)[21]			2.29			
Europe							
Denmark	Olsen *et al.* (1999)[18]	1.1			2.7		
Northern-Ireland	Giwa-Osagie *et al.* (1999)[17]		2.2				
Sweden	Flam *et al.* (1992)[16]	0.9	1.46		2	3	
Finland	Loukovaara *et al.* (2004)[7]					4	
North America							
USA (New Mexico)	Smith *et al.* (2003)[19]	1.19		1.42			
Africa							
South Africa	Moodley *et al.* (2003)[34]		1.2		2.4		3.9

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3

Linear regression of post-evacuation serum hCG concentrations predicts post-molar gestational trophoblastic neoplasia

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Abstract

Background Currently, hCG follow-up after evacuation of hydatidiform moles is essential to identify patients requiring chemotherapeutic treatment for gestational trophoblastic neoplasia (GTN). Here we propose a model based on linear regression of post-evacuation serum hCG concentrations for the prediction of post-molar GTN.
Methods A total of 113 patients with at least three serum samples from day 7-28 after evacuation were selected from the Dutch Central Registry for Hydatidiform Moles (1994-2009). The slopes of the linear regression lines of the first three log-transformed serum hCG and free beta hCG values were calculated. Receiver operating characteristic (ROC) curves were constructed to calculate Areas Under Curve (AUCs).
Results The slope of the hCG regression line showed an AUC of 0.906 (95% CI 0.845-0.967). GTN could be predicted in 52% of GTN patients at 97.5% specificity (cut-off -0,020). 21% of GTN patients could be predicted before diagnosis according to the FIGO 2000 criteria. The slope of free beta hCG showed an AUC of 0.844 (95% CI 0.752-0.935), showed 69% sensitivity at 97.5% specificity, and 38% of GTN patients could be predicted before diagnosis according to the FIGO criteria.
Conclusion The slope of the linear regression line of hCG proved to be a good test to discriminate between patients that achieve spontaneous disease remission and patients developing GTN. The slope of free beta hCG seems to be a better predictor for GTN than the slope of hCG. Although this model needs further validation for different assays, it seems a promising way to predict the more aggressive cases of GTN.

Introduction

Hydatidiform mole (HM) is a non viable form of pregnancy with a cystic appearance of chorionic villi, most often in the absence of an embryo. HM occurs in approximately 1-3 per 1000 pregnancies.[1] Following molar evacuation by uterine curettage, remaining trophoblastic tissue resolves spontaneously in most cases. However, 15% of all complete molar pregnancies (CHM) and 1-5% of partial moles (PHM) develop gestational trophoblastic neoplasia (GTN) which is a malignant condition.[2] According to the criteria of the International Federation of Gynecology and Obstetrics (FIGO), post-hydatidiform mole trophoblastic neoplasia (GTN) is defined as 1) a plateau in weekly serum hCG concentrations for four consecutive measurements, 2) an increase in serum hCG levels in three weekly consecutive measurements, 3) persistence of detectable hCG levels for more than 6 months after evacuation, and 4) the presence of a histologic diagnosis of choriocarcinoma.[3]

The pathogenesis of GTN remains to be elucidated; it is unknown why some HM patients develop GTN, whereas others achieve spontaneous disease remission following HM evacuation. Prognostic markers to predict GTN are lacking. Therefore, hCG follow-up after evacuation of HMs currently is essential for all patients to identify those requiring chemotherapeutic treatment for GTN. If treated instantly, the prognosis of post-molar GTN, including choriocarcinoma, is excellent, with an overall survival rate of more than 98%.[4]

Many efforts have been made to predict GTN at an early stage of the disease.[5-8] Although clinical and pathological features have been evaluated for their potential in defining a subset of patients at high risk of persistent disease, much of this research has focused on serum hCG as a tumour marker.[9-11] Previously, our group performed several studies showing that post-molar GTN can be predicted using ratios from pre- and post-evacuation serum hCG levels, ratios from post-evacuation hCG levels, hCG subunit ratios and chromatofocusing of hCG.[12-14] Up to date, no generally accepted method for prediction of GTN prior to the clinical detection by FIGO 2000 criteria, is available. In the present study we propose a model based on the slope of the linear regression line of three consecutive serum hCG measurements in the first weeks after evacuation for the prediction of GTN.

Methods

Patients

In the Netherlands, patients with gestational trophoblastic disease (GTD) are registered at the Dutch Central Registry for Hydatidiform Moles (DCRHM) residing at the Radboud University Nijmegen Medical Centre (RUNMC). This voluntary

registry serves as an epidemiological database and provides a national hCG assay service to gynaecologists. Patients diagnosed with a molar pregnancy and with at least three hCG values from day 7 up to day 28 after evacuation were selected from the DCRHM from 1994-2009 (n=113). Of these patients, 42 developed GTN whereas in 71 patients the hCG levels returned to normal after evacuation.

Immunoassays

All hCG concentrations are measured using an in-house developed radioimmunoassay (RIA) based on polyclonal antibody raised in rabbits.[15] This assay detects intact hCG and free β-subunit: hCG+hCGβ. Free beta hCG was measured using a radioimmunoassay based on a monoclonal antibody.[15] A highly purified hCG beta subunit preparation labelled with Iodine-125 was employed as a tracer for both assays. The RIAs for hCG+hCGβ and hCGβ were calibrated with the third International Standard Preparations for intact hCG and hCG beta subunit, respectively.

Statistics

Serum hCG levels and serum free beta hCG levels were analyzed as a function of time. Log transformation was performed on all hCG and free beta hCG values to obtain normal distribution of the data. After log-transformation, data in both groups were distributed normally (spontaneous normalisation group Kolmogorov–Smirnov p=0.200; GTN group Shapiro-Wilk p=0.246). The follow-up guidelines after evacuation of a molar pregnancy recommend weekly serum hCG measurements after evacuation until the hCG is normalised in three consecutive measurements. The first hCG measurement should be obtained one week after evacuation. The slope of the linear regression line of serum hCG was calculated using the linear least squares regression method ($y=ax+b$, with y representing the logarithm of the hCG concentration, x representing the time in days, a representing the slope, and b representing the intercept y) of the first three hCG concentrations from day 7 up to day 28 after evacuation. An upper limit of 28 days was chosen in order to be able to predict GTN earlier than diagnosis using FIGO 2000 criteria. The slopes of the linear regression lines of hCG were analyzed using receiver operating characteristic (ROC) curves to calculate Areas Under Curve (AUCs) and sensitivity and cut-off values at 95 and 97.5% specificity for both hCG and free beta hCG. GTN was the only endpoint used in the analysis. In addition, the specificity at 100% sensitivity was assessed to determine whether GTN could be ruled out.

For the patients in which GTN could be predicted at 97.5% specificity, the day of the third hCG or free beta hCG measurement was chosen as the day after evacuation at which GTN could be predicted. The time interval between the day of evacuation and the day GTN could be predicted was compared to the time interval between the day of evacuation and the day at which the GTN was diagnosed according to the

FIGO 2000 criteria. In addition, the number of chemotherapy courses administered (methotrexate (MTX) as well as additional multi-agent chemotherapy) was compared between patients that could be predicted and patients that could not be predicted as developing GTN by the slope of the linear regression line at 97.5% specificity, using the Mann-Whitney Test. *P* values of less than 0.05 were considered statistically significant.

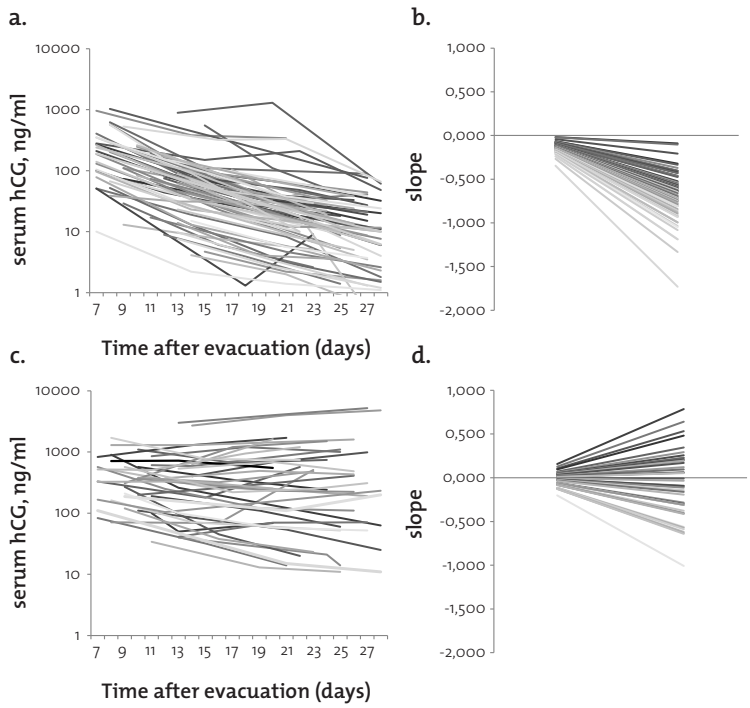
Results

One hundred thirteen patients with a minimum of three hCG values from day 7 up to day 28 after evacuation were selected. In the group of patients that showed spontaneous disease remission (n=71), 57 patients were diagnosed as having a complete HM, 12 patients had a partial HM, and 2 patients were diagnosed as having a HM without further specification into complete or partial HM. Of the patients that developed GTN (n=42), 26 patients were initially diagnosed with a complete HM, 1 patient had a partial HM, and 15 patients were diagnosed as having a HM without further specification into complete or partial HM. All GTN patients had low-risk disease and were initially treated with MTX, except for one patient who was treated with a hysterectomy. Seven patients required additional treatment with multi-agent chemotherapy.

Figure 1 illustrates regression curves and the slope of the first three hCG values from day 7-28 after evacuation from patients that showed spontaneous disease remission and from patients that developed GTN. Using the slopes of hCG (right panel), a clearer distinction can be made between patients showing spontaneous normalisation of hCG levels and patients developing GTN compared to using the regression curves (left panel). In all patients that showed spontaneous disease remission, the slope of hCG was negative (range -0.346 to -0.018). In patients that developed GTN, 24 patients had a negative slope, whereas 18 patients had a positive slope (for n=42 range -0.202 to 0.157). The slope of hCG and free beta hCG for patients showing spontaneous disease remission and for those developing GTN, and ROC curves for the slope of hCG and free beta hCG are shown in Figure 2. For hCG, the AUC was 0.906 (95% CI 0.845-0.967), and the slope of hCG could predict GTN in 52% of the GTN patients at 97.5% specificity (cut-off value -0,020). At 95% specificity, GTN could be predicted in 74% of GTN patients (cut-off value -0.063). For free beta hCG, the AUC was 0.844 (95% CI 0.752-0.935). Using the slope of free beta hCG, GTN could be predicted in 69% of GTN patients at 97.5% specificity (cut-off value -0,063) and in 69% of GTN patients at 95% specificity (cut-off value -0.067). In addition, the slope of the linear regression line was used to rule out GTN. Using the slope of hCG, 100% of GTN was predicted at a cut-off value of -0.205. In 10% of patients that

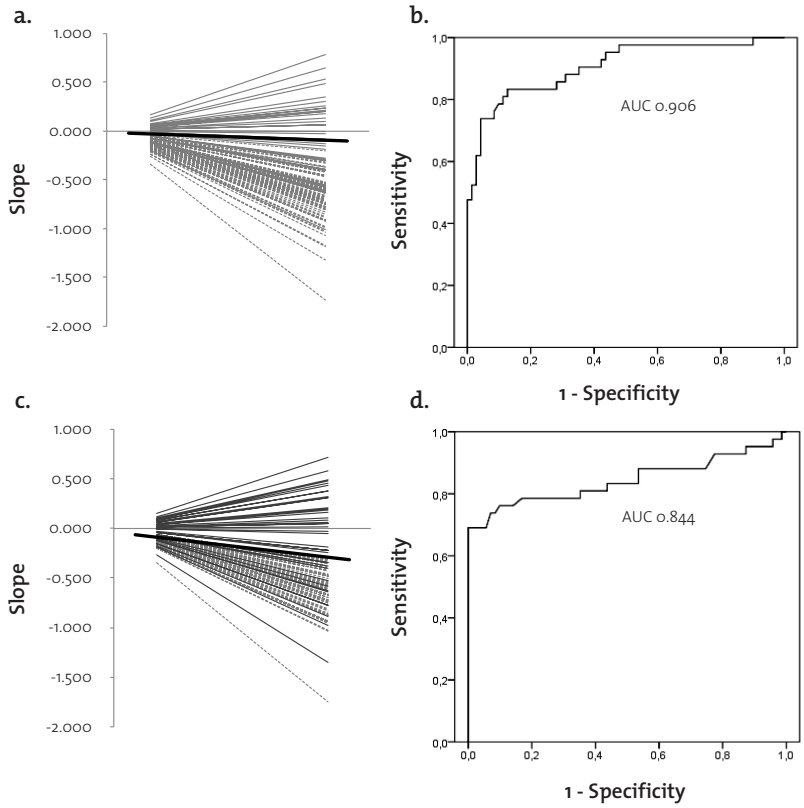
showed spontaneous disease remission the slope was smaller than -0.205 and GTN could be ruled out. Using the slope of free beta hCG, only 1% of patients that showed spontaneous disease remission could be ruled out from developing GTN (100% sensitivity; cut-off-0.310).

Figure 1 Regression curves (**a**) and the slopes of the linear regression lines of the first three hCG values (**b**) from day 7 up to day 28 after evacuation from patients showing spontaneous disease remission (n=71). Regression curves (**c**) and the slope of the first three hCG values (**d**) from day 7 up to day 28 after evacuation from GTN patients (n=42).



Of the patients that could be predicted at 97.5% specificity using the slope of the linear regression line, the day GTN could be predicted was compared to the day GTN was diagnosed using FIGO criteria. Table 1 shows patients predicted as developing GTN according to the slope of the linear regression line and time to diagnosis according to FIGO. Using the slope of hCG, 22 patients could be predicted as developing GTN. In 9/22 (41%) of these predicted GTN patients, the slope of hCG predicted GTN before diagnosis using the conventional FIGO criteria with a median

Figure 2 **a**. Slope of the linear regression line of hCG and **c**. of free beta hCG from day 7 up to day 28 after evacuation from patients showing spontaneous disease remission (n=71) and patients developing GTN (n=42). Thick line indicates the optimal cut-off level **b**. ROC curve for the slope of hCG, showing 52% sensitivity at 97.5% specificity and 74% sensitivity at 95% specificity **d**. ROC curve for the slope of free beta hCG, showing 69% sensitivity at 97.5% and 95% specificity



of 7 days before FIGO diagnosis (range 4-19). This corresponds to 21% (9/42) of all GTN patients that could be predicted before diagnosis according to the FIGO criteria. Using the slope of free beta hCG, 29 patients could be predicted as developing GTN. In 16/29 (55%) of these predicted GTN patients, the slope of free beta hCG predicted GTN before the conventional FIGO criteria with a median of 7 days before FIGO (range 4-49 days). This corresponds to 38% (16/42) of all GTN patients that could be predicted before diagnosis according to the FIGO criteria.

Table 1 Patients predicted as developing GTN according to the slope of the linear regression line of hCG and free beta hCG and time to diagnosis according to FIGO.

Patient	hCG (days)	Free beta hCG (days)	FIGO (days)	Time gained (days)
1	20	20	39	19
2	22	22	30	8
3	26	26	34	8
4	27	27	34	7
5	23	23	30	7
6	28	28	35	7
7	23	23	29	6
8	25	25	31	6
9	18	18	22	4
10	Not predicted	24	73	49
11	Not predicted	19	40	21
12	Not predicted	22	39	17
13	Not predicted	24	31	7
14	Not predicted	26	33	7
15	Not predicted	25	31	6
16	Not predicted	23	27	4
17	20	20	20	0
18	27	27	27	0
19	28	28	28	0
20	27	27	27	0
21	21	21	21	0
22	26	26	26	0
23	25	25	25	0
24	24	24	24	0
25	26	26	26	0
26	28	28	28	0
27	26	26	26	0
28	20	20	20	0
29	27	27	20	-7

To determine whether patients that could be predicted as developing GTN were the patients that had the more aggressive tumours and therefore required more courses of chemotherapy, we compared the number of chemotherapy courses administered between the patients that could be predicted as developing GTN at 97.5% specificity and the patients in whom GTN was not predicted using the slope of the linear regression line. The number of chemotherapy courses administered was known for all but one of the GTN patients. One patient received an abdominal hysterectomy instead of chemotherapy, after which the hCG levels regressed to normal. In the group of patients that could be predicted as developing GTN using the slope of hCG (n=22), the median number of chemotherapy courses administered was 6.5 (range 4-20 courses), including four patients requiring multi-agent chemotherapy due to MTX resistance. The group of patients in whom GTN was not predicted (n=20) was treated with a median number of 5 courses (range 2-16 courses) of chemotherapy, including three patients that required multi-agent chemotherapy (Mann Whitney p=0.033). In the group of patients that could be predicted as developing GTN using the slope of free beta hCG (n=28), the median number of chemotherapy courses administered was 6.5 (range 4-20 courses), including six patients requiring multi-agent chemotherapy due to MTX resistance. In the group of patients in whom GTN was not predicted (n=12) a median number of 4.0 courses (range 2-12 courses) of chemotherapy was administered, including three patients that required multi-agent chemotherapy (Mann-Whitney p=0.001).

Discussion

The present study aimed at identifying the value of a model based on the slope of the linear regression line of serum hCG or free beta hCG concentrations during the first weeks after evacuation for the prediction of GTN. Interpretations of the ROCAUC are based on the traditional academic point system, where 0.5 to 0.6 classifies the test as a fail, 0.6 to 0.7 as poor, 0.7 to 0.8 as fair, 0.8 to 0.9 as good, and 0.9 to 1.0 as excellent.[16] With AUCs of 0.906 and 0.844 for hCG and free beta hCG, respectively, this model proved to be a good test to discriminate between patients that achieve spontaneous disease remission following HM evacuation and patients that will develop GTN. We showed that at 97.5% specificity, 52% and 69% of patients that will eventually develop GTN could be predicted by the slope of the linear regression line of hCG or free beta hCG, respectively. In 21% and 38% of the GTN patients, respectively, the slope of hCG and free beta hCG predicted GTN before diagnosis using the FIGO criteria. The slope of free beta hCG seems to be a better test to predict GTN than the slope of hCG.

A possible explanation for the better performance of the slope of free beta hCG in predicting GTN might be that as tumours become more malignant, a relatively

higher amount of free beta hCG is produced.[17-19] Fan *et al.* concluded that the production of free beta hCG increases with the immaturity of the trophoblastic cell, and the degree of differentiation of trophoblastic cells may be reflected by the free beta hCG to hCG ratio.[17] Ozturk *et al.* showed that beta hCG to hCG ratios increased from normal pregnancy to complete HM to choriocarcinoma.[18] Harvey *et al.* showed that choriocarcinoma patients and PSTT patients had significantly higher free beta hCG concentrations than GTN patients.[20]

Previous studies have also attempted to predict GTN using hCG values. Some authors tried to find absolute hCG levels predictive of outcome.[10] However, estimating an absolute hCG cut-off to predict GTN is highly dependent on the assay used to determine the hCG values, and therefore seems unfeasible to apply in a wide setting. Other authors have developed normograms for spontaneous regression of serum hCG to predict GTN.[9;11;21-25] The use of normograms, however, also bears the disadvantage that each individual hCG assay requires the construction of its own normogram since the cut-off of hCG used for prediction of GTN will vary depending on the assay used due to differences in assay specificity and standardization. Our group therefore previously attempted to predict GTN using ratios of two hCG values in serum. Although serum concentrations of hCGα, hCGβ and hCG+hCGβ concentrations in pre-evacuation serum were significantly higher in patients with GTN compared with patients with spontaneous regression after evacuation, none of the calculated ratios, however, had adequate diagnostic accuracy for the prediction of persistent disease.[12] The hCG ratios obtained from post-evacuation hCG levels (dividing the first available hCG concentration of a specimen taken within the first 2 weeks after evacuation by the hCG concentration of a specimen taken in following weeks) showed an increasing diagnostic potential.[13] Recently, Kim *et al.* showed that GTN could be predicted by the hCG regression rate obtained by dividing the hCG by the initial hCG with a sensitivity of 48.0% and specificity of 89.5% (AUC 0.759) in the second week after evacuation, although only patients were selected with an initial hCG level of more than 100,000 IU/L.[26]

The prediction model presented in the current study is based on three hCG measurements, making the slope of the linear regression line more stable compared to a model based on two measurements. However, it is clear that the additional hCG value needed negatively affects early prediction of GTN. In the present study, in 21% (9/42) of GTN patients using the hCG model, and in 38% (16/42) of GTN patients using the free beta hCG model, GTN was predicted before diagnosis using the FIGO criteria. Unfortunately, since the protocol for follow-up after evacuation recommends weekly hCG measurement, our database did not contain sufficient hCG measurements for each patient to determine the time that can be gained maximally in predicting GTN using the slope of the linear regression line. Therefore, this model would have to be prospectively applied on more frequently obtained hCG measurements in

serum. Prediction of GTN at an earlier stage so that treatment can be started earlier, is particularly important for patients lost to follow-up by the time GTN would be diagnosed with the FIGO criteria, as is often the case in developing countries. In addition, early prediction of GTN allows start of treatment at a less advanced stage of the disease and also enables earlier permission for a renewed pregnancy, which is usually very important for these patients.

In total, 83 patients were diagnosed with a complete HM, 13 patients had a partial HM, and 17 patients were diagnosed as having a HM without further specification into complete or partial HM. Although we did not apply the slope of hCG on the separate histological groups due to the small number of patients, Smith *et al.* previously showed that no statistically significant differences were found when the coefficients of regression of hCG in patients with complete HM, partial HM and non-molar abortion were compared.[27] Kim *et al.* also showed that the hCG regression rate showed no significant difference between complete and partial moles in the spontaneous regression group.[26]

In the group of patients that could be predicted as developing GTN, the median number of chemotherapy courses administered was significantly higher compared to the group of patients in whom GTN was not predicted. It seems within reason that the patients with the most aggressive tumours show a less strong decline or even increase in their regression curve (with a corresponding less negative or even positive slope) and therefore could be predicted using the slope of the regression line, and that these are also the patients that required more chemotherapy courses to obtain remission.

Strengths of the study are that the proposed prediction model is not based on absolute hCG values but on the steepness of the regression curve. This may enable application of this model on multiple (commercial) hCG assays, although this needs to be confirmed in other studies. In addition, the prediction model is based on three hCG measurements, providing a reliable reflection of the course of the hCG level. A limitation is that retrospective hCG data were used that were supplied from clinical practice. More frequent blood sampling is needed after evacuation to determine the time that can be gained maximally in predicting GTN.

In conclusion, the slope of the linear regression line proved to be an accurate test to discriminate between patients that achieve spontaneous disease remission and patients that will develop GTN. The slope of free beta hCG seems to be a better test to predict GTN than the slope of hCG. Sixty-nine percent of patients that will eventually develop GTN could be predicted within 28 days after evacuation at 97.5% specificity by the slope of free beta hCG. In 38% of GTN patients the slope of free beta hCG predicted GTN before diagnosis using the FIGO criteria. In addition, patients that could be predicted as developing GTN required more courses of chemotherapy to obtain remission. Although this model needs further validation on different assays, it seems a promising way to predict the more aggressive cases of GTN.

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4

Relapse rates after two versus three consolidation courses of methotrexate in the treatment of low-risk gestational trophoblastic neoplasia

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Gynecologic Oncology 2012; 125: 576-579

Abstract

Background Methotrexate (MTX) alternating with folinic acid is a commonly used treatment regimen for low-risk gestational trophoblastic neoplasia (GTN). In the Netherlands, two courses of MTX are administered after Normalisation of serum human chorionic gonadotrophin (hCG) levels (consolidation courses), whereas in the United Kingdom, three consolidation courses are given. In a retrospective setting we compared relapse rates of women completing MTX therapy for low-risk GTN in the Netherlands and the UK.

Methods From 1980 to 2008, 351 patients were collected from the Dutch Central Registry for Hydatidiform Moles and records from the Dutch Working Party on Trophoblastic Disease. From the Charing Cross Hospital Trophoblast Disease Centre (London), 600 low-risk GTN patients were identified from 1992 to 2008.

Results In 4.0% of patients relapse occurred after MTX treatment with three consolidation courses, whereas 8.3% of patients relapsed after MTX treatment with two consolidation courses ($p=0.006$). Although patients from the Netherlands had a higher level of hCG ($p<0.001$) and more patients had metastases before the start of treatment ($p=0.012$), the number of courses of MTX to achieve a normal hCG did not differ significantly between patients from the Netherlands and the UK ($p=0.375$).

Conclusion Relapse rates were higher in patients treated with two consolidation courses of MTX. Although other factors might have influenced the observed difference in relapse rates, three courses of consolidation chemotherapy may be preferable to two in the treatment of low-risk GTN in order to decrease the risk of disease relapse. A prospective randomized study would be required to confirm these findings.

Introduction

A hydatidiform mole (HM) is an abnormal pregnancy with excessive proliferation of placental villi but severely stunted or absent embryonic development. This condition affects one to three per 1000 pregnancies in western countries [1;2] and can be classified into complete (CHM) or partial HM (PHM).[3] Management of both CHM and PHM is similar: surgical evacuation followed by regular measurement of serum human chorionic gonadotropin (hCG) until the levels have returned to normal. Normalisation occurs in the 80-90% of women. However, in the presence of three consecutive static or rising weekly hCG levels during follow-up, patients are defined as having gestational trophoblastic neoplasia (GTN). In the Netherlands, an additional criterion was added to this definition in 1993. At least one of the values should exceed the 95th percentile of the hCG regression corridor of uneventful decline as constructed by Yedema *et al*.[4]

GTN is an indication for chemotherapeutic treatment. Depending on the stage of the disease, patients are treated with either single-agent therapy for low-risk disease, or multi-agent therapy for high-risk disease.[5] In the Netherlands and the UK, low-risk patients receive intramuscular methotrexate (MTX) (1 mg/kg or 50 mg total, respectively) on days 1, 3, 5 and 7 alternating with oral folinic acid (FA) 15 mg on days 2, 4, 6, and 8, repeated every two weeks.[6-8] Treatment continues until the hCG level is normal and then for a further consolidation period. The number of courses of MTX administered after normalisation of serum hCG levels in order to eradicate the remaining tumour cells differs between the Netherlands and the UK. In the Netherlands, two consolidation courses are given, whereas in the UK, MTX treatment is consolidated over three further courses after normalisation of hCG levels.[4;8;9] Evidence exists that relapse rates after MTX treatment diverge between the Netherlands and the UK, although definitions of relapse differed between these studies.[10;11] Disease relapse requires treatment with multi-agent chemotherapy, which, in contrast to MTX, is associated with an increased incidence of secondary malignancies and increases the risk of an early menopause.[12-14] Therefore an additional course of MTX would be preferred if this decreases the chance of developing relapsed disease. In the present study we have retrospectively compared the percentage of disease relapse in a large cohort of women with low-risk GTN, treated with either two (the Netherlands) or three (UK) consolidation courses of MTX.

Methods

In the Netherlands, patients with gestational trophoblastic disease (GTD) are registered at the Dutch Central Registry for Hydatidiform Moles (DCRHM) residing at the Radboud University Nijmegen Medical Centre (RUNMC). This voluntary

registry serves as an epidemiological database and provides a national hCG assay service to gynaecologists. Between 1977 and 2010, 3983 patients were registered at the DCRHM. Patients are treated in different referral hospitals with the Dutch Working Party on Trophoblastic Disease having a coordinative and advisory function. Data on patients with low-risk GTN were collected retrospectively from 1980 to 2008 from the DCRHM and from records of the meetings of the Dutch Working Party. According to the Dutch guideline for classification of GTN, low-risk disease is defined as GTN with as antecedent pregnancy a mole or abortion, no metastases or metastases restricted to vagina or lungs, no previous chemotherapy administered, and an interval of ffi 12 months between the end of pregnancy and beginning of treatment. Patients who developed MTX resistant disease and therefore required additional treatment with multi-agent chemotherapy were not included. A total number of 401 low-risk GTN patients with normal hCG values after MTX treatment were selected. At the assay service of the DCRHM, all hCG concentrations are measured using an in-house developed radioimmunoassay (RIA) based on polyclonal antibody raised in rabbits. This assay detects intact hCG and free β-subunit: hCG+hCGβ. Using this assay, serum hCG concentrations of less than 2 ng/ml are considered normal. For patients whose serum hCG was measured in another hospital on a different assay, the reference values provided by the manufacturer of the assay were considered normal. After the first normalized hCG level the guidelines from the Dutch Society for Obstetrics and Gynaecology recommend two additional courses of MTX.[6] Patients who received less than two consolidation courses of MTX (n=19) or more than two consolidation courses of MTX (n=31) were excluded from further analysis. The remaining 351 patients were included in the study.

In the United Kingdom, all patients with GTD are registered with one of three centres for hCG monitoring and, if necessary, referred to one of the two centres for treatment (Charing Cross Hospital Trophoblast Disease Centre, London or Sheffield Trophoblastic Disease Centre, Sheffield). From the Charing Cross Hospital Trophoblast Disease Centre, 610 patients were identified who were successfully treated with MTX for low-risk GTN from 1992 to 2008. Before 1992, patients were stratified in three prognostic categories according to the Charing Cross scoring system, namely high (score>9), medium (score 6-9) or low risk (score 0-5). After 1992, patients were classified as either low-risk (score 0-8 on the Charing Cross scoring system; similar to score 0-6 on the revised prognostic scoring system of the International Federation of Gynecology and Obstetrics (FIGO)) or high risk (score >8, corresponding to a FIGO score of >6). In order to compare the same risk categories between the Netherlands and the UK, patients treated at the Charing Cross Hospital were included from 1992 onwards. Patients whose treatment protocol was changed due to MTX-resistance or toxicity were not included. According to the guidelines from the Royal College of Obstetricians and Gynaecologists (RCOG), three

consolidation courses of MTX should be administered after normal hCG levels are obtained. At the Charing Cross Hospital, hCG detection and monitoring is done with a non-commercial, one-site, in-house, competitive hCG radioimmunoassay that uses a rabbit polyclonal antibody. Serum hCG concentrations of less than 5 IU/L are considered normal. Patients who received less than a total of four courses of methotrexate (n=10) were also excluded from further analysis. Patients with the histology of a placental site trophoblastic tumour were excluded from the study. The remaining 600 patients were included in the study.

Indications for chemotherapy following a molar pregnancy are similar between the Netherlands and the UK. Indications for chemotherapy in the Netherlands include: a plateaued or rising hCG concentration in three consecutive weekly measurements after evacuation, or a histological diagnosis of choriocarcinoma. In addition, in the UK chemotherapy is started for a serum hCG concentration of more than 20,000 IU/L at 4 weeks or more after evacuation, lung or vaginal metastases more than 2 cm in diameter, or heavy vaginal bleeding, and until recently for a persistently raised but falling hCG at 6 months after evacuation.

Patient characteristics were compared between patients from the Netherlands and from the UK on maternal age, tumour histology, hCG level before the start of MTX, presence of lung metastases and metastases elsewhere. The hCG values before start of MTX treatment were categorized according to the FIGO 2000 prognostic scoring system into $<10^3$, 10^3-10^4, 10^4-10^5, and $>10^5$ IU/L. In addition, the number of courses of MTX required to achieve normal hCG levels were noted.

Disease relapse was defined as a rise in serum hCG values after termination of the consolidation courses of MTX, in the absence of a new pregnancy. This corresponds to six weeks after normalisation of serum hCG for the patients who received treatment in the UK, and four weeks after normalisation for patient treated in the Netherlands. Increasing serum hCG levels during consolidation therapy were not considered as relapsed disease. Statistical analyses were performed using SPSS 16.0 software. Differences in disease relapse between two and three consolidation courses of MTX were determined using Pearson Chi-square tests. Differences in number of courses to normalisation were compared using Mann-Whitney U Test. Statistical differences were considered significant at $p<0.05$.

Results

From 1980 to 2008, 351 women with low-risk GTN with normalisation of hCG levels after MTX treatment were collected from records from the DCRHM and the Dutch Working Party on Trophoblastic Disease. From the Charing Cross Hospital database, 600 low-risk patients successfully treated with MTX were identified from 1992 to

2008. Patient characteristics of these patients before the start of MTX treatment are shown in Table 1. Mean maternal age at diagnosis was 30.3 and 31.1 years in patients from both the Netherlands and from the UK, respectively (p=0.113). In the majority of patients from the Netherlands and from The UK, the tumour histology showed a molar pregnancy (95.8% and 94.6%, respectively). The level of serum hCG before start of MTX treatment was not the same between patients from The Netherlands and from The UK (p<0.001). More patients with an hCG level of >100,000 IU/L were found in the Netherlands compared to the UK (7.4% versus 2.2%, respectively). The percentage of patients with metastases prior to MTX chemotherapy was 9.9% in the Netherlands and 6.0% in The United Kingdom (p=0.012).

Table 1 Patient characteristics of low-risk GTN patients from the Netherlands and the UK before the start of MTX treatment.

	The Netherlands N=351	**United Kingdom** N=600	**Significance** *P*
Age at diagnosis (y)	(N=351)	(N=600)	0.113[#]
Mean (range)	30.3 (15-53)	31.1 (15-56)	
Histology	(N=351)	(N=600)	<0.001[*]
Complete HM	161 (45.9%)	491 (81.8%)	
Partial HM	54 (15.4%)	50 (8.3%)	
Unspecified mole[‡]	121 (34.5%)	27 (4.5%)	
Choriocarcinoma	9 (2.6%)	18 (3.0%)	
Non-molar abortion	6 (1.7%)	14 (2.3%)	
Serum hCG before start MTX (IU/L)	(N=324)	(N=600)	<0.001[*]
<1000	89 (27.5%)	184 (30.7%)	
1001-10,000	119 (36.7%)	193 (32.2%)	
10,001-100,000	92 (28.4%)	210 (34.9%)	
100,001-1,000,000	24 (7.4%)	13 (2.2%)	
Metastases	(N=304)	(N=600)[^]	0.012[*]
None	274 (90.1%)	565 (94.0%)	
Lungs	28 (9.2%)	27 (4.5%)	
Elsewhere (vagina)	2 (0.7%)	9 (1.5%)	

[‡] Pathologically confirmed mole, not further specified into complete or partial HM
[^] One patient had metastases in both lungs and vagina
[*] Chi-square
[#] T-test

The number of MTX courses to achieve a normal hCG level was compared between patients from the Netherlands (N=332) and the United Kingdom (N=464). In the UK, 3.94 courses were given on average to reach normal hCG levels (median 4 courses, range 1-12), compared to an average of 4.25 MTX courses to normalisation (median 4, range 1-18) in the Netherlands (Mann Whitney p=0.375). Table 2 shows relapse rates after two and three consolidation courses of MTX in the Netherlands and the United Kingdom, respectively. From the patients who were treated with two consolidation courses of MTX, 8.3% (29/351) developed disease relapse, compared to 4.0% (24/600) of the patients who were treated with three consolidation courses of MTX (p=0.006). In patients with non-metastatic disease, relapse rates were 8.0% (22/274) and 4.1% (23/564) in the Netherlands and the UK, respectively. In 13.3% (4/30) of patients with metastatic disease in the Netherlands, disease relapse occurred, compared to 2.9% (1/35) of patients with metastatic disease in the UK.

Table 2 Relapse rates in patients treated with two versus three consolidation courses of MTX for low-risk GTN.

	Two MTX consolidation courses (n=351)	**Three MTX consolidation courses** (n=600)	**Significance** *P*
Disease relapse	29 (8.3%)	24 (4.0%)	0.006*
No relapse	322 (91.7%)	576 (96.0%)	

*Chi-square

Discussion

The present study showed that 4.0% of low-risk GTN patients relapsed after MTX treatment with three consolidation courses after normalisation of hCG levels, whereas 8.3% of patients relapsed after MTX treatment with two consolidation courses after hCG normalisation. Several previous studies have examined relapse rates after MTX treatment for low-risk GTN, although some have combined patients normalizing on MTX with patients who had progressive disease on MTX and therefore had to switch to more aggressive chemotherapy.[10;11;15-19] Among 20 patients treated with MTX for low-risk GTN, including patients who showed MTX resistance, Matsui *et al.* found that one patient developed relapsed disease (5%).[19] For non-metastatic cases, one course of MTX consolidation treatment was given, whereas metastatic cases received two additional courses after normalisation of the hCG levels. Powles *et al.* found that 35 of 1397 (2%) low-risk patients relapsed.

Although these patients initially started with low-risk treatment, eight of the relapsed patients became resistant to MTX and changed to more intensive chemotherapy.[20] Ngan *et al*. found a relapse rate of 4.6% in 153 low-risk GTN patients treated with single-agent chemotherapy with MTX or etoposide. In this study, relapse was diagnosed if the serum hCG rose one month or later after cessation of chemotherapy.[16]Between 1999 and 2006, only 1.8% of the low-risk GTN patients who responded to MTX had recurrent disease after hCG normalisation in the French Trophoblastic Disease Reference Centre.[18]

Few studies have illustrated the importance of consolidation therapy. Yang *et al*. identified less than two courses of consolidation chemotherapy as a risk factor for relapsed disease.[21] In their study, the relapse rate of low-risk patients was 2.9%, although recurrence was defined as an increase of serum hCG values 3 months after complete remission. Mutch *et al.* found that no consolidation chemotherapy after the first normalized hCG level was related to development of relapsed disease.[15] Sun *et al*. reported a 2.4% recurrence rate in low-risk choriocarcinoma patients, and analyzed the factors associated with recurrence. Especially interesting was their finding that recurrence rates among patients without and with one course of consolidation chemotherapy were 6.1% (3/49) and 9.8% (6/61), respectively, while in 2, 3 and >3 courses of consolidation chemotherapy recurrence rates were 1.4% (1/70), 3.9% (2/51) and 3.1% (5/163), respectively.[17] However, this difference might be a chance finding because of the very small number of patients who relapsed. In the same study, a relapse rate of 2.5% was found in patients with non-metastatic disease who received one cycle of consolidation chemotherapy, compared to a 8.0% and 4.1% relapse rate in patients with non-metastatic disease in the Netherlands and the UK, respectively. However, patients with non-metastatic disease in the study by Mutch *et al*. were treated with single agent chemotherapy in the form of either methotrexate, methotrexate with folinic acid rescue, or actinomycin D.[15]

Relapse rates in low-risk GTN patients in the Netherlands and in the UK have been described previously. Kerkmeijer *et al*. found a relapse rate of 8.1% in 74 low-risk GTN patients treated with MTX/FA in the Netherlands.[10] McNeish *et al.* studied 324 low-risk GTN patients treated with MTX/FA in the UK and found a relapse rate of 3.4%.[11] However, definitions of relapse differed between these studies. McNeish *et al*. classified patients as having relapsed disease if the serum hCG value started to rise after completing the consolidation courses of MTX, whereas Kerkmeijer *et al*. also classified a raising hCG during consolidation therapy as relapsed disease. In order to compare relapse rates, the same definition of relapse should be applied to both patient groups, as performed in the present study.

Other factors than the number of consolidation courses might have influenced the difference in relapse rates found in the present study. First, the extra criterion in the definition of GTN added in the Netherlands in 1993 of one hCG value exceeding

the p95 of the normal regression curve, in addition to a plateau or rise in hCG serum concentrations in three subsequent weekly measurements, might have influenced the composition of the group of patients in the Netherlands, thereby possibly increasing the observed relapse rate. In addition, the broader indications for the start of chemotherapy in the UK (persistently raised but falling hCG at 6 months after evacuation, a serum hCG concentration of more than 20,000 IU/L at 4 weeks or more after evacuation, lung or vaginal metastases more than 2 cm in diameter, or heavy vaginal bleeding) might have contributed to the difference in the observed relapse rates.

Secondly, the level of hCG before the start of MTX treatment was higher in patients from the Netherlands compared to patients from the UK. More patients treated in the Netherlands had an hCG level higher than 100,000 IU/L. Since the response to treatment is known to be influenced by the burden of tumour as reflected by the level of hCG, [22;23] this discrepancy might contribute to the higher relapse rate in the Netherlands patients. However, different hCG assays were used in the Netherlands and in the UK to measure the hCG levels, which might have contributed to the observed difference in pre-treatment hCG levels.

Finally, the percentage of patients with metastases in the lungs or the vagina differed between the Netherlands and the UK. Patients from the Netherlands more often had metastatic disease before the start of MTX treatment. However, although patients from the Netherlands had a higher serum level of hCG and more patients had pulmonary metastases before the start of treatment, no significant difference was observed in the number of courses of MTX required to achieve normal hCG levels.

Other potential limitations of our study are that this is a retrospective analysis in which different scoring systems were employed in defining low-risk disease. Thus, unlike the FIGO 2000 scoring system, the Dutch guideline does not include scorings for age, pre-treatment serum hCG concentration, the number of metastases or largest tumour size.[6;22] Nevertheless, strengths of our study include its size and to the best of our knowledge, this is the first study in which relapse rates following two or three consolidation courses of MTX have been investigated.

In conclusion, the data from this large retrospective analysis showed that relapse rates were higher after completing treatment with two consolidation courses of methotrexate. Although other factors might have influenced the difference in relapse rates found in this study, three courses of consolidation chemotherapy may be preferable to two in the treatment of low-risk GTN in order to decrease the risk of development of disease relapse. A prospective randomized study would be required to confirm these findings.

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5

Fatal cases of gestational trophoblastic neoplasia over four decades in the Netherlands: a retrospective cohort study

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BJOG 2012; 119: 1465-72

Abstract

Background We described fatal cases of gestational trophoblastic neoplasia (GTN) over four decades in the Netherlands and evaluated whether treatment was given according to the protocol and revealed possible implications for future management.
Methods Records from the Dutch Central Registry for Hydatidiform Moles and the Working Party on Trophoblastic Disease were used to identify fatal cases of GTN from 1971 to 2011. Main outcome measures were disease extent, risk classification, treatment regimens and cause of death.
Results Twenty-six patients died from GTN. In five cases GTN developed after a hydatidiform mole and in 19 cases following term pregnancy. Half the number of patients died between 1971-1980 when patients were not yet classified as having low- or high-risk disease and were thus not yet treated accordingly. A major decline in the number of deaths was seen after the first decade, with a further decrease from 1981-2011. Early death occurred in nine patients. In four of these patients, death was treatment related. Patients that died more than four weeks after start of treatment mostly died from metastatic tumour (n=14).
Conclusion The yearly number of patients that died from GTN decreased considerably over the last four decades. Appropriate risk classification is essential to start optimal initial therapy and to prevent therapy resistance. Patients with post-term choriocarcinoma represented a large proportion of the deceased patients and we propose that these patients are considered as having high-risk disease.

Introduction

Gestational trophoblastic disease (GTD) is a condition in which there is abnormal proliferation of placental trophoblastic tissue. It encompasses the benign hydatidiform moles (HMs) and gestational trophoblastic neoplasia (GTN) including invasive mole, choriocarcinoma, placental site trophoblastic tumours (PSTT), and epithelioid trophoblastic tumour (ETT). HMs are premalignant non-viable pregnancies that develop because of a complication in fertilization. They may develop into post-molar GTN (also known as persistent trophoblastic disease) which requires chemotherapeutic treatment. However, GTN can occur after any kind of gestation.

Patients with GTN are classified as having a high or low risk of developing resistance to treatment with single-agent chemotherapy. During the years, a number of different classification systems were applied that resulted in the current FIGO 2000 staging system.[1] Low-risk disease is treated with single-agent methotrexate (MTX) or actinomycin D chemotherapy, whereas high-risk disease is treated with multi-agent chemotherapy. The most widely accepted initial treatment for high-risk trophoblastic tumour is EMA/CO (etoposide, MTX and actinomycin D, alternating with cyclophosphamide and vincristine).

Prognosis of GTN improved greatly over the past decades. In the 1950s, when MTX was introduced as a treatment for GTN, choriocarcinoma had a cure rate of approximately 25%.[2,3] Nowadays, patients still die from high-risk disease with survival rates of 87-91% after treatment with EMA/CO and adjuvant cisplatin-based chemotherapy, surgery and radiotherapy.[4-7] For low-risk disease, survival rates approach 100%.[8-10] Literature is limited on cases that fatally failed on treatment.[11-14] Reasons for unsuccessful treatment are late presentation and diagnosis, and drug resistance.[15,16] The present study reviews all patients who died from GTN over the last four decades in the Netherlands and aims to evaluate whether treatment was given according to the protocol or guideline at the time of diagnosis and to reveal possible implications for future management.

Methods

Patients

In the Netherlands, patients with GTD, and even GTN are treated in various referral hospitals. The Dutch Working Party on Trophoblastic Disease, founded in 1971, has a registering and advisory function.[16] Since its founding, 788 patients were discussed in the meetings of the Working Party. In 1977, the Dutch Central Registry for Hydatidiform Moles (DCRHM) was established at the Radboud University Nijmegen Medical Centre (RUNMC), which currently contains 4190 records of patients with GTD. Data from 34 patients with GTN who died from this disease were collected

retrospectively from 1971 to 2011 from the DCRHM and the database of the Working Party. After evaluation, eight patients were excluded because of diagnosis of non-gestational choriocarcinoma, based on suspicion of the clinicians.

For each patient, data were collected on demographics, disease extent, treatment regimens and cause of death. Patients were categorized as early death (within four weeks after the start of treatment before adequate chemotherapy could be given) or late death (more than four weeks after the start of treatment). The risk classification as stated at the time of diagnosis was registered. In addition, patients were classified retrospectively using the current Dutch classification system introduced in 1983.[17] This system defines high-risk disease by the presence of one or more of the following features: insufficient response to single-agent chemotherapy; metastasis in more than one organ; metastasis in liver, spleen, kidneys, gastro-intestinal tract, bones or brain; antecedent term pregnancy; and an interval of more than 12 months between the end of the antecedent pregnancy and the start of treatment. Absence of all of these features is defined as low-risk disease. Finally, for each patient we evaluated whether treatment was given according to the protocol or guideline at the time of diagnosis and if not, why treatment deviated from these protocols.

Protocols for the management of GTN

Until 1977, MTX was given to all patients and if resistance occurred, either actinomycin D or multi-agent chemotherapy as second line treatment was used.[2,18] From 1977 onwards patients were gradually classified into low-risk or high-risk disease.[19]

Treatment for low-risk disease always consisted of MTX with folinic acid rescue, although before 1982 different routes of administration and schedules were applied. From 1980 onwards, high-risk disease was treated with multi-agent chemotherapy. Various chemotherapy regimens were used in the treatment of high-risk GTN. Until 1982 most patients were treated with CHAMOCA (cyclophosphamide, hydroxyurea, actinomycin D, MTX, vincristine, adriamycin), VAC (vincristine, adriamycin, cyclophosphamide) or PLE(M)CA (cisplatin, etoposide, (MTX), cyclophosphamide, adriamycin).[16,20] Because of high toxicity of CHAMOCA, a platinum containing combination chemotherapy schedule (EMACP: etoposide, MTX, cyclophosphamide, actinomycin D and cisplatin) was introduced by the Working Party in 1982, aiming to create a schedule that could be repeated frequently with a short interval between two courses, causing less myelosuppression and containing the new agents etoposide and cisplatin. For similar reasons, the EMA/CO regimen was developed in the UK. In 1989, EMA/CO was introduced in the Netherlands because of seemingly less toxicity compared to cisplatin containing EMACP, and both regimens were used simultaneously.[21,22]

If relapse or resistance to multi-agent chemotherapy regimens occurred, a wide variety of salvage chemotherapy was given. Since 2004, platinum containing EMA/

EP therapy (etoposide, MTX and actinomycin D, alternating with etoposide and cisplatin) is recommended as salvage chemotherapy after failure of EMA/CO.[17,23] Beside chemotherapy, hysterectomy could be considered in patients with tumour confined to the uterus who did not wish to preserve fertility, as salvage therapy and in patients with life-threatening haemorrhage. Also, metastasectomy was used in some cases as salvage treatment.[17] Radiotherapy was used for lung and brain metastasis until 1986. Surgery for brain metastases at an early stage combined with multi-agent chemotherapy is the currently recommended treatment of choice. (Stereotactic) radiotherapy might still be used when the lesions do not respond to chemotherapy and cannot be removed neurosurgical.[17]

Results

Twenty-six patients died from GTN. Patient characteristics are presented in Table 1. The median age of women at the time of diagnosis was 30·5 years. In 21 patients histology showed a choriocarcinoma, whereas in five patients a histological diagnosis of a hydatidiform mole was made. Five patients developed GTN from antecedent HM and 19 patients developed GTN from an antecedent term pregnancy. In the case of two patients it was unclear whether the index pregnancy was a HM or previous term pregnancy. Seven patients were diagnosed with GTN within four months from the index pregnancy, ten patients had an interval from the index pregnancy of more than 12 months. Nineteen patients had an hCG level over 10,000 IU/L before start of treatment, of which eleven patients had hCG levels of more than 100,000 IU/L. Six patients had no evidence of metastatic disease at presentation. Twelve out of 20 patients with metastatic disease at presentation, had metastases in more than one organ. Two patients presented with metastases in the gastro-intestinal tract, four with brain metastases and three with liver metastases.

Figure 1 shows the number of fatal cases of GTN from 1971 to 2011 as well as changes in the Dutch protocols and guidelines of management of GTN during this period. Thirteen patients died during the first decade, before patients were classified into low- or high-risk disease and were treated accordingly. A major decline was seen after the first decade, with a further decrease between 1981-2011.

Figure 2 shows the causes of death of patients who died either within four weeks or after four weeks following the start of treatment. Early death occurred in nine of the 26 patients. In four of these patients, death was treatment related. The cause of early death of six patients was fatal tumour bleeding, related to treatment in one of them (haemorrhage from the lungs after MTX) and unrelated to treatment in five patients (uterus, brain and gastro-intestinal bleeding), since tumour bleeding occurred before the start of chemotherapy. Two early deaths resulted from sepsis as

Table 1 Patient characteristics.

	Number of patients (N=26)	Patient numbers
Age (years)		
Median (range)	30.5 (23-55)	
Parity		
Median (range)	2 (0-5)	
Histology		
Choriocarcinoma	21	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 19, 20, 21, 22, 23, 26
HM	5	5, 17, 18, 24, 25
Index pregnancy		
HM	5	5, 17, 18, 24, 25
Term	19	1, 2, 3, 4, 7, 8, 9, 11, 12, 13, 14, 15, 16, 19, 20, 21, 22, 23, 26
HM or term	2	6,10
Interval from index pregnancy (months)		
<4	7	5, 8, 18, 20, 22, 23, 24
4-6	5	1, 7, 12, 16, 21
7-12	3	15, 19, 26
>12	10	2, 3, 4, 6, 9, 10, 11, 13, 14, 25
Unknown	1	17
Pre-treatment hCG (IU/L)		
<1000	0	
1000-10,000	4	5, 19, 20, 21
10,000-100,000	8	2, 4, 11, 13, 14, 16, 24, 26
>100,000	11	3, 6, 7, 8, 10, 12, 15, 18, 22, 23, 25
Unknown	3	1, 9, 17
Metastasis site at presentation*		
None	6	1, 10, 13, 18, 21, 26
Lung	20	2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 17, 19, 20, 22, 23, 24, 25
Vaginal	3	2, 6, 25
Brain	4	3, 4, 12, 19
Liver	3	11, 15, 20
Spleen/kidney	1	9
Gastro-intestinal	2	9, 11

*Some patients had metastatic spread to several sites

Figure 1 Fatal cases of GTN in the Netherlands from 1971-2011 (n=26). Major changes in the Dutch protocols for management of GTN are indicated.

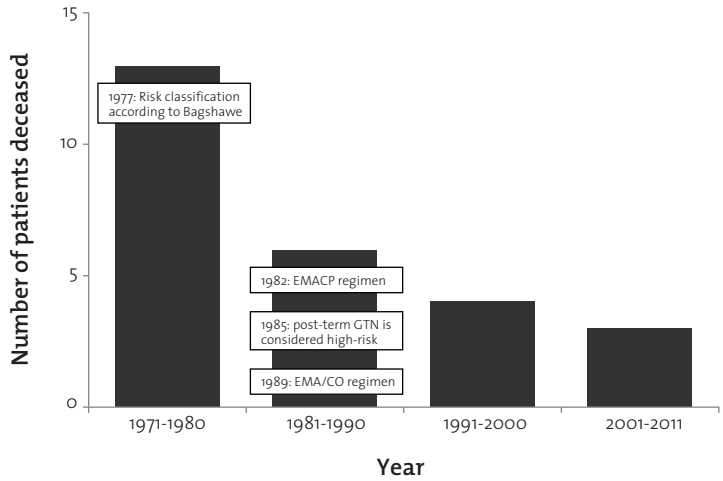
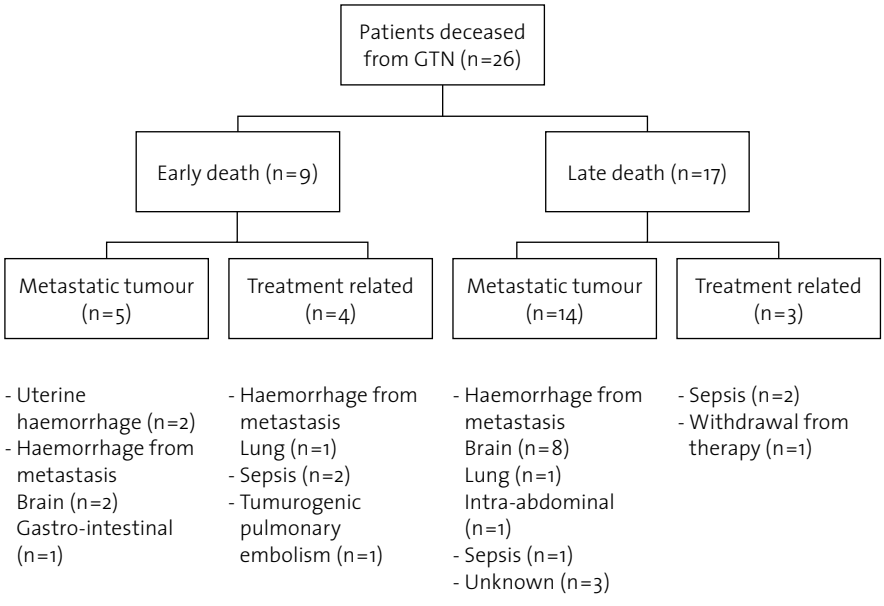


Figure 2 Causes of death of patients who died within four weeks after start of treatment and of patients who died more than four weeks after the start of treatment.



a complication of chemotherapy, one patient died from tumourogenic pulmonary embolism shortly after initiation of chemotherapy. The chemotherapy that was given shortly before early treatment related death was MTX (in one patient who died from lung haemorrhage and in one patient who died from sepsis) and EMACP (in one patient who died from sepsis, and in one patient with massive pulmonary obstruction due to carcinoma tissue plugs). The patients who died from haemorrhage from the lungs was not ventilated prior to the start of bleeding. Between the first two decades and the last two decades the relative number of early deaths was rather comparable (six out of 19 patients from 1971-1990 and two out of seven patients from 1991-2011).

Patients that died more than four weeks after start of treatment mostly died from metastatic tumour (14 out of 17). All these patients had treatment resistance to or disease relapse after at least three different chemotherapy regimens (multi-agent resistant disease). Ten patients died from fatal tumour bleeding, which included haemorrhage from brain metastasis in eight patients. The patient who died from haemorrhage from the lungs was not ventilated prior to the start of bleeding. One patient died of sepsis, caused by peritonitis after tumour growth through the bowel wall. The remaining three patients died at home after cessation of all therapy, thus causing uncertainty about the eventual cause of death. Treatment related death in patients that died late was due to sepsis from leucopoenia from EMA/CO chemotherapy in one patient, and severe leucopoenia from EP salvage therapy (for multi-agent resistant disease) in the other patient. One patient withdrew from therapy 6 weeks after diagnosis and died abroad.

Haemorrhage from brain metastasis was the eventual cause of death in ten out of the 26 deceased patients. Three patients had brain metastasis at presentation, in seven patients brain metastasis developed during treatment. Nine of these patients died in the first two decades of the study period, whereas only one patient died from brain metastasis in the last two decades of the study period.

Table 2 demonstrates risk classification and first treatment. Twelve out of 17 patients who were initially treated with MTX had high-risk disease according to the current Dutch risk classification. In retrospect, risk classification at time of diagnosis of nine of these patients was inconsistent with the revised risk classification. Six of the nine patients that would now be classified as high-risk were initially treated with MTX since they were diagnosed before 1978, when risk classification was not yet widely available (patients 2, 3, 4, 7, 8 and 10). The other three patients were classified as low-risk at time of diagnosis of GTN and would now be classified as high-risk (patients 13, 21 and 26). Risk classification was difficult for two of these patients because at the time of diagnosis it was uncertain whether an abortion or term pregnancy was the index pregnancy. In another patient, a choriocarcinoma after a term pregnancy was diagnosed, without metastases. According to the guidelines at

Table 2 Risk classification and first treatment in patients deceased from GTN. Patients with post-term GTN are marked^

Patient number	Year of diagnosis	Risk classification†	Revised risk classification‡	First treatment
1^	1970	Not available	Low-risk	MTX
2^	1972	Not available	High-risk	MTX
3^	1974	Not available	High-risk	MTX
4^	1974	Not available	High-risk	MTX
5	1975	Not available	Low-risk	MTX
6	1975	High-risk¨	High-risk	Multi-agent chemotherapy¨¨
7^	1976	Not available	High-risk	MTX
8^	1976	Not available	High-risk	MTX
9^	1977	High-risk	High-risk	MTX / actinomycin D
10	1977	Not available	High-risk	Hysterectomy / MTX
11^	1978	High-risk	High-risk	MTX
12^	1980	High-risk	High-risk	CHAMOCA
13^	1980	Low-risk	High-risk	MTX
14^	1980	High-risk	High-risk	MTX
15^	1984	High-risk	High-risk	EMACP
16^	1985	High-risk	High-risk	EMACP
17	1986	Low-risk	Low-risk	MTX
18	1987	Low-risk	Low-risk	MTX
19^	1987	High-risk	High-risk	EMACP
20^	1991	High-risk	High-risk	EMA/CO
21^	1991	Low-risk	High-risk	MTX
22^	1992	High-risk	High-risk	EMA/CO
23^	1999	High-risk	High-risk	EMACP
24	2000	Low-risk	Low-risk	MTX
25	2008	High-risk	High-risk	Hysterectomy
26^	2010	Low-risk	High-risk	MTX

†Risk classification according to Bagshawe available from 1977 onwards. Dutch classification system available from 1983 onwards.
‡Patients were classified according to the Dutch classification system
¨Based on long interval from index pregnancy, high serum hCG values and lung metastases
¨¨MTX, actinomycin D, adriamycin, cytarabin, vincristine, cyclophosphamide

that time, multi-agent chemotherapy should have been started, but instead MTX was started. The remaining three patients were classified as high-risk at the time, but MTX was the drug of choice in two patients because they were too ill to receive multi-agent chemotherapy (patients 9 and 11) and one patient refused multi-agent chemotherapy (patient 14).

Nineteen patients were diagnosed with post-term GTN. According to the current Dutch guidelines, these patients would all be classified as having high-risk disease. Seven patients were classified as high-risk and treated accordingly. Twelve patients were not treated with multi-agent chemotherapy for various reasons.

Discussion

This study provides an overview of patients who died from GTN in the Netherlands, and aimed to identify factors in the management that may have influenced the course of disease in these patients. A decline was observed in the yearly number of fatal cases over the last four decades, despite the increased incidence of GTD and GTN in the Netherlands.[24] Several changes in the management of GTN during the study period contributed to this decline. Risk classification of patients into low- and high-risk disease, and introduction of multi-agent chemotherapy for high-risk patients caused a major decline in fatal cases as seen from 1971-1990. In addition, the classification and corresponding treatment of patients with post-term chorio-carcinoma as high-risk patients most likely contributed to a decline in fatal cases.

Nineteen patients (73·1%) died from metastatic disease and in seven patients (26·9%) death was treatment related. Kingdon *et al.* reported comparable results from the United Kingdom with 68% death from metastatic tumour and 32% treatment related death.[25] Most important cause of death in their study was metastatic multi-agent resistant PSTT. Although incidences of PSTT are similar between the Netherlands and the UK, none of the deceased patients in the present study had PSTT.[24,26]

Previously, Lurain *et al.* stated that death from GTN could mainly be attributed to the presence of extensive disease at the time of initial treatment, lack of initial, appropriately aggressive treatment in high-risk patients, and failure of chemotherapy protocols used at the time to control advanced disease.[12] Appropriate risk classification is essential to start the right initial therapy and to prevent therapy resistance. In the present study, risk classification was sometimes difficult because of uncertain antecedent pregnancy. Some patients presented with an extensive time interval since the last diagnosed pregnancy and some of them additionally reported recent vaginal bleeding. This led to suspected, but undiagnosed abortion as the causative gestational event. Although rare, cases of GTN presenting many

years after the last pregnancy are described.[27,28] Therefore, to prevent these patient from being undertreated, these patients should be considered as having post-term GTN. Other women in our study had a history of HM, with or without GTN, and had abortions or term pregnancies afterwards, leading to difficulties in determining the causative pregnancy. Previously, other authors demonstrated using molecular genetic techniques that the immediate antecedent pregnancy may not be the causative conception in some cases of choriocarcinoma.[29;30] Genetic techniques are also used to differentiate gestational from non-gestational trophoblastic neoplasia. In our study, genetic techniques might have been helpful to differentiate between gestational and non-gestational tumours since clinical diagnosis often was not evident. Unfortunately, paraffin embedded tissue from these patients was not available.

Our study revealed that uterine haemorrhage or haemorrhage from metastases was the most common cause of death (16/26). Previously, Lurain *et al.* also reported haemorrhage from metastases as the most common cause of death (42%).[12] Early death occurred in nine patients and most of these patients presented with extensive disease. One patient died within four weeks from treatment related haemorrhage. Treatment related haemorrhage can occur in GTN because these tumours are highly vascularised and can easily start bleeding, for instance when a biopsy is taken or when fast necrosis occurs after administration of multi-agent chemotherapy.[15] One patient died from tumourogenic pulmonary embolism within eight hours after initiation of treatment with moderately high doses of MTX, etoposide and cyclophosphamide. Previously, other authors suggested that patients with large tumour loads might benefit from relatively mild initial treatment to prevent massive necrosis of the tumour leading to fatal haemorrhage or massive embolism.[11,15] This might especially be true for patients not previously exposed to this type of chemotherapy.[11] Seckl *et al.* suggested initial low dose etoposide and cisplatin (EP) for 2 days combined with dexamethasone to reduce tumour oedema in patients with extensive disease.[15] Alifrangis *et al.* demonstrated that after introduction of low-dose EP in 1995, 23·1% of high-risk patients (34/147) with a high disease burden (FIGO >8 and >6 metastases) had received EP-induction chemotherapy, with a reduction in early deaths in high-risk patients from 7·2% in the pre-1995 cohort to 0·7% (n=1) after 1995.[31] Other authors demonstrated the usefulness of angiographic embolisation in haemorrhage due to GTN.[32-34] Pelvic haemorrhage, either uterine or vaginal, may be controlled by embolisation of the internal iliac vessels, and embolisation of the hepatic arteries may be performed to control bleeding from liver metastases.[33,34] In acute hemorrhagic complications in GTN, this intervention should be considered to control bleeding.

Nineteen patients in our study died from post-term choriocarcinoma. In 1978 Hammond was the first to propose antecedent term pregnancy as a poor prognostic indicator and subsequently added this into a clinical classification system.[35,36] They

showed that post-term gestation classified as having a poor prognosis had a significantly lower cure rate (47%) than other patients classified as having a poor prognosis (75%, p<0.05).[36] Lurain *et al.* also showed that GTN after term pregnancy, abortion or ectopic pregnancy has a significant lower cure rate than after HM (64% vs. 84%, respectively).[12] In the Netherlands, patients with a choriocarcinoma after a term pregnancy are classified as high-risk.[17] Before the introduction of this Dutch classification system in 1983, patients with GTN after a term pregnancy might also be classified as low-risk and were treated accordingly with single-agent chemotherapy. In the FIGO 2000 system, patients with GTN after a term pregnancy are given two points. Since high-risk disease is defined as a score of fff7, these patients might still be classified as low-risk. In our study, two patients with post-term GTN would have been classified as low-risk according to the FIGO 2000 criteria. Lok *et al.* reported MTX resistance in 75% of patients with post-term choriocarcinoma treated with MTX primarily. A subgroup of patients in which single-agent chemotherapy would be a safe alternative could not be determined. Additionally, an overall survival rate of 85% was found in patients with post-term choriocarcinoma, which is comparable to survival rates of high-risk disease in general.[22] Therefore we propose that all patients with post-term GTN are classified as having high-risk disease. Lok *et al.* proposed the long interval between the index antecedent term pregnancy and start of treatment as an explanation for the high-risk status of patients with post term choriocarcinoma.[22] A longer time interval between the antecedent pregnancy and the start of treatment is associated with lower survival rates.[12] In addition, the long symptom free period may cause delay in diagnosis, because a possible diagnosis of choriocarcinoma may not be evident, especially since most physicians are relatively unfamiliar with choriocarcinoma.

Strengths of this nationwide study lie in the long time period studied and detailed analysis of fatal cases. A limitation is the possibility of an underestimation of the number of fatal cases in the Netherlands, because registration in the DCRHM and Dutch Working Party is not obligatory and therefore not all deceased patients might be registered. In addition, we did not include a comparative group of patients that did not die from GTN, since our goal was to establish a description of a unique group of patients that died from GTN, and compare the management of these cases with the protocol or guideline at the time of diagnosis.

In conclusion, we found that the yearly number of fatal cases of GTN decreased considerably over the last four decades, despite the increased incidence of GTD. Several improvements in management contributed to this decrease. Appropriate risk classification is essential to start optimal initial therapy and prevent therapy resistance. Patients with extensive disease may benefit from relatively mild initial treatment to prevent massive necrosis of the tumour leading to fatal haemorrhage or massive embolism. Patients with post-term choriocarcinoma represented a large

part of the patients deceased and we propose that these patients, as in Dutch classification system, are considered as having high-risk disease.

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Comparing cisplatin-based combination chemotherapy with EMA/CO chemotherapy for the treatment of high-risk gestational trophoblastic neoplasia

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European Journal of Cancer 2012

Abstract

Background: Cisplatin-based chemotherapy (etoposide 100mg/m^2 day 1-5, methotrexate 300mg/m^2 day 1, cyclophosphamide 600mg/m^2 day 1, actinomycin D 0.6 mg/m^2 day 2 and cisplatin 60 mg/m^2 day 4, EMACP) was compared to EMA/CO (etoposide 100mg/m^2 day 1-2, methotrexate 300mg/m^2 day 1 and actinomycin D 0.5 mg i.v. bolus day 1 and 0.5 mg/m^2 day 2, alternating with cyclophosphamide 600 mg/m^2 day 8 and vincristine 1 mg/m^2 day 8) for the treatment of high-risk gestational trophoblastic neoplasia (GTN).

Methods: In the Netherlands, 83 patients were treated with EMACP and 103 patients with EMA/CO. Outcome measures were remission rate, median number of courses to achieve normal human chorionic gonadotrophin (hCG) concentrations, toxicity, recurrent disease rate and disease specific survival.

Results: Remission rates were similar (EMACP 91.6%, EMA/CO 85.4%). The median number of courses of EMA/CO to reach hCG normalisation for single-agent resistant disease and primary high-risk disease were 3 and 5 courses, respectively, compared to 1.5 ($p=0.001$) and 3 ($p<0.001$) courses of EMACP. Patients treated with EMACP more often developed fever, renal toxicity, nausea and diarrhoea compared to patients treated with EMA/CO. Patients treated with EMA/CO more often had anaemia, neuropathy and hepatotoxicity.

Conclusion: EMACP combination chemotherapy is an effective treatment for high-risk GTN, with a remission rate comparable to EMA/CO. However, the difference in duration of treatment is only slightly shorter with EMACP. Cisplatin-based chemotherapy in the form of EMACP in this study was not proven more effective than EMA/CO.

Introduction

Gestational trophoblastic disease (GTD) comprises a spectrum of disorders, ranging from the premalignant complete and partial hydatidiform moles (CHM and PHM, respectively), to gestational trophoblastic neoplasia (GTN) consisting of invasive moles, choriocarcinoma, placental site trophoblastic tumours (PSTT) and the rare epithelioid trophoblastic tumour (ETT). Patients with GTN are classified as having low-risk or high-risk disease using the modified WHO prognostic scoring system as adapted by FIGO.[1] Patients with a score of 0-6 are defined as having low-risk disease. These patients are treated with single-agent chemotherapy, consisting of either methotrexate (MTX) or actinomycin D. For high-risk patients (prognostic score of 7 or more) single-agent chemotherapy is considered insufficient treatment and they are therefore treated with multi-agent chemotherapy.[2]

Before the introduction of multi-agent chemotherapy in the 1970s, only 31% of the high-risk patients would be cured with single-agent chemotherapy.[3] Throughout the late 1970s, the combination of MTX, actinomycin D, and cyclophosphamide or chlorambucil (MAC) became the preferred first-line chemotherapy, followed by the combination regimen of cyclophosphamide, hydroxyurea, actinomycin D, MTX, vincristine and doxorubicin (CHAMOCA) in the early 1980s.[2;4;5] In 1982, an alternative schedule to CHAMOCA was designed by the Dutch Working Party on Trophoblastic Disease, consisting of etoposide, MTX, actinomycin D, cyclophosphamide and cisplatin (EMACP), aiming to design a schedule that could be repeated frequently with a short interval between two courses, causing less myelosuppression and containing the new agents etoposide and cisplatin.[6;7] Today, the most widely accepted initial treatment for high-risk trophoblastic tumour is EMA/CO chemotherapy (etoposide, MTX and actinomycin D, alternating with cyclophosphamide and vincristine) introduced in 1979 by Newlands and Bagshawe, showing complete remission rates ranging from 69% to 86%.[8-11] However, due to the favourable outcome following treatment, some centres in the Netherlands preferred to continue application of the EMACP schedule after the introduction of EMA/CO. The aim of the present study was to evaluate the efficacy and safety of cisplatin-based combination chemotherapy (EMACP) as compared to the EMA/CO schedule for the treatment of high risk GTN.

Methods

Patients

In the Netherlands, patients with GTD are registered at the Dutch Central Registry for Hydatidiform Moles (DCRHM) residing at the Radboud University Nijmegen Medical Centre (RUNMC). This voluntary registry serves as an epidemiological

database and provides a national human chorionic gonadotrophin (hCG) assay service to gynaecologists. Patients with GTD, and even GTN are treated in various referral hospitals. The Dutch Working Party on Trophoblastic Disease, founded in 1971, has a registration and advisory function. The Dutch classification system for trophoblastic tumours scores for previous failure to chemotherapy, localisation of metastases, antecedent pregnancy and the interval between end of pregnancy and beginning of treatment (Table 1). Patients treated with EMACP or EMA/CO from 1982 to 2009 were identified from the databases of the DCRHM and the Dutch Working Party on Trophoblastic Disease. Patients treated with other multi-agent chemotherapy administered before the start of EMACP or EMA/CO, patients diagnosed with PSTT, and patients with a non-gestational tumour were excluded. In total, 83 patients treated with EMACP and 103 patients treated with EMA/CO were included in this study. Medical records of all patients were reviewed for age at diagnosis, antecedent pregnancy, date of evacuation, histology of the tumour, localisation of metastases, indication for treatment with first multi-agent chemotherapy and duration of follow-up.

Table 1 Risk classification of GTN by the Dutch Working Party on Trophoblastic Disease.

Low-risk GTN	Demands all of the following conditions: 1. Antecedent pregnancy: hydatidiform mole or abortion* 2. No metastases or metastases in vagina or lung 3. No previous chemotherapy 4. Interval between evacuation and start of chemotherapy less than 12 months
High-risk GTN	Demands one or more of the following conditions: 1. Failure of previous (mono-) chemotherapy 2. Metastases in more than one site (outside the uterus) 3. Metastases in one or more of the following organs: liver, spleen, kidney, gastrointestinal tract, bones or brain 4. Antecedent pregnancy: term 5. Interval between end of antecedent pregnancy and start of chemotherapy more than 12 months

* There is no consensus on classification of non-molar abortion as low- or high-risk disease

Treatment

The EMACP and EMA/CO chemotherapy regimen are shown in Table 2. In the EMACP regimen, the interval between courses is twenty-one days. After normalisation of the serum hCG concentration generally two courses of chemotherapy were given to

prevent disease relapse. In the EMA/CO regimen, interval between courses is 15 days. After normalisation of the serum hCG the national guideline advises three courses of consolidation chemotherapy.[12] Drug resistance was defined as steady or increasing serum hCG during treatment. When drug resistance occurred most patients were treated with surgery, salvage chemotherapy treatment or both.

Table 2 (**a**) EMACP regimen and EMA/CO regimen for high-risk gestational trophoblastic neoplasia and (**b**) the accumulated dosage per week. Interval between courses is 21 and 15 days, respectively.

2a

EMACP regimen			**EMA/CO regimen**		
Day	*Chemotherapy*	*Dose (i.v.)*	*Day*	*Chemotherapy*	*Dose (i.v.)*
1	Etoposide	100 mg/m²	1	Etoposide	100 mg/m²
	Methotrexate	300 mg/m²		Methotrexate	300 mg/m²
	Cyclophosphamide	600 mg/m²		Actinomycin D	0.5 mg
2	Etoposide	100 mg/m²	2	Etoposide	100 mg/m²
	Actinomycin D	0.6 mg/m²		Actinomycin D	0.5 mg
3	Etoposide	100 mg/m²	8	Vincristine	1 mg/m²
4	Etoposide	100 mg/m²		Cyclophosphamide	600 mg/m²
	Cisplatin	60 mg/m²			
5	Etoposide	100 mg/m²			

2b

EMACP regimen	**EMA/CO regimen**
Etoposide 166.7 mg/m²	Etoposide 100 mg/m²
Methotrexate 100 mg/m²	Methotrexate 150 mg/m²
Cyclophosphamide 200 mg/m²	Cyclophosphamide 300mg/m²
Actinomycin D 0.2 mg/m²	Actinomycin D 0.5 mg
Cisplatin 20 mg/m²	Vincristine 0.5 mg/m²

Outcome measures

Outcome measures were the percentage of patients that achieved a complete remission, defined as a normal hCG value after completion of treatment (without consolidation courses), and the median number of chemotherapy courses required

to achieve complete remission. Also, the percentage of patients with recurrent disease and the disease specific survival was registered after 24 months of follow-up from the start of treatment. The cut-off point of 24 months was chosen because the national guidelines recommend two years of follow-up after treatment for high-risk GTN, since the chance of relapsed disease is the highest within the first year of remission, and 85% of all episodes of disease relapse occur within 18 months of remission.[12;13] Short-term toxicities were registered according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Also long-term toxicities, including secondary malignancies were registered. Data on toxicity were obtained from the medical records and if used, from the Systemic Therapy Checklist designed by the EORTC.[14]

Statistical analysis

Statistical analysis was performed using SPSS software (version 18). Patient characteristics, remission rates and toxicity were compared using Chi-square test. Mean age was compared using Student's t-test. The median number of chemotherapy courses to hCG normalisation were compared using the non-parametric independent-samples median test. P values of less than 0.05 were considered statistically significant.

Results

Patient characteristics are shown in Table 3. In total, 83 patients were treated with EMACP, of which 16 patients received a modified schedule without MTX (ECAP). Patients treated with EMA/CO were significantly older (mean 32 years) than patients treated with EMACP (mean 29 years, p=0.008). The antecedent pregnancies from patients treated with EMACP were not significantly different from patients treated with EMA/CO. The indications for treatment were significantly different between patients treated with EMACP and those treated with EMA/CO (p=0.023). Patients treated with EMACP more often were treated for single-agent resistant disease (63.9%) compared to EMA/CO patients (43.7%). More patients treated with EMA/CO had primary high-risk disease (42.7%) compared to patients treated with EMACP (27.7%). Seven patients (8.4%) were treated with EMACP and 14 patients (13.6%) with EMA/CO because of disease relapse after single-agent chemotherapy. Median follow-up from the start of treatment was 33.3 months (range 3.9–246.9 months).

Acute toxicity

Table 4 shows toxicity profiles for EMACP and EMA/CO. Three patients died from sepsis related to treatment, of which one patient was treated with EMACP and two

Table 3 Patient characteristics.

	EMACP (n=83)	**EMA/CO** (n=103)	*p*
Age at diagnosis (y)			0.008
Mean (SD)	29 (6)	32 (6.2)	
Antecedent pregnancy			NS
Hydatidiform Mole	62 (74.7%)	59 (57.3%)	
Term pregnancy	17 (20.5%)	38 (36.9%)	
Non-molar abortion	2 (2.4%)	4 (3.9%)	
Uncertain	2 (2.4%)	2 (1.9%)	
Indication for treatment			0.023
Single-agent resistant disease	53 (63.9%)	45 (43.7%)	
Primary high-risk disease	23 (27.7%)	44 (42.7%)	
Disease relapse after MTX	7 (8.4%)	14 (13.6%)	

SD standard deviation
MTX Methotrexate

were treated with EMA/CO. Patients treated with EMACP more often developed fever (22.9% vs. 11.7%, p=0.041), renal toxicity (15.7% vs. 4.9%, p=0.013), nausea (48.2% vs. 28.2%, p=0.005) and diarrhoea (13.3% vs. 3.9% p=0.02) compared to patients treated with EMA/CO. Patients treated with EMA/CO more often had anaemia (28.8% vs. 7.2%, p<0.001), although the incidence of leucopenia and thrombocytopenia was not significantly different. In addition, they more often developed neuropathy (26.2% vs. 6.0%, p<0.001) and hepatotoxicity (16.5% vs. 6.0%, p=0.028).

The number of patients in whom the dose of chemotherapy had to be reduced was not significantly different between EMA/CO and EMACP (21.4% versus 27.7%), but in more patients treated with EMACP one or more courses had to be delayed due to toxicity or myelosuppression (36.1% versus 20.4%, p=0.017). In patients treated with EMACP, a total of 330 courses were administered. Patients treated with EMA/CO received a total of 688 courses. Dose reduction occurred in 39 (11.8%) of EMACP courses and in 60 (8.7%) of EMA/CO courses (mostly removal of vincristine due to neuropathy) (p=0.119). Delay of chemotherapy was required in 45 courses (13.6%) of EMACP, compared to 33 (4.8%) of EMA/CO courses that were given with delay (p<0.001).

Table 4 Toxicity profile for EMACP (n=83) and EMA/CO (n=103).

Toxicity	EMACP Number of patients (%)	EMA/CO Number of patients (%)	*P*
Myelosuppression			
Anaemia	6 (7.2%)	29 (28.2%)	<0.001
Leucopoenia	50 (60.2%)	50 (48.5%)	NS
Thrombocytopenia	6 (7.2%)	4 (3.9%)	NS
Fever	19 (22.9%)	12 (11.7%)	0.041
Neuropathy	5 (6.0%)	27 (26.2%)	<0.001
Renal toxicity	13 (15.7%)	5 (4.9%)	0.013
Hepatotoxicity	5 (6.0%)	17 (16.5%)	0.028
Gastro-intestinal			
Mucositis	16 (19.3%)	10 (9.7%)	NS
Nausea	40 (48.2%)	29 (28.2%)	0.005
Diarrhoea	11 (13.3%)	4 (3.9%)	0.02
Dose reduction	23 (27.7%)	22 (21.4%)	NS
Delay in courses	30 (36.1%)	21 (20.4%)	0.017
Secondary malignancy	3 (3.6%)	4 (3.9%)	NS
Death	1 (1.2%)	2 (1.9%)	NS

NS not significant

Late toxicity

In total, seven patients developed secondary malignancies, of whom two patients died. After EMA/CO, 4 patients developed a secondary malignancy (3.9%): 2 Acute Myeloid Leukaemia (AML), 1 patient developed Chronic Myeloid Leukaemia (CML), and 1 patient died from multiple myeloma sixteen years after treatment with EMA/CO. Three patients developed a secondary tumour after EMACP (3.6%). One developed myelodysplastic syndrome (refractory anaemia with excess blasts (RAEB)), and 2 patients developed AML, of which 1 patient died, 40 months after start of treatment. None of the patients had a cumulative etoposide dose exceeding 2g/m^2.

Response to treatment

Table 5 shows remission rates achieved with EMACP and EMA/CO for single-agent

resistant disease, primary high-risk disease, and relapsed disease after initial remission on single-agent chemotherapy. In total, 76 out of 83 patients achieved disease remission with EMACP (91.6%), compared to 88 out of 103 patients treated with EMA/CO (85.4%), which was not significantly different. Remission was achieved in 52 of 53 (98.1%) patients that were treated with EMACP after failure of initial single-agent chemotherapy, compared to 44 of 45 (97.8%) in patients treated with EMA/CO, which was not significantly different. Median number of EMACP courses to achieve normal serum hCG concentrations in these patients was 1.5 (range 1-5) compared to a median of 3 (range 1-6) courses of EMA/CO ($p=0.001$). In 78.3% (18 out of 23) and 72.7% (32 out of 44) of patients that were primarily classified as high-risk GTN, disease remission was achieved with EMACP and EMA/CO, respectively (not significant). They received a median of 3 EMACP courses (range 2–7) and 5 EMA/CO courses (range 3-13) to achieve normal serum hCG concentrations ($p<0.001$). In 85.7% (6 out of 7) of patients treated for disease relapse after single-agent chemotherapy disease remission was achieved with a median of 1 course of EMACP. Complete remission was also achieved in 85.7% (12 out of 14) of these patients treated with EMA/CO, but with a median of 2 (range 1-4) courses (not significantly different). The median duration of treatment to achieve disease remission for single-agent resistant disease, primary high risk disease and relapsed disease, respectively, is 6, 10 and 4 weeks with EMA/CO, compared to 4.5, 9 and 3 weeks with EMACP.

Of the 17 patients treated with EMACP for post-term GTN, 14 went into remission (82.4%) with a median of 3 courses. Of the 38 patients treated with EMA/CO that had an antecedent term pregnancy, 28 patients (73.7%) went in remission (data not shown). These patients received a median of 5 courses of EMA/CO to achieve normalisation of the hCG concentration (range 1-13). During follow-up, 10 patients out of 76 patients that initially went into remission with EMACP subsequently developed recurrent disease (13.2%), compared to 4 out of 88 patients that achieved disease remission with EMA/CO (4.5%, $p=0.049$).

Seven patients did not achieve disease remission with EMACP. Two patients had an additional hysterectomy and one patient was switched to EMA/CO due to severe toxicity of EMACP. One patient developed a severe sepsis and died after the first EMACP course. Another patient was wrongfully switched to EMACO due to presumed drug resistance, however, this was due to the use of another hCG assay. In 1 patient the hCG concentration was decreasing but upfront she agreed on performing a hysterectomy if after 4 courses of EMACP the hCG was not normalised. One patient initially treated with EMACP, died from metastatic disease 23 months after diagnosis. She presented with a choriocarcinoma after a term pregnancy with metastases to liver and lungs, and was treated with extensive chemotherapy and radiotherapy but subsequently developed bone and brain metastases.

Table 5 Remission rates in patients treated with EMACP and EMA/CO for single-agent resistant disease, primary high-risk disease and relapsed disease after initial remission on single-agent chemotherapy.

	EMACP			EMA/CO		
Treatment indication	n	CRR (%)	Number of courses to disease remission (median)	n	CRR (%)	Number of courses to disease remission (median)
Single-agent resistant disease	53	52 (98.1%)	1.5 (4.5 weeks)	45	44 (97.8%)	3 (6 weeks)
Primary high-risk disease	23	18 (78.3%)	3 (9 weeks)	44	32 (72.7%)	5 (10 weeks)
Disease relapse after MTX	7	6 (85.7%)	1 (3 weeks)	14	12 (85.7%)	2 (4 weeks)
Total	**83**	**76 (91.6%)**		**103**	**88 (85.4%)**	

CRR complete remission rates

Fifteen patients did not achieve disease remission with EMA/CO. Seven patients were switched to a platinum-containing chemotherapy regimen. Five patients were treated with a platinum-containing chemotherapy regimen and hysterectomy. Two patients died during EMA/CO due to sepsis, and 1 patient was cured with a hysterectomy only.

Five patients died from GTN or the given treatment within the 24 months of follow-up. Disease specific survival of EMACP patients was 96.4% (80 out of 83) and 98.1% (101 out of 103) of patients initially treated with EMA/CO.

Discussion

The present study showed that 91.6% of patients treated with EMACP achieved disease remission compared to 85.4% of patients treated with EMA/CO. Patients treated for primary high-risk disease, single-agent resistant disease and relapsed disease all required less courses of EMACP to achieve normalisation of serum hCG compared to patients treated with EMA/CO. Patients treated with EMACP more often developed fever, renal toxicity and gastro-intestinal toxicity, and significantly more courses had to be delayed compared to EMA/CO. However, patients treated with EMA/CO more often had anaemia, neuropathy and hepatotoxicity. The number of patients that died from sepsis during treatment and of patients that developed secondary tumours was comparable in EMA/CO en EMACP.

In previous studies evaluating EMA/CO combination chemotherapy, a remission rate of 69% to 86% was obtained.[8-10;15-18] The disease specific survival rate varies from 85% to 90.9%. [8;9;16;17] The remission rate and disease specific survival of all EMA/CO patients in our study were 85.4% and 98.1%, respectively. In the present study more patients treated with EMA/CO had primary high-risk disease compared to patients treated with EMACP. Remission rates were higher in patients who received prior single-agent chemotherapy compared to patients that received EMA/CO as first treatment for primary high-risk disease (97.8% versus 72.7% respectively). For comparison, Escobar et al. found that the complete response to EMA/CO was higher in patients who received EMA/CO as primary treatment (76%) than in patients who were treated secondarily with EMA/CO (65%).[18] In contrast, Bower et al. reported that the complete remission rate after EMA/CO was not different if women received prior chemotherapy (78% and 79% for first and second line treatment).[8] Lu et al. also found the same remission rates in patients receiving EMA/CO as first-line treatment and as secondary treatment (77.8%).[16] The differences found in our study might result from a difference in prior chemotherapy. Patients that received prior chemotherapy in the studies by Bower et al. and Lu et al. had received either single-agent MTX or combination chemotherapy, whereas all patients in our study only received previous single-agent MTX or actinomycin D.

Although less courses of EMACP than EMA/CO were needed to achieve normal serum hCG, EMACP is a three-weekly schedule whereas EMA/CO is a two-weekly schedule. Still, the duration of chemotherapeutic treatment, although minimal, is shorter with EMACP. However, each course of EMA/CO requires only one day of hospital admittance every two weeks for the administration of the EMA part of the course, since the CO part on day 8 can be given in the outpatient clinic. In contrast, administration of the EMACP schedule requires five days of hospital admittance (day 1 to 5) every three weeks, which is a higher burden for the patient and is less cost-effective. In this respect, EMA/CO therefore seems preferable over EMACP. If the difference in number of consolidation courses would be included, patients treated with EMACP, who received two consolidation courses, would have received 3.5, 5 and 3 courses for single-agent resistant disease, primary high risk disease and relapsed disease, respectively, compared to 6, 8 and 5 courses of EMA/CO, since for the EMA/CO regimen three consolidation courses are advised. However, significantly more patients developed recurrent disease after EMACP (13.2%) than after EMA/CO (4.5%), which may warrant the need for three courses of consolidation therapy of EMACP to obtain sustained remission.

The relatively high risk of developing secondary malignancy found is most likely brought about by etoposide.[19] Perdersen-Bjergaard et al. reported on 5 patients with leukemic complications among 82 patients who received a cumulative dose of more than 2 g/m^2 etoposide, whereas no leukaemia's were observed among 130 patients who had received up to 2 g/m^2.[20;21] Each course of EMA/CO contains 200 mg/m^2 of etoposide compared to 500 mg/m^2 in the EMACP schedule. Therefore the cumulative dose of 2g/m^2 is reached after four courses of EMACP and only after ten courses of EMA/CO. All three patients that developed secondary tumours after EMACP received four courses of EMACP. Of the four patients who developed secondary malignancies after EMA/CO, in none of the patients this dose was reached. However, more recent reports suggest that the risk estimate after moderate cumulative doses (1.5-3 g/m^2) was virtually identical to the risk estimate for patients who received etoposide at a dose of less than 2.0g/m^2.[22] Etoposide is a cell-cycle specific agent, and large differences have been reported for different schedules. A schedule of five consecutive daily infusions is much more active than 24-h infusion of the same total dose.[23] Similarly, increased leukemogenicity was suggested for these intermittent administration schedules.

Cisplatin has previously been identified as an active agent for high-risk GTN.[24] Currently, it is used in patients who have failed initial combination chemotherapy in particular, such as the EMA/EP regimen, substituting etoposide and cisplatin for cyclophosphamide and vincristine in the EMA/CO protocol. EP and EMA are alternated at weekly intervals.[24] In contrast to EMACP, EMA/EP does not contain cyclophosphamide. In addition, EMA/EP contains a cumulative dose of 250 mg/m^2 etoposide in

each course, compared to 500 mg/m^2 in the EMACP schedule. However, EMA/EP contains 75 mg/m^2 cisplatin in each course compared to 60 mg/m^2 cisplatin in the EMACP schedule. Previously, Newlands et al. reported significant toxicity of EMA/EP chemotherapy in patients who have relapsed after or who have become refractory to EMA/CO, with patients suffering from neutropenia (68%), thrombocytopenia (40%), anaemia (21%) and renal toxicity (41%). Myelosuppression caused delays in chemotherapy in 88% of EMA/EP patients and 38% of patients required dose reductions.[25] Mao et al. reported myelosuppression and gastro-intestinal problems as the main adverse effects in 18 patients treated with 74 cycles of EMA/EP. Because of myelosuppression and hepatotoxicity, 32 courses (43.2%) were delayed.[26] This is a much higher toxicity than the observed toxicity of EMACP in our study. Adding cisplatin after previous multi-agent chemotherapy leads to an impaired bone marrow reserve. It might therefore be advantageous to start chemotherapy with cisplatin earlier in the course of treatment.

In conclusion, EMACP combination chemotherapy is an effective treatment for high-risk GTN. The remission rate of EMACP was comparable to EMA/CO, and although less courses of EMACP were required to achieve disease remission, the difference in duration of chemotherapeutic treatment is only slightly shorter with EMACP. Short term toxicity was alternating different between EMACP and EMA/CO, but overall not significantly more common in either of them, whereas long-term toxicity was not different between both regimens. Cisplatin-based chemotherapy in the form of EMACP in this study was not proven more effective than EMA/CO. From the results of this study no arguments could be found to change the current standard with EMACO.

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Human chorionic gonadotropin (hCG) regression normograms for patients with high-risk gestational trophoblastic neoplasia treated with EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine) chemotherapy

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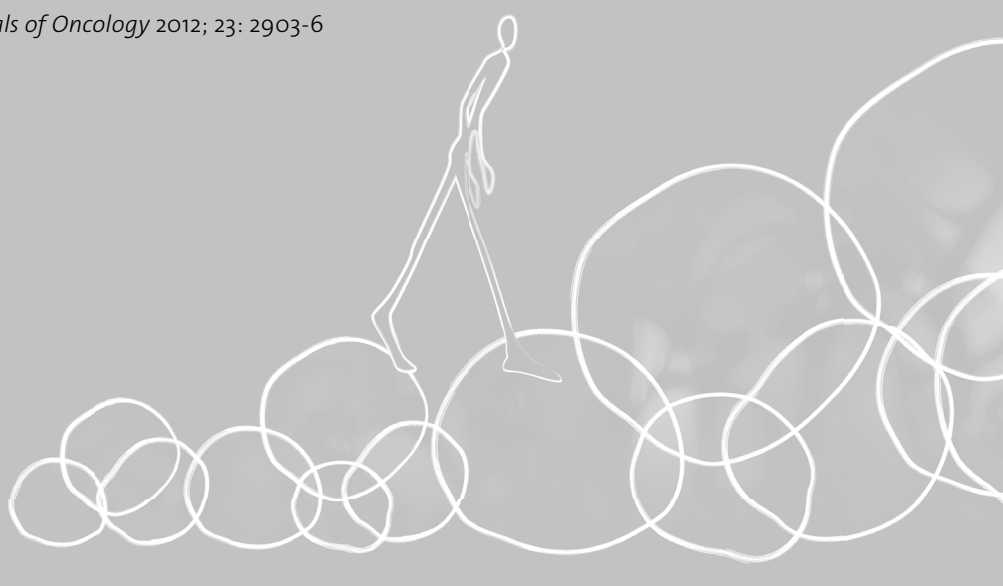
Annals of Oncology 2012; 23: 2903-6



Abstract

Background We present normograms for human chorionic gonadotropin (hCG) regression in patients with high-risk gestational trophoblastic neoplasia (GTN) successfully treated with multi-agent chemotherapy in order to predict treatment resistance.

Methods We collected data for 46 patients with high-risk GTN treated with EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine) who had hCG values available. Patients were classified as having methotrexate (MTX)-resistant disease (n=22) or primary high-risk disease (n=24). The 10th, 50th and 90th percentiles of the hCG before every chemotherapy course were calculated and plotted into normograms.

Results Half of the patients treated for MTX-resistant disease and primary high-risk disease had normal hCG levels before the third and sixth course of chemotherapy, respectively. In patients with MTX-resistant disease, the 90th percentile line fell below normal before the start of the fourth course, whereas in patients with primary high-risk disease this was not the case until the eighth course of chemotherapy.

Conclusion Resistance to EMA/CO treatment for high-risk GTN, as illustrated by examples, could be predicted using normograms for hCG resistance. Normograms differed depending on the indication for multi-agent chemotherapy due to much higher initial hCG values in patients with primary high-risk disease compared with those treated for MTX-resistant disease.

Introduction

Gestational trophoblastic neoplasia (GTN) represents a unique group of tumours which includes invasive mole, choriocarcinoma, placental-site trophoblastic tumour (PSTT) and epithelioid trophoblastic tumour. GTN may arise in association with any pregnancy event, although ~50% are diagnosed after a hydatidiform mole. Patients with GTN are classified as having low-risk or high-risk disease using the modified World Health Organisation prognostic scoring system as adapted by the International Federation of Gynaecology and Obstetrics.[1] Patients scoring ff7 are considered to be at high risk of developing resistance to single-agent methotrexate (MTX), and are therefore treated with multi-agent chemotherapy. Worldwide, the most frequently used chemotherapy is EMA/CO (etoposide, MTX and actinomycin D, alternated weekly with cyclophosphamide and vincristine). The remission rate of EMA/CO ranges from 71% to 86%.[2-5] Patients who progress during the EMA/CO therapy have a poor outcome with a reported 5-year survival of only 43% (95% confidence interval (CI) 12%-73%).[6]

Currently an international definition of resistance to chemotherapy is lacking. Previously, van Trommel *et al*. developed a normogram for the regression of human chorionic gonadotropin (hCG) concentrations during the treatment of low-risk GTN with MTX to identify resistance to MTX at an early stage.[7] This normogram allows the identification of 50% of patients needing alternative therapy with a specificity of 97.5% before the fourth course of MTX. The early identification of patients resistant to multi-agent chemotherapy is even more important, since these patients are at greater risk of a fatal outcome. For these patients, treatment options are limited. Salvage chemotherapy with etoposide and platinum drug regimens often combined with surgical excision of persistent tumour will result in remission in 70%-88% of patients resistant to EMA/CO.[2;8-11] We aimed at constructing normograms for hCG regression in patients with high-risk GTN successfully treated with EMA/CO chemotherapy in order to predict patients developing resistance to EMA/CO in an early phase of their treatment.

Methods

Patients

In the Netherlands, patients with gestational trophoblastic disease are registered voluntarily at the Dutch Central Registry for Hydatidiform Moles (DCRHM), which also provides a national hCG assay service to gynaecologists. A total of 4,190 patients were registered at the DCRHM from 1977 until 2012. Furthermore, patients with high-risk GTN are presented at the Dutch Working Party on Trophoblastic Disease.

Since 1983 in the Netherlands, a clinical classification system proposed by the Dutch Working Party has been used which defines high-risk disease as the presence of one or more of the following features: insufficient response to single-agent chemotherapy; metastasis in more than one organ; metastasis in liver, spleen, kidneys, gastro-intestinal tract, bones or brain; antecedent term pregnancy and an interval of >12 months between the end of the antecedent pregnancy and the start of treatment.[12]

Data for all patients with high-risk GTN according to Dutch guidelines and treated with EMA/CO chemotherapy were collected from the databases of the DCRHM and the Dutch Working Party and included in the study. Patients receiving EMA/CO for recurrent disease were excluded. In addition, patients for whom the serum hCG values during EMA/CO treatment were unavailable or measured on an assay other than the in-house developed radioimmunoassay (RIA) of the assay service of the DCRHM were excluded from further analysis. Eventually, a total number of 46 patients treated with EMA/CO and with available hCG values were included in this study.

Based on the indication for treatment with multi-agent chemotherapy, patients were classified as having single-agent MTX-resistant disease (n=22) or as having a high prognostic score at the time of diagnosis of GTN (n=24). MTX resistant disease was defined as a rise or plateau in the hCG concentration during treatment with MTX for low-risk disease. Three patients with primary high-risk disease were treated with a hysterectomy before EMA/CO treatment, and one patient had a hysterectomy during EMA/CO treatment since she no longer wished to conceive. Separate normograms were constructed for patients receiving multi-agent chemotherapy due to MTX resistance and patients treated for primary high-risk disease at diagnosis of GTN.

hCG measurements

All hCG concentrations were measured using an in-house developed RIA based on polyclonal antibody raised in rabbits.[13] This assay detects intact hCG and free β-subunit: hCG+hCGβ. A highly purified hCG β-subunit preparation labelled with iodine-125 was employed as a tracer for this assay. The RIA was calibrated with the third International Standard Preparations for intact hCG and hCG β-subunit. A value of 2 ng/ml is considered normal. hCG concentrations that were measured in serum obtained before the start of a chemotherapy course were used in the construction of the normogram. However, hCG concentrations that were measured in serum obtained during a chemotherapy course were excluded.

Statistics

All hCG measurements of the patients reaching complete remission after multi-agent chemotherapy without recurrent disease were used to construct the normograms

for MTX-resistant disease and for primary high-risk GTN. Log transformation was carried out on all hCG values to obtain normal distribution of the data. The hCG values were evaluated cross-sectionally for each chemotherapy course. The normograms were based on a minimum of 10 log-transformed hCG values for each chemotherapy course. Serum hCG concentrations were subsequently sorted for each chemotherapy course. The 10th, 50th and 90th percentiles for every chemotherapy course were calculated and plotted for each course in a normogram using Excel (Microsoft). Resistance to EMA/CO according to the normogram was defined as the hCG concentration exceeding the 90th percentile of the normogram. Statistical analysis was carried out using SPSS 18 software. Serum hCG concentrations before the first course of EMA/CO and the number of chemotherapy courses were compared using the Mann- Whitney U test.

Results

The characteristics of patients with MTX-resistant disease and patients with primary high-risk disease are shown in Table 1. The median follow-up was 26.1 months for patients with MTX-resistant disease and 26.0 months for patients with primary high-risk disease. No patients who achieved normal hCG concentrations with EMA/CO chemotherapy relapsed during follow-up.

The normogram for patients treated with EMA/CO for MTX-resistant disease (n=22) is shown in Figure 1(a). The median hCG concentration before the onset of the first chemotherapy course was 21 ng/ml (range 3-1300 ng/ml), and before the third EMA/CO course half of the patients had normal hCG levels. The 90th percentile was at 148 ng/ml before the start of treatment. Ninety percent of the patients with MTX-resistant disease had normal hCG values before the start of the fourth EMA/CO course. The 10th percentile is just above normal (3 ng/ml) before the start of the first course but decreases to normal levels before the second course.

The normogram for patients treated with EMA/CO for primary high-risk disease is shown in Figure 1(b). The 90th percentile before the start of treatment was 32,781 ng/ml. In 90% of patients, hCG concentrations regressed to normal before commencing the eighth course of EMA/CO. The median hCG level was 7059 ng/ml (range 300-60,000 ng/ml) at the start of the first course, and after five EMA/CO courses 50% of patients had a normal hCG concentration. The 10th percentile line started at an hCG concentration of 1520 ng/ml, and ten percent of patients showed disease remission before the fourth course.

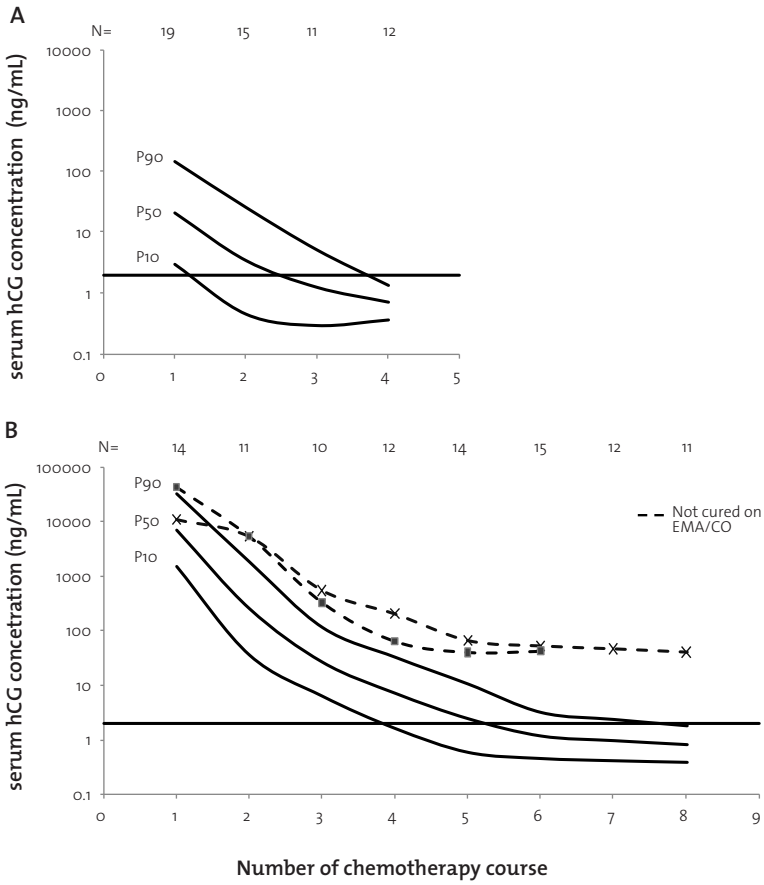
Table 1 Patient characteristics.

	Methotrexate (MTX)-resistant disease (n=22)	**Primary high-risk disease** (n=24)
Age (years)		
Median (range)	31 (23-43)	29 (22-54)
Antecedent pregnancy		
Hydatidiform mole	22 (100%)	3 (12.5%)
Term	0 (0%)	20 (83.3%)
Non-molar abortion	0 (0%)	1 (4.2%)
Site of metastases**		
None	18 (81.8%)	8 (33.3%)
Lungs	3 (13.6%)	15 (62.5%)
Vagina	0 (0.0%)	4 (16.7%)
Liver	1* (4.6%)	0 (0.0%)
Brain	0 (0.0%)	2 (8.3%)
Interval from index pregnancy to start EMA/CO treatment (months)		
<4	8 (36.4%)	16 (66.7%)
4-6	10 (45.4%)	2 (8.3%)
7-12	4 (18.2%)	1 (4.2%)
>12	0 (0.0%)	5 (20.8%)
Serum hCG before start EMA/CO treatment (ng/ml)		
Median (range)	21 (3-1300)	7059 (300-60,000)

*Dubious lesion on computed tomography scan
** Some patients had metastases at several sites

The median serum hCG concentration before the first course of EMA/CO was significantly less elevated (21 ng/ml) in the group of patients with MTX-resistant disease (n=19) compared with the group of patients with primary high-risk disease (7059 ng/ml, n=14) (p<0.001). Significantly fewer courses of EMA/CO were administered to the group of patients with MTX-resistant disease (n=22) (median 5 courses; range 2-10) compared with the primary high-risk patients (n=22) (median 8 courses; range 5-12; p<0.001).

Figure 1 Normogram for patients treated with EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine) chemotherapy for methotrexate (MTX)-resistant disease (**A**) and for primary high-risk disease (**B**). The lower, middle and upper lines indicate the 10th, 50th and 90th percentiles. The number of hCG values the percentiles were calculated from is shown above each course. Patients not cured on EMA/CO are plotted.



Two primary high-risk patients failed to achieve normal hCG concentrations with EMA/CO. In one patient, there was a plateau of serum hCG after five courses of EMA/CO. Subsequent chemotherapy consisted of three courses of EMA/EP, one course of VIP (etoposide, ifosfamide and cisplatin) and salvage surgery consisting of a hysterectomy and surgical excision of pulmonary metastases. Her hCG level subsequently regressed to normal. The hCG concentration at the onset of EMA/CO

was between the 50th and 90th percentile line, but the level exceeded the 90th percentile before the second course. The other patient also received five courses of EMA/CO, after which a plateau of serum hCG developed. Treatment was continued with four courses of VIP and a hysterectomy, after which the hCG level was normal. The serum hCG level in this patient exceeded the 90th percentile already before the start of treatment.

Discussion

In the present study, we constructed two normograms for the prediction of resistance to EMA/CO chemotherapy. As shown, normograms for hCG regression during multi-agent treatment differed depending on the indication for multi-agent chemotherapy. The initial hCG concentrations in the group of patients with primary high-risk disease are much higher due to the higher tumour load compared with those in the MTX-resistant group of patients, who already had tumour regression with MTX and subsequent decrease of the hCG concentration. It is therefore important that hCG concentrations of patients with MTX-resistant disease and patients with primary high-risk disease are not combined into one normogram.

Previous normograms for the prediction of postmolar GTN and for the prediction of resistance to MTX chemotherapy used upper percentiles of p95, p97.5 and p99, respectively.[7;14;15] Early prediction of patients who will not be cured by multi-agent chemotherapy is of utmost importance to avoid fatal outcomes by an early change to a platinum-containing regimen (usually EMA-EP). Therefore, the 90th percentile was chosen as the upper line of the normogram. We feel that early prediction of a larger portion of patients not showing disease remission on EMA/CO outweighs the fact that more people will unnecessarily be treated with more toxic platinum-containing chemotherapy regimens. Patients with an hCG level >90th percentile of the normogram before the start of EMA/CO might be considered to start a platinum-containing chemotherapy regimen.

We presented for the first time a normogram for hCG regression in patients with high-risk GTN successfully treated with EMA/CO chemotherapy in order to predict treatment resistance. In addition, we demonstrated examples of two patients who could have been predicted early as developing resistance to treatment. We showed that normograms for hCG regression differed depending on the indication for multi-agent chemotherapy due to higher hCG values in the group of patients with primary high-risk disease compared with those with MTX-resistant disease. Our findings need to be validated in a larger cohort of patients.

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8

General discussion

The main aim of the present thesis was to further optimise the management of patients with GTN by attempting to predict post-molar GTN and resistance to multi-agent chemotherapy.

Implications for the management of GTN

Staging: increasing role for antecedent term pregnancy

The current FIGO 2000 staging and risk factor scoring system results from a merge of the basic anatomic FIGO staging from 1992 and the risk factor scoring system of the World Health Organisation (WHO).[1-3] The latter had been adopted by the WHO in 1982 from the earlier system invented by Bagshawe.[4] Unlike the FIGO 2000 scoring system, the Dutch classification system does not include scorings for age, pre-treatment serum hCG concentration, the number of metastases and largest tumour size.[3,5] In contrast to the FIGO system, the Dutch classification system uses a number of absolute criteria and attaches more importance to a term pregnancy as the index pregnancy. This risk factor in itself has sufficient weight to classify the patient as having high-risk disease, whereas in the FIGO scoring system, only two points are allocated to patients with a term antecedent pregnancy. Since high-risk disease is defined as a score of ff17, these patients might still be classified as having low-risk GTN. In 1978, Hammond was the first to propose an antecedent term pregnancy as a poor prognostic indicator and subsequently added this into a clinical classification system.[6,7] He showed that 47% (7 out of 15) of patients with high-risk metastatic GTN with a post-term gestation were cured compared to 75% (48 of 63; $p<0.05$) of other patients with high-risk metastatic disease.[7] Lurain *et al.* also showed that GTN after term pregnancy, abortion or ectopic pregnancy had a significantly lower cure rate than after HM (64% vs. 84%, respectively).[8] In Chapter 5 we reported on 26 patients who died from GTN, of whom 19 patients were found to have a post-term choriocarcinoma. In our study, two patients with post-term GTN would have been classified as low-risk according to the FIGO 2000 criteria, and would subsequently have been treated with single-agent MTX. Lok *et al.* reported on MTX resistance in 75% of patients with post-term choriocarcinoma primarily treated with MTX. A subgroup of patients in which single-agent chemotherapy would be a safe alternative could not be determined. Additionally, an overall survival rate of 85% was found in patients with post-term choriocarcinoma, which is comparable to survival rates of high-risk disease in general.[9] In chapter 6 we showed that 14 out of the 17 patients treated with EMACP for post-term GTN went into remission (82.4%), and 28 out of the 38 patients with an antecedent term pregnancy (73.7%) treated with EMA/CO. This was lower than the overall disease remission rate that was observed with EMACP (91.6%) and EMA/CO (85.4%). We propose an increasing role for term

antecedent pregnancy in the FIGO prognostic scoring system. This might be manifested by allotting four points instead of the two points that are currently allotted to patients with a previous term pregnancy.

Low-risk GTN: three consolidation courses of MTX

Regarding the management of low-risk patients with two or three consolidation courses of MTX (Chapter 4), we reported that 4.0% of patients relapsed after MTX treatment with three consolidation courses after normalisation of hCG levels, whereas 8.3% of patients relapsed after MTX treatment with two consolidation courses. Although other factors might have influenced the difference in relapse rates found in this study, we stated that three courses of consolidation chemotherapy may be preferable to two in the treatment of low-risk GTN in order to decrease the risk of development of disease relapse. Obviously, a randomised prospective study to confirm our findings would be desirable. In general, caution needs to be exercised with retrospective studies because bias is more common than in prospective studies, and it may not always be possible to retrieve information on all parameters. However, retrospective studies have the ability to address research questions on diseases of low incidence, which would require extremely large cohorts in prospective studies and thus require a long time to complete. Therefore our results provide the best available data regarding the possible need for three as opposed to two courses of consolidation therapy in this disease setting. Results of our study were presented to the Dutch Working Party on Trophoblastic Disease and we proposed to alter the national guideline concerning the management of low-risk GTN from two to three consolidation courses of MTX after normalisation of the serum hCG concentration.

High-risk GTN: use of normograms for prediction of resistance to chemotherapy

Previously, our group developed a normogram for the regression of hCG concentrations during the treatment of low-risk GTN with MTX to identify resistance to MTX in an early stage.[10] This normogram has now been adopted by the Dutch Working Party for Trophoblastic Disease and is used by the advisory body of the Working party in their consultation on patients that are presented to them from throughout the country. Early identification of patients resistant to multi-agent chemotherapy seems even more important, since these patients are at risk for a fatal outcome, and further treatment options are limited. In Chapter 7 we constructed two normograms for the prediction of resistance to EMA/CO chemotherapy and demonstrated examples of patients that could in an early stage have been predicted as developing resistance to treatment. In addition, we showed that normograms for hCG regression under multi-agent treatment differed depending on the indication for multi-agent chemotherapy, with much higher initial hCG values in the group of patients with primary

high-risk disease compared to those treated with EMA/CO for MTX resistant disease. These normograms still need to be validated in a larger cohort of patients. Subsequently, these normograms can be of great value in the decision making of patients treated with EMA/CO chemotherapy, bringing forward the vitally important switch to EMA/EP chemotherapy in patients that will not be cured with EMA/CO. However, the normograms should only be used by gynaecologists experienced in the field of GTN.

Adjustments in the organisation of management of GTN

Centralisation

Besides improving the management of GTN by scientific research, much can be gained by adjustments in the organisation of management of GTN and GTD in general. Since the incidence of GTD is low, most general pathologists, gynaecologists and medical oncologists do not see many patients with GTN, which makes it difficult to build up expertise in this particular disease. We propose future referral of all hydatidiform moles to one of four yet to be assigned referral centres for further evaluation and determination of management in case of development of low-risk GTN. High-risk GTN patients would preferably have to be referred to one referral hospital. This is somewhat similar to the organisation of care of patients with GTD in the UK, where the first national service for GTD was established through a combined agreement of the Royal College of Obstetricians and Gynaecologists and the Department of Health's National Commissioning Group. Since 1973, all women with hydatidiform mole or other forms of GTD have been registered with one of three centres for hCG monitoring and, if subsequent treatment is necessary, they are treated at the Sheffield Trophoblastic Disease Centre (Sheffield, UK) or Charing Cross Hospital Trophoblast Disease Centre (London, UK).[11]

Uniformity in scoring systems

To facilitate and improve the comparison of data from various hospitals and various countries, different scoring systems to classify GTN should be harmonised.[12] Comparison of results between countries or to international literature is difficult due to the use of a classification system in the Netherlands that is different from the FIGO prognostic scoring system. Ngan previously stated that all physicians treating GTN should use the FIGO 2000 scoring system, so that data can be compared among different centres.[13] Retrospective allocation of the FIGO score is often difficult. Since the Dutch classification system does not include all items that are included in the FIGO prognostic scoring system, not all items required to retrospectively determine the FIGO score are recorded for the Dutch patients. Often data on the items 'number of metastases' and 'largest tumour size' cannot be retrieved. In 1986, Dijkema *et al.*

made a comparison of the ability of the Dutch classification and the scoring systems developed by Bagshawe and Goldstein to predict the effect of primary treatment with MTX (n=37).[14] They found there was hardly any difference in specificity and prognostic value between the Bagshawe score and the Dutch classification, but a somewhat lower sensitivity in the Dutch classification system was observed. This illustrates the need for uniformity in application of a scoring system. In the Netherlands we should adopt the internationally used FIGO scoring system. Preferably this would be a next (modified) version of the scoring system with a more prominent role assigned to an antecedent term pregnancy as a poor prognostic factor. This would simplify the comparison of patients using FIGO scores, which is of great importance in diseases with a low incidence such as GTD to enable large multi-centre studies with sufficient numbers of patients.

More uniform hCG measurement

Centralisation of care might result in more adherence to the guidelines in the management of patients and clinical expertise can be extended. In addition, it would dramatically reduce the number of different hCG assays used leading to more uniformity in the hCG measurements. As stated previously, the Nijmegen radioimmunoassay (RIA) detects intact hCG and free-beta subunit.[15] Most laboratories use sandwich-type assays as this type of assay can be performed automatically on a random access platform and is thus less labour-intensive, facilitating a high throughput of samples. Sandwich assays in general are highly specific and less molecular forms of hCG are detected than with a RIA. In patients with GTD, the predominant molecular form(s) of hCG present in blood might be different from the intact hCG molecule. Therefore, it is highly recommended to use an hCG assay that detects different/most forms of hCG for diagnosis and follow-up of GTD patients.[16]

International collaboration

On an international level, in 2009 the European Organisation for the Treatment of Trophoblastic Disease (EOTTD) was founded in order to improve management and subsequent registration of GTD in Europe.[17] In May 2010 the first meeting of the EOTTD was held in Lyon where over 50 gynaecologists, oncologists and clinical chemists where gathered. An inventory was made of the current organisation of care around GTD patients in different European countries, including the estimated disease incidence, registration systems and management of GTD. In addition, an agreement was signed to underline the importance of raising attention for GTD. Participants from 15 different countries reached a consensus on the need for education, taking steps toward centralisation of clinical management and registration of GTD cases in every participating country. To accomplish this, one of the goals of the EOTTD is to connect a gynaecologist, oncologist and expert

pathologist on gestational trophoblastic disease in every country, in order to be able to build clinical expertise on GTD, which would further improve outcomes in the management of the disease. In the long run, this would ideally also lead to uniformity in the type of hCG assay used by laboratories in these countries. An external quality assessments program should become available to harmonise the hCG assay results. A working party on hCG with prominent members was established to formulate further advice on the preferred hCG assay.

Better registration for research

Currently, registration in the Dutch Central Registry for Hydatidiform Moles (DCRHM) is not obligatory and therefore not all patients in the Netherlands will be registered. Due to the possibility of an underestimation of the number of patients, to establish the incidence of GTD in the Netherlands (Chapter 2) we used PALGA (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief), a unique nationwide network and archive that has complete coverage of all histopathological and cyto-pathological diagnoses made in the Netherlands.[18] Also, from many patients registered in the DCRHM no serum hCG concentrations are available obtained using the hCG RIA of the central laboratory of the working party. In developing the models for prediction of post-molar GTN (Chapter 3) and prediction of resistance to multi-agent chemotherapy in high-risk patients (Chapter 7), this resulted in small numbers of patients eligible for the study. Centralisation of care would facilitate and simplify a more complete registration of GTD patients and collection of serum and tissue for research purposes.

Future research

The findings in this thesis offer new possibilities for further research. First, to gather support for a more uniform classification of patients diagnosed with GTN, for instance by application of the FIGO 2000 classification system instead of the Dutch classification system, it would be of high interest to note in what proportion of patients treatment would be different using one classification system or the other. The collection of patients that would be classified and thus treated differently according to the FIGO 2000 system compared to the current Dutch classification system, and an analysis of the outcome of these patients would be valuable. This might also provide a solid ground for our proposal to attach more importance to an antecedent term pregnancy in the next version of the FIGO scoring system.

Secondly, up to now most of our efforts to predict GTN at an early stage of the disease has focused on serum hCG as a tumour marker.[19-21] It is well known that complete and partial hydatidiform moles are genetically distinct, but both

overexpress paternal genes. Clues to the causative genes in GTD have come from linkage studies in families with repetitive molar pregnancies, in which the mole is biparental in origin but pathologically indistinguishable from other complete HMs.[22-25] In these rare cases, mutations of the NLRP7 gene on chromosome 19 are thought to cause the disease in some patients. However, since not all familial cases could be explained by a mutation in this gene, other/more genes are probably involved. Besides using genetics in trying to understand more about the origin of HM, it would be interesting to explore possibilities of using genetics in the prediction of GTN.

In Chapter 6 we compared the efficiency, toxicity and survival rate of EMA/CO and EMACP in the treatment of high-risk GTN. Future chemotherapeutic agents might be found in the group of taxanes, such as paclitaxel and docetaxel.[26-28] Previous literature suggests that paclitaxel contributes significantly to the treatment of choriocarcinoma, mostly in the setting of tumours resistant to second-line EMA/EP therapy.[26;28] The integration of agents such as paclitaxel has not yet been subject to a randomised trial. As previously stated, randomised controlled trials can only be realised by means of international collaboration and equal risk classification of patients to ensure sufficient numbers of patients. Until then, a description of a large series of patients treated with a regimen containing paclitaxel or docetaxel would provide us with further information on the value of these agents in the management of GTN.

Finally, external validation of the normograms for patients with high-risk GTN (Chapter 7) in a larger cohort of patients is required before these tools can be applied in clinical practice. More patients that showed progression on EMA/CO are needed to investigate what proportion of patients not showing disease remission on EMA/CO can be predicted in an early phase of their treatment.

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9

Summary

Samenvatting

Summary

Chapter 1 describes the background and introduces research parameters of the studies performed within this thesis. Gestational trophoblastic neoplasia (GTN) represents the malignant forms of gestational trophoblastic disease (GTD), and comprises invasive hydatidiform moles that do not resolve spontaneously, choriocarcinoma, placental site trophoblastic tumour (PSTT) and the rare epithelioid trophoblastic tumour (ETT). Once the diagnosis of GTN is made, thorough assessment of the extent of disease as well as an evaluation for risk factors should be performed, in order to classify patients into low-risk or high-risk disease. Treatment of low-risk disease consists of single-agent chemotherapy, mostly methotrexate (MTX). High-risk patients are considered to be at high risk of developing resistance to single-agent MTX, and are therefore treated with multi-agent chemotherapy, mostly EMA/CO (etoposide, MTX and actinomycin D, alternating with cyclophosphamide and vincristine). Research parameters in this thesis include disease incidence, prediction of post-molar GTN and resistance to multi-agent chemotherapy, and evaluation of the treatment of low- and high-risk GTN.

The reported incidence of GTD varies widely in different regions of the world. In **chapter 2** we determined the incidence and time trends of GTD in the Netherlands using population-based data obtained from PALGA, a national archive containing all histopathology reports in the Netherlands. Overall incidence rates of hydatidiform mole (HM), choriocarcinoma and PSTT were 1.34 per 1,000 deliveries, 3.1 per 100,000 deliveries, and 1.0 per 100,000 deliveries, respectively. The incidence of GTD in the Netherlands increased significantly from 1995 to 2008. This can partially be explained by increased maternal age and increased proportion of live births of Asian descent. Part of the increase might result from improved diagnostic techniques. However, these factors do not seem to account for the total observed increase and part of the increase therefore remains unexplained.

Currently, serum human chorionic gonadotrophin (hCG) follow-up after evacuation of hydatidiform moles is essential to identify patients requiring chemotherapeutic treatment. **Chapter 3** described a model based on linear regression of post-evacuation serum hCG concentrations for the prediction of post-molar GTN. The slopes of the linear regression lines of the first three log-transformed serum hCG and free beta hCG values were calculated. Receiver operating characteristic curves were constructed to calculate Areas Under Curve (AUCs). The slope of free beta hCG seems to be a better predictor for post-molar GTN than the slope of hCG. Post-molar GTN could be predicted in 69% of patients at 97.5% specificity. Thirty-eight percent of post-molar GTN patients could be predicted prior to diagnosis according to the FIGO 2000 criteria. Although this model needs further validation for different assays, it seems a promising way to predict the more aggressive cases of post-molar GTN.

MTX alternating with folinic acid is a commonly used treatment regimen for low-risk GTN. In the Netherlands, two courses of MTX are administered after normalisation of serum hCG levels (consolidation courses), whereas in the United Kingdom, three consolidation courses are given. In **chapter 4** we compared in a retrospective setting relapse rates of women completing MTX therapy for low-risk GTN in the Netherlands and the UK. In 4.0% of patients relapse occurred after MTX treatment with three consolidation courses, whereas 8.3% of patients relapsed after MTX treatment with two consolidation courses (p=0.006). Although patients from the Netherlands had a higher level of hCG and more patients had metastases before the start of treatment, the number of courses of MTX to achieve a normal hCG did not differ significantly between patients from the Netherlands and the UK. We concluded that although other factors might have influenced the observed difference in relapse rates, three courses of consolidation chemotherapy may be preferable to two in the treatment of low-risk GTN in order to decrease the risk of disease relapse. A prospective randomised study would be required to confirm these findings.

In **chapter 5** we described fatal cases of GTN over four decades in the Netherlands and evaluated whether treatment was given according to the protocol at the time of diagnosis and aimed to reveal possible implications for future management. Data from patients who died from GTN from 1971 to 2011 were obtained from the DCRHM and the Dutch Working Party on Trophoblastic Disease. Twenty-six patients died from GTN. In five cases GTN developed after a hydatidiform mole and in 19 cases GTN occurred following term pregnancy. Half the number of patients died between 1971-1980 (n=13) when patients were not yet classified as having low- or high-risk disease and were thus not yet treated accordingly. The yearly number of patients that died from GTN decreased considerably over the last four decades. Twelve out of 17 patients who were initially treated with MTX had high-risk disease according to the current Dutch risk classification and should therefore have received combination chemotherapy. We concluded that appropriate risk classification is essential to start optimal initial therapy and to prevent therapy resistance. Patients with post-term choriocarcinoma represented a large proportion of the deceased patients and we proposed that these patients, as in Dutch classification system, are considered as having high-risk disease.

In **chapter 6** we evaluated the efficacy and safety of cisplatin-based combination chemotherapy (etoposide, MTX, actinomycin D, cyclophosphamide and cisplatin; EMACP) compared to the EMA/CO schedule for the treatment of high-risk GTN. 84 patients treated with 330 courses of EMACP and 103 patients treated with 688 courses of EMA/CO were identified from the databases of the DCRHM and the Dutch Working Party on Trophoblastic Disease. The remission rate was not significantly different with EMACP (91.6%) or with EMA/CO (85.4%). The median

number of courses to achieve normal serum hCG concentrations in patients treated with EMA/CO for single-agent resistant disease and primary high-risk disease were 3 and 5 courses, compared to 1.5 and 3 courses of EMACP, respectively. Patients treated with EMACP more often developed fever, renal toxicity, nausea and diarrhoea compared to patients treated with EMA/CO. Patients treated with EMA/CO more often had anaemia, neuropathy and hepatotoxicity. The number of patients that developed secondary tumours was comparable in EMA/CO (3.9%) and EMACP (3.6%). Dose reduction occurred in 39 EMACP courses (11.8%) and in 60 (8.7%) EMA/CO courses ($p=0.119$). 45 courses of EMACP were delayed (13.6%), compared to 33 (4.8%) EMA/CO courses ($p<0.001$). We concluded that EMACP combination chemotherapy is an effective treatment for high-risk GTN. However, the remission rate of EMACP was not significantly higher compared to EMA/CO, and although less courses of EMACP were required to achieve disease remission, the difference in duration of chemotherapeutic treatment was only slightly shorter with EMACP. Short term toxicity was alternating more common in EMACP and EMA/CO, whereas long-term toxicity was not different between both regimens. Therefore cisplatin-based chemotherapy in the form of EMACP does not seem preferable over EMA/CO as first-line treatment of high-risk GTN.

Early identification of patients with high-risk GTN resistant to multi-agent chemotherapy is highly important since they are at risk for a fatal outcome. In **chapter 7** we presented a normogram for hCG regression in patients with high-risk GTN successfully treated with multi-agent chemotherapy in order to predict treatment resistance. Using this normogram, patients could be predicted in an early stage as developing resistance to treatment as illustrated with examples. We showed that normograms for hCG regression under multi-agent treatment differed depending on the indication for multi-agent chemotherapy, with much higher initial hCG values in the group of patient with primary high-risk disease compared to those treated with EMA/CO for MTX resistant disease.

In **chapter 8**, we summarised the main findings of the studies in this thesis, we discussed methodological issues, addressed the importance of the organisation of the management of GTN, and postulated ideas for future research.

Samenvatting

In **hoofdstuk 1** beschreven we de achtergrond en de onderzoeksparameters van de studies die in dit proefschrift werden uitgevoerd. Trofoblasttumoren omvatten de maligne vormen van trofoblastziekte, en bestaan uit invasieve mola hydatidosa die niet spontaan in regressie gaan, choriocarcinomen, "placental site trophoblastic tumour" (PSTT) en de uiterst zeldzame epithelioide trofoblast tumoren (ETT). Als de diagnose trofoblasttumor gesteld wordt, is onderzoek naar de uitgebreidheid van de ziekte nodig, alsmede een evaluatie van risicofactoren, zodat patiënten kunnen worden ingedeeld als hebbende laag- of hoog-risico tumoren. De behandeling van laag-risico tumoren bestaat uit monochemotherapie, veelal in de vorm van methotrexaat (MTX). Van hoog-risico tumoren wordt aangenomen dat ze een hoog-risico hebben om resistentie te ontwikkelen tegen MTX, en worden daarom behandeld met polychemotherapie. Internationaal is de meeste ervaring opgedaan met het EMA/CO-schema (etoposide, MTX en actinomycine D, afgewisseld met cyclofosfamide en vincristine). In dit proefschrift worden de ziekte incidentie, het voorspellen van het ontstaan van trofoblasttumoren na een mola hydatidosa en het voorspellen van resistentie tegen polychemotherapie, alsmede de behandeling van laag- en hoog-risico trofoblasttumoren bestudeerd.

Er is grote variatie in de gerapporteerde incidentie van trofoblastziekten in verschillende delen van de wereld. In **hoofdstuk 2** beschreven we de incidentie en trends in de tijd van trofoblastziekten in Nederland met behulp van populatie gebaseerde data die werd verkregen uit PALGA, een landelijke databank met alle pathologie-uitslagen in Nederland. De totale incidentie van mola hydatididosa, choriocarcinoma en PSTT was respectievelijk 1.34 per 1000 bevallingen, 3.1 per 100,000 bevallingen, en 1.0 per 100,000 bevallingen. De incidentie van trofoblastziekten in Nederland nam significant toe van 1995 tot 2008. Dit kan deels worden verklaard door een toegenomen maternale leeftijd en een toegenomen aandeel van levendgeborenen met minimaal één ouder van Aziatische afkomst. Een deel van de toename kan mogelijk ook het gevolg zijn van verbeterde diagnostische technieken. Deze factoren verklaren echter niet de gehele toename in incidentie en een deel van de geobserveerde stijging blijft daarom onverklaard.

Momenteel is het vervolgen van het serum humaan choriongonadotrofine (hCG) na de curettage van een mola hydatidosa essentieel om patiënten te identificeren bij wie chemotherapeutische behandeling voor persisterende trofoblastziekte noodzakelijk is. In **hoofdstuk 3** beschreven we een model op basis van de lineaire regressie van serum hCG concentraties verkregen in de eerste weken na curettage voor het voorspellen van het ontstaan van persisterende trofoblastziekte. De helling van de lineaire regressie lijnen van de eerste drie log-getransformeerde serum hCG en vrij bèta hCG waarden werden berekend. Receiver Operating characteristic (ROC)

Curves werden geconstrueerd en de Areas Under Curve (AUCs) berekend. De helling van vrij bèta hCG bleek een betere voorspeller voor persisterende trofoblastziekte dan de helling van het hCG. Persisterende trofoblastziekte kon worden voorspeld bij 69% van de patiënten met 97.5% specificiteit. Achtendertig procent van de patiënten die persisterende trofoblastziekte ontwikkelde kon worden voorspeld vóór de diagnose volgens de FIGO 2000 criteria. Hoewel dit model gevalideerd moet worden voor verschillende hCG assays, lijkt het een veelbelovende manier om de meer agressieve gevallen van persisterende trofoblastziekte te voorspellen.

Methotrexaat met folinezuur is het meest gangbare behandelschema voor laag-risico trofoblasttumoren. In Nederland worden na normalisatie van het serum hCG nog twee kuren MTX gegeven (consolidatie kuren), terwijl in het Verenigd Koninkrijk drie consolidatie kuren worden gegeven. In **hoofdstuk 4** vergeleken we in een retrospectieve setting het percentage recidief trofoblasttumoren bij vrouwen die succesvol behandeld zijn met MTX voor een laag-risico trofoblasttumor in Nederland en in het Verenigd Koninkrijk. In 4.0% van de patiënten kwam een recidief trofoblasttumor voor na MTX behandeling met drie consolidatie kuren, terwijl in 8.3% van de patiënten die behandeld werden met twee consolidatiekuren een recidief trofoblasttumor werd geconstateerd. (p=0.006). Hoewel de Nederlandse patiënten hogere hCG concentraties hadden en meer patiënten metastasen hadden voor de start van de behandeling, verschilde het benodigde aantal kuren MTX om een normaal hCG te verkrijgen niet tussen de patiënten uit Nederland en uit het Verenigd Koninkrijk. We concludeerden dat hoewel andere factoren dan de consolidatiekuren mogelijk het geobserveerde verschil in percentage recidief trofoblasttumor kunnen hebben beïnvloed, drie kuren consolidatie chemotherapie mogelijk te prefereren is boven twee consolidatiekuren in de behandeling van laag-risico trofoblasttumoren om zo de kans op een recidief trofoblasttumor te verkleinen. Een prospectieve gerandomiseerde studie is nodig om deze bevindingen te bevestigen.

In **hoofdstuk 5** beschreven we de patiënten overleden aan een trofoblasttumor gedurende vier decennia in Nederland en evalueerden of behandeling in overeenstemming was met het protocol ten tijde van de diagnose, en probeerden hieruit mogelijke implicaties voor toekomstig beleid te formuleren. Data van patiënten die overleden als gevolg van een trofoblasttumor van 1971 tot en met 2011 werden verkregen uit de CMRN en de Werkgroep Trofoblast Tumoren. Zesentwintig patiënten overleden als gevolg van een trofoblasttumor. Bij vijf patiënten ontstond de trofoblasttumor na een mola hydatidosa en bij 19 patiënten ontstond deze na een voldragen zwangerschap. De helft van de patiënten overleed tussen 1971-1980 (n=13) voordat trofoblasttumoren werden geclassificeerd in laag- of hoog-risico tumoren en dus ook nog niet zodanig behandeld werden. Het jaarlijkse aantal patiënten dat overleed aan een trofoblasttumor daalde aanzienlijk gedurende de laatste vier decennia. Twaalf van de 17 patiënten die initieel behandeld werden met MTX hadden

een hoog-risico tumor volgens de huidige Nederlandse risico classificatie en zouden daarom nu behandeld zijn met polychemotherapie. We concludeerden dat een juiste risicoclassificatie essentieel is om te kunnen starten met een optimale behandeling en therapieresistentie te voorkomen. Patiënten met een trofoblasttumor na een voldragen zwangerschap vormden een groot deel van de overleden patiënten en wij stelden voor dat deze tumoren, net zoals in het Nederlandse classificatie systeem, worden beschouwd als hoog-risico tumoren.

In **hoofdstuk 6** vergeleken we de werkzaamheid en veiligheid van poly-chemotherapie met cisplatine (etoposide, MTX, actinomycine D, cyclofosfamide en cisplatine; EMACP) met het EMA/CO schema voor de behandeling van hoog-risico trofoblasttumoren. De 84 patiënten die samen werden behandeld met 330 kuren EMACP en de 103 patiënten die werden behandeld met 688 kuren EMA/CO werden geselecteerd uit de databases van de CMRN en de Werkgroep Trofoblast Tumoren. Het percentage patiënten dat in complete remissie kwam was niet significant verschillend voor patiënten die werden behandeld met EMACP (91.6%) of met EMA/CO (85.4%). Het mediane aantal kuren om tot een normale serum hCG concentratie te komen was 3 bij patiënten die met EMA/CO werden behandeld wegens MTX resistentie en 5 kuren bij patiënten met een primair hoog-risico trofoblasttumor. Bij patiënten die werden behandeld met EMACP was dit respectievelijk 1.5 en 3 kuren. Patiënten die werden behandeld met EMACP ontwikkelden vaker koorts, renale toxiciteit, misselijk en braken en diarree vergeleken met patiënten die werden behandeld met EMA/CO. Patiënten die werden behandeld met EMA/CO hadden vaker last van anemie, neuropathie en hepatotoxiciteit. Het aantal patiënten dat secundaire tumoren ontwikkelde was vergelijkbaar tussen patiënten behandeld met EMA/CO (n=4; 3.9%) en EMACP (n=3; 3.6%). Dosis reductie was noodzakelijk bij 39 EMACP kuren (11.8%) en bij 60 (8.7%) EMA/CO kuren (p=0.119). 45 EMACP kuren werden uitgesteld (13.6%), vergeleken met 33 (4.8%) EMA/CO kuren (p<0.001). We concludeerden dat polychemotherapie in de vorm van EMACP een effectieve behandeling is voor hoog-risico trofoblasttumoren. Echter, het percentage patiënten dat in complete remissie ging met EMACP was niet significant hoger vergeleken met EMA/CO, en hoewel er minder kuren EMACP nodig waren om remissie van de tumor te bereiken, was het verschil in duur van de chemotherapeutische behandeling slechts enigszins korter met EMACP. Korte termijn bijwerkingen kwamen afwisselend vaker voor bij EMACP and EMA/CO, terwijl het voorkomen van lange termijn bijwerkingen niet verschilde tussen beide schema's. Daarom heeft chemotherapie op basis van cisplatine in de vorm van EMACP, geen bewezen meerwaarde boven EMA/CO in de behandeling van patiënten met hoog-risico trofoblasttumoren.

Vroege identificatie van hoog-risico trofoblasttumoren die resistentie hebben ontwikkeld tegen polychemotherapie is van groot belang, aangezien deze patiënten het risico lopen op een fatale afloop. In **hoofdstuk 7** presenteerden we een normo-

gram voor hCG regressie in patiënten met hoog-risico trofoblasttumoren die succesvol behandeld werden met polychemotherapie. We lieten met voorbeelden zien dat met behulp van dit normogram, resistentie ontwikkeling tegen EMA/CO al in een vroege fase van de behandeling voorspeld kon worden. We lieten zien dat de normogrammen voor hCG regressie gedurende polychemotherapie verschilden afhankelijk van de indicatie voor polychemotherapie. Patiënten met primair hoog-risico tumoren hadden veel hogere hCG waarden bij start van EMA/CO dan patiënten die behandeld werden met EMA/CO voor MTX resistente tumoren.

In het laatste hoofdstuk vatten we de belangrijkste bevindingen van de studies in dit proefschrift samen, we bespraken methodologische discussiepunten, we behandelden het belang van de organisatie van zorg van trofoblasttumoren, en deden suggesties voor toekomstig onderzoek.

Dankwoord

Curriculum Vitae

Bibliography

Dankwoord

Eindelijk kan ik dan beginnen aan het meest gelezen hoofdstuk van dit proefschrift, het dankwoord. Een proefschrift dat nooit tot stand was gekomen zonder samenwerking met en hulp van velen. Een aantal mensen wil ik in het bijzonder bedanken.

Allereerst mijn beide promotoren, **Prof. Massuger** en **Prof. Sweep**. Wat vormen jullie een goed team.
Beste **Leon**; jouw waterval aan ideeën lijkt nooit op te drogen, en dat is maar goed ook. Als geen ander kun je mensen enthousiasmeren voor onderzoek, zoals je dat ook bij mij gedaan hebt. Je hebt me geleerd om "groter" te denken, en het grote geheel te zien. Ik ben blij dat je me alle kansen hebt gegeven om mee te doen aan de oprichting van de EOTTD en hoop onder jouw vleugels nog verder te kunnen groeien als onderzoeker.
Beste **Fred**; bedankt voor je hartelijkheid, interesse en fijne samenwerking. Ondanks dat je loeidruk was met het (her)vormen van de afdeling laboratoriumgeneeskunde, heb ik altijd het idee gehad dat je tijd had voor mij en het onderzoek. Dank daarvoor!

Mijn beide copromotoren: **dr. Thomas** en **dr. Ottevanger**.
Beste **Chris**, dat niemand zo goed is als jij in orde scheppen in grote excel bestanden waarin alles 'rijp en groen' is mag onderhand bekend zijn. Bedankt dat je ook na je pensionering de tijd bent blijven nemen om bij te dragen aan het onderzoek, tenslotte zou men zich ook kunnen voorstellen dat het na 40 jaar Radboud welletjes is geweest. Bedankt ook voor je persoonlijke verhalen en je interesse in de mijne.
Beste **Nelleke**, dankzij jouw inbreng kwam onder andere hoofdstuk 6 tot stand. Dank voor de plezierige samenwerking, de inspirerende discussies en het leerzame commentaar.

Leon, Fred, Chris en Nelleke, ik ben blij dat ik heb mogen profiteren van jullie ruime onderzoekservaring. Ik hoop nog veel met jullie te mogen samenwerken in de toekomst. Mooi om te zien hoe iedereen met z'n eigen achtergrond en expertise een waardevolle bijdrage levert aan het "trofoblastteam".

Alle **coauteurs** wil ik bedanken voor ieders bijdrage aan de afzonderlijke hoofdstukken.

Beste **Prof. van Krieken** en **Prof. Kiemeney**. Dank voor het zitting nemen in de manuscriptcommissie. Dear **Prof. Seckl,** thank you for taking seat in the manuscript committee, for giving me the opportunity to see how the Charing Cross Hospital Trophoblast Disease Service operates, and for the collaboration on Chapter 4 of this thesis.

Beste **Werkgroep Trofoblast Tumoren**; **Rene Verheijen, Marianne Ten Kate Booij,** en **Christianne Lok.** Dank voor het verzamelen van de vele notulen die bij een aantal studies in dit proefschrift een belangrijke bron van informatie vormden. Charles Schijf dank voor de (mede)oprichting van de Centrale Mola Registratie Nederland, een onmisbare schat aan informatie.

Beste **Arie Smits** en **Angelique Kooper**. Dank voor het eerste stappen in de samenwerking met de afdeling antropogenetica. Hopelijk kunnen we de ingeslagen weg (samen met Kim en Alex Hoischen) voortzetten!

Beste **Hans Bulten**, dank voor je hulp bij hoofdstuk 2, voor je persoonlijke interesse en verhalen.

Beste **Jeroen Dijkstra**, ik moest alle labtechnieken die nodig waren voor het isoleren van DNA nog leren, tot vermaak van jou. Helaas hebben die inspanningen nog niet geleid tot een artikel, maar ik ben ervan overtuigd dat dat nog gaat gebeuren.

Jan Hendriks en **Joanna in 't Hou**t dank voor de (brood)nodige statistische ondersteuning.

Dear **Rosemary Fisher**, **Dee Short,** and **Richard Harvey,** thanks for showing me your genetic techniques, your help with questionnaires in your (huge) database, and collecting serum samples from your freezer. Richard, you have a lovely sense of humour, I had a great laugh with you and hope we can continue to do so at future trophoblast conferences.

Ook al zat ik niet vaak bij jullie op het laboratorium; beste **Nicolai**, bedankt voor de tips t.a.v. fietsroutes en hoe het hoofd leeg te maken bij drukte. Dear **Jyotsna**, the road you and your husband have chosen isn't an easy one, but I'm sure it will all end well. The best of luck on finishing your (second!) PhD. Beste **Rob** en **Andre**, dank voor jullie hulp met de isovormen, ook al liep die studie op een gegeven moment spaak.

Beste stagiaires, **Fatima, Dorien** en **Kim**, dank voor jullie hulp bij hoofdstuk 4, 5 en 7.

Beste secretaresses; **Els**, **Ine** en **Hilde**: dank voor het bijhouden van de molaregistratie. Zonder dat zouden we niet het onderzoek kunnen doen wat we nu doen. Beste **Ingrid** en **Hannie**, dank voor alle kletsmomenten op dinsdag rond 13 uur. **Wilma** en **Yvonne** dank voor alle logistieke ondersteuning en het vinden van gaatjes in de agenda's.

Beste **Nienke van Trommel** en **Linda Kerkmeijer**. Weinig boekjes heb ik zo goed gelezen als die van jullie twee! Dank dat ik daarop enigszins kon voortborduren. Nienke ook bedankt voor je gezelligheid bij de EOTTD bijeenkomsten en je bemiddeling bij mijn stap naar het CWZ.

Beste **Kim Westerdijk**, wat ben ik blij dat de onderzoekslijn trofoblasttumoren voortgezet wordt. Je bent vlot van start gegaan, en er zijn nog ideeën genoeg. Hopelijk kunnen we met z'n allen nog heel veel stappen verder zetten!

Maatschap gynaecologen van het CWZ, dank voor het vertrouwen en het goede gevoel dat jullie mij gaven bij mijn eerste stappen als ANIOS.

Lieve **collega's uit de onderzoekstuin**; waar het me in het begin nog verstandiger leek om privé en werk gescheiden te houden, kwam ik er al snel achter dat het veel gezelliger is om dat niet te doen. Dank voor de vele gezellige borrels bij de Aesculaaf en St. Anneke, de leuke congressen, en het delen van frustraties en blijdschap om al die wetenschap gepubliceerd te krijgen. Ik had het niet willen missen.

Lieve **vrienden**. Dank voor jullie oprechte interesse in wat ik aan het doen was (al vanaf "zal ik het nou gaan doen, dat promoveren?") en voor jullie indirecte bijdrage in de vorm van de nodige afleiding op z'n tijd. Ik ben heel dankbaar en blij dat ik jullie om me heen heb.

Lieve paranimfen, lieve **Refika** en lieve **Thijs**.
Refika, lieve surrogaat grote zus! Wat was en ben je een heerlijk klankbord. Al vanaf dat je tegenover me zat in de tuin klikt het en daarna is het alleen nog maar veel gezelliger geworden. Ook zonder dit dankwoord weet je best hoe blij ik met jou ben.
Thijs, ik bewonder je toewijding aan het onderzoek. Jij had en hebt altijd een luisterend oor en je wist altijd de boel te relativeren als ik door de bomen het bos niet meer zag. Jullie zijn me zeer dierbaar. Ik ben blij dat jullie naast me staan op 24 januari!

Dan tot slot lieve **Pap en Mam**. Van jullie heb ik de allerbelangrijkste eigenschap om te promoveren gekregen: doorzettingsvermogen. Al vanaf de basisschool en later de middelbare school was altijd jullie adagium: "gewoon proberen, je kunt altijd nog stoppen, later alsnog starten is veel moeilijker". Bedankt voor alle kansen, en dat jullie deur echt altijd open staat. Jullie stabiele basis heeft mij gebracht waar ik nu sta.

Curriculum Vitae

Charlotte Lybøl werd geboren op 8 juni 1984 in het Achterhoekse Sint Jozefziekenhuis te Doetinchem, en groeide op in het nabijgelegen Etten. Zij volgde het profiel Natuur en Gezondheid aan het Almende College locatie Isala te Silvolde. In 2002 behaalde zij haar gymnasium diploma en kon meteen beginnen aan de studie geneeskunde aan de toenmalige Katholieke Universiteit Nijmegen. Tijdens het reguliere co-schap gynaecologie in het Slingeland ziekenhuis te Doetinchem werd haar interesse voor de gynaecologie gewekt. Een wetenschappelijke stage in het UMC St. Radboud op de afdeling Verloskunde en Gynaecologie (pijler gynaecologische oncologie) onder leiding van Prof. Dr. Leon Massuger naar de pathologie van het ovariumcarcinoom leidde er toe dat zij na het arts-examen in juni 2009 kon starten aan het promotie-onderzoek naar trofoblastziekten. De samenwerking tussen de afdelingen Verloskunde en Gynaecologie (Prof. dr. Leon Massuger) en Laboratoriumgeneeskunde (Prof. Dr. Fred Sweep en dr. Chris Thomas), later versterkt door de afdeling Medische Oncologie (dr. Petronella Ottevanger) leidde tot de studies die resulteerden in dit proefschrift. Sinds juni 2012 is Charlotte met veel plezier werkzaam als ANIOS op de afdeling Verloskunde en Gynaecologie van het Canisius Wilhelmina Ziekenhuis te Nijmegen. Per 1 april 2013 start zij met de opleiding tot gynaecoloog in het Jeroen Bosch Ziekenhuis te 's Hertogenbosch (opleider dr. H.P. Oosterbaan).

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