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Refinements in the Management of Persistent Trophoblastic Disease

NIENKE VAN TROMMEL

2006

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CHAPTER 1

General introduction

General introduction

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Introduction

Gestational Trophoblastic Disease (GTD) is a rare pre-malignant condition that occurs in approximately one in 500 to 2000 pregnancies. On pathologic examination, trophoblastic hyperplasia and hydropic swelling of the chorionic villi are predominantly present. These villous malformations of trophoblast can be subdivided in partial moles (both paternally and maternally derived) and complete moles (solely paternally derived). After evacuation of the hydatidiform mole, serum human Chorionic Gonadotropin (hCG) is closely monitored to detect the malignant development of Persistent Trophoblastic Disease (PTD). If PTD occurs, patients are classified as having low or high lethal risk and they are treated accordingly. Serum hCG is closely monitored in all patients with GTD and PTD after the evacuation of the hydatidiform mole. All patients with GTD are advised not to conceive within 6 to 12 months after normalisation of serum hCG. It remains to be elucidated which patients with GTD will develop PTD. Furthermore, treatment strategies need to be optimized in order to obtain cure in individual patients as soon as possible with a minimum of side effects of chemotherapy in a population of patients who are often anxious to conceive. The main objective of this thesis is the development of strategies for early identification of PTD and critical analysis of its treatment.

History

Aetius of Amida (502-575 AD), a physician from Mesopotamia who studied in Alexandria and became the private physician to emperor Justinian I in Byzantium, was the first to introduce the term 'mola hydatidosa' in his extensive compilation of Greek and Christian traditions of medicine. He described that women with a mole feel pregnant, have swollen breasts and that the menses are suppressed. The distinction with a real pregnancy could only be made on the basis of foetal movements. Aetius also knew the cause of a hydatidiform mole: 'an inflammation or strenuous walking'(1). In 1276, a 'Clerc uten laghen lande bi der see'(clerk from 'The Low Lands Near the Sea'; The Netherlands) reported that the Dutch Countess Margaretha van Henneberg on Good Friday 1276 gave

birth to 'as many children as days in a year' as a punishment by God after having insulted a poor beggar woman by saying she was promiscuous because she was carrying a twin: the Countess believed that a twin must have two different fathers (2-4). From the description of the 365 'children' (of which half were believed to be female and were named 'Elizabeth' and half male and were named 'John') it is clear that the pregnancy was a hydatidiform mole. The Countess died soon after giving birth to the 'children'. The 365 'babies' were baptised in a bowl. The basins were stored in the church of Loosduinen, near The Hague, which became a place of pilgrimage for women with fertility problems. In 1654, the poet Jacob Westerbaen wrote (3):

'Waerom hier menigh wijf gegaen komt of gereden
Die vruchtbaar hoopt te zyn en haest met kind gemaeckt
So maer haer handschoen een van dese beckens raeckt'

('Therefore many women come or drive here
Who hope to be fertile and are anxious to have a baby
And touch with her glove one of these bowls').

17th century French etching after a picture by the 17th century artist Petrus Kaerius, depicting Margaretha van Henneberg in bed on the right and the 365 'children' on a plate on the left. Picture kindly provided by Mr A. Molenkamp, Museum 'De Korenschuur', Loosduinen, The Netherlands

In 1895, Felix Marchand was the first to describe that the malignancy he called chorionepithelioma, was derived from placental villous trophoblast which emerged after a normal pregnancy, a spontaneous abortion, extra-uterine pregnancy or after a hydatidiform mole (5).

Clinical presentation

The clinical presentation of hydatidiform mole has changed considerably over the last decades (6). In the 1960-1970s, the mean time for diagnosis was 16 weeks (6) and classic presentations were, in declining frequency, vaginal bleeding (89-97%), uterine enlargement (38-51%) and theca-lutein cysts due to ovarian hyperstimulation by high serum hCG concentrations (20-46%) (7,8). Pre-eclampsia and hyperemesis occurred in 12-27% and 20-26% of patients, respectively (7,8), and 2% of patients with a hydatidiform mole presented with signs of hyperthyroidism (7). The clinical presentation changed to earlier detection at a gestational age of 10-12 weeks (9-11) over the recent years. Vaginal bleeding continues to be the most common presenting symptom occurring in 58- 84% of patients presenting with hydatidiform moles (9-11). Due to the availability of ultrasound and sensitive hCG tests the diagnosis is nowadays generally made before the other classic symptoms develop (6).

Incidence of hydatidiform mole

Incidence of hydatidiform mole is highest in South-East Asia, Indonesia, India and Turkey, with rates ranging from 2 to 12 per 1000 pregnancies. In North America and Europe, incidence is lower: 0.5 to 1 per 1000 pregnancies. Interestingly, significant reductions of incidence of hydatidiform mole have been recently reported in Korea, Japan and Taiwan to levels comparable to those in North America and Europe (12).

In the Netherlands, hydatidiform moles are registered at the Dutch Central Registry for Hydatidiform Moles (DCRHM). Since the first registration in 1977 until the 1st of January 2006, 3203 cases were registered. If a first or optionally a second histological specimen was diagnosed as choriocarcinoma, the case was registered as a choriocarcinoma irrespective of whether a previous diagnosis was a hydatidiform mole.

Table 1 provides an inventory of the cases of GTD registered with the DCRHM and compares the time periods from 1977 until end of 2003 with the time period 1994 until end of 2003 in which, respectively,

Table 1. Registered cases with the Dutch Central Registry for Hydatidiform Moles (DCRHM)

Histological diagnosis and development of PTD	DCRHM 1977 - 2003 n		%		DCRHM 1994 - 2003 n		%	
Complete mole, no PTD	521		18.5		244		17.6	
Complete mole, with PTD	257		9.1		146		10.5	
Complete mole, PTD unknown	650		23.0		165		11.9	
Subtotal complete mole		*1428*		*50.6*		*555*		*40.0*
Partial mole, no PTD	363		12.9		268		19.3	
Partial mole, with PTD	61		2.2		48		3.5	
Partial mole, PTD unknown	311		11.0		176		12.7	
Subtotal partial mole		*735*		*26.1*		*492*		*35.5*
Mole not specified, no PTD	72		2.6		70		5.0	
Mole not specified, with PTD	71		2.5		67		4.8	
Mole not specified, PTD unknown	105		3.7		106		7.6	
Subtotal mole not specified		*248*		*8.8*		*243*		*17.4*
Invasive mole	5		0.2		3		0.2	
Hydropic degeneration	55		1.9		12		0.9	
Placental site trophoblastic tumour	1		0.0		1		0.1	
Choriocarcinoma	58		2.1		29		2.0	
Remaining diagnoses	30		1.1		20		1.4	
Unknown	261		9.3		34		2.4	
Subtotal other diagnoses		*410*		*14.5*		*99*		*7.1*
Total	*2821*		*100*		*1389*		*100*	

2821 and 1389 patients were recorded. Subdivision into complete moles, partial moles, moles not specified, and other diagnoses of GTD (invasive mole, hydropic degeneration, placental site trophoblastic tumour, choriocarcinoma, remaining and unknown diagnoses) revealed percent rates for the period 1977-2003 of respectively 50.6%, 26.1%, 8.8%, an 14.5% and for the period 1994-2003 respectively 40.0%, 35.5%, 17.4% and 7.1%. In both time periods the number of cases without development of PTD following diagnosis of complete moles was approximately twice the number of cases of complete moles with subsequent development of PTD (18.5% and 9.1% in the period 1977-2003, and 17.6% and 10.5% in the period 1994-2003). In the case of partial moles, PTD developed approxi-

Table 2. Incidence of hydatidiform mole from population-based studies*

	Rate per 1000			
Country	pregnancies	deliveries	live births	Reference
Australia	0.57		0.7	(16)
Canada	0.83			(17)
England and Wales			1.54	(18)
Greenland		1.2		(19)
Italy	0.7			(20)
Japan	2.96		3.0	(14)
Netherlands			0.68	(21)
Northern Ireland	2.2			(22)
Paraguay	0.2			(15)
Singapore		1.2		(23)
Sweden	0.9			(24, 25)
Turkey	1.84	2.48		(26)
United Arab Emirates		2.0		(27)
USA	1.1			(28)
USA	0.8	(White Hawaiian)		(29)
	1.75	(Filippino)		
USA	1.14	(Non-Hispanic whites)	1.04	(30)
	1.14	(Hispanic whites)	1.34	
	2.23	(American Indians)	2.27	

* Adapted from Kim JS. Epidemiology. In: Hancock BW, Newlands ES, Berkowitz RS, editors. Gestational Trophoblastic Disease. First ed. London: Chapman & Hall; 1997. p. 27- 42; with kind permission from Springer Science and Business Media

mately six times less frequently than spontaneous regression of serum hCG.

Since hospital-based incidence studies invariably tend to have elevated incidence rates, especially those from regions of the world where uncomplicated pregnancies are not registered, population-based studies give more reliable data on incidence of hydatidiform mole (13). Table 2 shows a highest incidence of hydatidiform mole of 2.96 per 1000 pregnancies in Japan (14) and the lowest incidence of hydatidiform mole in Paraguay (15).

It is generally accepted that incidence of hydatidiform mole is highest among American Indians, Eskimos, and Asian populations (13). In studies performed at Hawaii, women of Japanese and Philippine descent have a significant higher risk for a mole pregnancy than women from Caucasian descent (13). However, the risk for a hydatidiform mole pregnancy in Hawaiian women from Japanese descent is lower than for women living in Japan (13). Two cytogenetic studies have reported the incidence of balanced translocations to be as high as 4.6% in patients with a hydatidiform mole compared to an incidence of 0.6% balanced translocations in unaffected populations (13). Furthermore, family clustering of hydatidiform moles has been described. These findings indicate a certain genetic factor to be involved in the development of hydatidiform mole (12,13). Next to race, geographical region and genetic factors, maternal age (<20 years or >40 years) and a previous history of hydatidiform mole (12,13) are factors associated with an increased risk for a hydatidiform mole pregnancy. No unequivocal association between the occurrence of a hydatidiform and blood group, diet, cigarette smoking or exposure to herbicides have been described (13).

The reported relative incidences of complete mole versus partial mole are conflicting, ranging from 75% complete mole and 25% partial mole (31,32) to exactly the opposite: 25% complete mole and 75% partial mole (31,33). The large variation in the reported frequency of partial and complete hydatidiform mole might be due to large inter- and intra-observer variations brought about by the histological diagnosis of a partial hydatidiform mole without adjuvant diagnostic aid. In a retrospective study in which all first trimester abortions were reanalyzed both on histological grounds and with flow cytometry, Jeffers *et al.* showed that the incidence of partial mole is far more common than complete mole: 76% partial mole and 24% complete mole (31). The reported frequency of PTD is 20 % in complete hydatidiform mole (34) and 0.5 % to 9.9 % in partial hydatidiform mole (35-38). Again, the large dispersion in the incidence of PTD after a partial hydatidiform mole might be caused by the difficult diagnosis on histological grounds of a partial mole.

Pathology

The development of a placenta is relatively new in the evolution and allows the development of a small number of foetuses within the protec-

tive maternal organism (39). The Dutch professor of Zoology, Ambrosius Arnold Willem Hubrecht, was the first to introduce the term 'trophoblast' in 1895 to designate those cells in the blastocyst that are not part of the embryo, but are essential for its nourishment (40). The syncytiotrophoblast develops from the trophoblastic stem cell or cytotrophoblast. These cells cover the chorionic villi (villous trophoblasts) and invade the endometrium and the proximal third part of the myometrium (extra villous trophoblast) (39,41,42).

A variety of pathologic entities is included in the diagnosis of GTD that are divided in villous and non-villous malformations of trophoblasts. Villous malformations of trophoblasts comprise hydatidiform mole which represents placentas with abnormally enlarged and oedematous chorionic villi which are covered with abnormally proliferative trophoblasts (43). Non-villous malformations of trophoblast are mainly choriocarcinoma, an extremely haemorrhagic tumour with no villi, and the placental site trophoblastic tumour, derived from intermediate trophoblasts and occurring at the implantation site of the placenta (43). Mola hydatidosa can be subdivided in complete and partial mole. This subdivision is important, since these two histological entities have different risks to develop malignant sequelae, i.e. PTD. A complete hydatidiform mole is a diploid, androgenic conception (46 XX or rarely XY) which is the result of an 'empty' oocyte (after degeneration of the nucleus) either being fertilized by one haploid sperm, followed by duplication of its chromosome (44-46), or by two spermatozoa (47). The syndrome of partial (incomplete) mole has an ascertainable foetus (alive or dead) and a triploid karyotype (69 XXX or XXY) after fertilization of a normal ovum by two spermatozoa (48). Distinction between a hydatidiform mole and an abortion with hydropic villi can be difficult. Similar to hydatidiform mole, a hydropic abortion may show villous edema with hydropic swelling, although a hydropic abortion lacks the marked trophoblastic hyperplasia (49).

Difficulty in proper diagnosing gestational trophoblastic disease

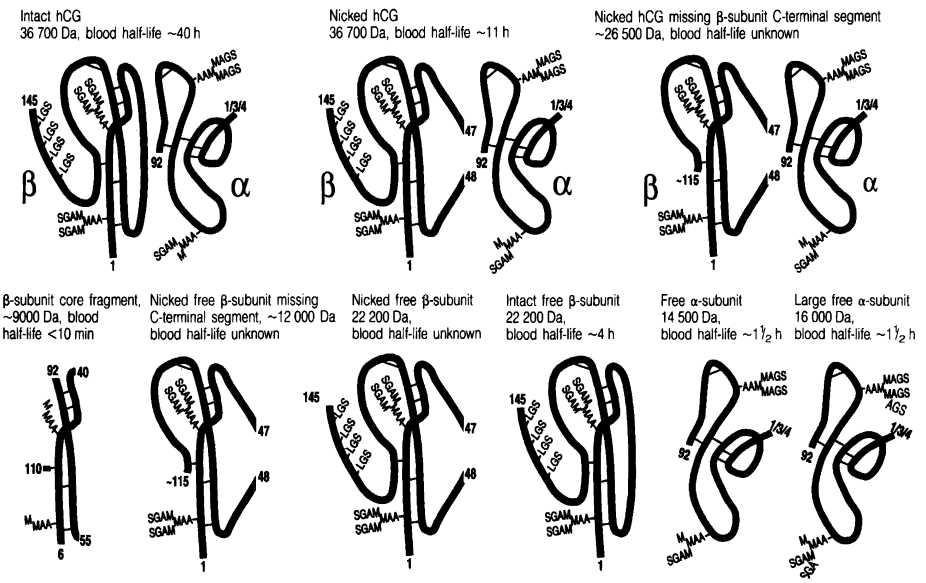
Howat *et al.* showed that out of 50 cases of partial mole, complete mole and hydropic degeneration, 30% of hydatidiform moles were misdiagnosed (50). More than 80% of misdiagnoses concerned the discrimination of partial mole from hydropic degeneration. Interestingly, Paradinas found upon reanalysis of 400 cases of partial mole that 50% of these par-

tial moles were misclassified, but the majority of misclassification in their study concerned complete mole which had been initially diagnosed as partial mole (51). Adjuvant diagnostic tools to distinguish complete mole, partial mole and hydropic degeneration have been developed. Triploid partial moles can be distinguished from diploid complete moles with flow cytometry or fluorescent in situ hybridization (FISH)(52,53). Cytometry or FISH does not contribute to make the distinction between complete hydatidiform mole and hydropic degeneration. Genomic imprinting refers to the phenomenon that for certain traits either the paternal or maternal copy of the gene is expressed and hence the transmission of the tract is dependent on the parental origin (54). To aid the distinction between complete hydatidiform mole and hydropic degeneration, a histopathological staining technique using P^{57kip2} has been developed which identifies the paternally imprinted and maternally expressed CDKN1C gene. The product of this gene is not present in a complete (diploid) hydatidiform mole and present in (diploid) hydropic degeneration (53). It is argued that besides pathological examination, other techniques like DNA flowcytometry, imunohistochemical analysis for P^{57kip2} expression and genetic analysis (to determine whether in partial mole two alleles were derived paternally or maternally) are helpful in establishing the proper diagnosis of hydatidiform mole (43,49,52,53).

Human chorionic gonadotropin

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone produced by trophoblastic tissue and therefore a key marker in pregnancy and GTD. HCG is composed of two non-covalently bound subunits (i.e. the α- and β-subunit) (Figure 1). The α-subunit of hCG comprises 92 amino acids and is identical to the α-subunit of the pituitary glycoprotein hormones Follicle Stimulating Hormone (FSH), Thyroid Stimulating Hormone (TSH) and Luteinizing Hormone (LH). The hCG β-subunit is composed of 145 amino acids, which distinguishes hCG from these other glycoproteins. Two variants exist of intact hCG: non-nicked and nicked hCG. Nicked hCG is cleaved either between amino acid 44 and 45 or 47 and 48 in the hCG β-subunit and has, unlike non-nicked hCG, little biological activity (55). In addition to intact hCG, free hCG α-subunit, non-nicked free hCG β-subunit, nicked free hCG β-subunit, and the hCG β-subunit core fragment are present in blood.

Figure 1. The heterogeneity of hCG*



* Adapted from Cole LA, Human Chorionic Gonadotropin assay. In: Hancock BW, Newlands ES, Berkowitz RS, editors. Gestational Trophoblastic Disease. First ed. London: Chapman & Hall; 1997. p. 77-94; with kind permission from Springer Science and Business Media

The main functions of serum hCG in uneventful pregnancy are antigonadotrophic (by inhibiting LH and FSH avoiding ovulation during pregnancy) and steroidogenic (by stimulating progesterone production of the corpus luteum in early pregnancy and the trophoblast at a later stage) (39).

In uneventful pregnancy concentrations of intact hCG and free hCGβ in blood double approximately every 2 days to reach a peak at 8-10 weeks of gestation. From week 10 to 20, these concentrations decline to levels comparable to those in early first trimester and from 20 weeks on they remain constantly low (56). In contrast, hCGα concentrations in blood increase steadily until the end of pregnancy (57). The production of subunits of hCG is under stringent physiological control in normal pregnancy, and is reported to be different in pathological states such as hydatidiform mole (58-60), although the literature data are not unequivocal. In

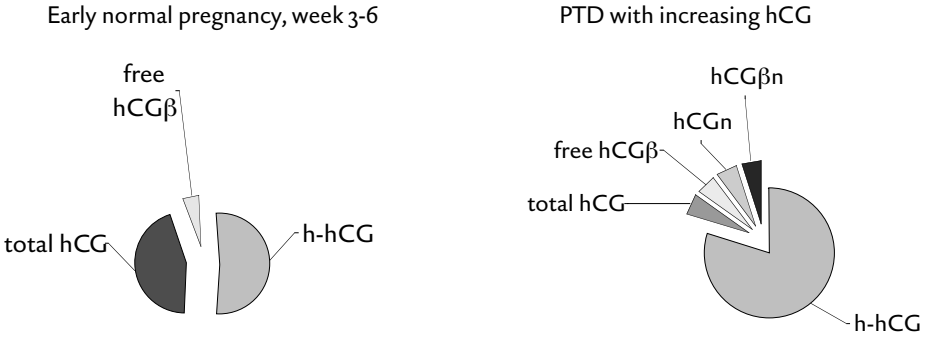
particular, the concentration of hCGβ and the ratio of hCGβ to total hCGβ (explanation: see page 20) is reported to be increased in molar as compared to normal pregnancy (61). Some studies, using a limited number of patients, reported that an increased ratio of hCGβ to total hCGβ identifies patients with molar pregnancy who are at risk for developing persistent twphoblastic disease (62-64), although other investigators did not report such an association (58).

Why hCGβ is synthesized more abundantly in complete hydatidiform mole than in partial moles or in uneventful pregnancy is unknown. hCG is synthesized in cytotrophoblasts and more abundantly in syncytiotrophoblasts (65,66). Messenger-RNA for hCGα is encoded by a single gene on chromosome 6 (67), whereas the β-subunit of hCG is encoded by four genes: hCGβ-3, -5, -7 and -8, located on chromosome 19q13.3 (67,68). hCG α- and β-subunits are thus synthesized separately, and their production is regulated independently during pregnancy and in trophoblastic disease (69,70). Berkowitz *et al.* (58) suggested that the percent free hCGβ level might reflect the level of differentiation and hyperplasia of the trophoblast, since the level of free hCGβ increases up to 0.5% at five weeks of normal gestation, to 1% in partial hydatidiform mole, to 2.4% in complete hydatidiform mole, and as high as 9.2% in choriocarcinoma. Hay (60) indeed proposed a regulatory mechanism to link irregularities in trophoblastic differentiation to altered biosynthesis of hCG. In proliferating syncytium formed by differentiating cytotrophoblasts, amplification of hCG genes results in excess mRNA for hCG β-subunits. All free hCGα will be used to produce hCG and the remaining hCGβ will be released together with intact hCG. In the same line it is feasible to think that in partial mole, which contains both normal trophoblast and hyperplastic trophoblasts, less free hCGβ is synthesized as compared to complete moles.

Hyperglycosylated human chorionic gonadotropin

Four N-linked and four O-linked sugar side chains are attached to each hCG molecule (72). Primarily mono- and biantennary N-linked oligosaccharides, and tri-and tetrasaccharide-type- O- linked sugar units were found in the β-subunit of hCG in normal and molar pregnancy. A small proportion of more complex triantennary N-linked oligosaccharides (0-30%) and larger hexasaccharide-type-O-linked sugar units (0-20%) were also found in normal and molar pregnancy preparations. In

Figure 2. Different hCG subtypes in normal early pregnancy and in PTD (71)



total hCG	intact hCG+free hCGβ-subunit
free hCGβ	free hCGβ-subunit
hCGn	nicked intact hCG
hCGβn	nicked free hCGβ-subunit
h-hCG	hyperglycosylated hCG

contrast, primary triantennary N-linked oligosaccharides (up to 100%) and hexasaccharide O-linked oligosaccharides (up to 100%) were found in choriocarcinoma hCG (73). hCG with these larger N- and O-linked oligosaccharides is called hyperglycosylated hCG (74).

In uneventful pregnancy, after implantation, hyperglycosylated hCG is gradually replaced by hCG; hyperglycosylated hCG accounts respectively for >80%, 63% and 50% in the first to third week after implantation (72). In the third trimester, hyperglycosylated hCG has diminished to 2% of total hCG (73). Kovalevskaya *et al.* showed that hyperglycosylated hCG is produced in placental stem cells or cytotrophoblasts and that the less glycosylated hCG form is synthesized mainly in syncytiotrophoblasts (75). Hyperglycosylated hCG accounted for virtually all hCG produced in five individuals with choriocarcinoma (73). The finding that hyperglycosylated hCG is produced by invasive normal pregnancy cells during the first week of gestation and by invasive or cancerous trophoblast cells in choriocarcinoma suggests that hyperglycosylated hCG is a product of distinct trophoblast cells and that it may be synthesized by invasive trophoblast cells (73).

Measurement of human chorionic gonadotropin

The first pregnancy test measuring hCG was developed by Selmar Aschheim and Bernard Zondek. In 1928, they described a bioassay in which young female mice were injected abdominally with urine of a pregnant woman. If 48 hours later on autopsy the ovaries of the mice were enlarged, the woman was pregnant (76). This test was considered to have a good accuracy. In 1956, Solomon Berson and Rosalyn Yalow introduced the concept of radioimmunoassay (RIA) (77). The principle of this new technique was based on the reaction of radioactive labelled analyte with an antibody directed against that analyte. The urine or serum sample in which the analyte (e.g., hCG) needs to be measured is added to this mixture. The more analyte is present in the sample the less radioactive analyte is captured by the antibodies and this leads to quantification of the analyte in a competitive setting. To date, these assays use an antiserum directed against the free hCG β-subunit as first described by Vaitukaitis *et al.* (78). In The Netherlands, the Dutch Central Registry for Hydatidiform Moles uses a comparable RIA which is based on privately raised hCGβ antisera (79). These RIAs are erroneously called hCGβ RIAs because they apply antisera raised against the free hCG β-subunit, and thus measure free hCG β-subunit with greater affinity than intact hCG. Instead, these assays should correctly be designated as hCG + hCGβ RIAs. Closely related to this 'total' hCG RIA is the RIA specifically measuring the free hCG β-subunit which employs a monoclonal antibody directed against an epitope on the hCG β-subunit. This epitope is only accessible in case that the α-subunit is not attached to the β-subunit, the reason why this kind of assay specifically measures the free hCG β-subunit.

More recently, in the 1980s, the concept of double-antibody 'sandwich'-type immunoassays was developed which now are widely used for many purposes. In this type of assay, two antibodies are used to bind the hCG molecule serving as the analyte; one antibody is bound to a solid surface of the reaction tube and captures the hCG analyte ('capture antibody') while the other antibody (binding the hCG analyte as well) is labelled with a (radioactive) tracer ('signal antibody'). The principle is based on an increase of signal with increasing concentrations of analyte ('proportional assay'). Application of 'sandwich'-type assay formats are primarily suited for pregnancy detection and these type of assays do not necessarily detect the aberrant hCG molecules found in trophoblastic disease (80).

Treatment of gestational trophoblastic disease

Hydatidiform moles should be evacuated as soon as possible after diagnosis using suction curettage (81). Medical induction of labour or hysterotomy is not indicated since it increases the risk for PTD (82,83). There is a theoretical concern that the use of strong oxytocic agents force trophoblastic tissue into the circulation. Therefore oxytocin should only be administered after the evacuation of the hydatidiform mole (82,84). The routine use of a second curettage after evacuation of a hydatidiform mole is advised by one author (81), but is advised against by others (82-84). Hysterectomy with preservation of the adnexa is an alternative therapy for hydatidiform mole in patients opting for sterilisation (81,83). This therapy seems to reduce the chance to develop PTD to 3-5% (83). After evacuation of a hydatidiform mole, hCG is monitored on a weekly basis in order to diagnose malignant sequelae as soon as possible (82,83,85).

Prophylactic chemotherapy

The use of prophylactic chemotherapy may be particularly beneficial in patients with high-risk complete hydatidiform mole who cannot be followed closely (86). The first descriptive studies on prophylactic chemotherapy after evacuation of a hydatidiform mole showed an overall incidence of PTD ranging from 4-12% with either Methotrexate (MTX) or Actinomycin D compared to an incidence of PTD without prophylactic chemotherapy of 20% (36). The differences in the occurrence of PTD between the groups were not tested statistically and no subdivision was made between patients at low or high-risk for the development of PTD. Kashimura *et al.* performed the only prospective trial in which it was concluded that prophylactic chemotherapy is beneficial for all patients with hydatidiform mole, irrespective whether patients had a high or low-risk for a malignant sequel (87). Their study dealt with 293 patients who were given chemoprophylaxis of 10 mg MTX that was administered daily for seven days within three weeks after evacuation. The control group comprised 127 patients who did not receive prophylactic treatment. In the study group, PTD occurred significantly less ($p<0.01$) (22 out of 293 patients, 7.5%) than in the control group (23 out of 127, 18.1%). No statistical difference in the occurrence of PTD was found in a prospective trial with 71 patients who received MTX chemoprophylaxis after evacuation and 42 patients who were not treated prophylacti-

cally (88). In subgroup analysis, however, patients with high-risk hydatidiform mole according to the NETDC (New England Trophoblastic Disease Center) criteria (89) were found to benefit from chemoprophylaxis. If PTD occurred, significantly more courses of chemotherapy were needed for cure of the patients treated with chemoprophylaxis. A retrospective study by Park *et al.* (90) confirmed the statistically significant reduction of PTD development following high-risk hydatidiform mole according to the NEDTC criteria in the case that prophylactic chemotherapy was given. Finally, in a double blind randomized controlled trial, Limpongsanurak showed that patients with high-risk hydatidiform mole according to the NETDC criteria and irrespective of maternal age, had a significant risk reduction of 72.4 % to develop PTD after prophylactic chemotherapy with Actinomycin D (13.8 % in the study group versus 50.0 % in the control group) (86).

Persistent trophoblastic disease

In the first papers on women with PTD successfully treated with MTX, the only criterion provided for PTD was 'a sustained elevation of gonadotropin titer for up to six months'(91-93). To date, an internationally accepted definition as proposed by the International Federation of Gynaecology and Obstetrics (FIGO) is used in which PTD is defined as a plateau in serum hCG concentrations for three weeks, a hCG rise for two consecutive weeks, the detection of hCG 6 months after evacuation, or the histological finding of a choriocarcinoma (94), as accepted by the majority of practitioners in the field (95). A persistent trophoblastic tumour may have the histological features of either a hydatidiform mole or a choriocarcinoma (6). When PTD develops, (new) tissue is often not obtained and precise histopathological diagnosis may, therefore, not be possible.

Several authors described serum hCG concentrations to be normalized in 50 % of patients between 6 and 14 weeks after evacuation (96-100). Using normal regression corridors, PTD as defined by exceeding the 95th percentile (P95) of normal regression was diagnosed two weeks earlier than in the case that the definition for PTD was derived from a plateau or rise for three consecutive measurements (98,100). In The Netherlands, the Dutch Society for Obstetrics and Gynaecology defines PTD as a hCG plateau or rise for three consecutive weekly measurements, with at least one measurement above P95 of a normal regression

curve as published by Yedema *et al.* (99). The reason for this extended definition is that it has been described by these authors that 15% of patients with a plateau or rise for three weeks, but without a serum hCG measurement exceeding P95 will still have spontaneous serum hCG remission (99). Also, upon using a normal regression corridor for serum hCG after evacuation of hydatidiform mole, Rotmensch *et al.* reported that in retrospect 6 out of 21 patients (29%) who were treated for PTD since serum hCG concentration was rising during two weeks or showed a plateau for three weeks, the serum hCG concentration was below the 90th percentile of normal regression of serum hCG when chemotherapy was started (96). In a previous study of our group it was found that in retrospect 8% of patients treated for PTD had serum hCG levels below the 95th percentile of normal regression (99). It has been hypothesized that the use of a normal hCG regression corridor together with the FIGO definition for PTD makes a more expectant attitude towards the start of treatment for PTD possible (99).

Classifications in high/low-risk persistent trophoblastic disease

In contrast to most other malignancies, in PTD the prognosis for patients with similar pathological staging may differ greatly (95,101). It is known that there are a number of clinical features beside extend of the disease that affects the prognosis (101). In 1965 the International Union against Cancer (UICC) proposed to adopt a clinical classification incorporating both histological and prognostic markers (95,101). In the same year, Ross *et al.* identified the following clinical features to be associated with poor prognosis in PTD: high hCG level, duration of disease longer than four months and the presence of liver and/or brain metastases (101). In line with this report, Hammond *et al.* proposed in 1973 to use a clinical classification, dividing patients with PTD in non-metastatic or metastatic. The metastatic group was further divided in patients with either a 'good' prognosis (urinary hCG<100 000 IU/24 hours or < 40 000 IU/L in serum, symptoms for less than 4 months, no brain or liver metastases, no prior chemotherapy and no term pregnancy preceding the PTD), or a 'bad' prognosis (urinary hCG>100 000 IU/24 hours or > 40 000 IU/L in serum, symptoms for more than 4 months, brain or liver metastases, failure to prior chemotherapy or a term pregnancy preceding the PTD) (102). In 1976, Bagshawe proposed a scoring system that weighs different prognostic factors (103). This extensive scoring system included age, par-

ity, antecedent pregnancy, histological diagnosis, interval between end of (mole) pregnancy and start of treatment, hCG concentration, ABO blood group in patient versus partner, number and site of metastases, largest tumour mass, lymphocyte infiltration of the tumour, immune status and relapse after chemotherapy (103). The WHO developed in 1983 a simplified Bagshawe score excluding the items parity, lymphocyte infiltration and immune status (104). In 1992, FIGO introduced a revised scoring which combined an anatomical staging (stage I: disease confined to uterus, stage II: disease outside uterus but within genital organs, stage III: disease extends to lungs, stage IV: disease at other metastatic sites) with prognostic risk factors of serum hCG (>10 000 IU/L) and interval between end of pregnancy and start of treatment >6 months (105). Several retrospective studies investigated the relationship between a clinical marker and poor prognosis in PTD. Soper *et al.* showed that hCG level and tumour size were not independent prognostic factors (106). Azab *et al.* demonstrated that only patients with an antecedent non-mole pregnancy, more than one metastatic site and resistance to previous chemotherapy, together with a histological diagnosis of choriocarcinoma had a poor prognosis (107). In 2000, a new FIGO staging (FIGO 2000) was introduced which combined the WHO prognostic scoring and the FIGO 1992 anatomical staging. The risk score for the ABO blood group was excluded and the risk factor for liver metastases was upgraded from two to the maximum of four points (94,95,108). The worldwide use of this system would be an important step forward in the management of PTD because results can be compared among different centres and it would facilitate enrolment of patients in large multi-centre trials (109). To date, however, different classifications systems are used next to each other.

The Dutch guideline for classification for PTD scores for antecedent pregnancy (low-risk: mole or non-molar abortion, high-risk: term choriocarcinoma), localisation of metastases (low-risk: no metastases or in vagina or lungs only, high-risk: more than one organ with metastases or remaining localisation of metastases), previous failure to chemotherapy (low-risk: no failure, high-risk: earlier failure to any chemotherapy) and the interval between end of pregnancy and beginning of treatment (low-risk: ≤ 12 months, high-risk: > 12 months) (95,110,111). Unlike FIGO 2000 (94), the Dutch guideline issued in 1999 does not include scorings for age, pre-treatment serum hCG concentration, the number of metastases

or largest tumour size. Items scored in the Dutch scoring system as 'low-risk' are given 0 points in FIGO 2000, except for the item antecedent pregnancy where the Dutch system classifies a non-molar abortion as 'low-risk', whereas FIGO 2000 scores one point for non-molar abortion. Although FIGO 2000 has been tested in 201 patients with persistent low hCG or high-risk GTD (112) against the WHO scoring system (95) and the in 1992 revised FIGO staging system (95), FIGO 2000 has not been tested against the Dutch classification for PTD (95,110,111).

Predicting persistent trophoblastic disease

Goldstein *et al.* from the New England Trophoblastic Disease Center published a list of risk factors for malignant sequelae after the evacuation of a hydatidiform mole (89). These risk factors comprise the existence of a pre-evacuation serum hCG > 100 000 IU/l, uterus large-for-date, theca lutein cysts > 6 cm, maternal age > 40 years and other factors like a history of GTD, hyperthyroidism, toxemia, trophoblastic embolisation and disseminated intravascular coagulation.

Biomarkers in the prediction of persistent trophoblastic disease

To identify in a very early stage (before evacuation) those patients who will develop PTD would be most challenging. Several studies have explored the potency of different biomarkers to enable early detection of PTD (58,62-64,113-123). Some studies, using a limited number of patients, reported that an increased ratio of hCGβ to total hCGβ identifies patients with molar pregnancy who are at risk for developing persistent disease (62-64). Two of these studies analyzed pre-evacuation serum samples (62,64). Neither study calculated a diagnostic accuracy of this test, although these studies reported a true positive rate of 83% and 91.2% and a true negative rate of 89% and 61.4% at a cut off ratio of free hCGβ over total hCGβ of 4 and 0.43, respectively (62,64). The third study included serum samples up to 113 days after curettage and is therefore not very meaningful in the early identification of PTD (63). Other investigators did not report an association between the ratio of free hCGβ to total hCGβ and PTD (58).

Inhibin may be an important regulator of foetal and human development (119). In clinical oncology, inhibins are used as markers for the diagnosis and follow-up of granulosa cell cancer (120). Yohkaichiya *et al.* were the

first to report that serum inhibin concentration was significantly more elevated seven days after evacuation of a hydatidiform mole in two patients who subsequently developed PTD as compared to four patients who did not (123). Other studies did neither find inhibin, nor its subforms inhibin A and B to be significantly different in a pre-evacuation serum sample from patients who either or not developed PTD (115,117). Inhibin is not considered to be of clinical relevance in the prediction or follow-up of PTD (117,121). In two studies serum progesterone concentrations have been found to be significantly elevated in pre-evacuation samples of patients who will develop PTD as compared to those who will not (115,122), whereas other studies did not report this finding (113,116). Because of these conflicting findings, the value of progesterone in predicting PTD needs to be established in a prospective trial (121).

The tumour marker CA-125 measured in pre-evacuation serum samples is not found to be of value in the prediction of PTD (64,114,118), nor are CEA, CA 15-3 and CA 19-9 (114). Finally, although Li *et al.* found that leptin and the leptin receptor are up-regulated in GTD, no differences were found between the group of patients who develop PTD and the group with spontaneous remission of serum hCG (124). No other serum biomarkers than hCG are reported to have clinical benefit in the detection and follow-up of PTD (121).

Molecular markers in gestational trophoblastic disease and persistent trophoblastic disease

Many cell cycle regulators like TP53, P21, RB, MDM2, DOC-2/hDab2, EGFR, C-erbB-2, C-erbB-3, C-erbB-4, Bcl-2, PTEN, and Cyclin E, are differently expressed in complete hydatidiform mole, choriocarcinoma and normal placenta (48,125-127). Cell adhesion molecules, e.g. Matrix Metalloproteinases 1 and 2 (MMP 1 and MMP 2) and Osteopontin (OPN), have significantly higher expression in choriocarcinoma than in normal placenta, partial and complete mole (128). Interestingly, the MMP inhibiting Tissue Inhibitor of Metalloproteinases (TIMPs) are less expressed in choriocarcinoma compared to normal placenta, partial mole and complete hydatidiform mole (128). The Heat Shock Protein 27 (HSP 27) which is involved in both placental differentiation and the acquisition of chemotherapeutic drug resistance, is found to be significantly down-regulated in choriocarcinoma as compared to normal placenta (129). Finally, Bcl-2 which is considered to be an antagonist of

apoptosis, is found to be strongly expressed in 84% of complete moles and 72.7% of choriocarcinoma as compared to absence of expression in normal placentas and expression of Bcl-2 in 5.8% of partial moles (130).

With respect to the development of PTD, several studies have identified a different expression pattern of anti-apoptotic-, onco- and tumour suppressor genes. The anti-apoptopic gene MCL-1 is found to be up-regulated in patients who will develop PTD (131). With respect to oncogenes, the Epidermal Growth Factor Receptor (EGFR) and its related tyrosine kinases c-erbB-2 and C-erbB-3 were found to be up-regulated in tissue of patients who would later be diagnosed with PTD (132,133). Another tyrosine kinase, c-erbB-4 was not found to be differentially expressed in tissue of patients who would later on develop PTD (133). The oncogene, c-ras, believed to have a cell regulating function in normal placenta (133), is found to have a decreased expression in PTD (133). Concerning tumour suppressor genes, the p53 gene products were found to be significantly more elevated in tissue of patients who would develop PTD as compared to that of patients with spontaneous regression of serum hCG (133). The DOC-2/hDab-2 gene is believed to be involved in the growth regulation of normal trophoblastic cells (134). This DOC-2/hDab-2 gene had a significantly decreased expression in a choriocarcinoma cell line and in vivo material of choriocarcinoma (134). Transfection of this DOC-2/hDab-2 gene in the choriocarcinoma cell line resulted in a significantly reduced growth rate (134). The product of the Nm23 gene has been proposed as a candidate metastatic tumour suppressor gene (133). The product of this gene was found to be significantly decreased in histological specimen from patients who would develop PTD as compared to patients who would not develop PTD (133).

Telomerase is a reverse transcriptase that stabilises the telomere ends of human chromosomes. With every DNA replication, the telomeres are shortened. Telomere shortening is believed to be a 'mitotic clock count': excessive shortening of telomere ends will trigger the onset of cell senescence (135). Most normal cells lack the telomerase activity. In hydatidiform moles of patients who developed PTD as compared to those with a spontaneous regression, the 'cell-life saving' telomerase activity was detectable in 75% versus 18.5% of cases, respectively ($P<0.01$)(135). Although differences in the expression of imprinted genes like P^{57kip} have been found between normal pregnancy and GTD (136), its value to predict PTD at an early stage remains to be elucidated (48).

Treatment of persistent trophoblastic disease

Second curettage

A repeat or second curettage for patients with PTD has been recommended by some authors (137,138), but the routine use of this procedure is advised against by others (82,139). Two studies on the curative effect of second curettage have been performed. The first retrospective study on 37 patients administered at the University of Southern California Women's Hospital between 1976-1980 and 1987-1989 showed a curative effect from a second curettage in 4 out of 23 (16%) patients with non-metastatic GTD. No curative effect was observed for the other patients in this study (with metastatic GTD or in retrospect no PTD) (139). The second, prospective study was executed by the Sheffield Trophoblastic Disease Centre (138). Between 1991 and 2000, 4075 patients with GTD were registered. Out of this cohort, 606 (15%) patients were diagnosed with PTD on the basis of elevated hCG (time course not mentioned), vaginal bleeding, abnormal tissue visible in utero on ultrasound or other gynaecological symptoms not specified. Of these 606 patients, 544 were treated with a second uterine evacuation. After this procedure, *no* further chemotherapeutic treatment was necessary in 68% of these patients. If PTD was diagnosed on elevated hCG only, 282 fulfilled the premises for PTD of which 171 (60%) needed *no* further chemotherapy for PTD. Unfortunately, the authors of this study did not specify the duration for which hCG should be elevated or rising before the criteria for the diagnosis of PTD were met. Upon request, the authors provided their criteria for PTD in a personal communication, stating that any patient whose serum hCG concentrations failed to normalize within 4-6 weeks after primary evacuation or had a rising hCG at any stage, were diagnosed with PTD. In 1993, our group showed that serum hCG after uneventful hydatidiform mole normalizes in 5% of patients 5 weeks evacuation, in 50% of patients after 11 weeks and in 95% after 26 weeks (99). The FIGO 2000 prognostic scoring system for PTD, clearly defines PTD if serum hCG shows a rise or plateau for three consecutive weeks (94). Failure of serum hCG to normalize within 6 weeks after first evacuation without plateau or rising in hCG (as defined by FIGO), in itself does not make trophoblastic disease persistent. Advising patients to undergo a second curettage 4-6 weeks after evacuation for "persistent trophoblastic disease" according to the criteria which were applied in the study by the Sheffield Trophoblastic Disease Centre (138) will put patients at risk

of complications of this procedure, which is unnecessary in the majority of patients.

Chemotherapy for persistent trophoblastic disease

Until the discovery by Hertz *et al.* and Li *et al.* in the 1950s that MTX is highly effective to cure PTD, this condition was highly lethal (91,93). Once diagnosed and scored, low-risk PTD is nowadays typically treated with single-agent chemotherapy (MTX or Actinomycin-D) (108,140). If staged as high-risk PTD, multi-agent chemotherapy with EMA-CO (Etoposide, MTX, Actinomycin-D, Cyclophosphamide and Vincristine) is most widely used (141). Patients with high- risk PTD who are treated with the EMA-CO regimen and whose serum hCG initially normalize but show recurrence or persistent low hCG levels afterwards, should be treated with the EMA-EP regimen (Etoposide, MTX, Actinomycin-D,Cyclophosphamide and Vincristine) (142). If patients with high-risk PTD fail to respond to Methotrexate containing regimens, cure has been reached with different kinds of platinum and etoposide containing regimens like BEP (Bleomycin, Etoposide, and Cisplatin), VIP (Etoposide, Ifosfamide and Cisplatin) and ICE (Ifosfamide, Carbosplatin and Etoposide) (142).

Approximately 9-33% of patients treated with single-agent therapy for low-risk PTD, will require multi-agent chemotherapy since they become resistant to the first-line drug or that toxic side effects occur (142-144). Repeated administration of MTX can induce MTX resistance (145), mainly due to down-regulation of the reduced folate carrier which is responsible for transport of MTX into the cell or amplification of the gene for dihydrofolate reductase. This enzyme reduces dihydrofolate in tetrahydrofolate which is essential in DNA synthesis (146,147). To date, an internationally accepted definition for resistance to first-line chemotherapy is lacking. In some clinics resistance to first-line chemotherapy is defined as a plateau or rise in serum hCG and/or development of new metastases (96,98,142-144). Others define resistance to single-agent chemotherapy more quantitatively, a less than 10% decrease of serum hCG concentrations within two weeks, or less than one log decrease of serum hCG within six weeks (148,149).

After single-agent chemotherapy with MTX, no increase in the number of second primary tumours has been observed (150). In contrast, patients with PTD who are treated with Etoposide containing multi-

agent chemotherapy have a 50% increased Relative Risk to develop secondary malignancies, in particular myeloid leukaemia, colon carcinoma and breast cancer, as compared to an age-matched control group (151). Furthermore, multi-agent chemotherapy hastens the occurrence of menopause by three years as compared to patients treated with single-agent MTX (152) and can be accompanied by the occurrence of alopecia and gastro-intestinal symptoms.

Regression corridors during treatment for persistent trophoblastic disease

Two studies have described the serum hCG regression pattern in patients treated with first-line single agent therapy responding to this therapy and in patients who do not. In the first study, Rotmensch *et al.* described the only normogram for normalization of hCG in 21 patients with non-metastatic trophoblastic disease treated with MTX, of whom 2 patients were treated with multi-agent chemotherapy after serum hCG failed to normalize after first-line single-agent therapy (96). They reported that the median time for serum hCG concentrations to normalize in successfully treated patients with PTD is 50 days. The second study included 72 patients with non-metastatic low-risk GTD (148). Sixty-nine patients were treated with Actinomycin-D as a first-line treatment, one patient was treated with MTX and two were treated with Etoposide. Of these patients, 64 responded to single- agent therapy and 8 needed multi-agent therapy since resistance occurred. In this study the median time to normalization were four, two weekly courses (56 days). Rotmensch *et al.* stated that in successfully treated patients with low-risk PTD, the median time to normalization is comparable to patients with uncomplicated regression of serum hCG after hydatidiform mole (50 days) (96). Serum hCG in two patients in the study by Rotmensch *et al.* failed to normalize after primary chemotherapy (96). Serum hCG of these two patients was above the P90 of the 'normal regression with MTX' at the start of the MTX treatment. These investigators did not explore the diagnostic accuracy of serum hCG levels to detect resistance to single-agent chemotherapy in low-risk PTD (96) nor did the authors of the second study (148).

The main objectives of this thesis are to assess:

1. The diagnostic accuracy of different serum hCG analytes and parameters to predict PTD (Chapters 2, 3, 4 and 5)
2. The value of second curettage in the management of PTD (Chapter 6)

3. The development of a regression curve for the early detection of resistance to first-line single-agent MTX therapy in patients treated for low-risk PTD (Chapter 7)

References

1. Ricci JV. Gynaecology during the Byzantine Period. Fourth to the Seventh Century. The Genealogy of Gynaecology. Second ed. Philadelphia/Toronto: The Blakiston Company; 1950. p. 145-80.
2. Bondeson J, Molenkamp A. The Countess Margaret of Henneberg and her 365 children. J R Soc Med 1996; 89:711-6.
3. Meertens Institute. Margaretha van Henneberg. Ref Type: Internet Communication. Online publication available from: http://www.meertens.knaw.nl/bol/fulltext_detail.php?id=1246
4. Ober WB, Fass RO. The early history of choriocarcinoma. J Hist Med 1961; 16:49-73.
5. Marchand F. Ueber die sogenannten "decidualen" Geschwülste im Anschluss an normale Geburt, Abort, Blasenmole und Extrauterinischzwangerschaft. Monatschrift für Geburtshilfe und Gynäkologie 1895; 1:419-38.
6. Berkowitz RS, Goldstein DP. Chorionic tumors. N Engl J Med 1996; 335:1740-8.
7. Curry SL, Hammond CB, Tyrey L, Creasman WT, Parker RT. Hydatidiform mole: diagnosis, management, and long-term follow up of 347 patients. Obstet Gynecol 1975; 45:1-8.
8. Kohorn EI. Molar pregnancy: presentation and diagnosis. Clin Obstet Gynecol 1984; 27:181-91.
9. Soto-Wright V, Bernstein M, Goldstein DP, Berkowitz RS. The changing clinical presentation of complete molar pregnancy. Obstet Gynecol 1995; 86:775-9.
10. Gemer O, Segal S, Kopmar A, Sassoon E. The current clinical presentation of complete molar pregnancy. Arch Gynecol Obstet 2000; 264:33-4.
11. Lindholm H, Flam F. The diagnosis of molar pregnancy by sonography and gross morphology. Acta Obstet Gynecol Scand 1999; 78:6-9.
12. Steigrad SJ. Epidemiology of gestational trophoblastic diseases. Best Pract Res Clin Obstet Gynaecol 2003; 17:837-47.
13. Smith HO, Kim SJ: Epidemiology, in: Hancock BW, Newlands ES, Berkowitz R, Cole L (ed): Gestational Trophoblastic Disease, online book. 2003, pp 39-76. http://www.isstd.org/gtd/03%20word%20for%20print.pdf
14. Takeuchi S. Incidence of gestational trophoblastic disease by regional registration in Japan. Hum Reprod 1987; 2:729-34.

15. Rolon PA, de Lopez BH. Epidemiological aspects of hydatidiform mole in the Republic of Paraguay (South America). Br J Obstet Gynaecol 1977; 84:862-4.
16. Olesnicky G, Quinn M. Molar pregnancy after artificial insemination (donor). Lancet 1984; 1:1296.
17. Yuen BH, Cannon W. Molar pregnancy in British Columbia: estimated incidence and postevacuation regression patterns of the beta subunit of human chorionic gonadotropin. Am J Obstet Gynecol 1981; 139:316-9.
18. Bagshawe KD, Dent J, Webb J. Hydatidiform mole in England and Wales 1973-83. Lancet 1986; 2:673-7.
19. Nielsen NH, Hansen JP. Trophoblastic tumors in Greenland. J Cancer Res Clin Oncol 1979; 95:177-86.
20. Mazzanti P, La VC, Parazzini F, Bolis G. Frequency of hydatidiform mole in Lombardy, Northern Italy. Gynecol Oncol 1986; 24:337-42.
21. Franke HR, Risse EK, Kenemans P, Vooijs GP, Stolk JG. Epidemiologic features of hydatidiform mole in the Netherlands. Obstet Gynecol 1983; 62:613-6.
22. Giwa-Osagie MO, Okwerekwu G. Epidemiology of molar pregnancies in Northern Ireland. Int J Gynaecol Obstet 1999; 66:175-7.
23. Teoh ES, Dawood MY, Ratnam SS. Epidemiology of hydatidiform mole in Singapore. Am J Obstet Gynecol 1971; 110:415-20.
24. Flam F, Rutqvist LE. Under-registration of gestational trophoblastic disease in the Swedish Cancer Registry. Eur J Epidemiol 1992; 8:683-6.
25. Flam F, Lundstrom-Lindstedt V, Rutqvist LE. Incidence of gestational trophoblastic disease in Stockholm County, 1975-1988. Eur J Epidemiol 1992; 8:173-7.
26. Mungan T, Kuscu E, Dabakoglu T, Senoz S, Ugur M, Cobanoglu O. Hydatidiform mole: clinical analysis of 310 patients. Int J Gynaecol Obstet 1996; 52:233-6.
27. Graham IH, Fajardo AM. The incidence and morphology of hydatidiform mole in Abu Dhabi, United Arab Emirates, 1978-1986. Br J Obstet Gynaecol 1988; 95:391-2.
28. Hayashi K, Bracken MB, Freeman DH, Jr., Hellenbrand K. Hydatidiform mole in the United States (1970-1977): a statistical and theoretical analysis. Am J Epidemiol 1982; 115:67-77.
29. Matsuura J, Chiu D, Jacobs PA, Szulman AE. Complete hydatidiform mole in Hawaii: an epidemiological study. Genet Epidemiol 1984; 1:271-84.
30. Smith HO, Qualls CR, Prairie BA, Padilla LA, Rayburn WF, Key CR. Trends in gestational choriocarcinoma: a 27-year perspective. Obstet Gynecol 2003; 102:978-87.

31. Jeffers MD, O'Dwyer P, Curran B, Leader M, Gillan JE. Partial hydatidiform mole: a common but underdiagnosed condition. A 3-year retrospective clinicopathological and DNA flow cytometric analysis. Int J Gynecol Pathol 1993; 12:315-23.
32. Womack C, Elston CW. Hydatidiform mole in Nottingham: a 12-year retrospective epidemiological and morphological study. Placenta 1985; 6:93-105.
33. Vassilakos P, Riotton G, Kajii T. Hydatidiform mole: two entities. A morphologic and cytogenetic study with some clinical consideration. Am J Obstet Gynecol 1977; 127:167-70.
34. Berkowitz RS, Goldstein DP. Gestational trophoblastic disease. Cancer 1995; 76:2079-85.
35. Bagshawe KD, Lawler SD, Paradinas FJ, Dent J, Brown P, Boxer GM. Gestational trophoblastic tumours following initial diagnosis of partial hydatidiform mole. Lancet 1990; 335:1074-6.
36. Berkowitz RS, Goldstein DP, Bernstein MR. Natural history of partial molar pregnancy. Obstet Gynecol 1985; 66:677-81.
37. Goto S, Yamada A, Ishizuka T, Tomoda Y. Development of postmolar trophoblastic disease after partial molar pregnancy. Gynecol Oncol 1993; 48:165-70.
38. Rice LW, Berkowitz RS, Lage JM, Goldstein DP, Bernstein MR. Persistent gestational trophoblastic tumor after partial hydatidiform mole. Gynecol Oncol 1990; 36:358-62.
39. Bischof P, Irminger-Finger I. The human cytotrophoblastic cell, a mononuclear chameleon. Int J Biochem Cell Biol 2005; 37:1-16.
40. Hubrecht AAW. Die Phylogenese des Amnions und die Bedeutung des Trophoblastes. Amsterdam: Johannes Muller; 1895.
41. Pollheimer J, Knofler M. Signalling pathways regulating the invasive differentiation of human trophoblasts: a review. Placenta 2005; 26 Suppl A: S21-S30.
42. Shih I, Kurman RJ. Molecular basis of gestational trophoblastic diseases. Curr Mol Med 2002; 2:1-12.
43. Bentley RC. Pathology of gestational trophoblastic disease. Clin Obstet Gynecol 2003; 46:513-22.
44. Kajii T, Ohama K. Androgenetic origin of hydatidiform mole. Nature 1977; 268:633-4.
45. Lawler SD, Pickthall VJ, Fisher RA, Povey S, Evans MW, Szulman AE. Genetic studies of complete and partial hydatidiform moles. Lancet 1979; 2:580.

46. Wake N, Takagi N, Sasaki M. Androgenesis as a cause of hydatidiform mole. J Natl Cancer Inst 1978; 60:51-7.
47. Ohama K, Kajii T, Okamoto E, Fukuda Y, Imaizumi K, Tsukahara M, et al. Dispermic origin of XY hydatidiform moles. Nature 1981; 292:551-2.
48. Matsuda T, Wake N. Genetics and molecular markers in gestational trophoblastic disease with special reference to their clinical application. Best Pract Res Clin Obstet Gynaecol 2003; 17:827-36.
49. Cheung AN. Pathology of gestational trophoblastic diseases. Best Pract Res Clin Obstet Gynaecol 2003; 17:849-68.
50. Howat AJ, Beck S, Fox H, Harris SC, Hill AS, Nicholson CM, et al. Can histopathologists reliably diagnose molar pregnancy? J Clin Pathol 1993; 46:599-602.
51. Paradinas FJ. The diagnosis and prognosis of molar pregnancy: the experience of the National Referral Centre in London. Int J Gynaecol Obstet 1998; 60 Suppl 1:S57-S64.
52. Hulsbergen-van der Kaa CA. DNA analysis of hydatidiform mole. PhD thesis, Catholic University Nijmegen, 1997.
53. Petignat P, Billieux MH, Blouin JL, Dahoun S, Vassilakos P. Is genetic analysis useful in the routine management of hydatidiform mole? Hum Reprod 2003; 18:243-9.
54. Li HW, Tsao SW, Cheung AN. Current understandings of the molecular genetics of gestational trophoblastic diseases. Placenta 2002; 23:20-31.
55. Cole LA, Kardana A, Park SY, Braunstein GD. The deactivation of hCG by nicking and dissociation. J Clin Endocrinol Metab 1993; 76:704-10.
56. Thomas CM, Reijnders FJ, Segers MF, Doesburg WH, Rolland R. Human choriogonadotropin (hCG): comparisons between determinations of intact hCG, free hCG beta-subunit, and "total" hCG + beta in serum during the first half of high-risk pregnancy. Clin Chem 1990; 36:651-5.
57. Elegbe RA, Pattillo RA, Hussa RO, Hoffmann RG, Damole IO, Finlayson WE. Alpha subunit and human chorionic gonadotropin in normal pregnancy and gestational trophoblastic disease. Obstet Gynecol 1984; 63:335-7.
58. Berkowitz RS, Ozturk M, Goldstein DP, Bernstein MR, Hill L, Wands JR. Human chorionic gonadotropin and free subunits' serum levels in patients with partial and complete hydatidiform moles. Obstet Gynecol 1989; 74:212-6.
59. Gaspard UJ, Reuter AM, Deville J-L, Vrindts-Gevaert Y, Bagshawe KD, Franchimont P. Serum concentration of human chorionic gonadotropin and its alpha and beta subunits. 2. Trophoblastic tumours. Clin Endocrinol (Oxf) 1980; 13:319-29.

60. Hay DL. Histological origins of discordant chorionic gonadotropin secretion in malignancy. J Clin Endocrinol Metab 1988; 66:557-64.
61. Ozturk M, Berkowitz R, Goldstein D, Bellet D, Wands JR. Differential production of human chorionic gonadotropin and free subunits in gestational trophoblastic disease. Am J Obstet Gynecol 1988; 158:193-8.
62. Khazaeli MB, Hedayat MM, Hatch KD, To AC, Soong SJ, Shingleton HM, et al. Radioimmunoassay of free beta-subunit of human chorionic gonadotropin as a prognostic test for persistent trophoblastic disease in molar pregnancy. Am J Obstet Gynecol 1986; 155:320-4.
63. Khazaeli MB, Buchina ES, Pattillo RA, Soong SJ, Hatch KD. Radioimmunoassay of free beta-subunit of human chorionic gonadotropin in diagnosis of high-risk and low-risk gestational trophoblastic disease. Am J Obstet Gynecol 1989; 160:444-9.
64. Mungan T, Kuscu E, Ugur M, Dabakoglu T, Senses E, Cobanoglu O. Screening of persistent trophoblastic disease with various serum markers. Eur J Gynaecol Oncol 1998; 19:495-7.
65. Shi QJ, Lei ZM, Rao CV, Lin J. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. Endocrinology 1993; 132:1387-95.
66. Hoshina M, Boothby M, Boime I. Cytological localization of chorionic gonadotropin alpha and placental lactogen mRNAs during development of the human placenta. J Cell Biol 1982; 93:190-8.
67. Naylor SL, Chin WW, Goodman HM, Lalley PA, Grzeschik KH, Sakaguchi AY. Chromosome assignment of genes encoding the alpha and beta subunits of glycoprotein hormones in man and mouse. Somatic Cell Genet 1983; 9:757-70.
68. Policastro PF, Daniels-McQueen S, Carle G, Boime I. A map of the hCG beta-LH beta gene cluster. J Biol Chem 1986; 261:5907-16.
69. Hussa RO. Biosynthesis of human chorionic gonadotropin. Endocr Rev 1980; 1:268-94.
70. Jameson JL, Hollenberg AN. Regulation of chorionic gonadotropin gene expression. Endocr Rev 1993; 14:203-21.
71. Thomas CMG. Nieuwe ontwikkelingen bij bepaling van hCG, een overzicht. Ned Tijdschr Klin Chem Labgeesk 2005; 30:8-16.
72. Cole LA, Butler S. Detection of hCG in trophoblastic disease. The USA hCG reference service experience. J Reprod Med 2002; 47:433-44.
73. Cole LA, Shahabi S, Oz UA, Bahado-Singh RO, Mahoney MJ. Hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen) immunoassay: A new basis for gestational Down syndrome screening. Clin Chem 1999; 45:2109-19.

74. Elliott MM, Kardana A, Lustbader JW, Cole LA. Carbohydrate and peptide structure of the alpha- and beta-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine 1997; 7:15-32.
75. Kovalevskaya G, Genbacev O, Fisher SJ, Caceres E, O'Connor JF. Trophoblast origin of hCG isoforms: cytotrophoblasts are the primary source of choriocarcinoma-like hCG. Mol Cell Endocrinol 2002; 194:147-55.
76. Asscheim S, Zondek B. Hypofysenvorderlappen und Ovarialhormon im Harn von Schwangeren. Klin Wochenschr 1927; 6:1322.
77. Berson SA. Yalow SA., Bauman A., Rothschild MA., Newerly K. Insulin-I131 metabolism in human subjects: demonstration of insulin binding globulin in the circulation of insulin treated subjects. J Clin Invest 1956; 35:170-90.
78. Vaitukaitis JL, Braunstein GD, Ross GT. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am J Obstet Gynecol 1972; 113:751-8.
79. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. Ann Clin Biochem 1985; 22:236-46.
80. Cole LA, Kohorn EI, Kim GS. Detecting and monitoring trophoblastic disease. New perspectives on measuring human chorionic gonadotropin levels. J Reprod Med 1994; 39:193-200.
81. Sasaki S. Clinical presentation and management of molar pregnancy. Best Pract Res Clin Obstet Gynaecol 2003; 17:885-92.
82. Royal College of Obstetricians and Gynaecologists. The Management of Gestational Trophoblastic Neoplasia, guideline no 38. Available from: http://www.rcog.org.uk/resources/Public/pdf/Gestational_Troph_Neoplasia_No038.pdf
83. Soper JT, Mutch DG, Schink JC. Diagnosis and treatment of gestational trophoblastic disease: ACOG Practice Bulletin No. 53. Gynecol Oncol 2004; 93:575-85.
84. Dutch Society for Obstetrics and Gynaecology. Guideline 21: Hydatidiform mole and Persistent Trophoblastic Disease. Available from: http://www.nvog.nl
85. Dutch Society for Obstetrics and Gynaecology. Molazwangerschap 1.1. Available from: http://www.oncoline.nl
86. Limpongsanurak S. Prophylactic actinomycin D for high-risk complete hydatidiform mole. J Reprod Med 2001; 46:110-6.
87. Kashimura Y, Kashimura M, Sugimori H, Tsukamoto N, Matsuyama T, Matsukuma K, et al. Prophylactic chemotherapy for hydatidiform mole. Five to 15 years follow-up. Cancer 1986; 58:624-9.

88. Kim DS, Moon H, Kim KT, Moon YJ, Hwang YY. Effects of prophylactic chemotherapy for persistent trophoblastic disease in patients with complete hydatidiform mole. Obstet Gynecol 1986; 67:690-4.
89. Goldstein DP, Berkowitz RS, Bernstein MR. Management of molar pregnancy. J Reprod Med 1981; 26:208-12.
90. Park TK, Kim SN, Lee SK. Analysis of risk factors for postmolar trophoblastic disease: categorization of risk factors and effect of prophylactic chemotherapy. Yonsei Med J 1996; 37:412-9.
91. Hertz R, Li MC, Spencer DB. Effect of methotrexate therapy upon choriocarcinoma and chorioadenoma. Proc Soc Exp Biol Med 1956; 93:361-6.
92. Hertz R, Ross GT, Lipsett MB. Primary chemotherapy of nonmetastatic trophoblastic disease in women. Am J Obstet Gynecol 1963; 86:808-14.
93. Li MC, Hertz R, Bergenstal DM. Therapy of choriocarcinoma and related trophoblastic tumors with folic acid and purine antagonists. N Engl J Med 1958; 259:66-74.
94. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000: FIGO Oncology Committee. Int J Gynaecol Obstet 2002; 77:285-7.
95. Hancock BW. Staging and classification of gestational trophoblastic disease. Best Pract Res Clin Obstet Gynaecol 2003; 17:869-83.
96. Rotmensch J, Rosenshein NB, Block BS. Comparison of human chorionic gonadotropin regression in molar pregnancies and post-molar nonmetastatic gestational trophoblastic neoplasia. Gynecol Oncol 1988; 29:82-6.
97. Schlaerth JB, Morrow CP, Kletzky OA, Nalick RH, D'Ablaing GA. Prognostic characteristics of serum human chorionic gonadotropin titer regression following molar pregnancy. Obstet Gynecol 1981; 58:478-82.
98. Shigematsu T, Kamura T, Saito T, Kaku T, Nakano H, Kinugawa N. Identification of persistent trophoblastic diseases based on a human chorionic gonadotropin regression curve by means of a stepwise piecewise linear regression analysis after the evacuation of uneventful moles. Gynecol Oncol 1998; 71:376-80.
99. Yedema KA, Verheijen RH, Kenemans P, Schijf CP, Borm GF, Segers MF, et al. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. Am J Obstet Gynecol 1993; 168:787-92.
100. Behtash N, Ghaemmaghami F, Honar H, Riazi K, Nori A, Modares M, et al. Is normal beta-hCG regression curve helpful in the diagnosis of persistent trophoblastic disease? Int J Gynecol Cancer 2004; 14:980-3.

101. Ross GT, Goldstein DP, Hertz R, et al. Sequential use of methotrexate and actinomycin D in the treatment of metastatic choriocarcinoma and related trophoblastic diseases in women. Am J Obstet Gynecol 1965; 93:223-9.
102. Hammond CB, Borchert LG, Tyrey L, Creasman WT, Parker RT. Treatment of metastatic trophoblastic disease: good and poor prognosis. Am J Obstet Gynecol 1973; 115:451-7.
103. Bagshawe KD. Risk and prognostic factors in trophoblastic neoplasia. Cancer 1976; 38:1373-85.
104. Gestational trophoblastic diseases. Report of a WHO Scientific Group.World Health Organ Tech Rep Ser 1983; 692:7-81.
105. FIGO Oncology Committee Report (1992). Int J Gynecol Obstet 1992; 39:149-50.
106. Soper JT, Evans AC, Conaway MR, Clarke-Pearson DL, Berchuck A, Hammond CB. Evaluation of prognostic factors and staging in gestational trophoblastic tumor. Obstet Gynecol 1994; 84:969-73.
107. Azab MB, Pejovic MH, Theodore C, George M, Droz JP, Bellet D, et al. Prognostic factors in gestational trophoblastic tumors. A multivariate analysis. Cancer 1988; 62:585-92.
108. Ng TY, Wong LC. Diagnosis and management of gestational trophoblastic neoplasia. Best Pract Res Clin Obstet Gynaecol 2003; 17:893-903.
109. Ngan HY. The practicability of FIGO 2000 staging for gestational trophoblastic neoplasia. Int J Gynecol Cancer 2004; 14:202-5.
110. Ma H-K, Wong L-C, Ngan H. Staging and classification systems. In: Hancock BW, Newlands ES, Berkowitz RS, editors. Gestational Trophoblastic Disease. 1 ed. London: Chapman & Hall; 1997. p. 95-108.
111. Nederlandse Vereniging voor Obstetrie en Gynaecologie. Molazwangerschap en persisterende trofoblast. http://www nvog nl/files/21_molazwang_persist_trofo pdf 1999(21)
112. Hancock BW, Welch EM, Gillespie AM, Newlands ES. A retrospective comparison of current and proposed staging and scoring systems for persistent gestational trophoblastic disease. Int J Gynecol Cancer 2000; 10:318-22.
113. Dawood MY. The significance of serum progesterone and serum unconjugated oestradiol-17beta in unaborted hydatidiform mole. Acta Endocrinol (Copenh) 1975; 79:729-39.
114. Hegab HM, Schindler AE, Rizk M, Ramadan M. Evaluation of tumour markers in molar pregnancy. Arch Gynecol Obstet 2003; 268:151-4.
115. Hegab HM, Schindler AE. The prognostic value of serum inhibin, 17 beta-estradiol and progesterone in cases of hydatidiform mole. Gynecol Endocrinol 2004; 18:107-13.

116. Ho PC, Wong LC, Ma HK. Plasma prolactin, progesterone, estradiol, and human chorionic gonadotropin in complete and partial moles before and after evacuation. Obstet Gynecol 1986; 67:99-106.
117. Kato T, Seki K, Matsui H, Sekiya S. Circulating inhibin forms in patients with hydatidiform mole. Gynecol Obstet Invest 2002; 54:114-7.
118. Kohorn EI. Measurement of CA-125 in trophoblastic disease. Gynecol Oncol 2000; 78:39-42.
119. Riley SC, Wathen NC, Chard T, Groome NP, Wallace EM. Inhibin in extra-embryonic coelomic and amniotic fluids and maternal serum in early pregnancy. Hum Reprod 1996; 11:2772-6.
120. Robertson DM, Pruysers E, Burger HG, Jobling T, McNeilage J, Healy D. Inhibins and ovarian cancer. Mol Cell Endocrinol 2004; 225:65-71.
121. Seki K, Matsui H, Sekiya S. Advances in the clinical laboratory detection of gestational trophoblastic disease. Clin Chim Acta 2004; 349:1-13.
122. Vasilev SA, Schlaerth JB, Ahn C, Sauer MV. Serum progesterone monitoring in post-molar surveillance. Gynecol Oncol 1994; 54:288-91.
123. Yohkaichiya T, Fukaya T, Hoshiai H, Yajima A, de Kretser DM. Inhibin: a new circulating marker of hydatidiform mole? BMJ 1989; 298:1684-6.
124. Li RH, Yu MM, Cheung AN, Wong YF. Expression of leptin and leptin receptors in gestational trophoblastic diseases. Gynecol Oncol 2004; 95:299-306.
125. Fulop V, Mok SC, Berkowitz RS. Molecular biology of gestational trophoblastic neoplasia: a review. J Reprod Med 2004; 49:415-22.
126. Kim YT, Cho NH, Ko JH, Yang WI, Kim JW, Choi EK, et al. Expression of cyclin E in placentas with hydropic change and gestational trophoblastic diseases: implications for the malignant transformation of trophoblasts. Cancer 2000; 89:673-9.
127. Chen H, Ye D, Xie X, Lu W, Zhu C, Chen X. PTEN promoter methylation and protein expression in normal early placentas and hydatidiform moles. J Soc Gynecol Investig 2005; 12:214-7.
128. Vegh GL, Selcuk TZ, Fulop V, Genest DR, Mok SC, Berkowitz RS. Matrix metalloproteinases and their inhibitors in gestational trophoblastic diseases and normal placenta. Gynecol Oncol 1999; 75:248-53.
129. Vegh GL, Fulop V, Liu Y, Ng SW, Tuncer ZS, Genest DR, et al. Differential gene expression pattern between normal human trophoblast and choriocarcinoma cell lines: downregulation of heat shock protein-27 in choriocarcinoma in vitro and in vivo. Gynecol Oncol 1999; 75:391-6.

130. Fulop V, Mok SC, Genest DR, Szigetvari I, Cseh I, Berkowitz RS. c-myc, c-erbB-2, c-fms and bcl-2 oncoproteins. Expression in normal placenta, partial and complete mole, and choriocarcinoma. J Reprod Med 1998; 43:101-10.
131. Fong PY, Xue WC, Ngan HY, Chan KY, Khoo US, Tsao SW, et al. Mcl-1 expression in gestational trophoblastic disease correlates with clinical outcome: a differential expression study. Cancer 2005; 103:268-76.
132. Tuncer ZS, Vegh GL, Fulop V, Genest DR, Mok SC, Berkowitz RS. Expression of epidermal growth factor receptor-related family products in gestational trophoblastic diseases and normal placenta and its relationship with development of postmolar tumor. Gynecol Oncol 2000; 77:389-93.
133. Yang X, Zhang Z, Jia C, Li J, Yin L, Jiang S. The relationship between expression of c-ras, c-erbB-2, nm23, and p53 gene products and development of trophoblastic tumor and their predictive significance for the malignant transformation of complete hydatidiform mole. Gynecol Oncol 2002; 85:438-44.
134. Fulop V, Colitti CV, Genest D, Berkowitz RS, Yiu GK, Ng SW, et al. DOC-2/hDab2, a candidate tumor suppressor gene involved in the development of gestational trophoblastic diseases. Oncogene 1998; 17:419-24.
135. Cheung AN, Zhang DK, Liu Y, Ngan HY, Shen DH, Tsao SW. Telomerase activity in gestational trophoblastic disease. J Clin Pathol 1999; 52:588-92.
136. Chilosi M, Piazzola E, Lestani M, Benedetti A, Guasparri I, Granchelli G, et al. Differential expression of p57kip2, a maternally imprinted cdk inhibitor, in normal human placenta and gestational trophoblastic disease. Lab Invest 1998; 78:269-76.
137. Fisher PM, Hancock BW. Gestational trophoblastic diseases and their treatment. Cancer Treat Rev 1997; 23:1-16.
138. Pezeshki M, Hancock BW, Silcocks P, Everard JE, Coleman J, Gillespie AM, et al. The role of repeat uterine evacuation in the management of persistent gestational trophoblastic disease. Gynecol Oncol 2004; 95:423-9.
139. Schlaerth JB, Morrow CP, Rodriguez M. Diagnostic and therapeutic curettage in gestational trophoblastic disease. Am J Obstet Gynecol 1990; 162:1465-70.
140. Osborne R, Gerulath A. What is the best regimen for low-risk gestational trophoblastic neoplasia? A review. J Reprod Med 2004; 49:602-16.
141. Lurain JR. Advances in management of high-risk gestational trophoblastic tumors. J Reprod Med 2002; 47:451-9.
142. Lurain JR. Treatment of Metastatic Gestational Trophoblastic Tumors. In: Hancock BW, Newlands ES, Berkowitz R, editors. Gestational Trophoblastic Disease. First ed. London: Chapman & Hall; 1997. p. 199-209.

143. McNeish IA, Strickland S, Holden L, Rustin GJ, Foskett M, Seckl MJ, et al. Low-risk persistent gestational trophoblastic disease: outcome after initial treatment with low-dose methotrexate and folinic acid from 1992 to 2000. J Clin Oncol 2002; 20:1838-44.
144. Matsui H, Suzuka K, Yamazawa K, Tanaka N, Mitsuhashi A, Seki K, et al. Relapse rate of patients with low-risk gestational trophoblastic tumor initially treated with single-agent chemotherapy. Gynecol Oncol 2005; 96:616-20.
145. Sobrero A, Aschele C, Rosso R, Nicolin A, Bertino JR. Rapid development of resistance to antifolates in vitro: possible clinical implication. J Natl Cancer Inst 1991; 83:24-8.
146. Moscow JA, Gong M, He R, Sgagias MK, Dixon KH, Anzick SL, et al. Isolation of a gene encoding a human reduced folate carrier (RFC1) and analysis of its expression in transport-deficient, methotrexate-resistant human breast cancer cells. Cancer Res 1995; 55:3790-4.
147. Gorlick R, Goker E, Trippett T, Waltham M, Banerjee D, Bertino JR. Intrinsic and acquired resistance to methotrexate in acute leukemia. N Engl J Med 1996; 335:1041-8.
148. Lertkhachonsuk R, Limpongsanurak S. Serum human chorionic gonadotropin regression pattern in persistent trophoblastic disease during chemotherapy. J Med Assoc Thai 2001; 84 Suppl 1:S352-S359.
149. Homesley HD. Single-agent therapy for nonmetastatic and low-risk gestational trophoblastic disease. J Reprod Med 1998; 43:69-74.
150. Seckl MJ, Rustin GJ. Late Toxicity after Therapy of Gestational Trophoblastic Tumors. In: Hancock BW, Newlands ES, Berkowitz R, Cole L, editors. Gestational Trophoblastic Disease, online book. Second edition ed. 2003. p. 367-79.
151. Rustin GJ, Newlands ES, Lutz JM, Holden L, Bagshawe KD, Hiscox JG, et al. Combination but not single-agent methotrexate chemotherapy for gestational trophoblastic tumors increases the incidence of second tumors. J Clin Oncol 1996; 14:2769-73.
152. Bower M, Rustin GJ, Newlands ES, Holden L, Short D, Foskett M, et al. Chemotherapy for gestational trophoblastic tumours hastens menopause by 3 years. Eur J Cancer 1998; 34:1204-7.

CHAPTER 2

Diagnosis of hydatidiform mole and persistent trophoblastic disease: diagnostic accuracy of total human chorionic gonadotropin (hCG), free hCG α- and β-subunits, and their ratios

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Abstract

Objective: Human Chorionic Gonadotropin (hCG) is widely used in the management of hydatidiform mole and persistent trophoblastic disease (PTD). Predicting PTD after molar pregnancy might be beneficial since prophylactic chemotherapy reduces the incidence of PTD.

Design: A retrospective study based on blood specimen collected in the Dutch Registry for Hydatidiform Moles. A group of 165 patients with complete (of which 43 had PTD) and 39 patients with partial moles (of which 7 had PTD) were compared with 27 pregnant women with uneventful pregnancy.

Methods: Serum samples from patients with hydatidiform mole with or without PTD were assayed using specific (radio)immunoassays for free α-subunit (hCGα), free β-subunit (hCGβ) and 'total' hCG (hCG + hCGβ). In addition, we calculated the ratios hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ, and hCGα/hCGβ. Specificity and sensitivity were calculated and paired in receiver-operating characteristic (ROC) curve analysis, resulting in areas under the curves (AUCs).

Results: hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ show AUCs ranging between 0.922 and 0.999 and, therefore, are excellent diagnostic tests to distinguish complete and partial moles from normal pregnancy. To distinguish partial from complete moles the analytes hCGβ, hCG + hCGβ and the ratio hCGα/hCGβ have AUCs between 0.7 and 0.8. Although hCGα, hCGβ and hCG + hCGβ concentrations are significantly elevated in patients who will develop PTD compared with patients with spontaneous regression after evacuation of their moles, in predictiong PTD, these analytes and parameters have AUCs <0.7.

Conclusions: Distinction between hydatidiform mole and normal pregnancy is best shown by a single blood specimen with hCGβ, but hCGβ/hCG + hCGβ and hCGα/hCGβ are also excellent diagnostic parameters. To predict PTD, hCGα, hCGβ, hCG + hCGβ and hCGα/hCGβ are moderately accurate tests, although they are not accurate enough to justify prophylactic chemotherapy treatment for prevention of PTD.

Introduction

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone produced by trophoblastic tissue and therefore is a key marker in pregnancy and gestational trophoblastic disease (GTD) (1). A variety of pathological types of trophoblast are included in GTD comprising villous malformations of trophoblast: hydatidiform mole, subdivided in complete and partial hydatidiform moles, and non-villous malformations of which choriocarcinoma is the most frequent (2). Genetically, complete moles are diploid (46 XX or XY) which is the result of an 'empty' oocyte (after degeneration of the nucleus) being fertilized by one haploid sperm, followed by duplication of its chromosome, or fertilization of an empty egg by two spermatozoa (3). The syndrome of partial (incomplete) mole has an ascertainable fetus (alive or dead) and a triploid karyotype (69 XXX or XXY) after fertilization of a normal ovum by two spermatozoa (3). Incidence of hydatiform mole is highest in South-East Asia, Indonesia, India and Turkey with rates ranging from 2 to 12 per 1000 pregnancies. In North America and Europe, incidence is lower: 0.5 to 1 per 1000 pregnancies. Interestingly, significant reductions in the incidence of hydatidiform mole have recently been reported in Korea, Japan and Taiwan to levels comparable to those in North America and Europe (4). In persistent trophoblastic disease (PTD), trophoblastic activity remains after evacuation of the hydatidiform mole as shown by subsequent unaltered high or even rising hCG concentrations in blood. The reported frequency of PTD is 20% in complete hydatidiform mole (5) and 0.5 to 9.9% in partial hydatidiform mole (6-9). In order to prevent complications from metastatic disease, PTD needs to be treated. Prophylactic chemotherapy (started immediately after evacuation of the mole) reduces the incidence of PTD to 4-12% (10,11). Because of the large proportion of patients who will show spontaneous remission of molar pregnancy after evacuation and because of the side effects of chemotherapy, clinicians are reluctant to use prophylactic chemotherapy. It would therefore be helpful to identify patients at risk for developing PTD. hCG is composed of two noncovalently bound α- and β-subunits. The α-subunit of hCG comprises 92 amino acids and is identical to the α-subunit of the pituitary glycoprotein hormones follicle-stimulating hormone (FSH), thyrotropin (TSH) and luteinizing hormone (LH). The β-subunit is composed of 145 amino acids, which distinguish hCG from these other glycoproteins. In addition to intact hCG, free hCG α-subunit, free hCG β-subunit, and the

hCG β-subunit core fragment are present in blood. Since most hCG radioimmunoassays detect both intact hCG and free hCGβ, these tests are designated 'total hCG' or 'hCG + hCGβ' assays. In normal pregnancy, concentrations of intact hCG, hCG + hCGβ and hCGβ in blood double approximately every 2 days to reach a peak at 8 to 10 weeks of gestation. From week 10 to 20, these concentrations decline to levels comparable to those in early first trimester and from 20 weeks on they remain constantly low (12). In contrast, hCGα concentrations in blood increase steadily until the end of pregnancy (13). The production of subunits of hCG is under stringent physiological control in normal pregnancy, and is reported to be different in pathological states such as hydatidiform mole (14-16), although the literature data are not unequivocal. In particular, the concentration of hCGβ and the ratio of hCGβ to total hCGβ is reported to be increased in molar as compared with normal pregnancy (17). Some studies, using a limited number of patients, reported that an increased ratio of hCGβ to total hCGβ identifies patients with molar pregnancy who are at risk for developing persistent disease (18-20), although other investigators did not report such an association (14).

The present retrospective study includes measurement of hCGα, hCGβ, hCG + hCGβ and calculates the ratios of hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ in blood taken before evacuation from 203 patients with hydatidiform mole of which 43 developed PTD. The aim of this study is to evaluate the clinical utility of the six hCG parameters by receiver-operating characteristic (ROC) curve analysis to distinguish normal pregnancy from hydatidiform mole, partial from complete mole, and to predict the occurrence of PTD.

Materials and Methods

Patients

In The Netherlands, patients with hydatidiform mole can be registered, after informed consent, at the Dutch Central Registry for Hydatidiform Moles residing at the Radboud University Nijmegen Medical Centre (RUNMC). We have included 1630 patients in this database. We excluded all patients with a histological diagnosis of choriocarcinoma. In 692 registered patients with hydatidiform mole, hCG in blood was analyzed in our department at the RUNMC. After collection, blood samples were centrifuged and serum was sent to our institute and kept at -20 °C until

assayed. Of these 692 patients, 430 had to be excluded since no blood specimen was taken prior to evacuation. Another 58 patients were excluded to match the gestational age in the control group. This led to the selection of 204 patients (mean age 27.9 years, range 16 to 54 years). Of those 204 patients, 129 had a complete hydatidiform mole with normal serum hCG regression (Group 1), as derived from a normal regression curve constructed by Yedema *et al.* (21). Another 36 patients with a complete mole developed PTD after evacuation (Group 2) while 32 patients had a partial mole with normal hCG regression (Group 3) whereas 7 patients with a partial hydatidiform mole developed PTD after evacuation (Group 4). The pathology institution of the Dutch Central Registry reviewed the histology of all patients with a hydatidiform mole. The control group comprised 27 women (mean age 28.2 years, range 23 to 39 years) with uneventful pregnancies, whose blood was collected on a weekly basis between 7 and 16 weeks of gestation except in weeks 13 and 15.

Immunoassays

All the measurements of 'total' hCG (i.e. intact hCG and free β-subunit, hCG + hCGβ) or its free α- or β-subunit in serum were performed with sensitive and specific radioimmunoassays (RIAs) that have been developed in our laboratory. The RIAs of hCG + hCGβ and hCGβ have been described previously (22), while the hCGα RIA was developed recently. Polyclonal anti-rabbit antisera were used in the RIAs of hCG + hCGβ and hCGα, and a monoclonal antibody (23) in the RIA for hCGβ. A highly purified hCG β-subunit preparation labeled with iodine-125 (NaI^{125}, Amersham plc, Amersham, Bucks, UK) was employed as a tracer in the RIAs of hCG + hCGβ and hCGβ, while the RIA of hCGα used I^{125}-labeled hCG α-subunit as a tracer. The RIAs were calibrated with the third International Standard (IS) Preparations for intact hCG, hCG α-subunit or hCG β-subunit (WHO third IS hCG 75/537, hCGα 75/569 or hCGβ 75/551 respectively, all obtained from the National Institute for Biological Standards, Potters Bar, Herts, UK).

Conversion factors are as follows: hCGα: 1μg/l is 0.0714 nmol/l and equivalent to 1 IU/l; hCGβ: 1μg/l is 0.0426 nmol/l and equivalent to 1 IU/l; hCG: 1 μg/l is 0.0267 nmol/l and equivalent to 9.29 IU/l. The measuring ranges of the assays were 0.027-2.14 nmol/l (1-80 μg/l, equivalent to 9.29-743 IU/l) for hCG + hCGβ, 0.0036-0.43 nmol/l (0.05-6.0 IU/l or μg/l) for hCGα, and 0.0033-0.107 nmol/l (0.078-2.50 IU/l or μg/l) for hCGβ. All

the RIAs applied the same assay protocol. In brief, the procedure comprised the following steps. To increase the sensitivity of the RIAs, the mixtures of standard material or serum specimen together with the antiserum were incubated for 18 h at 20 °C, and after addition of labeled analyte this was followed by a second incubation (6 h at 20 °C). Antibody-bound and free analyte were separated by applying second antibody donkey anti-rabbit IgG coupled to cellulose (Sac-Cel, The Wellcome Foundation Ltd, Dartford, Kent, UK) in the case of the hCG + hCGβ and hCGα RIAs, and by donkey anti-mouse IgG coupled to cellulose in the free hCGβ RIA. The RIA developed for hCGα cross-reacted 100% (on a molar basis at 50% displacement) with the α-subunits of LH, FSH and TSH, and 2%, 3.6%, 13% and 17% with the WHO International Reference Preparations (IRP) of native hCG, TSH, LH, and FSH respectively.

Intact hCG and the free hCG β-subunit are abundantly present in serum samples from normal or mole pregnancies (unlike LH and FSH which both are suppressed during pregnancy while TSH concentrations are very low). For this reason, it was necessary to conduct an affinity chromatography procedure to eliminate intact hCG and free hCG β-subunit from each serum sample prior to determining its free hCG α-subunit concentration by this RIA. Thus, 100 µl of serum sample were transferred to a HiTrap N-hydroxy-succinimide (NHS)-activated column (Pharmacia, Uppsala, Sweden) coupled to polyclonal antiserum against free hCG β-subunit raised in rabbit, and the column effluent was checked by RIA for absence of the intact hCG and free hCG β-subunit present in the serum sample prior to chromatography. Next, the free hCG α-subunit concentrations were determined with the hCGα RIA. The free hCG β-subunit RIA showed a cross-reactivity with intact hCG of 0.35% (on a mass basis, equivalent to 0.55% on a molar basis) as tested with the WHO third IS 75/537 of hCG, 1.1% on a molar basis with nicked hCG (hCGn, WHO 99/642 Reference Reagent) and 0.4% with hCGβn (WHO 99/692 Reference Reagent) while the hCG + hCGβ RIA cross-reacted 100% on a molar basis with intact hCG and 1000% with hCG β-subunit, and 228% with hCGn and 507% with hCGβn (which is of minor practical importance because these nicked forms of hCG mainly occur in urine). The 95th percentile of the reference interval of healthy non-pregnant controls for the hCG + hCGβ assay was established at 0.053 nmol/l (2 µg/l or 18.6 IU/l of the WHO third IS hCG 75/537) (22), 0.286 nmol/l (4.0 µg/l or IU/l of the WHO third IS hCGα 75/569) with the

hCGα RIA, and 0.0085 nmol/l (0.20 µg/l or IU/l of the WHO third IS hCGβ 75/551) with the hCGβ RIA (24). The intra- and interassay coefficients of variation (CV_w, CV_b) for means of duplicate measurements for two serum pools (mean: 0.267 nmol/l (10 µg/l or 93 IU/l) and 1.50 nmol/l (56 µg/l or 520 IU/l) were 7.3% and 12% for the hCG + hCGβ RIA, 3.3% - 5.6% (CV_w) and 7.2% - 8.4% (CV_b) with two serum pools (mean: 0.510 nmol/l (7.1 µg/l or IU/l) and 3.07 nmol/l (43 µg/l or IU/l)) in the case of the hCGα RIA, and 5.2% - 5.8% (CV_w) and 9.5% - 9.9% (CV_b) with two serum pools (mean: 0.014 nmol/l (0.33 µg/l or IU/l) and 0.041 nmol/l (0.96 µg/l or IU/l)) in the hCGβ RIA.

Statistics

Calculation of reference values for normal pregnancy

To construct reference values for normal pregnancy, we collected blood samples in our control group on a weekly basis from week 7 to 16 of gestational age except in weeks 13 and 15. We determined the longitudinal patterns ('response curve') of each experimentally determined hCG analyte (hCG + hCGβ, hCGα, and hCGβ, all expressed in nmol/l), as well as for the calculated molar ratios of hCG α- and β-subunits to hCG + hCGβ (hCGα/hCG + hCGβ and hCGβ/hCG + hCGβ) and the ratio of hCGα to hCGβ (hCGα/hCGβ). In order to obtain normal Gaussian distributed data for the control group consisting of 27 pregnant women, we performed log transformations of all the determined hCGα concentrations (nmol/l) and calculated molar ratio of hCGα/hCGβ, while we performed square root transformation of the serum concentrations of hCGβ (nmol/l), hCG + hCGβ (nmol/l) and its calculated molar ratio. Next, by subsequent pooling (over the weeks) of variances (25), we calculated P5, P50, and P95 for these transformed data (i.e. mean(log or square root) ± 1.645 S.E. (log or square root)) to become, after back transformation, the reference values (the 5th, 50th, and 95th percentiles, P5, P50, P95) separately for each week of the gestational period for the three analytes and three molar ratios.

Statistical comparison of pregnancy controls with molar pregnancy subsets

Each individual log- or square root-transformed hCG serum analyte or parameter from the 27 control pregnancies were expressed as a multiple of the median (MoM) for each gestational week. Because the longitudinal patterns for each individual patient revealed rather constant MoMs

along the gestational period studied, we could calculate the mean MoM (mMoM) for each patient over the entire pregnancy period studied - 7 to 16. From these mMoMs for each patient we calculated the 'grand' mMoM ± S.E. Next, we calculated the MoMs for each of the available individual log- or square root-transformed hCG serum analytes of all the molar pregnancies. This was done for each mole by dividing the measured log- or square root-transformed blood concentration (or calculated ratios) of analyte or molar ratio by the corresponding P50 value of the control pregnancies as calculated and matched for the corresponding gestational week. Statistical significance of differences was tested with the Mann-Whitney U-test between the mean MoMs of all control pregnancies (the 'grand' mMoM) versus the calculated mean MoM of all molar pregnancies or versus the various subsets of moles (complete moles, partial moles), as well as between complete versus partial moles, or the presence or absence of PTD in case of molar pregnancies. The calculated MoM values of all six hCG analytes and parameters of control and study groups were utilized to construct ROC curves and to calculate areas under the curve (AUC) for assessment of diagnostic accuracy of the test. All calculations were conducted with SPSS (version 12.0) for Microsoft Windows XP (SPSS, Chicago, IL, USA).

ROC curves represent the full spectrum of possible sensitivity-specificity pairs for a test in a clinical application (26). Usually, it is assumed that the study group has higher values of the tested parameters than the control group, resulting in an AUC between 0.5 and 1.0. Conversely, if results are lower in the study group than in the control group, an AUC between 0.0 and 0.5 is found (27) but this can easily be circumvented by reversing the state variable of control and study group.

Results

Serum hCG parameters in hydatidiform molar pregnancy

Median serum hCGα concentrations increased steadily in the control pregnancies from 1.79 nmol/l (25 IU/l) at 7 weeks of gestation to 11.4 nmol/l (160 IU/l) at 16 weeks of gestation. Serum hCGβ and hCG + hCGβ concentrations were 0.248 nmol/l (5.8 IU/l) and 37.6 nmol/l (13100 IU/l) respectively at week 7, showed a peak at 8 - 9 weeks of gestation (0.417 nmol/l (9.8 IU/l) and 59.6 nmol/l (21000 IU/l) respectively) and then subsequently decreased to 0.032 nmol/l (0.75 IU/l) and 18.7 nmol/l (6500 IU/l) respectively at week 16 of gestation. Fig. 1 shows the

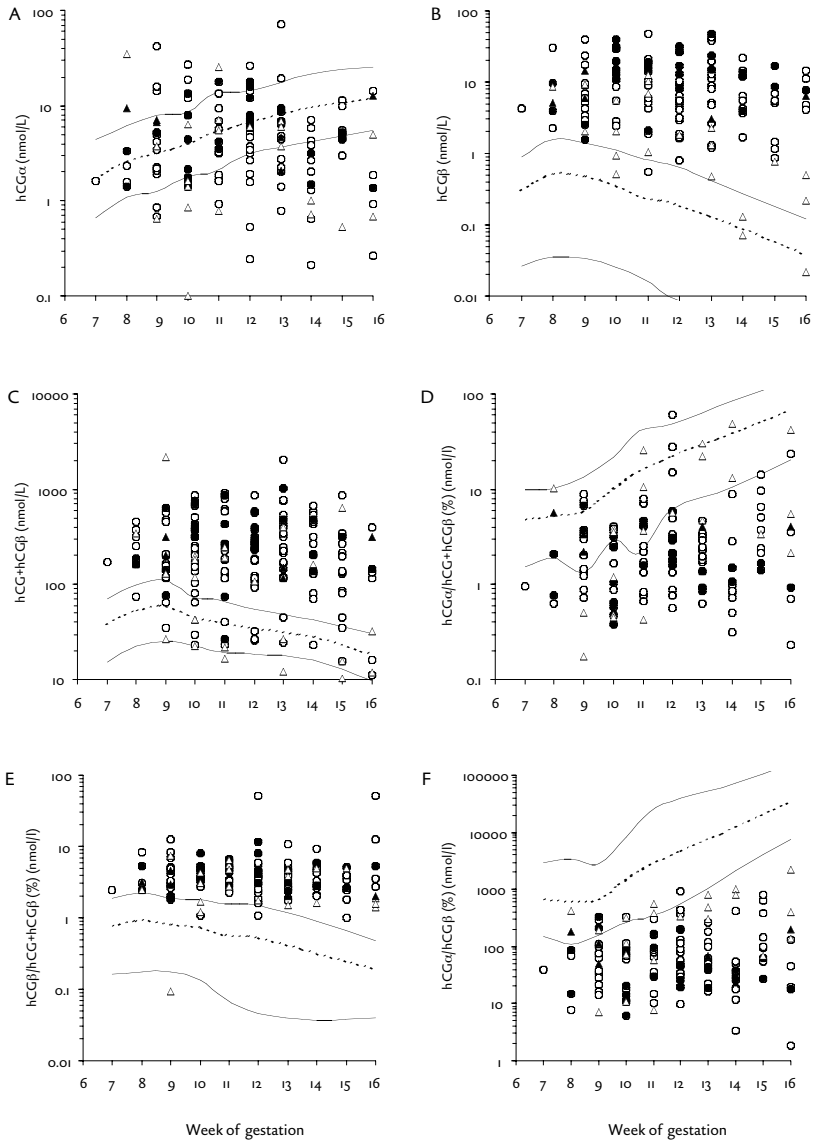

Figure 1. hCGα, hCGβ, hCG + hCGβ, hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ in partial or complete mole with or without persistent trophoblastic disease compared with normal pregnancy (*n*=27). The two solid lines represent the 5th and the 95th percentile, whereas the dotted line represents the 50th percentile of normal pregnancy. Group 1 (○), complete mole without PTD (*n*=129); Group 2 (●), complete mole with PTD (*n*=36); Group 3 (△), partial mole without PTD (*n*=32); Group 4 (▲), partial mole with PTD (*n*=7). The ratios of hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ are given in mol/mol, %.

serum concentrations of hCGα, hCGβ, hCG + hCGβ and its calculated ratios (hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ) in complete and partial moles with or without PTD compared with the values in normal pregnancy between weeks 7 and 16 of gestation. In many molar pregnancies, hCGα concentrations in serum (Fig. 1A) are below the P50 levels of normal pregnancy. The hCGα/hCG + hCGβ ratio in the majority of molar pregnancies is below P5 (Fig. 1D) while almost all hCGα/hCGβ ratios are below P5 of normal pregnancy (Fig. 1F). In contrast to this, hCGβ and hCG + hCGβ concentrations, and the ratio hCGβ/hCG + hCGβ are mostly above the corresponding P95 levels of normal pregnancy (Fig. 1B, 1C and 1E).

Comparison of serum hCG analytes and parameters in hydatidiform molar vs normal pregnancy

For each of the six hCG analytes and parameters investigated, we calculated the mean MoM (mMoM) values and their standard errors (S.E.) for the total and the individual groups of complete and partial molar pregnancy as compared with normal pregnancy (Table 1). This table also shows the comparisons between complete and partial moles, as well as all moles (complete and partial) without vs all moles with PTD.

The mean MoMs calculated for the concentrations of hCGβ, hCG + hCGβ and the ratio hCGβ/hCG + hCGβ were significantly higher in all molar pregnancies as well as in the complete molar pregnancy group compared with values in normal pregnancies (Table 1A and 1B). Comparable differences were also observed in the case of partial molar vs normal pregnancy except for hCG + hCGβ value, which was not significantly different (Table 1C). The mMoMs for the ratios of hCGα/hCG + hCGβ and hCGα/hCGβ in the all moles group and in its two subset groups were significantly lower than in normal pregnancy (Table 1A-C). The increase of the mMoMs observed for the group of all molar pregnancies (Table 1A), as well as for the subsets of complete (Table 1B) or partial (Table 1C) moles was highest in the case of hCGβ, followed by the ratio of hCGβ/hCG + hCGβ (Table 1A-C). The decrease of the mMoMs was highest in the case of the ratio of hCGα/hCG + hCGβ (Table 1A-C).

Table 1. Mean multiple of the median (mMoMs)* for six hCG analytes and parameters compared in subsets of moles vs normal pregnancy, complete vs partial moles, and in moles without vs with persistent trophoblastic disease (PTD) (first column represents number of controls in A, B and C and number of patients in D and E).

Parameter	Controls/ Patients (*n*)	mMoM	S.E.	Patients (*n*)	mMoM	S.E.	*P* value
A Normal pregnancies vs all molar pregnancies							
hCGα	27	0.999	0.343	136	0.804	0.765	NS
hCGβ	27	1.016	0.417	135	6.466	4.030	<0.001
hCG + hCGβ	27	1.001	0.137	204	2.430	1.265	<0.001
hCGα/hCG + hCGβ	27	0.997	0.254	136	0.449	0.295	<0.001
hCGβ/hCG + hCGβ	27	1.009	0.323	135	2.883	1.624	<0.001
hCGα/hCGβ	27	0.997	0.131	132	0.524	0.168	<0.001
B Normal pregnancies vs complete molar pregnancies							
hCGα	27	0.999	0.343	108	0.833	0.667	NS
hCGβ	27	1.016	0.417	109	7.145	4.010	<0.001
hCG + hCGβ	27	1.001	0.137	165	2.586	1.205	<0.001
hCGα/hCG + hCGβ	27	0.997	0.254	108	0.414	0.264	<0.001
hCGβ/hCG + hCGβ	27	1.009	0.323	109	2.991	1.745	<0.001
hCGα/hCGβ	27	0.993	0.131	106	0.498	0.156	<0.001
C Normal pregnancies vs partial molar pregnancies							
hCGα	27	0.999	0.343	28	0.689	1.071	NS
hCGβ	27	1.016	0.417	26	3.618	2.670	<0.001
hCG + hCGβ	27	1.001	0.137	39	1.769	1.315	NS
hCGα/hCG + hCGβ	27	0.997	0.254	28	0.583	0.368	<0.001
hCGβ/hCG + hCGβ	27	1.009	0.323	26	2.431	0.848	<0.001
hCGα/hCGβ	27	0.993	0.131	26	0.632	0.174	<0.001
D Complete mole pregnancies vs partial molar pregnancies							
hCGα	108	0.833	0.667	28	0.689	1.071	NS
hCGβ	109	7.145	4.010	26	3.618	2.670	<0.001
hCG + hCGβ	165	2.586	1.205	39	1.769	1.315	<0.001
hCGα/hCG + hCGβ	108	0.414	0.268	28	0.583	0.368	0.036
hCGβ/hCG + hCGβ	109	2.991	1.745	26	2.431	0.848	NS
hCGα/hCGβ	106	0.498	0.156	26	0.632	0.174	<0.001
E Complete and partial moles without PTD vs complete and partial moles with PTD							
hCGα	101	0.717	0.817	35	1.052	0.526	0.003
hCGβ	100	5.807	3.873	35	8.349	3.924	0.001
hCG + hCGβ	161	2.284	1.277	43	2.977	1.067	<0.001
hCGα/hCG + hCGβ	101	0.467	0.311	35	0.395	0.238	NS
hCGβ/hCG + hCGβ	100	2.917	1.819	35	2.787	0.866	NS
hCGα/hCGβ	99	0.534	0.168	33	0.493	0.164	NS

* mMoM and S.E. represent the natural logarithmic values of mMoM and S.E. in case of hCGα and hCGα/hCGβ, while with all other analytes and parameters mMoM and S.E. apply to square root mMoM and S.E. values.
NS, not significant.

Comparison of serum hCG parameters in groups of hydatidiform molar pregnancy

The comparison between the complete and the partial molar pregnancies (Table 1D) showed that the mMoMs of both hCGα and the ratio of hCGβ/hCG + hCGβ were not different, whereas the mMoMs for hCGβ and hCG + hCGβ in the partial moles were significantly lower than in the complete molar pregnancies ($p<0.001$). In contrast to this, the mMoM of the ratios of hCGα/hCG + hCGβ and hCGα/hCGβ in partial moles were significantly higher than those of complete molar pregnancy (Table 1D). In the case of all molar pregnancies with PTD as compared with all molar pregnancies without PTD, the three calculated ratios (hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ) were not significantly different, whereas the mMoM of all measured hCG analytes (hCGα, hCGβ and hCG + hCGβ) were significantly higher in those cases where women developed PTD as compared to those who did not (Table 1E).

Diagnostic accuracy

We established diagnostic accuracy by calculating specificity and sensitivity for the comparisons already presented in Table 1. Based on these data we constructed the corresponding ROC curves for each of the three hCG analytes determined in blood specimens collected prior to evacuation as well as for the three ratios derived from these measurements (Fig. 2). We then calculated the corresponding AUCs for the six hCG analytes and parameters (Table 2). The ROC curves of all molar pregnancies (Fig. 2A) vs normal pregnancy show AUCs ranging between 0.856 and 0.987 for all hCG analytes and parameters except for hCGα whose AUC was 0.618. hCGα/hCGβ, hCGβ/hCG + hCGβ and hCGβ showed sensitivities of, respectively, 79%, 93%, and 95%, all at 100% specificity. We also found that hCG + hCGβ and hCGα/hCG + hCGβ displayed sensitivities of 82% and 85% at 90% specificity.

In the case of hCG + hCGβ and hCGα/hCG + hCGβ the subset of complete moles (Table 2B, Fig. 2B) revealed higher AUCs (0.911 and 0.932) and the subset of partial moles revealed lower AUCs (0.621 and 0.788) (Table 2C, Fig. 2C) as compared with all molar pregnancies vs normal pregnancy (AUCs of 0.856 and 0.902) (Table 2A, Fig. 2A). Both in complete and partial hydatidiform mole, hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ were excellent diagnostic tests with AUCs ranging

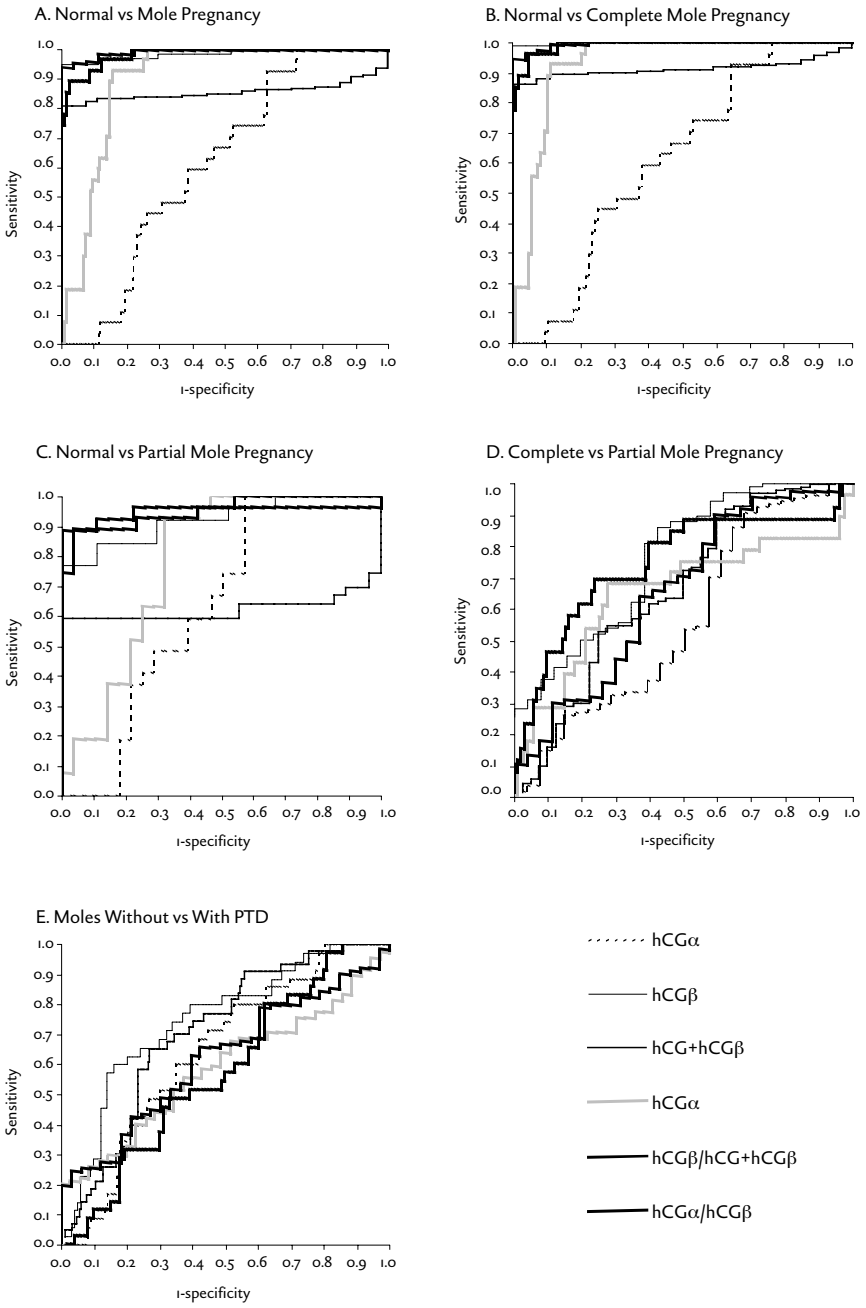

Figure 2. ROC analysis for six hCG analytes and parameters compared in all moles (A) and in subsets of moles (B, C) versus normal pregnancy, and compared in complete versus partial moles (D) and in all moles with PTD versus all moles without PTD (E).

Table 2. Area under the curve (AUC) for six hCG analytes and parameters compared in subsets of moles vs normal pregnancy, in subsets of complete vs partial moles, and in moles without vs with persistent trophoblastic disease (PTD) (first column represents number of controls in A, B and C, number of patients with complete moles in D and number of patients without PTD in E; second column represents number of patients in A, B and C, number of patients with partial moles in D and number of patients with PTD in E).

Parameter	Controls/Complete/ No PTD (*n*)	Patients/Partial/ PTD (*n*)	AUC	95% Confidence Interval
A Normal pregnancy vs all moles				
hCGα	27	136	0.618 *	0.524-0.713
hCGβ	27	135	0.984	0.968-0.999
hCG + hCGβ	27	204	0.856	0.809-0.902
hCGα/hCG + hCGβ	27	136	0.902 *	0.856-0.948
hCGβ/hCG + hCGβ	27	135	0.985	0.968-1.00
hCGα/hCGβ	27	132	0.987 *	0.973-1.00
B Normal pregnancies vs complete moles				
hCGα	27	108	0.614 *	0.513-0.715
hCGβ	27	109	0.999	0.996-1.00
hCG + hCGβ	27	165	0.911	0.870-0.952
hCGα/hCG + hCGβ	27	108	0.932 *	0.890-0.973
hCGβ/hCG + hCGβ	27	109	0.994	0.985-1.00
hCGα/hCGβ	27	106	0.994 *	0.985-1.00
C Normal pregnancies vs partial moles				
hCGα	27	28	0.634 *	0.481-0.788
hCGβ	27	26	0.922	0.848-0.995
hCG + hCGβ	27	39	0.621	0.474-0.768
hCGα/hCG + hCGβ	27	28	0.788 *	0.662-0.914
hCGβ/hCG + hCGβ	27	26	0.947	0.871-1.00
hCGα/hCGβ	27	26	0.962 *	0.917-1.00
D Complete vs partial moles				
hCGα	108	28	0.549 *	0.413-0.686
hCGβ	109	26	0.797 *	0.703-0.891
hCG+hCGβ	165	39	0.719 *	0.621-0.816
hCGα/hCG + hCGβ	108	28	0.629	0.504-0.754
hCGβ/hCG + hCGβ	109	26	0.602 *	0.477-0.726
hCGα/hCGβ	106	26	0.730	0.610-0.850
E All moles without vs with PTD				
hCGα	101	35	0.666	0.569-0.764
hCGβ	100	35	0.698	0.595-0.801
hCG + hCGβ	161	43	0.688	0.605-0.772
hCGα/hCG + hCGβ	101	35	0.568 *	0.461-0.675
hCGβ/hCG + hCGβ	100	35	0.520	0.411-0.630
hCGα/hCGβ	99	33	0.591	0.478-0.704

* reversal of study and control groups reveals AUC>0.5.

between 0.922 and 0.999 to distinguish these subsets from normal pregnancy (Table 2B,C, Fig. 2B,C). In the comparison of complete vs partial moles, five out of six hCG analytes and parameters tested (i.e. hCGβ, hCG + hCGβ, hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ) showed AUCs ranging between 0.602 and 0.797, while hCGα showed an AUC of 0.549 (Table 2D, Fig. 2D). In the comparison of moles without or with PTD, hCGα, hCGβ, hCG + hCGβ and hCGα/hCGβ showed AUCs ranging between 0.591 and 0.698, whereas the other two parameters revealed even lower AUCs (hCGβ/hCG + hCGβ: 0.520, and hCGα/hCG + hCGβ: 0.568) (Table 2E, Fig. 2E).

Discussion

The aim of our retrospective study was to explore the significance of six hCG analytes and parameters to distinguish hydatidiform mole from normal pregnancy, to distinguish complete from partial mole, and to assess whether any of these analytes and parameters is able to predict PTD. Specificity and sensitivity of all six hCG analytes and parameters in the various subsets of patient groups were explored in ROC curve analysis and the various AUCs thereof were compared for diagnostic accuracy. To distinguish hydatidiform molar from normal pregnancy, hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ proved to be excellent diagnostic tests with AUCs>0.9 in the all moles group as well as in its mole subsets. The comparison of the group of all moles vs normal pregnancy showed AUCs of 0.856 with hCG + hCGβ and 0.902 for the ratio hCGα/hCG + hCGβ. The diagnostic accuracy of free hCGα was less with AUCs of 0.6 in case of all the comparisons of moles with normal pregnancy. To distinguish complete from partial moles, five out of the six analytes and parameters tested (i.e. hCGβ, hCG + hCGβ, hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ) were found to have AUCs in the range of 0.6 to 0.8, whereas hCGα showed even a lower AUC of 0.549. To predict PTD after evacuation of a hydatidiform mole, we found hCGα, hCGβ, hCG + hCGβ and hCGα/hCGβ having AUCs in the range of 0.6 to 0.7. The other two parameters proved to have even lower AUCS ((between 0.5 and 0.6). Our finding that the values of hCGβ, hCG + hCGβ, and the ratio of hCGβ/hCG + hCGβ in blood withdrawn prior to evacuation are significantly higher, and the ratios of hCGα/hCG + hCGβ and hCGα/hCGβ are significantly lower in patients with hydatidiform mole as compared with normal pregnancy is

in accordance with an earlier report which included only 5 patients with a hydatidiform mole (15). Other studies, with limited numbers of patients with hydatidiform mole, also showed that hCGβ concentration (17) and the ratio hCGβ/hCG + hCGβ (17,19) were significantly higher while hCGα concentrations were not different in hydatidiform mole (17). Berkowitz *et al.* (14) reported significantly higher concentrations of hCGα, hCGβ, percentage hCGα and percentage hCGβ (i.e. the free subunit to total subunit ratios), and no effect on the hCGβ/hCGα ratio in partial moles (*n=8*) compared with normal pregnancy. Our data on hCGα/hCG + hCGβ and hCGα/hCGβ ratios are not in line with that study as we found a significant decrease in these levels. Interestingly, in the case of the complete mole subgroup comprising more patients (*n=20*), the same authors reported significantly lower levels of hCGα to intact hCG ratio and significantly higher concentrations of the hCGβ/hCGα which is in line with our data. It has to be noted that Berkowitz *et al.* (14) used intact hCG to calculate the ratios of free α and free β to hCG, whereas we measured total hCG immunoreactivity, which included intact hCG as well as free hCG β-subunit. Evidently, the marked increased free hCGβ concentrations in molar pregnancy will affect this ratio for which no correction could be made in the hCG + hCGβ assay because of the very different affinities of the polyclonal antiserum towards the free hCG β-subunit and the hCGβ as a part of the holo-hCG molecule. Very specific assays for all forms of hCGβ have been described (28). The use of a specific, more generally used, holo-hCG assay would have yielded different ratios. Nevertheless, we have used a hCG + hCGβ assay because it is superior in the diagnosis of trophoblastic tumors. To our knowledge the present study is the first to evaluate the diagnostic accuracy of the 6 hCG analytes and parameters in a substantial number of samples, showing that hCGβ, hCGβ/hCG + hCGβ and hCGα/hCGβ are excellent diagnostic tests as indicated by AUCs above 0.9 to distinguish partial and complete hydatidiform molar from normal pregnancy. The sensitivities of these three diagnostic tests to distinguish hydatidiform molar molar pregnancy from normal pregnancy were, respectively, 95%, 93%, and 79%, all at 100% specificity, while we found sensitivities of 82% and 85% at 90% specificity with hCG + hCGβ and hCGα/hCG + hCGβ.

We found significantly lower mMoMs of hCGβ and hCG + hCGβ and significantly higher hCGα/hCG + hCGβ and hCGα/hCGβ ratios

in partial moles as compared with complete moles. hCGα and hCGβ/hCG + hCGβ were not different in this comparison. Irrespective of the presence or absence of statistical significance, none of these six analytes or parameters proved to have meaningful AUCs (range: 0.549-0.797). In a smaller study comprising 20 complete moles and 8 partial moles, Berkowitz *et al.* (14) reported similar results for hCGα, hCGβ and hCGα/hCGβ. These investigators found no significant difference in intact hCG concentrations when distinguishing partial from complete moles. Why hCGβ is synthesized more abundantly in complete hydatidiform mole than in partial moles or in normal pregnancy is unknown. hCG is synthesized in cytotrophoblasts and more abundantly in syncytiotrophoblasts (29,30). Messenger-RNA for hCGα is encoded by a single gene on chromosome 6 (31), whereas the β-subunit of hCG is encoded by four genes - hCGβ-3, -5, -7, and –8 - located on chromosome 19q13.3 (31,32). hCG α- and β-subunits are thus synthesized separately, and their production is regulated independently during pregnancy and in trophoblastic disease (33,34). Berkowitz *et al.* (14) suggested that the percentage free hCGβ level might reflect the level of differentiation and hyperplasia of the trophoblast, since the level of free hCGβ increases up to 0.5% at five weeks of normal gestation, to 1% in partial hydatidiform mole, to 2.4% in complete hydatidiform mole, and as high as 9.2% in choriocarcinoma. Indeed, Hay (16) proposed a regulatory mechanism to link irregularities in trophoblastic differentiation to altered biosynthesis of hCG. In proliferating syncytium formed by differentiating cytotrophoblasts, amplification of hCG genes results in excess mRNA for hCG β-subunits. All free hCGα will be used to produce hCG and the remaining hCGβ will be released together with intact hCG. In the same way, it is feasible to think that in partial mole, which contains both normal trophoblast and hyperplastic trophoblasts, less free hCGβ is synthesized as compared to complete moles.

Identifying patients with increased risk for developing PTD after evacuation of hydatidiform mole is of great interest. Approximately 20% of patients with a complete hydatidiform mole will suffer from PTD that requires chemotherapy treatment. Prophylactic chemotherapy reduces the incidence of PTD to 4 - 12% (10,11). Optimism about prophylactic chemotherapy is tempered by a prospective randomized controlled trial by Kim *et al.* (35) who found that prophylactic chemotherapy indeed reduced the incidence of PTD in high risk patients (from 31% to 10%), but

these patients needed more courses of chemotherapy (mean 2.5 ± 0.5 (S.E.) vs 1.4 ± 0.5 courses, $p<0.005$), suggesting that prophylactic therapy increases tumor resistance and morbidity by selection and proliferation of cells that are resistant to the effect of chemotherapy. To prevent unnecessary treatment as well as resistance to chemotherapy, it is challenging to find out an adequate diagnostic modality that identifies patients at increased risk for developing PTD. We found that serum concentrations of hCGα, hCGβ, and hCG + hCGβ were significantly higher in patients with PTD as compared with those in which spontaneous regression after evacuation occurred. These hCG analytes showed only moderate diagnostic accuracy (with AUCs in the range of 0.6 to 0.7) indicating a limited clinical relevance of the observed differences in hCG concentrations between these groups. None of the calculated ratios were significantly different and consequently all had a poor diagnostic accuracy to predict PTD. In contrast to our data, a number of studies, all with limited patient samples, reported that the hCGβ/total hCG ratio is a predictor of PTD (18-20). These studies included patients with unspecified hydatidiform (18,19) or complete (20) hydatidiform moles. Berkowitz *et al.* (14) reported that none of the evaluated hCG parameters, equal to those in our study, were associated with the development of PTD. Our study showed that hCGα, hCGβ, hCG + hCGβ and the ratio hCGα/hCGβ with AUCs in the range of 0.6 to 0.7 are of less value in the prediction of PTD, and it is unlikely that a clinician will start prophylactic chemotherapy on the basis of the results obtained with these tests. Therefore, new modalities should be explored to find a reliable predictor for PTD. Amongst these is the serum concentration of hCG β-core fragment which was found to be rising prior to intact hCG in patients with hydatidiform mole who developed PTD as compared with patients with hydatidiform mole with remission of hCG after mole evacuation (hydatidiform mole, *n=14* of which 4 patients had PTD) (36). Another potentially interesting parameter is hyperglycosylated hCG which was found to rise from 25% to 80% of total serum hCG when patients with persistent low levels of hCG developed gestational trophoblastic neoplasm (37). The clinical value of this test in predicting PTD after hydatidiform mole remains to be elucidated.

In summary, our study based on a large patient sample showed that hCGβ in serum, and the ratios of hCGβ/hCG + hCGβ and hCGα/hCGβ are excellent diagnostic tests and parameters to make the distinc-

tion biochemically between hydatidiform mole and normal pregnancy at the 100% specificity level with more than 90% sensitivity. For practical use, the hCGβ assay is recommended. The distinction between complete and partial hydatidiform mole cannot reliably be made with any of the tested analytes or parameters because diagnostic accuracy of these tests is at best qualified as moderately applicable. Finally, we found that although hCGα, hCGβ and hCG + hCGβ concentrations are significantly elevated in the case of PTD, none of the six investigated hCG parameters had adequate diagnostic accuracy to permit the clinician to advise patients to undertake prophylactic chemotherapy after evacuation of a hydatidiform mole.

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References

1. Hussa, R. O. In The Clinical Marker hCG. New York: Praeger Publishers, 1987
2. Bentley RC. Pathology of gestational trophoblastic disease. Clinical Obstetrics and Gynecology 2003; 46: 513-522.
3. Matsuda T and Wake N. Genetics and molecular markers in gestational trophoblastic disease with special reference to their clinical application. Best Practice & Research Clinical Obstetrics & Gynaecogy 2003; 17: 827-836.
4. Steigrad SJ. Epidemiology of gestational trophoblastic diseases. Best Practice & Research Clinical Obstetrics & Gynaecogy 2003; 17: 837-847.
5. Berkowitz RS and Goldstein DP. Gestational trophoblastic disease. Cancer 1995; 76: 2079-2085.
6. Bagshawe KD, Lawler SD, Paradinas FJ, Dent J, Brown P, Boxer GM. Gestational trophoblastic tumours following initial diagnosis of partial hydatidiform mole. Lancet 1990; 335: 1074-1076.
7. Berkowitz RS, Goldstein DP, Bernstein MR. Natural history of partial molar pregnancy. Obstetrics & Gynecology 1985; 66: 677-681.
8. Goto S, Yamada A, Ishizuka T, Tomoda Y. Development of postmolar trophoblastic disease after partial molar pregnancy. Gynecologic Oncology 1993; 48: 165-170.
9. Rice LW, Berkowitz RS, Lage JM, Goldstein DP, Bernstein MR. Persistent gestational trophoblastic tumor after partial hydatidiform mole. Gynecologic Oncology 1990; 36: 358-362.
10. Goldstein DP, Berkowitz RS , Bernstein MR. Management of molar pregnancy. Journal of Reproductive Medicine 1981; 26: 208-212.
11. Curry SL, Hammond CB, Tyrey L, Creasman WT, Parker RT. Hydatidiform mole: diagnosis, management, and long-term follow up of 347 patients. Obstetrics & Gynecology 1975; 45: 1-8.
12. Thomas CM, Reijnders FJ, Segers MF, Doesburg WH, Rolland R. Human choriogonadotropin (hCG): comparisons between determinations of intact hCG, free hCG beta-subunit, and "total" hCG + beta in serum during the first half of high-risk pregnancy. Clinical Chemistry 1990; 36: 651-655.
13. Elegbe RA, Pattillo RA, Hussa RO, Hoffmann RG, Damole IO, Finlayson WE. Alpha subunit and human chorionic gonadotropin in normal pregnancy and gestational trophoblastic disease. Obstetrics & Gynecology 1984; 63: 335-337.
14. Berkowitz RS, Ozturk M, Goldstein DP, Bernstein MR, Hill L, Wands JR.

Human chorionic gonadotropin and free subunits' serum levels in patients with partial and complete hydatidiform moles. Obstetrics & Gynecology 1989; 74: 212-216.

15. Gaspard UJ, Reuter AM, Deville J-L, Vrindts-Gevaert Y, Bagshawe KD, Franchimont P. Serum concentration of human chorionic gonadotropin and its alpha and beta subunits. 2. Trophoblastic tumours Clinical Endocrinology 1980; 13: 319-329.
16. Hay DL. Histological origins of discordant chorionic gonadotropin secretion in malignancy. Journal of Clinical Endocrinology and Metabolism 1988; 66: 557-564.
17. Ozturk M, Berkowitz R, Goldstein D, Bellet D, Wands JR. Differential production of human chorionic gonadotropin and free subunits in gestational trophoblastic disease. American Journal of Obstetrics and Gynecology 1988; 158: 193-198.
18. Khazaeli MB, Hedayat MM, Hatch KD, To AC, Soong SJ, Shingleton HM, Boots LR, LoBuglio AF. Radioimmunoassay of free beta-subunit of human chorionic gonadotropin as a prognostic test for persistent trophoblastic disease in molar pregnancy. American Journal of Obstetrics and Gynecology 1986; 155: 320-324.
19. Khazaeli MB, Buchina ES, Pattillo RA, Soong SJ, Hatch KD. Radioimmunoassay of free beta-subunit of human chorionic gonadotropin in diagnosis of high-risk and low-risk gestational trophoblastic disease. American Journal of Obstetrics and Gynecology 1989; 160: 444-449.
20. Mungan T, Kuscu E, Ugur M, Dabakoglu T, Senses E, Cobanoglu O. Screening of persistent trophoblastic disease with various serum markers. European Journal of Gynaecological Oncology 1998; 19: 495-497.
21. Yedema KA, Verheijen RH, Kenemans P, Schijf CP, Borm GF, Segers MF & Thomas CM Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. American Journal of Obstetrics and Gynecology 1993; 168: 787-792.
22. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. Annals of Clinical Biochemistry 1985; 22: 236-246.
23. Khazaeli MB, England BG, Dieterle RC, Nordblom GD, Kabza GA, Beierwaltes WH. Development and characterization of a monoclonal antibody which distinguishes the beta-subunit of human chorionic gonadotropin (beta hCG) in the presence of the hCG. Endocrinology 1981; 109: 1290-1292.
24. Thomas CM and Segers MF. Quantification of choriogonadotropin: differential cross-reactivities of the free hCG beta-subunit with eight different monoclonal

antibody-based hCG and (hCG + beta) "sandwich"-type assays. Clinical Chemistry 1989; 35: 1791-1792.
25. Kletzky OA, Nakamura RM, Thorneycroft IH, Mishell DR, Jr. Log normal distribution of gonadotropins and ovarian steroid values in the normal menstrual cycle. American Journal of Obstetrics and Gynecology 1975; 121: 688-694.
26. Zweig MH, Broste SK, Reinhart RA. ROC curve analysis: an example showing the relationships among serum lipid and apolipoprotein concentrations in identifying patients with coronary artery disease. Clinical Chemistry 1992; 38: 1425-1428.
27. Zweig MH and Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clinical Chemistry 1993; 39: 561-577.
28. Alfthan H, Haglund C, Dabek J & Stenman UH Concentrations of human choriogonadotropin, its beta-subunit, and the core fragment of the beta-subunit in serum and urine of men and nonpregnant women. Clinical Chemistry 1992; 38: 1981-1987.
29. Shi QJ, Lei ZM, Rao CV, Lin J. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. Endocrinology 1993; 132: 1387-1395.
30. Hoshina M, Boothby M, Boime I. Cytological localization of chorionic gonadotropin alpha and placental lactogen mRNAs during development of the human placenta. Journal of Cell Biology 1982; 93: 190-198.
31. Naylor SL, Chin WW, Goodman HM, Lalley PA, Grzeschik KH, Sakaguchi AY. Chromosome assignment of genes encoding the alpha and beta subunits of glycoprotein hormones in man and mouse. Somatic Cell Genetics 1983; 9: 757-770.
32. Policastro PF, Daniels-McQueen S, Carle G, Boime I. A map of the hCG beta-LH beta gene cluster. Journal of Biological Chemistry 1986; 261: 5907-5916.
33. Hussa RO. Biosynthesis of human chorionic gonadotropin. Endocrine Reviews 1980; 1: 268-294.
34. Jameson JL and Hollenberg AN. Regulation of chorionic gonadotropin gene expression. Endocrine Reviews 1993; 14: 203-221.
35. Kim DS, Moon H, Kim KT, Moon YJ, Hwang YY. Effects of prophylactic chemotherapy for persistent trophoblastic disease in patients with complete hydatidiform mole. Obstetrics & Gynecology. 1986; 67: 690-694.
36. Okamoto T, Matsuo K, Niu R, Osawa M, Suzuki H. Human chorionic gonadotropin (hCG) beta-core fragment is produced by degradation of hCG or free hCG beta in gestational trophoblastic tumors: a possible marker for early

detection of persistent postmolar gestational trophoblastic disease. Journal of Endocrinology 2001; 171: 435-443.

37.Khanlian SA, Smith HO, Cole LA. Persistent low levels of human chorionic gonadotropin:A premalignant gestational trophoblastic disease. American Journal of Obstetrics and Gynecology 2003; 188: 1254-1259.

CHAPTER 3

Early identification of persistent trophoblastic disease with serum hCG concentration ratios

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submitted

Abstract

Objective: To assess the diagnostic potential of serum hCG concentration ratios obtained at different intervals after evacuation of hydatidiform mole to diagnose Persistent Trophoblastic Disease (PTD) and to compare its diagnostic accuracy with the current FIGO 2000 criteria for PTD.

Methods: A retrospective cohort study was performed on patients registered with the Dutch Central Registry for Hydatidiform Moles between 1977-2004. After exclusion for various reasons, serum hCG concentrations were available in 204 cases (86 with and 118 without PTD). hCG concentrations after evacuation were used for calculation of hCG ratios. ROC curve analysis was performed to calculate specificity, sensitivity and Areas Under Curves (AUCs).

Results: hCG ratios showed an increasing diagnostic potential. The hCG ratio obtained in week 1 and 5 after evacuation showed AUCs of 0.568 and 0.935, respectively, and identified 20% and 79% of patients with PTD at the 95% specificity level. The median time to diagnose PTD with the use of a hCG ratio was 3.0 weeks versus 4.7 weeks if FIGO 2000 criteria were applied.

Conclusions: A ratio of two serum hCG concentrations obtained after evacuation of the mole identifies patients with PTD approximately 2 weeks earlier than the internationally accepted FIGO 2000 criteria. This ratio makes it possible to identify more than 75% of patients who develop PTD by the fifth week after evacuation. The hCG ratio calculated from two post molar serum hCG concentrations provides a new quantitative measure which substantially improves the qualitative FIGO 2000 'increase' and 'plateau' criteria.

Introduction

A variety of pathologic subtypes of trophoblastic neoplasms are included in gestational trophoblastic disease, comprising villous malformations, hydatidiform mole -subdivided in complete and partial hydatidiform mole-, and nonvillous malformations of which choriocarcinoma is the most frequent (1). In Persistent Trophoblastic Disease (PTD), trophoblastic activity remains after evacuation of a hydatidiform mole as shown by subsequent unaltered high or even increasing serum hCG concentrations. The reported frequency of PTD is 20% in complete hydatidiform mole (2) and 0.5% to 9.9% in partial hydatidiform mole (3-6). The International Federation of Gynaecology and Obstetrics (FIGO) defines PTD as a plateau in serum hCG for three weeks, or a increase for two consecutive weeks (7). To a plateau or increase for 3 weeks the Dutch Society for Obstetrics and Gynaecology added to their definition for PTD the condition that at least one serum hCG measurement should exceed the 95th percentile (P95) of a normal hCG regression curve after hydatidiform mole (8-10) since utilization of a normal hCG regression corridor prevents over-treatment (9,11). Although several authors described normal serum hCG regression corridors after uneventful regression (9,11-14), it is not feasible to construct a normogram for every hCG assay procedure. Thus, such a normogram is not available to every physician who is involved in the follow-up of a patient with hydatidiform mole.

Our hypothesis is that there is a substantial difference in the calculated ratio from two serum hCG concentrations obtained after evacuation of a mole in patients with PTD versus the ratio in patients without PTD. In order to generate a more quantitative criterion for diagnosing PTD instead of the qualitative concepts of 'increase' and 'plateau' which are incorporated in the FIGO 2000 definition, the aim of the present study was to assess the ability of serum hCG ratios obtained after evacuation of the mole to distinguish patients who will develop PTD from those patients who will not.

Materials and Methods

Patients

Between 1977 and 2004, 2872 patients were registered with the Dutch Central Registry for Hydatidiform Moles. After informed consent, patients are registered through their referring clinician. In the Nether-

lands, after a histological diagnosis of mola hydatidosa or choriocarcinoma, PTD is diagnosed by means of a serum hCG plateau or increase for three consecutive weekly measurements, with at least one measurement exceeding the 95th percentile of an hCG regression curve established for uneventful postmolar regression (9). We evaluated all patients' data in our database and included all patients with available serum hCG data obtained after evacuation of the mole and assayed in our institution. In retrospect we classified the hCG regression according to the FIGO 2000 guideline (7) (i.e., mola hydatidosa with serum hCG plateauing for three consecutive weeks or increase for two consecutive weekly measurements) and confined all the patients with proven PTD and according to these criteria to the study group (n=86, 84 of which were treated with chemotherapy or a hysterectomy and two were cured by a second curettage only). Those with spontaneous regression of serum hCG after evacuation of the mole according to FIGO 2000 criteria were assigned to the control group (n=118). Additionally, to be included in the evaluation, the first hCG measurement should have been performed in a serum specimen taken within the first two weeks after evacuation of the mole. Reasons for exclusion were (a) the histological diagnosis of choriocarcinoma for which chemotherapy was initiated without awaiting a plateau or increase in post molar serum hCG concentrations, or (b) persistence of low hCG serum levels around 10 ng/mL after spontaneous regression. For each patient in the study group we scored the week that PTD was diagnosed according to FIGO 2000 criteria. The week in which therapy for PTD was started was scored as well and data were censored from that time point onwards.

Immunoassays

A privately developed ("in-house") radioimmunoassay (RIA) that measured 'total' hCG (i.e., intact hCG and free β-subunit, hCG + hCGβ) was used exclusively in the authors' laboratory (15). Thus, this assay has been utilized centrally for all measurements in sera sent to the Dutch Central Registry for Hydatidiform Moles and was used in the development of a normal hCG regression corridor for uneventful hydatidiform mole (9). The RIA was calibrated with the third International Standard for intact hCG (WHO third IS hCG 75/537 obtained from the National Institute for Biological Standards, Potters Bar, UK). The measuring range for the standard line of the assay was 1-80 μg/L (0.027-2.14 nmol/L, equivalent

to 9.29-743 IU/L of the WHO third IS hCG 75/537)(15). The hCG + hCGβ-RIA cross-reacts 100% on a mol/mol basis with intact hCG and 1000% with hCG β-subunit. Serum hCG concentrations were considered to be normalized if below 2 μg/L (0.053 nmol/L or 18.6 IU/L of the WHO third IS hCG 75/537). The within- and between-assay coefficients of variation (CV_w, CV_b) for means of duplicate measurements were at a level of 10 μg/L (equivalent to 0.267 nmol/L or 93 IU/L) 7.5% and 10.3%, respectively, and at a level of 56 μg/L (equivalent to 1.50 nmol/L or 520 IU/L), 7.3% and 12%.

Statistics

For each patient we calculated the ratio of the hCG concentrations obtained in weeks 1 through 11 after evacuation. The hCG ratio of week 1 after evacuation was calculated by dividing the first by the second available hCG concentration taken in week 1. In week 2, the hCG ratio was calculated by dividing the first available hCG concentration of weeks 1 or 2 by the hCG concentration of a specimen taken in week 2. From week 3 onwards until week 11, the hCG ratios were calculated by dividing the first available hCG concentration of a specimen taken within the first two weeks by the hCG concentration of a specimen taken in that particular week (these hCG ratios were designated hCG ratio week 1, 2, ..,11). In the study group, the hCG ratios were excluded from analysis onwards from the week that the curative therapy (chemotherapy, hysterectomy or a curative second curettage) was initiated or performed. The accuracy to diagnose PTD as derived from the calculated hCG ratios week 1 through 11 was investigated by Receiver Operating Characteristic (ROC) curve analysis of the hCG ratios per week and resulted in calculations of AUCs. Because the FIGO 2000 criteria to diagnose PTD are not suitable for ROC curve analysis, we compared for each week the number of patients identified by FIGO 2000 criteria as having PTD with those identified by hCG ratios per week. To make a direct comparison between the diagnostic accuracy of either identification procedure, we calculated the cumulative percent rate of identified patients with PTD as a function of the week after evacuation of the mole. All statistical analyses were performed using the SPSS statistical software package version 12.0.1 (SPSS Inc., Chicago, Il, USA).

Results

Table 1 depicts the ROC curve analyses of all available hCG ratios of weeks 1 through 11 for the study and control group. For each week analysed, the number of patients included in the PTD group ranged from 15 to 71 and in the control group from 33 to 89 patients. The AUC of the hCG ratio was 0.568 in week 1 and increased steadily to a plateau ≥ 0.9 by week 5. The calculated sensitivities at 95% specificity increased from 20% in week 1 to 79% in week 5 and declined to 48-52% in weeks 10-11. The cut-off values represent the hCG ratios below which PTD can be diagnosed with 95% specificity in a given week after evacuation. Figure 1 shows hCG ratios of weeks 1, 5 and 10. The study group fulfilled the criteria for the diagnosis PTD at a median time point of 4.7 weeks when using FIGO 2000 criteria. The median time point to diagnose PTD by using the hCG ratio was 3.0 weeks.

In Table 2 we show at weekly intervals the proportion of patients with a diagnosis of PTD (i.e., the study group) as identified with FIGO 2000 criteria as well as with the hCG ratio. In this cross-sectional analysis we scored the number of patients that were identified with either test as having PTD and expressed these as a proportion of the total number of PTD patients with an hCG ratio in that week. FIGO 2000 criteria identified zero out of 15 (0%) patients in week 1, while the hCG ratio of week 1 detected 3 patients (20%) to have PTD. In week 3 the FIGO 2000 criteria identified 11 (16%) and the hCG ratio 35 patients (52%) out of 67 patients with PTD. In week 7 FIGO 2000 criteria identified 70% (30/43) and the hCG ratio 32 patients (74%) out of 43 patients with PTD. Afterwards, the diagnostic ability of FIGO 2000 criteria increased steadily up to 89% in week 11, whereas the hCG ratios detected 48-63% of PTD patients in weeks 10-11 after evacuation of the mole.

Figure 2 depicts the cumulative number of patients with PTD once identified by FIGO 2000 criteria, and by the hCG ratio of the corresponding week. Out of the 86 patients with PTD, FIGO 2000 criteria cumulatively identified zero patients in week 1 and 12 patient in week 3 (14%) to reach 60% by week 5, and more than 90% in weeks 10-11 after evacuation. The hCG ratios of week 1 already identified 3.5% (3 cases) of the patients with PTD and up to 51% in week 3 (44 cases). The maximum cumulative detection rate (83%) of hCG ratio was reached in week 8.

Table 1. ROC curve analysis of available hCG ratios per week

	Number of patients Persistent trophoblastic disease		Area under curve	Standard error	95-% confidence interval	Sensitivity at 95% specificity	hCG ratio cut-off
Week	absent	present					
1	33	15	0.568	0.090	0.391-0.744	20	1.0
2	67	44	0.740	0.047	0.647-0.833	34	2.7
3	87	67	0.826	0.033	0.761-0.892	52	2.8
4	89	63	0.891	0.026	0.841-0.941	59	4.0
5	76	71	0.935	0.020	0.897-0.974	79	11
6	74	56	0.931	0.021	0.889-0.973	75	15
7	69	43	0.946	0.021	0.905-0.986	74	27
8	65	33	0.902	0.031	0.842-0.963	58	27
9	56	28	0.909	0.030	0.850-0.969	61	33
10	64	25	0.895	0.033	0.830-0.960	48	34
11	49	23	0.922	0.031	0.861-0.983	52	40

Table 2. Comparison of number of patients identified by FIGO 2000 criteria and hCG ratios per week as having PTD

Time after evacuation Week	No. of PTD patients with hCG ratio available	Positive scoring rates for FIGO 2000 criteria		hCG ratio	
		No. of patients	%	No. of patients	%
1	15	0	0	3	20
2	44	1	2.3	15	34
3	67	11	16	35	52
4	63	24	38	37	59
5	71	47	66	56	79
6	56	37	66	42	75
7	43	30	70	32	74
8	33	24	73	19	58
9	28	20	71	17	61
10	25	20	80	12	48
11	23	17	89	12	52

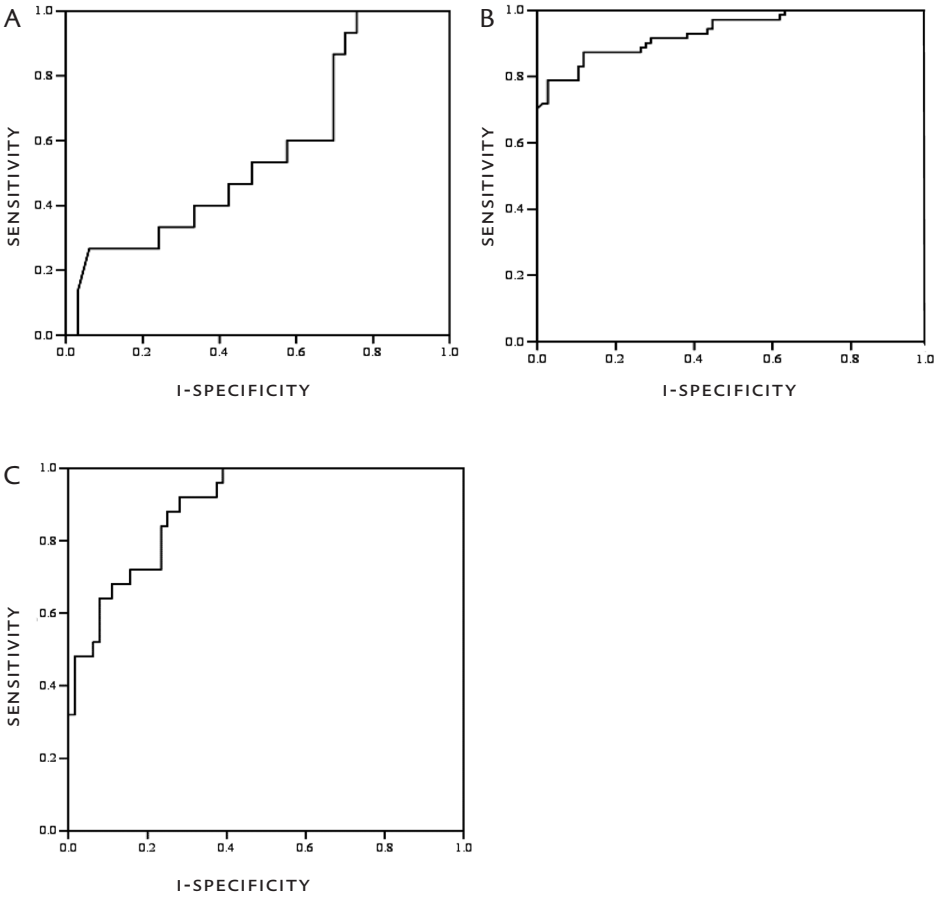

Figure 1. Examples of the ROC curves for study (PTD+) versus control group (PTD-): A) hCG ratio week 1; B) hCG ratio week 5; C) hCG ratio week 10

Discussion

The aim of our retrospective study was to explore the value of serum hCG concentration ratios to distinguish patients who will develop PTD from patients who will have spontaneous regression of serum hCG after evacuation of a hydatidiform mole and to compare the diagnostic potential of these hCG ratios with the current 'gold standard': PTD as diagnosed according to criteria provided by FIGO 2000. Specificity and sensitivity of ratios obtained from hCG measurements after evacuation

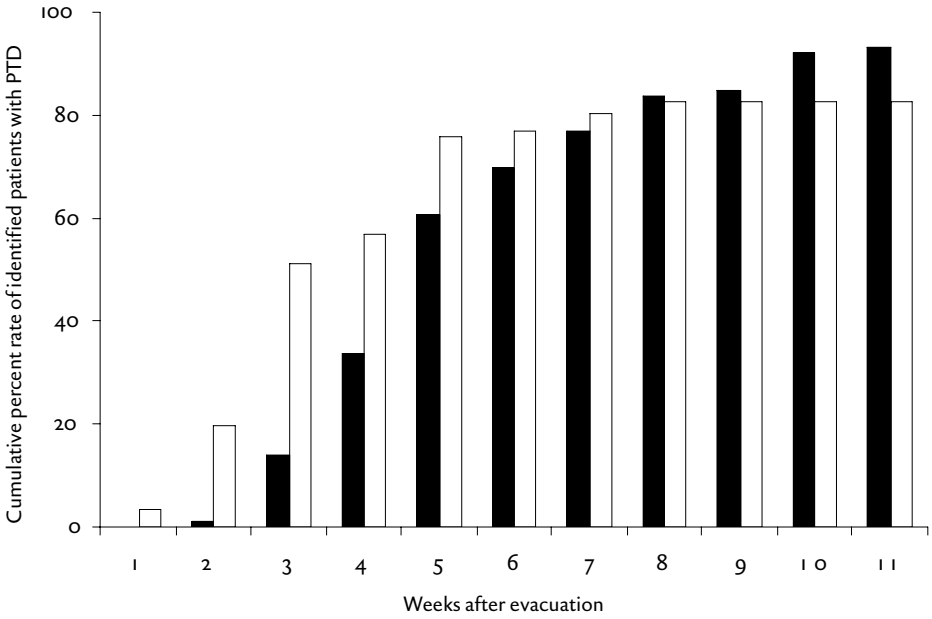

Figure 2. The cumulative distribution of patients with PTD (n=86) as identified on a weekly basis by FIGO 2000 criteria (black bars) and the hCG ratio of the corresponding week (open bars).

were explored in ROC curve analysis and the various AUCs thereof were compared for diagnostic accuracy. The hCG ratios obtained in week 1 through week 11 displayed an increasing diagnostic potential from week 1 onwards until a plateau was reached in week 5 after evacuation. For each week after evacuation a cut-off value for this ratio was calculated, below which PTD can be diagnosed at 95% specificity. The AUC of the hCG ratios increased from 0.568 in week 1 to 0.935 in week 5 and stabilized at AUCs of ≥0.9 afterwards. The hCG ratio and FIGO 2000 criteria identified 52% and only 16%, respectively, of patients to have PTD in week 3 after evacuation. In week 7 both the hCG ratio and the FIGO 2000 criteria identified ≥ 70% of patients with PTD.

To our knowledge no studies have been performed on the potential of hCG ratios in the diagnosis of PTD. In this study we analyzed at which time point after evacuation the criteria for PTD were met according to the internationally accepted FIGO 2000 criteria. We found that the median time to reach the diagnosis of PTD in the study group was 4.7

weeks when using FIGO 2000 criteria for PTD whereas the median time to diagnose PTD by using the hCG ratio was 3.0 weeks. Thus, the hCG ratio identified patients earlier than FIGO 2000 criteria.

The qualitative terms 'increase' and 'plateau' in serum hCG to confirm or reject the diagnosis PTD after evacuation of a hydatidiform mole according to the FIGO criteria may be subject to inter- and intra observer bias. To add a quantitative measure to the diagnosis of PTD, several normograms for spontaneous regression of serum hCG have been developed (9,11-14). The use of these normograms has been advocated since their use may be an aid to prevent over-treatment of patients (9,11), but bears the disadvantage that each individual hCG assay requires the construction of its own specific normogram. Another qualitative, though arbitrary addition to the definition of the FIGO 2000 criteria for PTD was put forward by Kohorn who proposed to define an 'increase' of hCG as an increase in terms of 10% or more for three values or more over a period of at least 2 weeks (16). However, up till now no universal agreement has been reached on this proposal (17). We now provide quantitative data, that is, hCG ratios calculated from already available serum hCG concentrations, that improve the accuracy for diagnosis of PTD. It remains to be investigated in a prospective setting whether combining these hCG ratios with the FIGO 2000 criteria improves the accuracy for diagnosis of PTD.

It is still unclear why some hydatidiform moles develop into PTD while others show a spontaneous regression. It is not very likely that residual tissue after evacuation is the only cause to develop PTD. Normally, extra villous trophoblasts invades the myometrium up to one-third of the entire uterine wall (18). With the commonly used suction curettage for the evacuation of a hydatidiform mole, it is highly unlikely that this molar tissue bearing the extra villous trophoblasts will be completely removed. The study by Lao et al. compared the 'histology result' (trophoblastic tissue or no trophoblastic tissue) of a routinely performed second curettage in patients with a hydatidiform mole with the subsequent need for chemotherapy for PTD (19). The authors reported absence of such a correlation, meaning that this result contradicts the hypothesis that residual tissue after evacuation of a hydatidiform mole is the entirely unique cause of PTD.

The search for genetic factors that can identify hydatidiform moles at risk to develop into PTD is continuing (20). Recently, several cell growth

regulating factors like MCL-1, EGFR, c-erbB-2 and c-erbB-3, c-ras, Nm-23, p53, Cyclin E and telomerase (21-25) were found to have different expression patterns in hydatidiform mole, which will develop PTD as compared to hydatidiform mole with spontaneous regression of serum hCG. This may lead to the development of new biochemical tools in the future that will aid the diagnosis of patients who will develop PTD. At present, no such tools are available.

In this retrospective study we found that the ratio of two serum hCG measurements identified more than 50% of patients with PTD at the 95% specificity level in week 3 after evacuation, whereas FIGO 2000 identified not more than 16% of patients with PTD at that week. With an hCG ratio we were able to identify up to 79% of patients by week 5 after evacuation at the 95% specificity level. These promising results justify that the diagnostic utility of this hCG ratio in the context of the present FIGO 2000 criteria and applicability in the definition for PTD should be further explored in a prospective study.

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References

1. Bentley RC. Pathology of gestational trophoblastic disease. Clin Obstet Gynecol 2003; 46:513-22.
2. Berkowitz RS, Goldstein DP. Gestational trophoblastic disease. Cancer 1995; 76:2079-85.
3. Bagshawe KD, Lawler SD, Paradinas FJ, Dent J, Brown P, Boxer GM. Gestational trophoblastic tumours following initial diagnosis of partial hydatidiform mole. Lancet 1990; 335:1074-6.
4. Berkowitz RS, Goldstein DP, Bernstein MR. Natural history of partial molar pregnancy. Obstet Gynecol 1985; 66:677-81.
5. Goto S, Yamada A, Ishizuka T, Tomoda Y. Development of postmolar trophoblastic disease after partial molar pregnancy. Gynecol Oncol 1993; 48:165-70.
6. Rice LW, Berkowitz RS, Lage JM, Goldstein DP, Bernstein MR. Persistent gestational trophoblastic tumor after partial hydatidiform mole. Gynecol Oncol 1990; 36:358-62.
7. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000. Int J Gynaecol Obstet 2002; 77:285-7.
8. Hancock BW. Staging and classification of gestational trophoblastic disease. Best Pract Res Clin Obstet Gynaecol 2003; 17:869-83.
9. Yedema KA, Verheijen RH, Kenemans P, Schijf CP, Borm GF, Segers MF, Thomas CM. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. Am J Obstet Gynecol 1993; 168:787-92.
10. Dutch Society for Obstetrics and Gynaecology: Richtlijn Molazwangerschap 1.1 (Guideline Mole Pregnancy 1.1). 2005. www.oncoline.nl
11. Rotmensch J, Rosenshein NB, Block BS. Comparison of human chorionic gonadotropin regression in molar pregnancies and post-molar nonmetastatic gestational trophoblastic neoplasia. Gynecol Oncol 1988; 29: 82-6.
12. Schlaerth JB, Morrow CP, Kletzky OA, Nalick RH, D'Ablaing GA. Prognostic characteristics of serum human chorionic gonadotropin titer regression following molar pregnancy. Obstet Gynecol 1981; 58: 478-82.
13. Shigematsu T, Kamura T, Saito T, Kaku T, Nakano H, Kinugawa N. Identification of persistent trophoblastic diseases based on a human chorionic gonadotropin regression curve by means of a stepwise piecewise linear regression

analysis after the evacuation of uneventful moles. Gynecol Oncol 1998; 71:376-80.
14. Behtash N, Ghaemmaghami F, Honar H, Riazi K, Nori A, Modares M, et al. Is normal beta-hCG regression curve helpful in the diagnosis of persistent trophoblastic disease? Int J Gynecol Cancer 2004; 14:980-3.
15. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. Ann Clin Biochem 1985; 22:236-46.
16. Kohorn EI. The new FIGO 2000 staging and risk factor scoring system for gestational trophoblastic disease: description and critical assessment. Int J Gynecol Cancer 2001; 11:73-7.
17. Ng TY, Wong LC. Diagnosis and management of gestational trophoblastic neoplasia. Best Pract Res Clin Obstet Gynaecol 2003; 17:893-903.
18. Huppertz B, Hemmings D, Renaud SJ, Bulmer JN, Dash P, Chamley LW. Extravillous trophoblast apoptosis-a workshop report. Placenta 2005;26 Suppl A:S46-S48.
19. Lao TT, Lee FH, Yeung SS. Repeat curettage after evacuation of hydatidiform mole. An appraisal. Acta Obstet Gynecol Scand 1987; 66:305-7.
20. Fisher R: Genetics, in: Hancock BW, Newlands ES, Berkowitz R, Cole L (ed): Gestational Trophoblastic Disease, online book. 2003, pp 6-38. http://www.isstd.org/gtd/02%20word%20for%20print.pdf
21. Cheung AN, Zhang DK, Liu Y, Ngan HY, Shen DH, Tsao SW. Telomerase activity in gestational trophoblastic disease. J Clin Pathol 1999; 52:588-92.
22. Fong PY, Xue WC, Ngan HY, Chan KY, Khoo US, Tsao SW, et al. Mcl-1 expression in gestational trophoblastic disease correlates with clinical outcome: a differential expression study. Cancer 2005; 103:268-76.
23. Fukunaga M. Immunohistochemical characterization of p57(KIP2) expression in early hydatidiform moles. Hum Pathol 2002; 33:1188-92.
24. Tuncer ZS, Vegh GL, Fulop V, Genest DR, Mok SC, Berkowitz RS. Expression of epidermal growth factor receptor-related family products in gestational trophoblastic diseases and normal placenta and its relationship with development of postmolar tumor. Gynecol Oncol 2000; 77:389-93.
25. Yang X, Zhang Z, Jia C, Li J, Yin L, Jiang S. The relationship between expression of c-ras, c-erbB-2, nm23, and p53 gene products and development of trophoblastic tumor and their predictive significance for the malignant transformation of complete hydatidiform mole. Gynecol Oncol 2002; 85:438-44.

CHAPTER 4

Comparison of hCG + hCGβ and ITA disappearance rates in serum after evacuation of molar pregnancy

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Abstract

Background: After evacuation of hydatidiform mole, the spontaneous regression or the persistent trophoblastic disease (PTD) needing chemotherapy is monitored by determination of serum human Chorionic Gonadotropin (hCG) concentrations. Hyperglycosylated hCG (Invasive Trophoblast Antigen, ITA) has been suggested to be of clinical value in the diagnosis and follow-up of gestational trophoblastic disease including PTD.

Methods: To further document the relationship between ITA and hCG in spontaneous post-molar regression and during chemotherapy treatment of PTD, we used distinct immunoassays to measure the concentrations of hCG + hCGβ and ITA in serum from three groups of patients after evacuation of moles. For each group (uneventful post molar hCG regression (group 1), PTD treated with Methotrexate (single-agent chemotherapy, group 2), and PTD with MTX and multiagent chemo-therapy (EMA-CO, group 3)), we compared the time course of the serum concentrations after evacuation, and determined the disappearance rates (half-life) within and between treatment groups.

Results: Significantly longer mean serum half-lifes for hCG + hCGβ and ITA were found in the multiagent-chemotherapy (Group 3: 3.02 and 2.51 weeks) as compared to the single-agent chemotherapy group (Group 2: 0.96 and 0.90 weeks) and the uneventful regression group (0.81 and 0.66 weeks) (all p=0.003), but no differences were observed between the single-agent chemotherapy and the uneventful regression group. Significantly shorter mean half-lifes for ITA than those calculated for hCG + hCGβ were observed in all three groups of patients.

Conclusions: The implication and the possible clinical value of faster regression of ITA to baseline levels as compared to hCG + hCGβ remain to be investigated prospectively.

Introduction

The glycoprotein hormone human chorionic gonadotropin (hCG) is produced by trophoblastic tissue and therefore is a key marker in pregnancy and gestational trophoblastic disease (GTD). The disappearance of serum hCG concentrations is monitored by constructing a hCG regression curve after mole evacuation (1). A treated molar pregnancy may persist and may either lead to persistent trophoblastic disease (PTD) or to development of choriocarcinoma. The reported frequency of PTD is 15 to 20 % in complete hydatidiform mole (2), 4-9 % in partial hydatidiform mole (3, 4), while choriocarcinoma occurs less frequently. In order to prevent complications from metastatic disease, PTD and choriocarcinoma need to be treated with chemotherapy. Once diagnosed and classified, low-risk PTD is typically treated with single-agent therapy (Methotrexate (MTX) or Actinomycin-D) (5, 6) while high-risk PTD is most widely treated with combination therapy (EMA-CO: Etoposide, MTX, Actinomycin-D, Cyclophosphamide and Vincristine) (5). PTD is recognised by plateauing, or even increasing levels of hCG in three subsequent weekly blood samples, with one or more hCG values above the 95th percentile of the hCG-regression curve (1).

The glycoprotein hormone hCG (M_r 37,500) is composed of two non-covalently bound subunits, α and β, with MW of 14,000 and 23,500, respectively. The α-subunit of hCG is homologous with that of the heterodimeric pituitary glycoprotein hormones LH, FSH and TSH. The β-chains distinguish these hormones from each other and they determine the biological activity. Eight carbohydrate chains are attached to hCG and comprise about one-third of the molecular mass of intact hCG: two N-linked carbohydrate chains are attached to the hCG α-subunit, two others to the hCG β-subunit, while four O-linked sugar side chains are attached to hCGβ. Whereas normal and molar pregnancy show only limited proportions of more complex triantennary N-linked oligosaccharides (0 - 30 %) and larger hexasaccharide-type-O-linked carbohydrates (0 – 20 %), choriocarcinoma amounts up to 100 % of these larger N- and O-linked oligosaccharides called hyperglycosylated hCG (7).

In normal pregnancy, hyperglycosylated hCG is gradually replaced by hCG. Hyperglycosylated hCG (M_r 42,000) accounts for >80 % of total hCG in the first week, to 50 % in the third week after implantation, while in the third trimester it has diminished to 2 % (8). Kovalevskaya *et al.* showed that hyperglycosylated hCG is produced in placental stem cells

or cytotrophoblasts and that the less glycosylated hCG form is synthesized mainly in syncytiotrophoblasts (9). Hyperglycosylated hCG is produced by invasive or cancerous trophoblast cells in choriocarcinoma. This suggests that hyperglycosylated hCG is a product of separate trophoblast cells and that it may be synthesized in invasive trophoblast cells, the reason why hyperglycosylated hCG is also referred to as Invasive Trophoblast Antigen (ITA) (8).

It has been suggested that ITA might be of clinical value in the diagnosis and follow up of GTD (10). In women with persistent low levels of hCG following evacuation without signs of GTD, the proportions of ITA account for less than 25 % of total hCG immuno-reactivity (11). ITA was considered to be effective in detecting invasive trophoblastic disease in patients with persistent low levels of hCG who experienced a sudden steep rise in their serum hCG concentrations after years of persistent low serum hCG concentrations because ITA then accounted for 81-100 % of the highly increased total hCG concentrations (11). However, it was not explicitly stated that determination of ITA is able to exclude presence of invasive trophoblastic disease in the reported cases with persistent low levels of hCG without signs of PTD.

To further document the relationship between ITA and hCG in PTD, we compared the time course and disappearance rate (half-life) in serum of ITA with those of already available hCG + hCGβ data determined longitudinally in serum from three groups of patients following evacuation of moles. These groups comprised uneventful post molar hCG regression, management of PTD with MTX (single-agent chemotherapy), and management of PTD with MTX and multiagent chemotherapy (EMA-CO).

Material and Methods

Patients

All the patients participating in the present study gave, voluntary, their informed consent before registration at the Dutch Central Registry for Hydatidiform Moles residing at the Radboud University Nijmegen Medical Centre (RUNMC). After collection, blood samples were centrifuged and serum was sent to our institute where it was assayed for "total" hCG and kept at -20 °C until further analysis. From this database, we randomly selected 20 patients who were all registered between 2000 and 2002, and from whom longitudinally collected serum samples were

available for ITA analysis. Of these 20 patients, 7 patients had a hydatidiform mole with uneventful hCG regression after evacuation, as derived from the normal hCG regression curve (1). Thirteen patients with a hydatidiform mole developed PTD after evacuation for which either single-agent chemotherapy (with Methotrexate, MTX, n = 7), or single-agent followed by multiagent chemotherapy (with EMA-CO, n = 6) was indicated.

Immunoassays

The measurements of 'total' hCG (i.e., intact hCG and free β-subunit, hCG + hCGβ) in serum were performed with a sensitive and specific radioimmunoassay (RIA) that had been developed in our laboratory and was described previously (12). The RIA used a polyclonal anti-rabbit antiserum. A highly purified hCG β-subunit preparation labeled with Iodine-125 (NaI^{125}, Amersham plc, Amersham, England) was used as tracer. The RIA was calibrated with the third International Standard Preparations for intact hCG (WHO third IS hCG 75/537, obtained from the National Institute for Biological Standards, Potters Bar, England). The measuring range for the standard dose-response curve of the hCG + hCGβ RIA was 0.027-2.14 nmol/L (equivalent to 9.29-743 IU/L). The intra- and interassay coefficients of variation (CV_w, CV_b) for means of duplicate measurements for two serum pools (mean: 0.267 nmol/L (93 IU/L) and 1.50 nmol/L (520 IU/L)) were 7.3 % and 12 %. The hCG + hCGβ-RIA cross-reacted 100 % with intact hCG and 1000 % with hCGβ. The 95th percentile of the reference interval of healthy control pregnancies for the hCG + hCGβ assay was established at 0.053 nmol/L (18.6 IU/L of the WHO third IS hCG 75/537) (12).

ITA was measured with the Nichols Advantage® Invasive Trophoblast Antigen Assay (Nichols Institute Diagnostics, San Clemente, CA 92673, USA) and the assay was performed according to the instructions provided by the manufacturer. The ITA assay is a two-step, two-site immunochemiluminometric assay that uses two monoclonal antibodies. The capture antibody (B152) is biotin labeled, while the second antibody (B207) is labeled with acridinium ester and used for detection. The analytical sensitivity of the assay is <0.005 nmol/L (<0.2 µg/L). Cross reactivity as documented by the supplier, is <1 % with recombinant hCG (Sigma C6322), 5.4 % with nicked hCG, 4.5 % with non-nicked hCG, 1.5 % with nicked free βhCG, and 1.0 % with non-nicked free βhCG.

Statistics

Both hCG + hCGβ and ITA concentrations were determined in the same serum samples. Half-lifes for hCG + hCGβ and ITA were calculated for each individual patient from linear regression analysis of log-transformed concentrations against time. The first time-point preceding the final phase of decreasing concentrations was taken as the starting point. In the patients with persistent disease this coincided with the start of the medication. Except for the first one encountered, values below the detection limits were excluded from calculation. Half-lifes were obtained for 7 patients with uneventful regression, and 13 patients with PTD, of which 7 patients received single-agent chemotherapy and 6 patients receiving single-agent chemotherapy followed by multiagent chemotherapy. Half-lifes of hCG + hCGβ and ITA were compared by paired t-test within the three subgroups. Between the subgroups, the half-lifes of hCG + hCGβ and ITA, respectively, were compared by the Kruskal-Wallis test (SPSS, version 12.1).

Results

Table 1 shows characteristics of assay results and chemotherapy courses of 7 patients with uneventful regression (Group 1), and of patients with PTD treated either with MTX (Group 2, n=7) or with EMA-CO (Group 3, n=6). The total observation periods of the patients comprising groups 1, 2 and 3, respectively, ranged between 11 and 95, 13 and 93, and 38 and 129 weeks. During these follow-up periods, the total number of hCG + hCGβ and ITA measurements ranged between 9-17 and 6-12 in Group 1, between 9-23 and 6-10 for Group 2, and between 17-37 and 13-32 for Group 3, respectively. Table 1 also shows that the observed maximum concentrations of the two analytes tested increased in the order uneventful regression (Group 1), single-agent (Group 2), multiagent chemotherapy (Group 3). The maximum concentration observed for hCG + hCGβ was 5.1 nmol/L in Group 1, 75 nmol/L in Group 2, and 590 nmol/L in Group 3. In case of ITA, these figures were 1.1 nmol/L in Group 1, 45 nmol/L in Group 2, and 110 nmol/L in Group 3.

The number of MTX courses administered to the patients receiving single-agent chemotherapy (Group 2) ranged between 3 and 9 courses administered throughout postevacuation weeks 3 and 19. The 6 patients comprising Group 3 received MTX courses ranging in number between 6 and 10 courses which were administered in the period of weeks 3 to 23

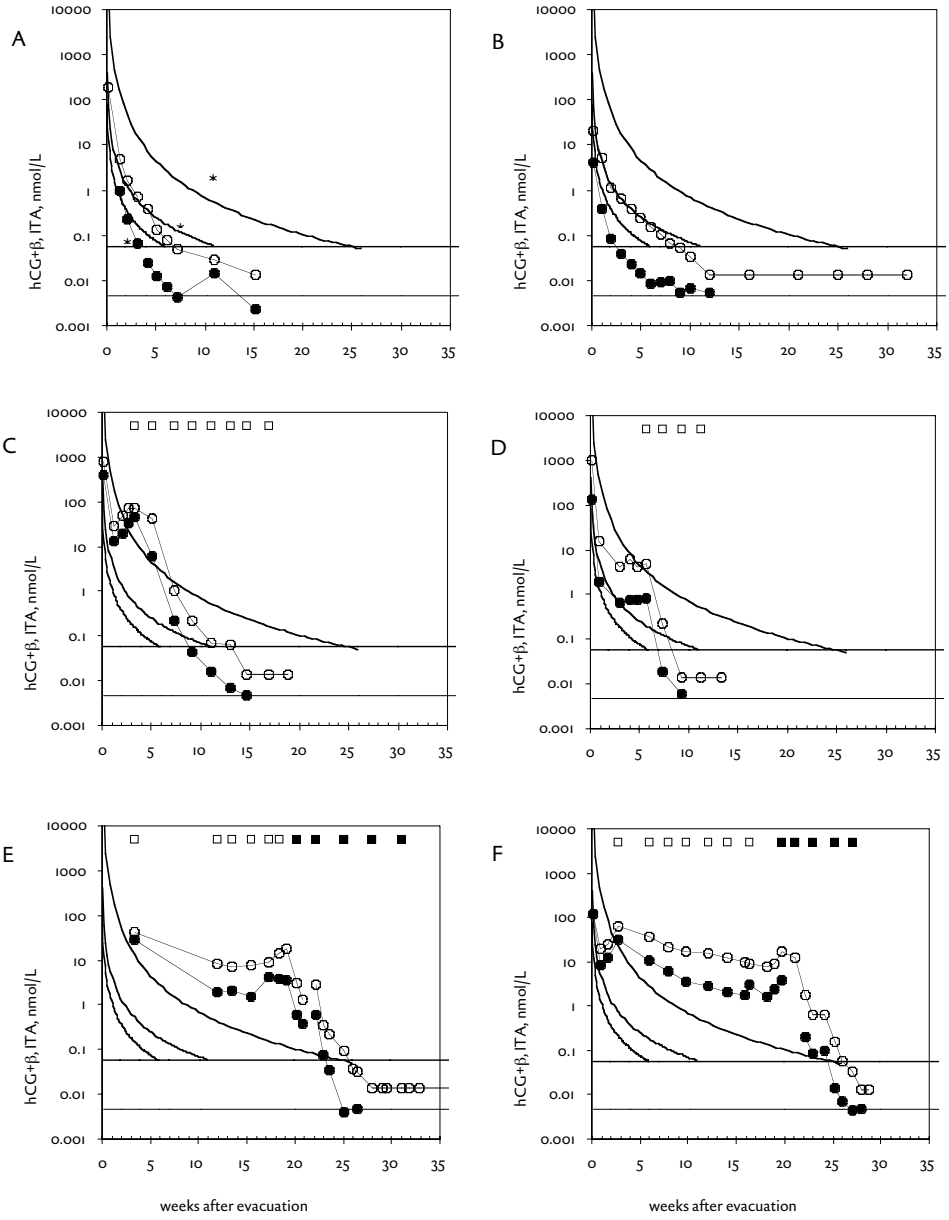
Table 1. Treatment characteristics of patients after evacuation of molar pregnancy: Observation period, number of specimens tested (No.), concentration ranges of the compared two analytes, and number (n) and time period (start-end) of the chemotherapy courses administered

Group/ Case	Period, weeks	hCG+hCGβ, No. (min-max) nmol/L	ITA No. n (start-end) weeks	Single-agent CT MTX, n (start-end) weeks	Multiagent CT EMA-CO,
1/1	15	10 (<0.027 - 4.8)	9 (<0.005 - 0.95)	none	none
1/2	32	14 (<0.027 - 2.6)	6 (<0.005 - 0.81)	none	none
1/3	20	9 (<0.027 - 4.5)	7 (<0.005 - 1.1)	none	none
1/4	29	15 (<0.027 - 3.5)	12 (<0.005 - 0.33)	none	none
1/5	11	11 (<0.027 - 4.0)	11 (<0.005 - 0.60)	none	none
1/6	32	17 (<0.027 - 5.1)	11 (<0.005 - 0.39)	none	none
1/7	95	11 (<0.027 - 2.9)	6 (<0.005 - 0.93)	none	none
2/1	93	16 (<0.027 - 75)	10 (<0.005 - 15)	7 (5 - 16)	none
2/2	66	23 (<0.027 - 75)	10 (<0.005 - 45)	8 (3 - 17)	none
2/3	13	9 (<0.027 - 16)	7 (<0.005 - 1.9)	4 (6 - 11)	none
2/4	59	18 (<0.027 - 4.3)	6 (<0.005 - 1.1)	6 (11 - 19)	none
2/5	73	21 (<0.027 - 29)	9 (0.007 - 24)	9 (3 - 19)	none
2/6	24	9 (<0.027 - 12)	8 (<0.005 - 1.4)	4 (4 - 12)	none
2/7	68	17 (<0.027 - 6.7)	10 (0.006 - 1.4)	3 (12 - 18)	none
3/1	129	37 (<0.027 - 290)	19 (<0.005 - 86)	7 (5 - 21)	4 (23 - 32)
3/2	38	22 (<0.027 - 43)	14 (<0.005 - 27)	6 (3 - 18)	5 (20 - 31)
3/3	61	17 (<0.027 -590)	16 (<0.005 -110)	10 (5 - 23)	1 (25)
3/4	41	34 (<0.027 - 250)	32 (<0.005 -110)	9 (4 - 19)	3 (24 - 29)
3/5	73	24 (<0.027 - 24)	13 (<0.005 - 13)	7 (6 - 19)	3 (21 - 34)
3/6	95	37 (<0.027 - 61)	20 (<0.005 - 31)	7 (3 - 16)	5 (20 - 27)

Group 1 Uneventful regression after evacuation of the molar pregnancy

Group 2 Persistent trophoblastic disease after evacuation of the molar pregnancy and treatment with single-agent chemotherapy (Methotrexate, MTX)

Group 3 Persistent trophoblastic disease after evacuation of the molar pregnancy and treatment with multiagent chemotherapy (EMA-CO) following MTX


A
B
C
D
E
F
hCG+β, ITA, nmol/L
10000
1000
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1
0.1
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weeks after evacuation

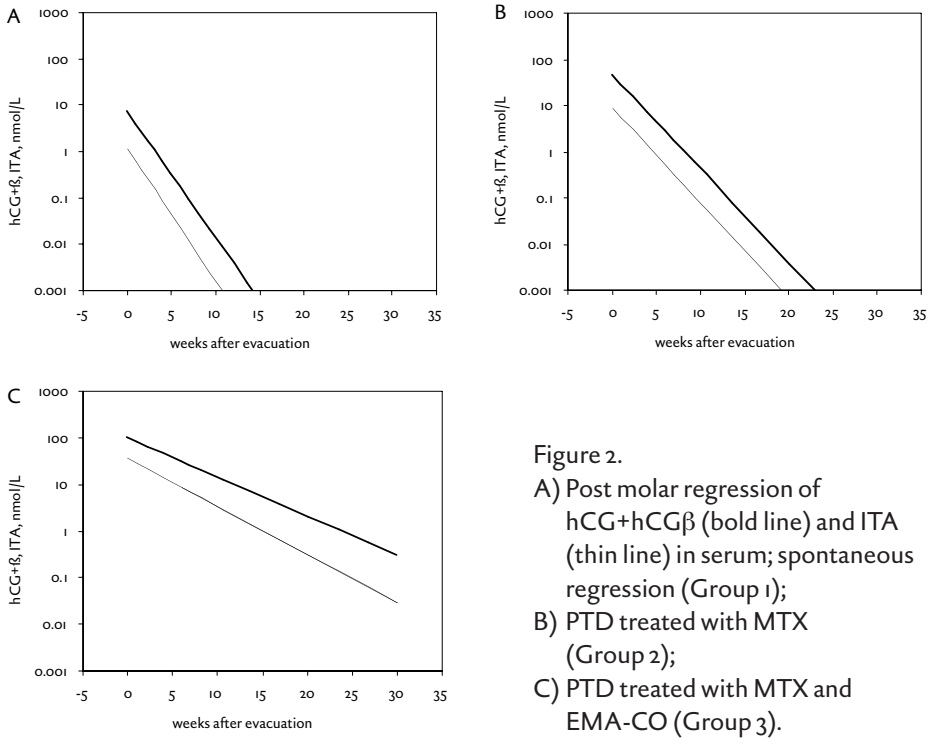

Figure 2.
A) Post molar regression of hCG+hCGβ (bold line) and ITA (thin line) in serum; spontaneous regression (Group 1);
B) PTD treated with MTX (Group 2);
C) PTD treated with MTX and EMA-CO (Group 3).

←Figure 1. Serum concentrations of hCG + hCGβ (solid line with ○) and ITA (solid line with ●) after evacuation of molar pregnancy followed by spontaneous regression (panels A, B), or by persistent trophoblastic disease, treated either with single-agent chemotherapy (Methotrexate, MTX) (panels C, D, □), or with MTX (□) followed by multiagent chemotherapy (EMA-CO) (panels E, F, ■).
The three solid lines (*) represent the 5th, 50th, and the 95th percentile of uneventful regression of hCG + hCGβ.
The 95th percentile of the reference interval of healthy control pregnancies for the hCG + hCGβ assay (fat line) was established at 0.053 nmol/L (18.6 IU/L of the WHO third IS hCG 75/537). Because the 95th percentile of the reference interval of healthy control pregnancies for the ITA assay was not established, the analytical sensitivity of the ITA assay (<0.005 nmol/L) is indicated (solid line).
Conversion factors used are: hCG + hCGβ: 1µg/L, equivalent to 9.29 IU/L, equivalent to 0.0267 nmol/L; ITA: 1µg/L, equivalent to 0.0238 nmol/L.

Table 2. Mean (min-max) half-lifes ($\tau_{1/2}$) of hCG + hCGβ and ITA after evacuation of hydatidiform mole followed by spontaneous regression, single-agent chemotherapy (MTX), or multiagent chemotherapy (EMA-CO) following MTX

	Group 1 Uneventful regression (PTD-)	Group 2 Single-agent chemotherapy (PTD+MTX)	Group 3 Multiagent chemotherapy (PTD+MTX +EMA-CO)	Significance* Group 1 vs 2 *p*	Significance* Group 1 vs 3 *p*	Significance* Group 2 vs 3 *p*
Number of cases	7	7	6			
$\tau_{1/2}$ of hCG+hCGβ (weeks)	0.81 (0.39-1.4)	0.96 (0.66-1.4)	3.02 (2.2-3.9)	NS	0.003	0.003
$\tau_{1/2}$ of ITA (weeks)	0.66 (0.27-1.0)	0.90 (0.57-1.2)	2.51 (1.5-3.1)	NS	0.003	0.003
Significance ** *p* $\tau_{1/2}$ of hCG+hCGβ vs ITA	0.010	0.012	0.006			

NS not significant (p>0.05)

* Kruskal-Wallis test

** Student's t-test

after evacuation of the molar pregnancy. Next to MTX treatment, Group 3 patients received multiagent chemotherapy in courses ranging between 1 and 5 that were administered in weeks 20 through 34 after evacuation of their mole. Figure 1 depicts the time course of hCG + hCGβ and ITA regression of two typical examples of each group studied.

Figure 2 depicts the mean time course of hCG + hCGβ and ITA concentrations as obtained for the three groups. The mean semi-logarithmic regression lines for either analyte in case of uneventful regression (Group 1) are given in Figure 2A, while those for the patients receiving single-agent chemotherapy (Group 2), or single-agent chemotherapy followed by multiagent chemotherapy (Group 3) are given in Figures 2B and 2C, respectively. Based on these data, we calculated the mean serum half-lifes (disappearance rates) for the two analytes (Table 2). Significant 3- to 4-fold increases of mean serum half-lifes for either parameter was observed in the group who received multiagent chemotherapy (Group 3) as compared with Group 1 (uneventful regression, both $p=0.003$) or with Group 2 (single- agent chemotherapy, both $p=0.003$). No significant differences of mean serum half-life for either parameter were observed between Group 1 and Group 2. Significantly shorter mean half-lifes for ITA than those calculated for hCG + hCGβ were observed in the three groups of patients. The ratios of ITA/hCG + hCGβ (given in mol/mol, %) of Groups 1, 2 and 3 were 29.3, 28.2 and 35.4 mol/mol %, respectively, and did not differ significantly between the three groups tested.

Discussion

The aim of the present study was to compare ITA with hCG + hCGβ measurements in serum in the course of uneventful regression after evacuation of hydatidiform mole as well as throughout the follow-up period of single-agent, or single-agent and multiagent chemotherapy treatment in cases where persistent trophoblastic disease occurred after evacuation of the molar pregnancy. Disappearance rates of the two analytes were calculated for the three groups of patients and the resulting half-lifes were compared between the three groups. The mean serum half-lifes for the two parameters were significantly 3 to 4 times longer in the group receiving single-agent and multiagent chemotherapy as compared to the groups with either single-agent chemotherapy or uneventful regression, while no significant differences were observed between the group with single-agent chemotherapy and the one with uneventful

regression. Significantly shorter mean half-lifes for ITA than those calculated for hCG + hCGβ were observed in all three groups of patients.

Determination of disappearance rates of hCG + hCGβ and ITA revealed that the half-life of hCG + hCGβ in the circulation was significantly longer than that of ITA, irrespective of whether it regards uneventful post molar regression, PTD treated with single-agent chemotherapy, or PTD treated with single-agent followed by multiagent chemotherapeutic agents. The longer half-life for hCG + hCGβ may be due to the rather complex multi-exponential decay of intact hCG (median half-times between 3.6 - 53 h) and free hCG β-subunit (1 - 194 h) as reported in serum of six women with term pregnancies throughout a period of 21 days after delivery (13). Although the data are not fully comparable with those presented here for disappearance of hCG + hCGβ during follow-up and treatment of PTD, these data indicate that presence of various molecular forms of hCG, all with different half-life times, may affect the observed disappearance rates of total hCG immuno-reactivity as measured with our hCG + hCGβ-RIA. Such data on half-lifes of ITA are as yet not available.

The different degrees of (hyper)glycosylation of hCG and ITA may also contribute to the differences in disappearance rates of these analytes. About one-third of the molecular mass of hCG consists of carbohydrates attached to six glycosylation sites on hCGβ and two on hCGα. The carbohydrate chains contain 8 – 15 terminal sialic acids, and therefore, hCG displays extensive heterogeneity (isoforms) (14). It has been reported that hyperglycosylated hCG can vary greatly in sialic acid content, with molecules having between 0 and 19 sialic acid residues (7, 15, 16). Hyperglycosylated hCG generally contains less of this acidic sugars than regular hCG (7). As terminal sialic acid protects the galactose residues of the carbohydrate chains against metabolic degradation by neuraminidases (17, 18, 19), and hCG has a higher sialic acid content than ITA, regular hCG might be better protected against metabolic breakdown than ITA resulting in a longer half-life in the circulation of hCG than of ITA. We also observed significantly longer half-lifes of hCG + hCGβ or ITA in the PTD group treated with multi-agent chemotherapy (Group 3) as compared with the two other groups. The presence of severely persisting trophoblastic tissue in these patients unequivocally requiring multi-agent chemotherapeutic treatment may be responsible for this. Similar disappearance rates for hCG + hCGβ and ITA are found

in the group with uneventful, spontaneously regressing trophoblast, and in the PTD group responding to single-agent chemotherapy. Likewise, the time needed for complete regression to normal serum concentrations also are not different. Thus, the PTDs curable with single-agent chemotherapy could be designated as 'moderate persistent trophoblasts', and those requiring multiagent chemotherapy as 'severe persistent trophoblasts'.

The question whether ITA is a better marker of PTD than hCG + hCGβ parallels the discussion by Okamoto *et al.* (20) regarding the usefulness of hCG β-core fragment (hCGβcf) for early prediction of subsequent development of post-molar PTD. These authors reported that, due tot its short half-life, serum hCGβcf rapidly declined and became undetectable after evacuation of the mole with subsequent spontaneous resolution. A similar observation is obtained in the present study for ITA. Okamoto *et al.* further argued that after evacuation, serum hCGβcf remained elevated or started to rise before PTD was diagnosed based on the rise of hCG serum levels. They concluded that hCGβcf in the serum after mole evacuation may indicate persistence of viable trophoblasts more sensitive than hCG. Our present data are too limited to conclude whether the same conclusion would hold for ITA. Okamoto *et al.* further reported that, once chemotherapy has started, hCGβcf became more rapidly undetectable than hCG because of its relatively low levels in serum and the faster clearance from the circulation than hCG. Therefore, it seems that hCGβcf may not be suitable for the follow-up of patients receiving chemotherapy.

The significance of persistent low levels of hCG in serum of non pregnant patients without clinical evidence of PTD and its relationship with ITA was studied by Khanlian *et al.* (11). These authors concluded that either the absence of detectable ITA (i.e., serum concentrations for ITA<0.005 nmol (<0.2 μg/L), or the presence of insignificant amounts of ITA (accounting for <5 % of the total hCG immuno-reactivity determined with their total hCG assay) were indicative for the absence of invasive cytotrophoblastic cells and that "quiescent PTD" was present. Because low, significantly increased hCG serum concentrations were present, these patients were treated, but despite treatment, low hCG levels persisted. In 4 out of 38 cases, the low hCG concentrations suddenly rose sharply, and the proportion of ITA in such samples then increased to more than 80 % up to 100 % of the total circulating hCG. Similarly,

this was also observed in specimens from 15 other cases with proven GTD (choriocarcinoma or placental site trophoblastic tumor) (10, 11). In the present study, we observed sharply decreasing hCG + hCGβ and ITA concentrations in the post evacuation follow-up of patients with uneventful regression, and in PTD treated with single-agent, or single-agent followed by multiagent chemotherapy, respectively, although the profiles of the serum ITA regression curves compared with those of total hCG + hCGβ immunoreactivity did not seem to be different in the various treatment groups tested. Thus, it may be questioned whether absence of invasive trophoblastic disease (Group 1), or presence of this disease with proven response to chemotherapy treatment (Groups 2 and 3) is accurately reflected by the proportion of ITA accounting for total hCG immuno-reactivity. In this respect, it would be of interest to investigate in a prospective randomised setting the effect of additional courses of chemotherapy in the course of treatment at the time point where serum ITA concentrations become nondetectable whereas hCG + hCGβ determinations are as yet not normalised, but still declining and significantly above the detection limit of the assay.

In conclusion, we have studied a series of patients with uneventful post-molar hCG + hCGβ regression in a longitudinal setting and compared these results with those of ITA. Similar comparisons were made in groups of patients who developed post-molar PTD and normalised upon treatment with either single-agent, or single-agent and subsequent multiagent chemotherapeutic agents. Serum half-lifes were calculated and revealed lower disappearance rates for hCG + hCGβ than for ITA. Multiagent chemotherapy treatment of post-molar PTD displayed significantly a longer half-life than single-agent chemotherapy treatment or spontaneous regression. The implication and the possible clinical value of faster regression of ITA to baseline levels as compared to hCG + hCGβ remain to be investigated prospectively.

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References

1. Yedema KA, Verheijen RH, Kenemans P, Schijf CP, Borm GF, Segers MF, Thomas CM. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. Am J Obstet Gynecol 1993; 168:787-92.
2. Khazaeli MB, Hedayat MM, Hatch KD, To AC, Soong SJ, Shingleton HM, et al. Radioimmunoassay of free beta-subunit of human chorionic gonadotropin as a prognostic test for persistent trophoblastic disease in molar pregnancy. Am J Obstet Gynecol 1986; 155:320-4.
3. Berkowitz RS, Goldstein DP, Bernstein MR. Natural history of partial molar pregnancy. Obstet Gynecol 1985; 66:677-81.
4. Szulman AE, Surti U. The clinicopathologic profile of the partial hydatidiform mole. Obstet Gynecol 1982; 59:597-602.
5. Ng TY, Wong LC. Diagnosis and management of gestational trophoblastic neoplasia. Best Pract Res Clin Obstet Gynaecol 2003; 17:893-903.
6. Osborne R, Gerulath A. What is the best regimen for low-risk gestational trophoblastic neoplasia? A review. J Reprod Med 2004; 49:602-16.
7. Elliott MM, Kardana A, Lustbader JW, Cole LA. Carbohydrate and peptide structure of the alpha- and beta-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine 1997; 7:15-32.
8. Cole LA, Shahabi S, Oz UA, Bahado-Singh RO, Mahoney MJ. Hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen) immunoassay: A new basis for gestational Down syndrome screening. Clin Chem 1999; 45:2109-19.
9. Kovalevskaya G, Genbacev O, Fisher SJ, Caceres E, O'Connor JF. Trophoblast origin of hCG isoforms: cytotrophoblasts are the primary source of choriocarcinoma-like hCG. Mol Cell Endocrinol 2002; 194:147-55.
10. Cole LA, Shahabi S, Butler SA, Mitchell H, Newlands ES, Behrman HR, Verrill HL. Utility of commonly used commercial human chorionic gonadotropin immunoassays in the diagnosis and management of trophoblastic diseases. Clin Chem 2001; 47:308-15.

11. Khanlian SA, Smith HO, Cole LA. Persistent low levels of human chorionic gonadotropin: A premalignant gestational trophoblastic disease. Am J Obstet Gynecol 2003; 188:1254-9.
12. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. Ann Clin Biochem 1985; 22:236-46.
13. Korhonen J, Alfthan H, Ylostalo P, Veldhuis J, Stenman UH. Disappearance of hCG and its alpha- and beta-subunits after term pregnancy, Clin Chem 1997; 43:2155-63.
14. Stenman UH, Alfthan H, Hotakainen K, Human chorionic gonadotropin in cancer [review]. Clin Biochem 2004; 37:549-61.
15. Imamura S, Armstrong EG, Birken S, Cole LA, Canfield RE. Detection of desialylated forms of human chorionic gonadotropin. Clin Chim Acta 1987; 163:339-49.
16. Nishimura R, Endo Y, Tanabe K, Ashitaka Y, Tojo S. The biochemical properties of urinary human chorionic gonadotropin from the patients with trophoblastic disease. J Endocrinol Invest. 1981; 4:349-58.
17. Morell AG, Gregoriadis G, Scheinberg IH. The role of sialic acid in determining the survival of glycoprotiens in the circulation. J Biol Chem 1971; 246:1461-7.
18. Hoermann R, Kubota K, Amir SM. Role of subunit sialic acid in hepatic binding, plasma survival rate, and in vivo thyrotropic activity of human chorionic gonadotropin. Thyroid 1993; 3:41-7.
19. Ashwell G, Harford J. Carbohydrate-specific receptors of the liver. Ann Rev Biochem 1982; 51:531-54.
20. Okamoto T, Matsuo K, Niu R, Osawa M, Suzuki H. Human chorionic gonadotropin (hCG) beta-core fragment is produced by degradation of hCG or free hCG beta in gestational trophoblastic tumors: a possible marker for early detection of persistent postmolar gestational trophoblastic disease. J Endocrinology 2001; 171:435-43.

CHAPTER 5

Clinical utility of hyperglycosylated hCG in serum taken before hydatidiform mole evacuation to predict persistent trophoblastic disease

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Abstract

Objective: Human chorionic gonadotropin (hCG) is widely used in the management of hydatidiform mole and persistent trophoblastic disease (PTD). Studies on hyperglycosylated human chorionic gonadotropin (Invasive Trophoblast Antigen, ITA) in PTD are limited. In serum samples taken before evacuation of molar pregnancies we measured the concentrations of free hCG β-subunit (free hCGβ), "total" hCG (hCG + hCGβ) and ITA, and we determined whether ITA, the two other hCG analytes, or the calculated ratios of hCGβ/hCG + hCGβ, hCGβ/ITA and hCG + hCGβ/ITA could predict the later development of PTD.

Design: A retrospective study based on blood specimens collected in the Dutch Central Registry for Hydatidiform Moles. The study group comprised 97 patients with hydatidiform mole who did not develop PTD after mole evacuation, and 33 patients who did develop PTD.

Methods: Serum samples from 130 patients with hydatidiform mole with or without PTD were assayed using specific (radio)immunoassays for free hCGβ, total hCG, and ITA. From these analytes we also calculated the ratios hCGβ/hCG + hCGβ, hCGβ/ITA, and hCG + hCGβ/ITA. To predict development of PTD from these analytes and parameters we performed receiver-operating characteristic (ROC) curve analysis resulting in areas under the curve (AUCs), that represented the diagnostic accuracy which was rated in a range from excellent (AUC>0.9 or <0.1) to poor (AUC 0.4-0.6).

Results: The diagnostic accuracy of ITA was moderate (0.618) and not different from that of free hCGβ (0.610) and hCG + hCGβ (0.622).

Conclusions: ITA as well as the other analytes and parameters in serum taken prior to evacuation from patients with molar pregnancies cannot be used to predict the subsequent development of persistent trophoblastic disease.

Introduction

Gestational trophoblastic disease (GTD) is an aberration of the trophoblast during pregnancy. It consists of a spectrum of diseases including molar pregnancies, persistent invasive mole, gestational choriocarcinoma and placental-site trophoblastic tumor. Molar pregnancy is the most common form of GTD, with a reported incidence of 0.5 to 2.5 out of 1000 pregnancies (1). The incidence varies according to regional, ethnic, socioeconomic and age differences. Molar pregnancy may be classified as either a complete or partial, based on gross morphology, histopathology and karyotype (2). Surgical uterine evacuation in the form of suction curettage is considered to be the management of choice in newly diagnosed GTD. PTD is seen in only 4-9% of cases after evacuation of a partial mole (2, 3). In case of complete molar pregnancies, the risk of PTD is thought to be 15-20% (4).

The human chorionic gonadotropin molecule is composed of an α- and β-subunit. The α-subunit is composed of 92 amino acids with two N-linked oligosaccharide side chains. The β-subunit comprises 145 amino acids with two N-linked oligosaccharides and four O-linked sugar structures on the C-terminal extension. A total of eight carbohydrate chains comprising about one-third of the molecular mass of intact hCG is attached to its α- and β- subunits. In comparison with normal pregnancy, the glycosylation pattern of the hCG β-subunits of choriocarcinoma is increased several fold (4). Whereas hCG during normal and molar pregnancy shows only small proportions of more complex carbohydrates, hCG molecules in choriocarcinoma amount up to 100% of these larger N- and O-linked oligosaccharides, called hyperglycosylated hCG (5), also referred to as Invasive Trophoblast Antigen (ITA) (6). Cole *et al.* suggest that hyperglycosylated hCG is produced by invasive trophoblast cells during early pregnancy or in case of malignant trophoblast transformation as in choriocarcinoma (6).

Based on the use of a monoclonal antibody (B-152) raised against the hyperglycosylated choriocarcinoma hCG, a hyperglycosylated isoform produced by first-trimester cytotrophoblast cells can be detected in maternal blood and urine during the first 6-7 weeks of pregnancy (7, 8). Based on the findings of Khanlian *et al.*, the USA hCG Reference Service indicates that measurement of hyperglycosylated hCG may be used to distinguish quiescent GTD from invasive disease (9, 10). It is the aim of the present study to evaluate the clinical utility of ITA as a tumor marker

in the prediction of PTD prior to evacuation of a molar pregnancy. The present retrospective study includes measurements of hCGβ, hCG+ hCGβ and ITA, and calculates the ratios of hCGβ/hCG + hCGβ, hCGβ/ITA and hCG + hCGβ/ITA in blood taken before evacuation from 130 patients with hydatidiform moles.

Materials and Methods

Patients

In the Netherlands hydatidiform moles are registered at the Dutch Central Registry for Hydatidiform Moles at the Radboud University Nijmegen Medical Centre (RUNMC). The registry was established in 1977 and until 2004 we registered 2821 cases. In 692 registered patients, hCG in blood was analysed in our department at the RUNMC. After collection, blood samples were centrifuged and serum was sent to our institute and kept at -20 °C until assayed. Of these 692 patients, 562 could not be analysed because we had not received a serum specimen taken prior to evacuation, or the blood sample was exhausted and therefore no longer available for further analysis. We analysed hCGβ, hCG + hCGβ and ITA in blood of the remaining 130 patients. Of these 130 patients, 97 had a normal serum hCG regression, as derived from a normal regression curve constructed by Yedema *et al.* (11). These patients did not develop PTD. The remaining 33 patients ultimately developed PTD after evacuation.

Immunoassays

We used two in-house developed radioimmunoassays (RIAs) that measured free hCG β-subunit (hCGβ) and "total" hCG (i.e., intact hCG and free β-subunit, hCG + hCGβ) (12). The hCG + hCGβ assay is utilized for all measurements in sera sent to the Dutch Central Registry for Hydatidiform Moles. Based on this assay we developed a normal hCG regression corridor for uneventful hydatidiform mole (11). The RIAs of hCGβ and hCG + hCGβ were calibrated with the third International Standard (IS) preparations for hCG β-subunit or intact hCG (WHO third IS hCGβ 75/551 or hCG 75/537, respectively, obtained from the National Institute for Biological Standards, Potters Bar, Herts, UK). The measuring range for the standard line of the hCGβ assay was 0.078 – 2.50 μg/L or IU/L (equivalent to 0.0033-0.107 nmol/L) and for the hCG + hCGβ assay 1-80 μg/L (0.027-2.14 nmol/L, equivalent to 9.29-743 IU/L of the WHO third

IS hCG 75/537 (12). The free hCG β-subunit RIA showed cross- reactivity with intact hCG of 0.35% (on a mass basis, equivalent to 0.55% on a molar basis) as tested with the WHO third IS 75/537 of hCG, 1.1% on a molar basis with nicked hCG (hCGn) (WHO 99/642 Reference Reagent) and 0.4% with nicked hCGβ (hCGβn) (WHO 99/692 Reference Reagent). The hCG + hCGβ-RIA cross-reacted 100% on a mol/mol basis with intact hCG and 1000% with hCG β-subunit, and 228% with hCGn (WHO 99/642 Reference Reagent) and 507% with hCGβn (WHO 99/692 Reference Reagent) this is of minor practical importance because these nicked forms of hCG mainly occur in urine. Serum hCG concentrations were considered to be normalized if below 2 μg/L (0.053 nmol/L or 18.6 IU/L of the WHO third IS hCG 75/537) which equals the 95th percentile of the reference interval of healthy non-pregnant controls. In the case of the hCGβ RIA, this was 0.20 μg/L or IU/L (0.0085 nmol/L of the WHO third IS hCGβ 75/551). The within- and between-assay coefficient of variation (CV_w, CV_b) for the means of duplicate measurements in the hCG + hCGβ RIA were at a level of 10 μg/L (equivalent to 0.267 nmol/L or 93 IU/L) 7.5% and 10.3%, respectively, and at a level of 56 μg/L (equivalent to 1.50 nmol/L or 520 IU/L), 7.3% and 12%. In the case of the hCGβ RIA, two serum pools (mean:0.014 nmol/L (0.33μg/L or IU/L) and 0.041 nmol/L (0.96 μg/L or IU/L)) revealed 5.2%-5.8% (CV_w) and 9.5%-9.9% (CV_b).

ITA was measured with the Nichols Advantage Invasive Trophoblast Antigen Assay (Nichols Institute Diagnostics, San Clemente, CA 92673, USA) and the assay was performed according to the instructions provided by the manufacturer. The ITA assay is a two-step, two-site immunochemiluminometric assay that uses two monoclonal antibodies. The capture antibody (B152) is biotin labelled while the second antibody (B207) is labelled with acridinium ester and used for detection. The analytical sensitivity of the assay is <0.048 nmol/L (<0.2 μg/L). Cross reactivity as documented by the supplier is <1 % with recombinant hCG (Sigma C6322), 5.4 % with nicked hCG, 4.5 % with non-nicked hCG, 1.5 % with nicked free βhCG, and 1.0 % with non-nicked free βhCG.

Statistics

The molar concentrations of hCGβ, hCG + hCGβ and ITA of mole with PTD and mole without PTD were utilized to construct receiver-operating characteristic (ROC) curves and to calculate areas under the curves

Table 1. AUC for six hCG analytes and parameters compared in moles without and with PTD

	PTD-	PTD+	AUC	95% CI	DA
Parameter	n	n			
free hCGβ	91	33	0.610	0.500-0.719	±
hCG+hCGβ	97	33	0.622	0.512-0.732	±
ITA	96	33	0.618	0.509-0.727	±
hCGβ/hCG + hCGβ	91	33	0.526	0.412-0.639	-
hCGβ/ITA	91	33	0.485	0.368-0.602	-
hCG + hCGβ/ITA	95	33	0.461	0.342-0.580	-

PTD- absence of Persistent Trophoblastic Disease
PTD+ presence of Persistent Trophoblastic Disease
AUC Area-Under-Curve
n number of patients
95%CI 95% Confidence Interval
DA Diagnostic Accuracy; ± moderate; - poor

(AUCs) for assessment of diagnostic accuracy of the test. All calculations were conducted with SPSS (version 12.0) for Microsoft Windows XP (SPSS, Chicago, IL, USA).

ROC curves represent the full spectrum of possible sensitivity-specificity pairs for a test in a clinical application (13). We present conventional ROC curves (with x-axis representing '1-specificity' and y-axis as 'sensitivity'), irrespective of whether AUC is <0.5 or >0.5. However, in those cases where AUC<0.5, the x-axis should read "specificity" and the y-axis '1-sensitivity' for correct interpretation of the ROC curve. To rank the accuracy of a diagnostic test, we interpreted AUC values of 0.91-1.00 and 0.0-0.09 as excellent accuracy (++), 0.81-0.90 and 0.10-0.19 as good accuracy (+), 0.61-0.80 and 0.20-0.39 as moderate accuracy (±), and 0.40-0.60 as poor accuracy (-).

Results

We established diagnostic accuracy by calculating specificity and sensitivity. We constructed ROC curves for hCGβ, hCG + hCGβ and ITA analytes determined in blood specimens collected prior to evacuation as

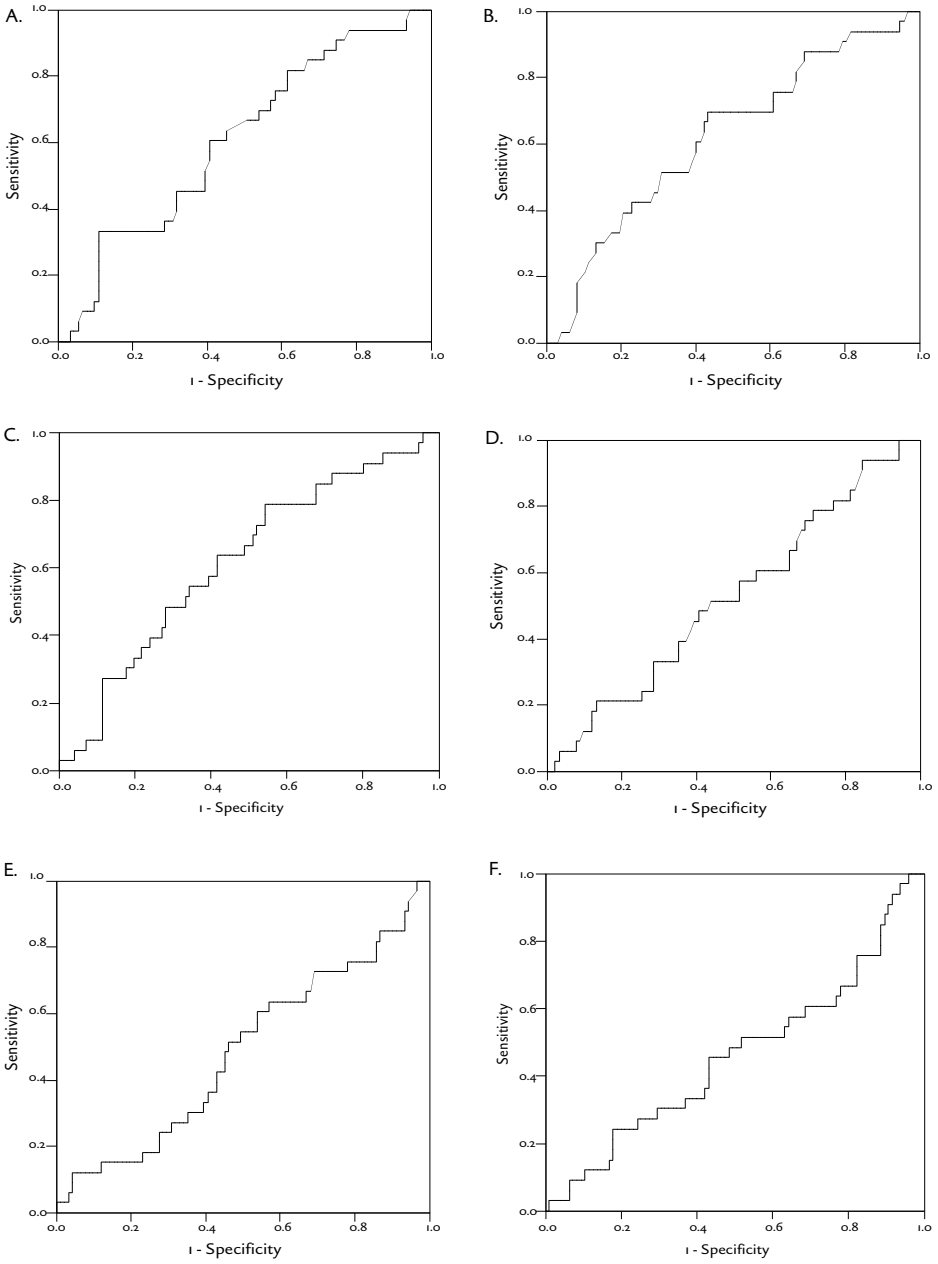

Figure 1. ROC curve analysis for hCG parameters compared in study group (all moles with PTD) versus control group (all moles without PTD)

A) free hCGβ B) hCG + hCGβ C) ITA

D) hCGβ/hCG + hCGβ E) hCGβ/ITA F) hCG + hCGβ/ITA

well as for the three ratios derived from these measurements (Figure 1, A-F). Table 1 gives the corresponding AUCs for hCGβ, hCG + hCGβ and ITA, analytes and the three molar ratios hCGβ/hCG + hCGβ, hCGβ/ ITA and hCG + hCGβ/ITA.

The AUCs of hCGβ (0.610), hCG + hCGβ (0.622) and ITA (0.618) from the groups of moles with PTD versus moles without PTD show moderate (±) diagnostic accuracy. Thus, the diagnostic accuracy of the two hCG and ITA analytes are not different. For the ratios hCGβ/ hCG + hCGβ (0.526), hCGβ/ITA (0.485) and hCG + hCGβ/ITA (0.461) the diagnostic accuracy was poor (-).

Discussion

The aim of our retrospective study was to determine whether serum ITA before evacuation of a molar pregnancy could predict the later development of PTD. The specificity and sensitivity of hCGβ, hCG + hCGβ and ITA in serum samples taken prior to evacuation in the groups of molar pregnancy with PTD and molar pregnancy without PTD were explored by ROC curve analysis, resulting in AUCs for the three analytes and the three calculated ratios hCGβ/hCG + hCG, hCGβ/ITA and hCG + hCGβ/ITA. Our study does not support that ITA is a reliable marker for predicting which patients with molar pregnancies are at high risk of developing persistent disease after evacuation. ITA has only moderate diagnostic accuracy in predicting PTD in blood samples taken prior to evacuation. The results were in the same range as with the analytes hCGβ and hCG + hCGβ. The three ratios derived from the three analytes only show poor diagnostic accuracy.

The incidence of PTD, which is the most important sequela of hydatidiform mole, was found to vary between 18.7% and 31.1% (14). Several prognostic factors in predicting the development of PTD have been described. Most of these were clinical features, i.e., age, uterine size, and the presence of theca-lutein cyst. A laboratory test that could predict the later development of PTD, would facilitate early appropriate management. To establish the clinical utility of hyperglycosylated hCG or invasive trophoblastic antigen, the use of these isoforms of hCG was explored in the prenatal diagnosis of Down syndrome, the prediction of pre-eclampsia and the management of quiescent GTD (9). With regard to the possible role of hyperglycosylated hCG in hydatidiform mole, ITA was found to rise from 25% to 80% of total serum hCG when patients

with persistently low levels of hCG developed gestational trophoblastic neoplasm (9). The source of hyperglycosylated hCG in pregnancy was reported to originate from cytotrophoblast tissue.

Kovaleskaya *et al.* reported that hCG is produced and/or accumulated in the syncytiotrophoblast cells and that the cytotrophoblast cells are the predominant source of the hyperglycosylated hCG isoform (8). Cole *et al.* found that the hyperglycosylated hCG is the predominant form of chorionic gonadotropin in very early pregnancy (16). The concentrations of hyperglycosylated hCG diminish rapidly and are replaced by the hCG isoform characteristic of later first trimester of pregnancy which predominates throughout the remainder of pregnancy (7, 8). High level of hyperglycosylated hCG is associated with aggressive trophoblast invasion in early pregnancy, at three to four weeks'gestation (7, 15) and trophoblastic disease (6). Khanlian *et al.* evaluated 114 cases with persistent low levels of hCG, all of whom were referred to the USA hCG Reference Service for consultation (9). In four cases with malignant disease, the proportions of ITA accounted between 81 to 100% of the detected hCG immunoreactivity. Based on these results, the USA hCG Reference Service indicated that measurement of hyperglycosylated hCG (ITA) may be used to distinguish quiescent GTD from invasive disease (9). The Service measures hyperglycosylated hCG as part of their routine consultation by means of the Nichols Advantage automated ITA test with JEG-3-derived standard, which only detects hyperglycosylated hCG (9, 10). As the present study also used the Nichols Advantage automated ITA assay for determination of ITA, the observed differences in the results cannot be explained by differences in assay procedures. The poor results from measurement in sera collected prior to evacuation reported here, as derived from the diagnostic accuracy by calculating specificity and sensitivity for the analytes hCGβ, hCG + hCGβ and ITA, and the ratios of hCGβ/hCG + hCGβ, hCGβ/ITA and hCG + hCGβ/ITA, do not support the idea that ITA is a powerful predictor of PTD. Still, it could be interesting to study hyperglycosylated hCG in molar pregnancies at later time points after the evacuation to predict patients with "high risk" disease or to identify patients who are likely to require chemotherapy for persistent trophoblastic tumor. In conclusion, the ITA concentrations in serum samples taken prior to evacuation as well as those of the other hCG analytes and parameters tested show very limited diagnostic accuracy in the prediction of subsequent development of PTD.

References

1. Palmer JR. Advances in the epidemiology of gestational trophoblastic disease. J Reprod Med 1994; 39: 155-62.
2. Szulman AE, Surti U. The clinicopathologic profile of the partial hydatidiform mole. Obstet Gynecol 1982; 59: 597-602.
3. Berkowitz RS, Goldstein DP, Bernstein MR. Natural history of partial molar pregnancy. Obstet Gyneco 1985; 66: 677-681.
4. Khazaeli MB, Hedayat MM, Hatch KD, et al. Radioimmunoassay of free beta-subunit of human chorionic gonadotropin as a prognostic test for persistent trophoblastic disease in molar pregnancy. Am J Obstet Gynecol 1986; 155: 320-324.
5. Elliott MM, Kardana A, Lustbader JW, Cole LA. Carbohydrate and peptide structure of the alpha- and beta-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine 1997; 7: 15-32.
6. Cole LA, Shahabi S, Oz UA, Bahado-Singh RO, Mahoney MJ. Hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen) immunoassay: A new basis for gestational Down syndrome screening. Clin Chem 1999; 45: 2109-2119.
7. Kovalevskaya G, Birken S, Kakuma T, et al. Differential expression of human chorionic gonadotropin (hCG) glycosylation isoforms in failing and continuing pregnancies: preliminary characterization of the hyperglycosylated hCG epitope. J Endocrin 2002; 172: 497-506.
8. Kovalevskaya G, Genbacev O, Fisher SJ, Caceres E, O'Connor JF. Trophoblast origin of hCG isoforms: cytotrophoblasts are the primary source of choriocarcinoma-like hCG. Mol Cell Endocrinol 2002; 194: 147-155.
9. Khanlian SA, Smith HO, Cole LA. Persistent low levels of human chorionic gonadotropin: A premalignant gestational trophoblastic disease. Am J Obstet Gynecol 2003; 188: 1254-1259.
10. Cole LA, Sutton JM. HCG tests in the management of gestational trophoblastic diseases. J Reprod Med2003; 46: 523-540.
11. Yedema KA, Verheijen RH, Kenemans P, et al.Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. Am J Obstet Gynecol 1993; 168: 787-792.
12. Thomas CM, Segers MF, Houx PC: Comparison of the analytical

characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. Ann Clin Biochem 1985; 22: 236-246.
13. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993; 39: 561-577.
14. Morrow CP, Townsend DE. Tumors of the placental trophoblast. In: Morrow CP, Townsed eds. Synopsis Gynecology Oncology, 3rd ed. New York: Wiley Medical, 1987, 367.
15. O'Connor JF, Ellish N, Kakuma T, Schlatterer J, Kovalevskaya G. Differential urinary gonadotrophin profiles in early pregnancy and early pregnancy loss. Prenat Diagn 1998; 18: 1232-1240.
16. Cole LA, Khanlian SA, Sutton JM, Davies S, Stephens ND. Hyperglycosylated hCG (Invasive Trophoblast Antigen, ITA) a key antigen for early pregnancy detection. Clin Biochem 2003; 36: 647-655.

CHAPTER 6

The curative effect of a second curettage in persistent trophoblastic disease: A retrospective cohort survey

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Abstract

Objective: To assess the curative effect of a second curettage in patients with low-risk Persistent Trophoblastic Disease (PTD) after molar pregnancy.

Methods: A retrospective cohort survey was performed on 2122 patients registered with the Dutch Central Registry for Hydatidiform Moles between 1987-2003. Of these, 422 patients developed PTD. For various reasons, 128 patients were excluded. The study group comprised 85 patients with, according to the Dutch guidelines, low-risk PTD who underwent a second therapeutic curettage as a part of the treatment for PTD. The control group consisted of 209 patients with low-risk PTD who did not undergo a second curettage. Patients in the study and control group were classified for high/low-risk PTD according to the internationally accepted FIGO 2000 guidelines. Primary outcome measure was the need for chemotherapy and if applicable, the number of chemotherapy courses.

Results: After second curettage, eight out of 85 patients (9.4 %) did not need additional chemotherapy which significantly differs from the 209 patients in the control group who all needed chemotherapy ($p<0.001$). A debulking effect of the second curettage was observed: a median of 6 courses (interquartile range 3 courses) in the control group versus 5 courses (interquartile range 3 courses) in the study group ($p=0.036$). Four out of the 85 (4.8%) patients with a second curettage had a major complication (uterine perforation or hemorrhage), which was managed conservatively.

Conclusions: A second curettage cured 9.4% of patients with PTD in this historical cohort and reduces the number of courses of chemotherapy. A second curettage seems to benefit only a limited number of patients with PTD. A randomised controlled prospective trial is needed to confirm this observation.

Introduction

In Persistent Trophoblastic Disease (PTD), trophoblastic activity remains after evacuation of a hydatidiform mole as shown by subsequent unaltered high or even rising hCG concentrations in blood. A variety of pathologic types of trophoblast are included in Gestational Trophoblastic Disease (GTD), comprising villous malformations of trophoblast: hydatidiform mole, subdivided in complete and partial hydatidiform moles, and non-villous malformations of which choriocarcinoma is the most frequent (1). The reported frequency of PTD is 20% in complete hydatidiform mole (2) and 0.5 to 9.9% in partial hydatidiform mole (3-6). For PTD, several staging classifications and prognostic scoring systems have been developed (7). In The Netherlands, the Dutch Working Party on Trophoblastic Tumours utilises a classification system based on the following items: antecedent pregnancy (i.e., the pregnancy preceding PTD development), location of metastases, previous chemotherapy and the duration of the interval between end of pregnancy and the start of treatment (7-9). To date, the FIGO 2000 staging and scoring system is advocated by the Committee of the International Society for the Study of Trophoblastic Diseases (ISSTD) and the International Society for Gynaecological Cancer (IGCS) in order to generate univocal data (10). Once diagnosed and scored, low-risk PTD is typically treated with single-agent chemotherapy (Methotrexate or Actinomycin-D) (11,12). If staged as high-risk PTD, multi-agent chemotherapy with EMA-CO (Etoposide, Methotrexate, Actinomycin-D, Cyclophosphamide and Vincristine) is most widely used (13). Although there are some patients with persistent hemorrhage with anemia who require a second curettage, most investigators are reluctant to recommend routine second curettage in case of PTD (14,15).

Whether a second therapeutic curettage in PTD is of curative benefit has recently been prospectively investigated: 171 out of 282 patients with PTD (60%) were cured after the second curettage (16). The authors defined PTD as a plateau or rise in hCG levels, but unlike the FIGO guideline did not define the time period (10). The 60 % cure rate after second curettage is in contrast with the only available retrospective study dealing with the curative effect of a second curettage in patients with PTD (15). Four patients in a subgroup of 23 patients (16%) with non-metastatic PTD (out of a group of 37 patients) were cured by the second curettage. No therapeutic effect was noticed in the remaining patients

(with metastatic PTD or in retrospect no PTD) in this study. In three (8.1%) out of the 37 patients included in this study, a uterine perforation occurred during the second curettage. More studies have been performed on the diagnostic utility of a second curettage (16-19): two studies found the histological finding of mole tissue in the second curettage beneficial to predict the response to chemotherapy (17) or need for chemotherapy (16) in patients with PTD, whereas the remaining two studies did not find a second curettage to be beneficial in this respect (18,19) .

The primary outcome measure of the present study, in which we investigated the curative effect of second curettage only, was whether or not chemotherapy was needed after the second curettage as compared to a control group without a second curettage. To investigate a possible debulking effect of the second curettage, the number of courses of chemotherapy needed for complete cure and consolidation was compared between patients with and without second curettage. Patient characteristics at enrolment for high/low-risk PTD according to the Dutch classification (7-9) were reconfirmed and in retrospect analyzed according to the FIGO 2000 (10) classification and compared between the study and control group.

Material and Methods

Patients

In The Netherlands, patients with hydatidiform mole are registered with the Dutch Central Registry for Hydatidiform Moles residing at the Radboud University Nijmegen Medical Centre (RUNMC). Between 1987 and 2003, 2122 patients were registered (Fig. 1). We found 1178 (55.5%) patients to be eligible for analysis. From these eligible patients, we selected all 422 (35.8%) patients with proven PTD according to Dutch guidelines (hCG plateau or rise for three consecutive weekly measurements, with at least one measurement >P95 of normal regression according to an uneventful hCG regression curve published earlier (20)). Of these 422 patients, treatment outcome was unknown in 78 cases (18.5%) and therefore these patients were excluded. In the remaining 344 (81.5%) patients with PTD, 103 (29.9%) underwent a second curettage as part of the treatment for PTD (study group). The remaining 241 (70.1%) patients did not undergo a second curettage (control group). Eighteen patients (17.5%) of the study group and 32 patients (13.3%) of the control group were exclud-

ed. Reason for exclusion in either group were: choriocarcinoma (11 and 30, respectively), high-risk PTD according to the Dutch classification for PTD ((7-9) (one patient in the study group), placental site trophoblastic tumor (one case in the study group). One patient in the study group and two patients in the control group were excluded because a hysterectomy was performed at the patient's request at the start of treatment of the PTD. Four patients in the study group were treated first with chemotherapy before second curettage and therefore excluded. Overall, 85 patients were included in the second curettage group (study group) and 209 patients in the group who did not undergo a second curettage (control group). Patients were scored according to the Dutch (7-9) and the FIGO 2000 (10) classification as high/low-risk at the moment PTD disease was diagnosed.

Immunoassays

Serum hCG measurements were either performed in the Registry's laboratory (n = 61) or in the referring hospitals (n = 75). An in-house RIA that measured "total" hCG (i.e., intact hCG and free β-subunit, hCG + hCGβ) was applied (21). The RIA was calibrated with the third International Standard Preparations for intact hCG (WHO third IS hCG 75/537 obtained from the National Institute for Biological Standards, Potters Bar, England). The measuring range for the standard line of the assays was 0.027-2.14 nmol/L (equivalent to 9.29-743 IU/L).

The hCG + hCGβ-RIA cross-reacted 100% with intact hCG and 1000% with hCG β-subunit. The 95th percentile of the interval of non-pregnant individuals for the hCG + hCGβ assay was established at 0.053 nmol/L (equivalent to 18.6 IU/L of the WHO third IS hCG 75/537) (21). The intra- and inter-assay coefficients of variation (CV_w, CV_b) for means of duplicate measurements for two serum pools (mean: 0.267 nmol/L (93 IU/L) and 1.50 nmol/L (520 IU/L)) were 7.3 % and 12 %. If no serum was sent to the Registry's laboratory, data on hCG levels were provided by the referring hospital.

Statistics

All statistical analyses were performed using the SPSS statistical software package version 12.0.1 (SPSS Inc). Normality of distributions were explored by Kolmogorov-Smirnov testing. Differences in numerical data between the study and control group were tested non- parametrically

(Mann-Whitney U). Differences in categorical distributions between study and control group were tested using the Pearson χ^2 test. All tests were considered significantly different at $p<0.05$.

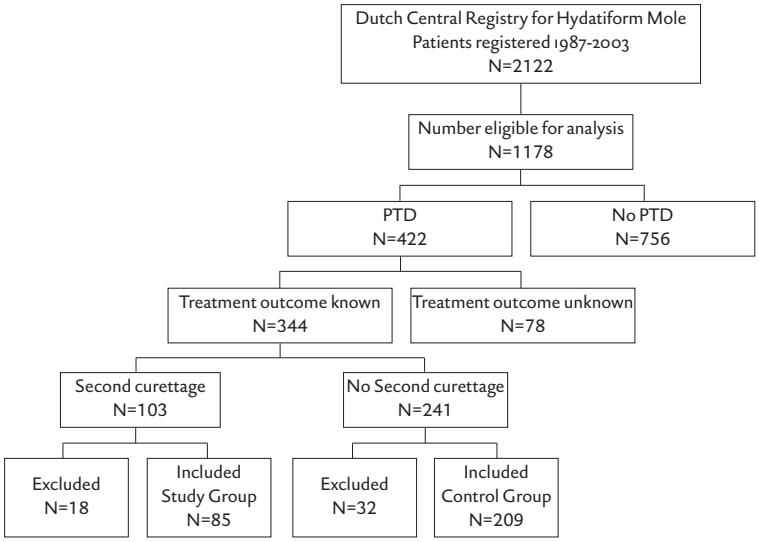
Results

Table 1 gives the patient characteristics of the study and control group. Between study and control group no significant differences were observed with respect to age, gravidity, parity, serum concentration of pre-evacuation hCG and interval between end of pregnancy and start of treatment. The median number of courses of first-line chemotherapy was significantly lower in the study group as compared to the control group (5 courses of Methotrexate (MTX) with folinic rescue (leucovorin) in the study and 6 courses in the control group, p= 0.036).

The Dutch classification for high/low-risk PTD takes into account site of metastases, antecedent pregnancy, interval between end of pregnancy and start of treatment, and previously failed chemotherapy (7-9). The classification of all patients to have low-risk PTD according to the Dutch guideline was reconfirmed by analysis of patient records in our registry.

An effort was made to score patients according to the FIGO 2000 classification for high/low-risk PTD in retrospect. In this classification, the distribution of items is significantly different between study and control group in antecedent pregnancy (study group: 8 out of 84 pregnancies non-mole abortion, control group: 4 out of 209 non-mole abortion), site and number of metastases (study group: one patient with one lung metastasis, control group: 19 patients with 1-4 lung metastases). Data were missing on the following items in more than 30% of cases: pre-treatment hCG, tumor size, interval between end of pregnancy and start of treatment, and the site and number of metastases (Table 2). Data on tumor size were missing in more than 95% of cases and therefore not shown in this table.

The main outcome measure of this study was the curative effect of a second curettage in patients with PTD. Table 3 shows that 8 of 85 patients (9.4%) were cured after the second curettage given as first-line therapy, while 76 (89.4%) needed further chemotherapy. In the case of one patient (1.2%), the treatment after second curettage was unknown. The need for chemotherapeutic treatment was significantly more frequently observed in the control group ($P < 0.001$). The final therapy (scored as: no other treatment than first, multi-agent chemotherapy only, hysterec-



Exclusion criteria
Treatment outcome unknown
Choriocarcinoma
placental site trophoblastic
Hysterectomy at the start of PTD treatment
High risk PTD according to Dutch guidelines
Chemothearapy preceding second curettage

Figure 1. Selection of study group (second curettage) and control group (no second curettage) from Dutch Central Registry for Hydatidiform Mole, 1987-2003.

tomy, or hysterectomy after multi-agent chemotherapy) leading to cure in 100% of cases was not differently distributed between the groups. In the study group, response to first-line MTX chemotherapy failed in 25.9% of patients and 20.6% of patients in the control group. Two patients (2.4%) in the study group and two in the control group (1%) were resistant to multi-agent chemotherapy, for which hysterectomy was performed.

Two patients (2.4%) had severe blood loss of more than 1000 mL after the second curettage, and 2 other patients (2.4%) had a uterine perforation during the second curettage which could be treated conservatively. In the 85 patients in the study group, a second curettage was performed in 51 cases (60%) for blood loss only or in conjunction with other reasons and in 32 (37.6%) cases for other reasons than blood loss (rise or plateau in serum hCG levels, abnormal tissue visible in utero).

Table 1. Patient characteristics, pre-evacuation hCG, interval between end of pregnancy and start of treatment, and number of courses of chemotherapy in study group (second curettage) and control group (no second curettage)

	Study group (*n* = 85) 2nd curettage: yes		Control group (*n* = 209) 2nd curettage: no		*P* [a]
	Median (interquartile range)[b]	Number of cases	Median (interquartile range)[b]	Number of cases	
Age (years)	29 (6)	85	30 (7)	209	0.128
Gravidity	2 (2)	83	2 (1)	192	0.124
Parity	1 (1)	83	0 (1)	189	0.365
Pre-evacuation hCG (IU/L)	$1.8x10^5$ ($3.5x10^5$)	28	$2.0x10^5$ ($3.2\ 10^5$)	108	0.974
Interval (months)	2 (2)	66	2 (2)	126	0.095
Number of courses MTX	5 (3)	67	6 (3)	145	0.036

[a] Mann-Whitney *U* test.

[b] Interquartile range: P25-P75.

Discussion

In the present study, we evaluated whether a second curettage in patients with low- risk PTD might be of clinical benefit. We tested the hypotheses that a second curettage would cure patients (resulting in no additional need for chemotherapeutic treatment after the second curettage) and/or that a second curettage would have a "debulking" effect with less courses of chemotherapy to obtain remission as a result. From the 2122 patients registered between 1987 and 2003 at the Dutch Central Registry for Hydatidiform Moles, we included 85 patients with low-risk PTD who underwent a second curettage with or without chemotherapy, and 209 patients with low-risk PTD who received only chemotherapy for treatment of established PTD. In order to assess whether baseline character-

Table 2. Baseline risk factors for low/high risk according to FIGO 2000 in study group (second curettage) and control group (no second curettage)

	Study group (*n* = 85) 2nd curettage: yes		Control group (*n* = 209) 2nd curettage: no		*p* [a]
Age (years)	Number	Rate, %	Number	Rate, %	
< 40 [b]	82	96.5	195	93.3	0.291
≥ 40 [c]	3	3.5	14	6.7	
Missing data	0	0.0	0	0.0	
Total	85	100	209	100	
Antecedent pregnancy					
Mole [b]	76	89.4	205	98.1	0.004
Non-mole abortion [c]	8	9.4	4	1.9	
Term [d]	0	0.0	0	0.0	
Missing data	1	1.2	0	0.0	
Total	84	99	209	100	
Interval end of pregnancy-start treatment (months)					
< 4 [b]	53	62.3	109	52.2	0.493
4-≤ 7 [c]	10	11.8	14	6.7	
7-≤ 13 [d]	3	3.5	3	1.4	
> 13 [e]	0	0	0	0.0	
Missing data	19	22.4	83	39.7	
Total	85	100	209	100	
Pre-treatment hCG (IU/L)					
< 1000 [b]	1	1.2	6	2.9	0.745
< 10000 [c]	1	1.2	1	0.5	
< 100000 [d]	6	7.1	24	11.5	
≥ 100000 [e]	20	23.5	77	36.6	
Missing data	57	67.1	101	48.3	
Total	85	100	209	100	
Site of metastases					
No metastases [b]	60	70.6	117	56.0	0.008
Lungs [b]	1	1.2	19	9.1	
Spleen/Kidney [c]	0	0.0	0	0.0	
Gastro-intestinal [d]	0	0.0	0	0.0	
Liver/Brain [e]	0	0.0	0	0.0	
Missing data	24	28.2	73	34.9	
Total	85	100	209	100	
Number of metastases					
No metastases [b]	60	70.6	117	56.0	0.008
1-4 [c]	1	1.2	19	9.1	
5-8 [d]	0	0.0	0	0.0	
>8 [e]	0	0.0	0	0.0	
Missing data	24	28.2	73	34.9	
Total	85	100	209	100	
Previous failed chemotherapy					
No [b]	85	100	209	100	1.00
Single drug [d]	0	0.0	0	0.0	
2 or more drugs [e]	0	0.0	0	0.0	
Missing data	0	0.0	0	0.0	
Total	85	100	209	100	

Total FIGO score of 6 or less: low risk; total FIGO score of 7 or more: high risk

[a] Pearson Chi-square, missing data excluded for analysis
[b] FIGO: no points [c] FIGO: 1 point [d] FIGO: 2 points [e] FIGO: 4 points

Table 3 Treatment results and complications in study group (second curettage) and control group (no second curettage)

	Study group (n = 85) 2nd curettage: yes		Control group (n = 209) 2nd curettage: no		p [a]
Age (years)	Number	Rate, %	Number	Rate, %	
First-line therapy					
MTX	76	89.4	209	100	<0.001
Multi-agent chemotherapy	0	0.0	0	0.0	
Second curettage only	8	9.4	0	0.0	
Missing data	1	1.2	0	0.0	
Total	85	100	209	100	
Final therapy					
No other treatment than first	60[b]	70.6	164	78.5	0.320
Multi-agent chemotherapy	19	22.4	39	18.7	
Hysterectomy	4	4.7	4	1.9	
Multi-agent chemotherapy and hysterectomy	2	2.4	2	1.0	
Missing data	0	0.0	0	0.0	
Total	85	100	209	100	
Multi-agent chemotherapy					
Yes	21	24.7	41	19.6	0.332
No	64	75.3	168	80.4	
Missing data	0	0.0	0	0.0	
Total	85	100	209	100	
Failed chemotherapy					
No	60	70.6	165	78.9	0.119
MTX	22	25.9	43	20.6	
Multi agent chemotherapy	2	2.4	1	0.5	
Missing data	1	1.2	0	0.0	
Total	85	100	209	100	
Reason 2nd curettage					
Bloodloss	51	60.0			
Other	32	37.6			
Missing data	2	2.4			
Total	85	100			
Complications with 2nd curettage					
No complications			81	95.2	
Fluxus with 2nd curettage			2	2.4	
Perforation with 2nd curettage			2	2.4	
Missing data			0	0.0	
Total			85	100	

[a] Pearson Chi-square, missing data excluded for analysis.
[b] Eight patients second curettage only and 52 patients cured following first line MTX treatment.

istics were comparable in study and control group, we reconfirmed that all patients had low-risk PTD according the Dutch classification for persistent trophoblastic disease (7-9). When classified according to the FIGO 2000 scoring system for high/low-risk PTD (10), patient baseline characteristics were significantly different between study and control group with respect to three out of eight parameters. The hypothesis that a second curettage is of curative benefit in PTD was confirmed by complete remission as reflected by normalized serum hCG directly after the second curettage without receiving any other treatment in case of eight patients (9.4%, $P < 0.001$). A debulking effect of a second curettage was observed in this study since significantly less courses of chemotherapy were needed in the study group as compared to the control group. The clinical benefit of second curettage was accompanied by 2 uterine perforations which could be managed conservatively and by 2 cases of blood loss >1000 mL.

Whether a second curettage could have a curative effect, is preferably investigated in groups with equal baseline characteristics for high- or low-risk PTD. The Dutch guideline for classification for PTD scores for antecedent pregnancy (low-risk: mole or non-molar abortion, high risk: term choriocarcinoma), localisation of metastases (low-risk: no metastases or in vagina or lungs only, high risk: more than one organ with metastases or remaining localization of metastases), previous failure to chemotherapy (low-risk: no failure, high risk: earlier failure to any chemotherapy), and the interval between end of pregnancy and beginning of treatment (low-risk: ≤ 12 months, high risk: >12 months) (7-9). Unlike FIGO 2000 (10), the Dutch guideline issued in 1999 does not include scorings for age, pre-treatment serum hCG concentration, the number of metastases, or largest tumor size. Items scored in the Dutch scoring system as "low-risk" are given 0 points in FIGO 2000, except for the item antecedent pregnancy where in the Dutch classification a non-molar abortion is classified as "low-risk" whereas FIGO 2000 scores one point for non-molar abortion. To meet international criteria as recommended by FIGO and the International Society for the Study of Trophoblastic Diseases (ISSTD), we scored our patients according to the FIGO 2000 classification (10). If this system were to be used worldwide, it would be an important step forward in the management of gestational trophoblastic neoplasia because results can be compared among different centres and it would facilitate enrolment in large multicenter trials

(22). Using the FIGO 2000 classification, we found significant differences between study and control group for antecedent pregnancy, tumor size and number and site of metastases although, due to differences in the two scoring systems, a substantial proportion of these parameters were missing. It may be possible that gynecologists are more willing to perform a second curettage in a patient with a non-mole histological diagnosis from the first curettage but with persistent vaginal bleeding and/or abnormal hCG regression. The number and localisation of metastases were significantly distributed between the groups. The distribution of the remaining FIGO items (age, pre-treatment serum hCG and tumor size) was not significantly different between the groups. Although FIGO 2000 has been tested in 201 patients with persistent low hCG or high-risk GTD (23) against the WHO scoring system (1983) (7) and the revised FIGO staging system (1992) (7), FIGO 2000 has not been tested against the Dutch classification for persistent trophoblastic disease (7-9). If staged according to FIGO 2000, we found significant differences between the study and control groups. These differences can be explained by unequally distributed amounts of missing data between the groups or due to a true difference between the groups.

Although there are some patients with PTD with continued hemorrhage and consequent anemia who require a second curettage, most investigators are reluctant to recommend routine curettage (14,15). One retrospective study by Schlaerth *et al.* (15) showed a curative effect in patients with non-metastatic gestational trophoblastic disease in approximately 16%. Enthusiasm about this curative effect was tempered by the drawback that 8% of these patients had an uterine perforation with the second curettage, for which in two cases a hysterectomy had to be performed (15). The curative effect described by Schlaerth *et al.* is comparable to our results: cure of PTD after second curettage by 9.4% at a complication rate of 2.4% uterine perforations and 2 cases (2.4%) of blood loss > 1000 mL. Schlaerth *et al.* and our results are in contrast with the first prospective study on the curative effect of a second curettage in patients with PTD by Pezeshki *et al.* (16). This study from the Weston Park Hospital in Sheffield, showed a second curettage-only to be curative in 60% of 282 patients with proven PTD. In this prospective study, criteria for the diagnosis of PTD are given as a plateauing or rising hCG level, without mentioning the period of plateauing. Upon request, the corresponding author of this study communicated that PTD was diagnosed if

serum hCG was not normalised between 4 and 6 weeks after the first evacuation, or if serum hCG was increasing at any stage. In 1993, our group constructed a normal regression curve for serum hCG in patients with a hydatidiform mole with uneventful regression to normal after evacuation (n = 129). We found a cumulative normalisation of serum hCG in 5% of patients within 6 weeks after first evacuation, 50% within 11 weeks, and 95% within 25 weeks (21). Failure of serum hCG to normalize within 6 weeks after first evacuation without plateau or rising in hCG (as defined by FIGO (10)), as such does not make trophoblastic disease persistent. Advising patients to undergo a second curettage 4-6 weeks after evacuation for "persistent trophoblastic disease" according to Pezeshki's criteria will put patients at risk for complications of this procedure, which is unnecessary in the majority of patients. Interestingly, the number of complications accompanying the second curettage is not mentioned in the study by Pezeshki *et al.* In this study, chemotherapy was needed in 206 out of 4075 patients (5%) with a hydatidiform mole. It is unknown, whether this is significantly less than the 7.75% of patients who needed chemotherapy for GTD in a study performed in England and Wales between 1973-1983 (24).

In conclusion, we have tested the hypothesis that a second curettage would cure or act as a "debulking" agent in PTD which would result in less patients needing chemotherapy for their PTD and if chemotherapy is needed, less courses of chemotherapy. We observed a curative effect in 9.4% patients who were cured after second curettage only. A "debulking" effect was noted, because of a reduction of a median of six chemotherapy courses in the control group to five courses in the study group. Although this reduction in the number of single agent chemotherapy courses will not be the primary reason to perform a second curettage, it confirms our biological hypothesis that a second curettage has a debulking effect. The second curettage was accompanied by a uterine perforation in 2 patients and blood loss >1000 mL in 2 cases. In this retrospective cohort, we reconfirmed that all patients had low-risk PTD in both study group (second curettage) and control group (no second curettage) when classified according the guideline by the Dutch Society of Obstetrics and Gynaecology (7-9). We did found differences between the study and control group when staging according to FIGO 2000. This study shows that a second curettage might have a curative and debulking effect in patients with PTD. A randomised prospective controlled trial

which takes into account the baseline risk of a patient of a subversive outcome of their PTD classified according to an internationally accepted classification for high/low-risk PTD, is needed to investigate this curative effect. In the meantime, gynecologists can expect a small curative effect by performing a second curettage in patients with PTD.

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References

1. Bentley RC. Pathology of gestational trophoblastic disease. Clin Obstet Gynecol 2003; 46:513-22.
2. Berkowitz RS, Goldstein DP. Gestational trophoblastic disease. Cancer 1995; 76:2079-85.
3. Bagshawe KD, Lawler SD, Paradinas FJ, Dent J, Brown P, Boxer GM. Gestational trophoblastic tumours following initial diagnosis of partial hydatidiform mole. Lancet 1990; 335:1074-6.
4. Berkowitz RS, Goldstein DP, Bernstein MR. Natural history of partial molar pregnancy. Obstet Gynecol 1985; 66:677-81.
5. Goto S, Yamada A, Ishizuka T, Tomoda Y. Development of postmolar trophoblastic disease after partial molar pregnancy. Gynecol Oncol 1993; 48:165-70.
6. Rice LW, Berkowitz RS, Lage JM, Goldstein DP, Bernstein MR. Persistent gestational trophoblastic tumor after partial hydatidiform mole. Gynecol Oncol 1990; 36:358-62.
7. Hancock BW. Staging and classification of gestational trophoblastic disease. Best Pract Res Clin Obstet Gynaecol 2003; 17:869-83.
8. Ma HK, Wong LC, Ngan HY. Staging and classification systems. In: Hancock BW, Newlands ES, Berkowitz RS, editors. Gestational Trophoblastic Disease. 1 ed. London: Chapman&Hall; 1997. p. 95-108.
9. Nederlandse Vereniging voor Obstetrie en Gynaecologie. Molazwangerschap en persisterende trofoblast. http://www nvog nl/files/21_molazwang_persist_trofo pdf 1999 (21)
10. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000: FIGO Oncology Committee. Int J Gynaecol Obstet 2002; 77:285-7.
11. Ng TY, Wong LC. Diagnosis and management of gestational trophoblastic neoplasia. Best Pract Res Clin Obstet Gynaecol 2003; 17:893-903.
12. Osborne R, Gerulath A. What is the best regimen for low-risk gestational trophoblastic neoplasia? A review. J Reprod Med 2004; 49:602-16.
13. Lurain JR. Advances in management of high-risk gestational trophoblastic tumors. J Reprod Med 2002; 47:451-9.
14. Soper JT. Role of surgery and radiation therapy in the management of gestational trophoblastic disease. Best Pract Res Clin Obstet Gynaecol 2003; 17:943-57.

15. Schlaerth JB, Morrow CP, Rodriguez M. Diagnostic and therapeutic curettage in gestational trophoblastic disease. Am J Obstet Gynecol 1990; 162:1465-70.
16. Pezeshki M, Hancock BW, Silcocks P, Everard JE, Coleman J, Gillespie AM, et al. The role of repeat uterine evacuation in the management of persistent gestational trophoblastic disease. Gynecol Oncol 2004; 95:423-9.
17. Berkowitz RS, Desai U, Goldstein DP, Driscoll SG, Marean AR, Bernstein MR. Pretreatment curettage-A predictor of chemotherapy response in gestational trophoblastic neoplasia. Gynecol Oncol 1980; 10:39-43.
18. Flam F, Lundstrom V. The value of endometrial curettage in the follow-up of hydatidiform mole. Acta Obstet Gynecol Scand 1988; 67:649-51.
19. Lao TT, Lee FH, Yeung SS. Repeat curettage after evacuation of hydatidiform mole. An appraisal. Acta Obstet Gynecol Scand 1987; 66:305-7.
20. Yedema KA, Verheijen RH, Kenemans P, Schijf CP, Borm GF, Segers MF, et al. Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. Am J Obstet Gynecol 1993; 168:787-92.
21. Thomas CM, Segers MF, Houx PC. Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. Ann Clin Biochem 1985; 22:236-46.
22. Ngan HY. The practicability of FIGO 2000 staging for gestational trophoblastic neoplasia. Int J Gynecol Cancer 2004; 14:202-5.
23. Hancock BW, Welch EM, Gillespie AM, Newlands ES. A retrospective comparison of current and proposed staging and scoring systems for persistent gestational trophoblastic disease. Int J Gynecol Cancer 2000; 10:318-22.
24. Bagshawe KD, Dent J, Webb J. Hydatidiform mole in England and Wales 1973-83. Lancet 1986; 2:673-7.

CHAPTER 7

Early Identification of Resistance to First-Line Single-Agent Methotrexate in Patients With Persistent Trophoblastic Disease

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Abstract

Purpose: A generally accepted definition for resistance to first-line single-agent chemotherapy for persistent trophoblastic disease (PTD) is lacking. In the present study, a normogram for serum human chorionic gonadotropin (hCG) from patients with normalization of serum hCG after first-line single-agent chemotherapy for PTD was constructed in order to identify patients resistant to this first-line single-agent chemotherapy.

Patients and Methods: Between 1987 and 2004, data from 2132 patients were registered at the Dutch Central Registry for Hydatidiform Moles. A normal serum hCG regression corridor was constructed for 79 patients with low-risk PTD who were cured by single-agent methotrexate (MTX) chemotherapy (control group). Another group of 29 patients with low-risk PTD needed additional alternative therapies (Actinomycin-D and multiagent chemotherapy) for failure of serum hCG to normalize with single-agent chemotherapy (study group).

Results: Serum hCG measurement preceding the fourth and sixth single-agent chemotherapy course proved to have excellent diagnostic accuracy for identifying resistance to single-agent chemotherapy, with an area under the curve (AUC) for receiver operating characteristic curve analysis of 0.949 and 0.975, respectively. At 97.5% specificity, serum hCG measurements after 7 weeks showed 50 % sensitivity.

Conclusions: In the largest study to date, we describe the regression of serum hCG levels in patients with low-risk PTD successfully treated with MTX. At high specificity, hCG levels in the first few courses of MTX can identify half the number of patients who are extremely likely to need alternative chemotherapy to cure their disease and for whom further treatment with single-agent chemotherapy will be ineffective.

Introduction

A variety of pathologic types of trophoblast neoplasms are included in gestational trophoblastic disease, comprising villous malformations of trophoblast, hydatidiform mole subdivided in complete and partial hydatidiform mole, and nonvillous malformations of which choriocarcinoma is the most frequent (1). In persistent trophoblastic disease (PTD), trophoblastic activity remains after evacuation of a hydatidiform mole, as shown by subsequent unaltered high or even increasing serum human chorionic gonadotropin (hCG) concentrations. The internationally accepted definition released by the International Federation of Gynecology and Obstetrics (FIGO) defines PTD as a plateau in serum hCG for 3 weeks, or a rise for 2 consecutive weeks (2). In The Netherlands, the Dutch Society for Obstetrics and Gynecology added to this definition for PTD the condition that at least one serum hCG measurement should exceed the 95th percentile (P95) of normal hCG regression after hydatidiform mole (3-5).

The reported frequency of PTD is 20% in complete hydatidiform mole (6) and 0.5% to 9.9 % in partial hydatidiform mole (7-10). For patients with PTD, several staging classifications and prognostic scoring systems have been developed (3). To date, the FIGO 2000 scoring system for high- and low-risk PTD is advocated by The International Society for the Study of Trophoblastic Diseases and the International Society for Gynecological Cancer to generate univocal data (2). This scoring includes serum hCG, age, antecedent pregnancy, interval between end of pregnancy and start of treatment, previous failure of chemotherapy and the localization and number of metastases (2). The Dutch classification for high- and low-risk PTD takes the same items into account, except for the number of metastases and serum hCG concentration (3,11).

Once diagnosed and scored, low-risk PTD is typically treated with single-agent chemotherapy (MTX or Actinomycin-D) (12,13). If staged as high-risk PTD, multiagent chemotherapy with Etoposide, Methotrexate, Actinomycin-D, Cyclophosphamide, and Vincristine (EMA-CO) is the most widely used therapy (12).

Approximately 9% to 33% of patients treated with single-agent chemotherapy for low-risk PTD will require multiagent chemotherapy because resistance to the first-line drug or toxic adverse effects occurred (14-16). Repeated administration of MTX could induce MTX resistance (17), mainly as a result of downregulation of the expression levels of the

reduced folate carrier that is responsible for transport of MTX into the cell or amplification of the gene for dihydrofolate reductase. This enzyme reduces dihydrofolate in tetrahydrofolate which is essential in DNA synthesis (18,19). To date, an internationally accepted definition for resistance to first-line chemotherapy is lacking. In some clinics, resistance to first-line chemotherapy is defined as a plateau or increase in serum hCG and/or development of new metastases (14-16,20,21).

After single-agent chemotherapy with MTX, no increase in the number of second tumors has been observed (22). In contrast, patients with PTD who are treated with etoposide-containing multiagent chemotherapy have a 50% increased relative risk to developing secondary malignancies, in particular myeloid leukemia, colon carcinoma, and breast cancer, compared with an age-matched group (23). Furthermore, multiagent chemotherapy hastens the occurrence of menopause by 2 years compared with patients treated with single-agent MTX (24) and can be accompanied by the occurrence of alopecia and GI symptoms. To identify patients not responding to single-agent chemotherapy in an early stage and, at the same time, to prevent unnecessary multiagent chemotherapy, we constructed a normal serum hCG regression corridor for patients successfully treated with single-agent chemotherapy.

Patients and Methods

Patients

Between 1987 and 2004, 2132 patients were registered at the Dutch Central Registry for Hydatidiform Moles. After informed consent, patients were registered by their referring clinician. The present study included, in retrospect, all low-risk patients with proven PTD according to Dutch guidelines (histological diagnosis mola hydatidosa or choriocarcinoma with serum hCG plateau or increase for 3 consecutive weekly measurements, with at least one measurement > P95 of normal regression according to an uneventful hCG regression published earlier (4) if serum was available in our registry. In the Netherlands, patients with low-risk PTD are treated with MTX 1 mg/kg on day 1, 3, 5, and 7 and leucovorin 15 mg rescue on days 2, 4, 6, and 8, and this schedule is repeated every 14 days (13). Exclusion criteria were hysterectomy when PTD was diagnosed; cure after a second curettage; need for alternative therapies for MTX toxicity (none in this cohort); persistent low serum hCG levels (2 to 10 μg/L) after spontaneous regression or persistent low serum hCG

after MTX treatment, for which no multiagent chemotherapy was administered; and recurrence of PTD after single-agent chemotherapy. Of the 108 eligible patients, serum hCG normalized under single-agent (MTX) treatment in 79 patients (73%; responders). The remaining 29 patients (27%) were treated with multiagent chemotherapy after the clinician decided that the disease failed to respond to single-agent chemotherapy.

Immunoassays

A privately developed (in-house) radioimmunoassay (RIA) that measured total hCG (ie, intact hCG and free beta-subunit (hCG + hCGβ) was used exclusively in the authors' laboratory (25). Thus, this assay has been utilized centrally for all measurements in sera sent to the Dutch Central Registry for Hydatidiform Moles and was used in the development of a normal hCG regression corridor for uneventful hydatidiform mole (4). The RIA was calibrated with the third International Standard (IS) Preparations for intact hCG (WHO third IS hCG 75/537 obtained from the National Institute for Biological Standards, Potters Bar, England, United Kingdom). The measuring range for the standard line of the assay was 1 to 80 μg/L (0.027 to 2.14 nmol/L, equivalent to 9.29-743 IU/L of the WHO third IS hCG 75/537) (25). The hCG + hCGβ-RIA cross reacts 100% on a mol/mol basis with intact hCG and 1,000% with hCG β. Serum hCG concentrations were considered to be normalized if they were less than 2 μg/L (0.053 nmol/L or 18.6 IU/L of the WHO third IS hCG 75/537). The intra- and interassay coefficients of variation for means of duplicate measurements for two serum pools (means: 0.267 nmol/L, or 10 μg/L or 93 IU/L, and 1.50 nmol/L, or 56 μg/L or 520 IU/L) were 7.3% and 12%, respectively.

Statistics

All statistical analyses were performed using the SPSS statistical software package version 12.0.1 (SPSS Inc, Chicago, IL). Normality of distributions was explored by Kolmogorov-Smirnov testing. Differences in numerical data between the control and study groups were tested nonparametrically (Mann-Whitney U test) or parametrically (Student's t test). Within each patient group, serum hCG results (obtained before the start of a new MTX course) were sorted by week from the start of MTX treatment. To approximate normal distributions, serum hCG measure-

ments were log transformed and sorted within each week. The percentiles of 2.5% (P2.5), 50% (P50), and 97.5% (P97.5) and the standard deviation were calculated. In the control group, serum P2.5, P50 and P97.5 were plotted for each week into a normogram. To describe the complete serum hCG response of each patient, we calculated the serum hCG half-life as derived from serum hCG measurements preceding the first single-agent course until the point of normalization (cutoff, 2 μg/L). After 17 weeks (before the ninth MTX course), statistics were censored because of small numbers of patients (12 patients in control group and 11 in study group). The hCG half-life and hCG concentration in a certain week from the start of MTX treatment of the control and study groups were used to construct receiver operating characteristic (ROC) curves and to calculate areas under the curve (AUCs). All tests were considered significantly different at $P < 0.05$.

Results

Table 1 lists age, prechemotherapy serum hCG, number of courses of single-agent chemotherapy, hCG serum half-life and, if applicable, number of courses of multiagent chemotherapy in both the control (n = 79) and study (n = 29) groups. No difference was observed between the groups for age (median age, 30 years in both groups). Expectedly, serum hCG concentration before the first course of single-agent chemotherapy was significantly less elevated in the control group compared with the study group (251 v 2,098 μg/L, respectively; $P < 0.001$). Significantly fewer courses of single-agent chemotherapy were administered in the control group (median, five courses; range, three to 17 courses) compared with the study group (median, seven courses; range, three to 16 courses; P = 0.003). As expected, the median serum hCG half-life was significantly shorter in the control group (1.02 weeks; 95% CI, 0.91 to 1.13) compared with the study group (1.92 weeks; 95% CI, 1.63 to 2.27 weeks; $P < 0.001$). The median number of courses of multiagent chemotherapy administered in the study group was four (range, three to seven courses).

For each single-agent treatment course, the P2.5, P50 and P97.5 of serum hCG concentration in the control group was calculated, and the results were plotted into a normogram (Fig 1A). In the control group, 2.5% of patients were cured after 2 weeks (after one MTX course), and 50% were cured after 8 weeks (after four MTX courses). After 17 weeks, the P97.5 of serum hCG in the control group remained at 4.3 μg/L and,

Table 1. Age, Prechemotherapy hCG Concentration, Number of Courses and hCG Half-Life During First-Line Single-Agent Chemotherapy in the Study (no regression after MTX) and Control Groups (regression after MTX)

	Complete Remission After First-Line Single-Agent Chemotherapy		
	Yes (n = 79)	No (n = 29)	*P*
Age, years			NS*
No. of patients	78	29	
Median	30	30	
Range	17-50	24-43	
hCG before chemotherapy, μg/L			< 0.001*
No. of patients	76	29	
Median	251	2,098	
Range	6.2-12000	62-16000	
Number of MTX courses			< 0.003*
No. of patients	78	27	
Median	5	7	
Range	3-17	3-16	
hCG half-life during MTX treatment, weeks			< 0.001†
No. of patients	78	29	
Median	1.02	1.92	
95% CI	0.91 to 1.13	1.63 to 2.27	
Number of courses of multiagent chemotherapy			NA
No. of patients	NA	24	
Median		4	
Range		3.0-7.0	

Abbreviations: hCG, human chorionic gonadotropin; MTX, methotrexate; NS, not significant; NA, not applicable.
* Mann-Whitney *U* test.
† Student's *t* test.

thus, not less than the cutoff level of 2 μg/L. This is because, after 17 weeks, there were still some patients who needed additional MTX courses who were, at that time point, not yet normalized.

In Figure 1B, the hCG levels of the two weekly intervals for the 29 patients in the study group were plotted into the normogram derived from the serum hCG measurements of the control group. Before the

Table 2. AUC, Sensitivity at 97.5% Specificity for ROC of hCG Half-Life, Before Start of MTX Treatment, After 7 Weeks (before the fourth MTX course), and After 11 Weeks (before the sixth MTX course)

Measure	ROC for hCG Half-Life	ROC Week 0	ROC Week 7	ROC Week 11
Study group, No. of patients	29	29	26	26
Control group, No. of patients	79	77	73	73
AUC	0.833	0.828	0.949	0.975
95% CI	0.753 to 0.914	0.742 to 0.914	0.910 to 0.989	0.947 to 1.00
Sensitivity. %		14	50	60
hCG cutoff, µg/L		9600	56.0	24.0

Abbreviations: AUC, area under the curve; ROC, receiver operating characteristic; hCG, human chorionic gonadotropin; MTX, methotrexate.

start of the first MTX course, serum hCG in the study group exceeded the P97.5 of the control group in four (13.8%) of 29 patients. After 7 weeks (before the fourth MTX course), serum hCG in the study group exceeded the P97.5 of the control group in 22 (76 %) of 29 patients. After 17 weeks (before the ninth MTX course), serum hCG in the study group exceeded the P97.5 of the control group in 25 (86 %) of 29 patients. These results are in line with the finding of a significantly shorter median serum hCG half-life in the control group compared with the study group, as stated previously.

Figure 2 shows the normogram for serum hCG + hCGβ for the study group. This normogram was plotted in the normogram for serum hCG + hCGβ derived from the control group. As shown, 2.5 % of patients in the study group had normalization of serum hCG (ie, < 2 µg/L) after 9 weeks (before the fifth MTX course). These patients were administered multiagent chemotherapy for persistent low hCG levels after treatment with MTX. After 17 weeks (before the ninth MTX course), the P50 in the study group was 13.8 µg/L, and the P97.5 was 122 µg/L. The overlapping area between the study and control groups, is darkly hatched. The area with hCG more than P97.5 of regression in the control group and with hCG less than P97.5 of the study group, is lightly hatched.

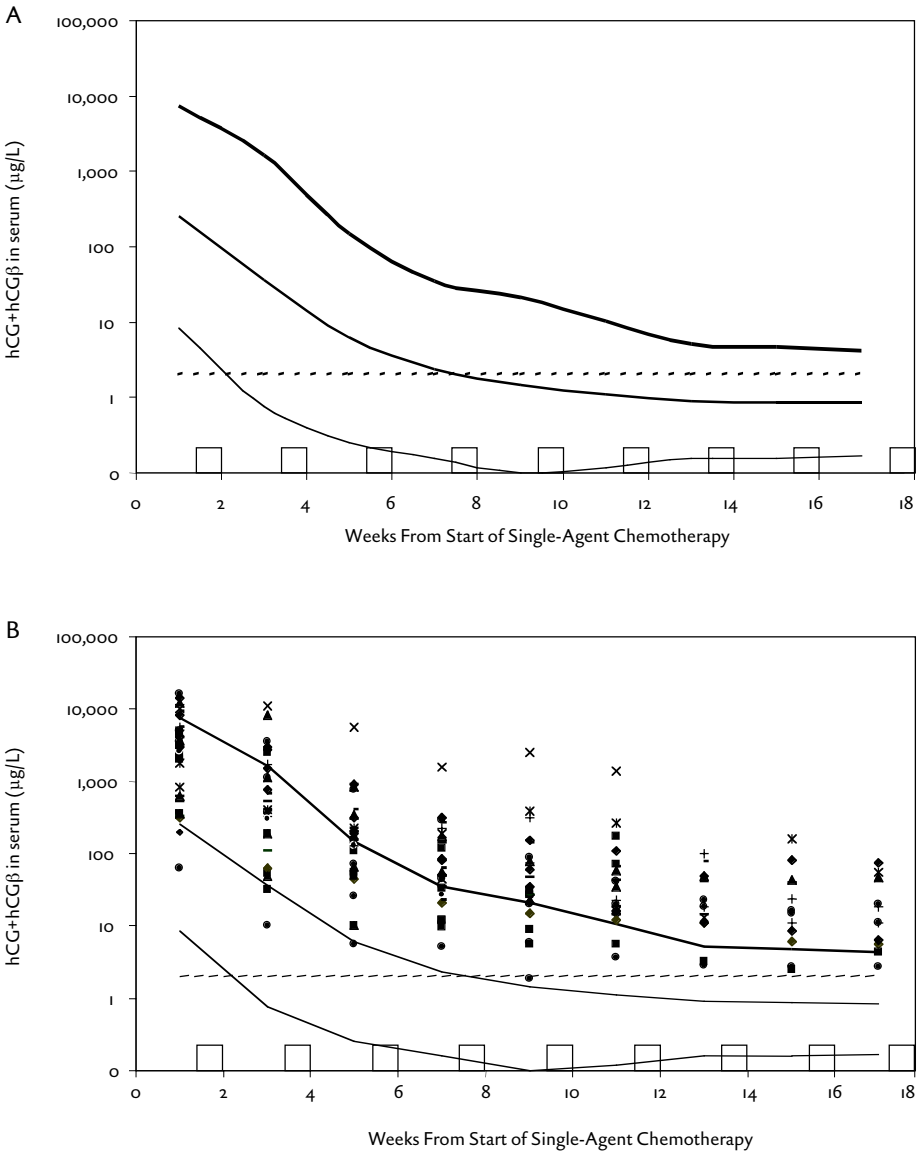

Figure 1. A) Normal serum human chorionic gonadotropin (hCG) regression curve under methotrexate (MTX) in the control group (n = 79). B) Normal serum hCG regression under MTX in control group (n = 79), with all individual measurements in the study group (n = 29). The different shapes refer to longitudinal serum hCG patterns of individual patients.

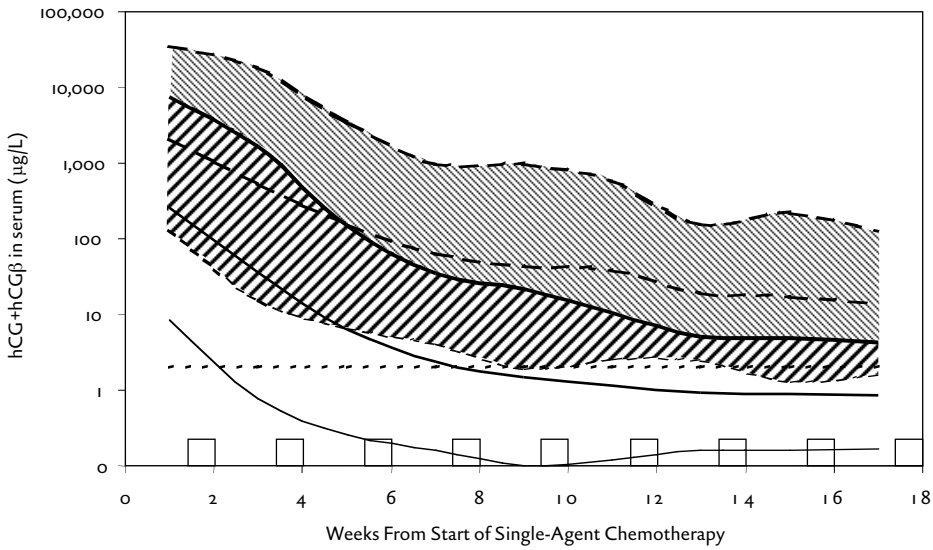

Figure 2. Regression of hCG in serum in control group (n = 79) versus the study group (n = 29)

Diagnostic accuracy of serum hCG half-life was derived from the calculation of an AUC of a ROC curve (Fig. 3A). In addition, the diagnostic accuracies of serum hCG levels before the first (Fig. 3B), fourth (Fig. 3C), and sixth (Fig. 3D) first-line single-agent chemotherapy course were established by the calculation of the AUCs of these ROC curves. The corresponding AUCs for these ROC curves are listed in Table 2. The AUC for serum hCG half-life throughout single-agent treatment was 0.833. The AUC of the serum hCG concentration preceding the first-single chemotherapy course was 0.828, which increased to 0.975 preceding the sixth course (after 11 weeks) of single-agent chemotherapy. This means that, at that time point, an excellent diagnostic accuracy was obtained because, after 11 weeks, 60 % of the patients needing alternative therapy could be identified at the 97.5% specificity level.

Using serum hCG before the start of single-agent chemotherapy as a diagnostic tool to identify patients who will need alternative chemotherapeutic treatment for PTD, resulted in the identification of 14% of

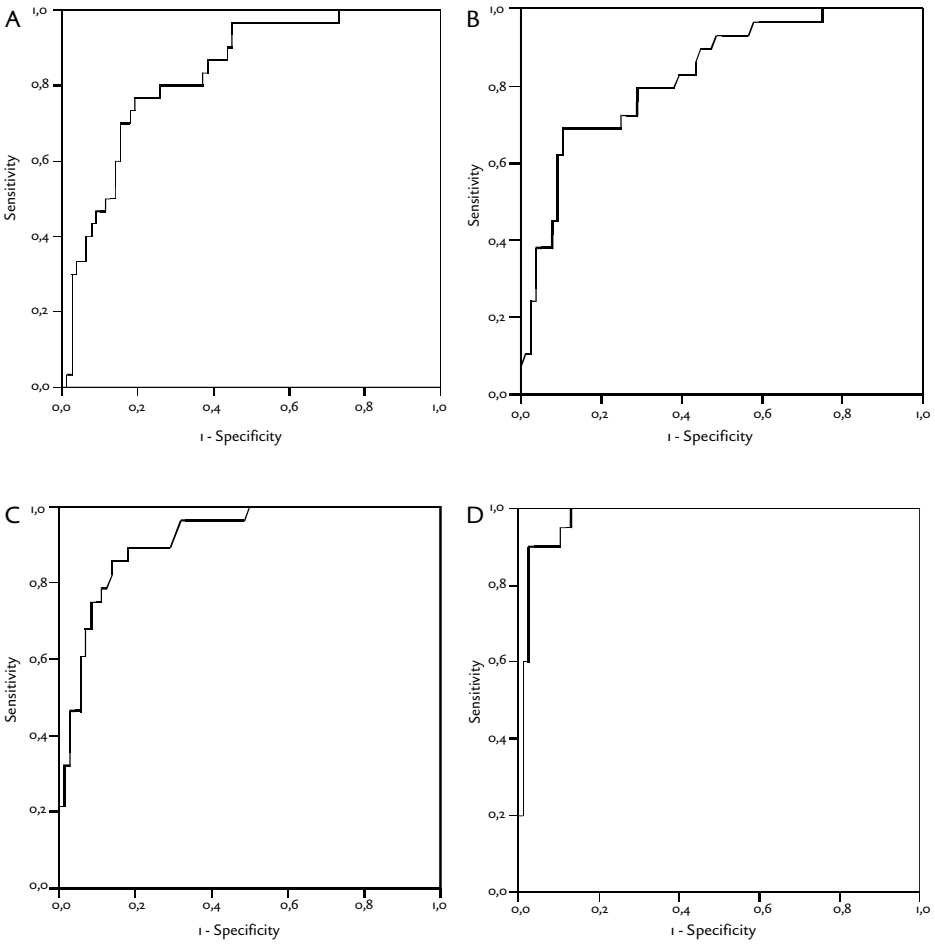

Figure 3. Receiver operating characteristic (ROC) analysis of the study versus control group for A) serum human chorionic gonadotropin (hCG) half-life (T1/2) and B) serum hCG concentration before the start of methotrexate (MTX) treatment (MTX1), C) after 7 weeks (before the fourth MTX course; MTX4), and D) after 11 weeks (before the sixth MTX course; MTX6).

patients who needed alternative therapy at the 97.5% specificity level (hCG serum cutoff, 9600 µg/L). Serum hCG levels obtained before the start of the fourth course to predict the need for alternative therapy, would identify as much as 50% of patients at the 97.5% specificity level (cutoff, 56 µg/L; Table 2).

Discussion

The objective of this study was to clarify whether it is possible to identify, at an early stage and from a single hCG measurement, patients with PTD who will not be cured by single-agent chemotherapy. For this reason, we established a serum hCG normogram with data from 79 patients with low-risk PTD who were cured by single-agent MTX chemotherapy. This normogram was applied to hCG measurements from 29 PTD patients who needed alternative therapy after single-agent chemotherapy failed to establish normalization of serum hCG. Using this normogram, we found that it is possible to identify 14% of patients who will need alternative therapy before the start of first-line single-agent chemotherapy with 97.5% specificity. Likewise, on the basis of serum hCG measurements obtained just before the fourth MTX course, we were able to identify 50% of patients who would not respond to single-agent chemotherapy at 97.5% specificity.

The international criteria used to diagnose PTD include an abnormal serum hCG regression, with a plateau for 3 weeks or an increase for two weeks (2). Normal serum hCG regression corridors for uneventful hydatidiform moles have been constructed, with 50% of patients having normalized serum hCG measurements between 6 and 14 weeks after evacuation (4,20,21,26,27). The Dutch Society for Obstetrics and Gynecology included the extra condition in the diagnosis for PTD that, in addition to a serum hCG plateau or increase for 3 consecutive weeks, at least one serum hCG measurement should exceed the P95 of normal hCG regression, as derived from an hCG normogram constructed from data of 130 patients with uneventful hydatidiform mole (4). There are two reasons for the inclusion of this additional condition. First, PTD, as defined by exceeding the P95 of normal regression, is diagnosed 2 weeks earlier than if the definition for PTD of an hCG plateau or increase for 3 consecutive measurements is used (21,27). Second, use of a normal hCG regression corridor prevents overtreatment. One study described that 15% of patients with a plateau or increase for 3 weeks but without a serum hCG measurement exceeding the P95 will have spontaneous serum hCG remission afterwards (4). Two retrospective studies showed that, in patients treated for PTD on the basis of a plateau or increase of serum hCG for 3 consecutive weeks, 29% had serum hCG measurements less than the P90 of normal regression (20), and 8% had measurements less than the P95 of normal regression (4).

Although firm criteria for the diagnosis of PTD exist and the use of a normogram for normal serum hCG regression identifies patients with PTD earlier and prevents overtreatment, no firm criteria for the diagnosis of resistance to first-line single-agent therapy exist. A generally accepted definition for resistance to first-line chemotherapy is lacking, although some clinics define resistance to chemotherapy by a plateau or increase in serum hCG or by detection of (new) metastases (14,15,20,21).

Rotmensch *et al.* (20) described the only normogram for normalization of hCG in 21 patients with nonmetastatic trophoblastic disease treated with MTX. They reported that the median time for serum hCG to normalize in successfully treated patients with PTD is 50 days. The authors stated that, in successfully treated patients with low-risk PTD, the median time to normalization is comparable to patients with uncomplicated regression of serum hCG after hydatidiform mole (50 days). Our results are in line with their results because we found that serum hCG normalizes in 50% of patients after five, 2-week courses of MTX. Our group previously developed a normogram for serum hCG regression after uneventful hydatidiform mole (4). In that study, median time for normalization was 11 weeks, which is comparable to the five 2-week courses that 50% of patients needed for normalization of hCG after successful single-agent chemotherapy.

Serum hCG in two patients in the study by Rotmensch *et al.* (20) failed to normalize after primary chemotherapy. Serum hCG of these two patients was greater than the P90 of the normal regression with MTX at the start of the MTX treatment. These investigators did not explore the diagnostic accuracy of serum hCG levels to detect resistance to single-agent chemotherapy in low-risk PTD. Shigematsu *et al.* (21), in their study comprising 24 patients with PTD, indicated that women who would show resistance to single-agent chemotherapy later on (as concluded from increasing hCG for 2 weeks, a plateau for 3 weeks, or the development of new metastases) exceeded the P95 of normal hCG regression significantly earlier than patients who would respond well to single-agent chemotherapy.

With the presented serum hCG regression corridor for patients treated with first-line single-agent MTX, we have an excellent diagnostic tool to identify patients with resistance to first-line single-agent chemotherapy at an early stage with great certainty. Before the fourth course of sin-

gle-agent chemotherapy (after 7 weeks), serum hCG identifies 50% of patients needing alternative therapy at the 97.5% specificity level.

The birthrate in the Netherlands is approximately 200000 persons per year. With an estimated incidence of hydatidiform mole of 1 per 1000 to 2000 pregnancies (28) the 18-years period of our study could yield 1800 to 3600 patients with hydatidiform mole, of whom we would expect 180 to 360 to have PTD. Registration with the Dutch Central Registry for Hydatidiform Moles is on a voluntary basis, and we registered 2132 patients with hydatidiform mole throughout the entire study period, of whom 108 patients with PTD were eligible for inclusion in our study. Because of the low incidence of PTD and, in particular, the need for alternative therapy, it is not feasible to perform a prospective study, and we used our retrospective data for a proposal for a new treatment regimen for patients with PTD.

In analogy with the regression curve for serum hCG after evacuation of uneventful hydatidiform mole as earlier published by our group (4) we developed a curve for the early detection of resistance to first-line single-agent MTX chemotherapy. Because overtreatment with multiagent chemotherapy is highly undesirable, we increased the cutoff from P95 to P97.5 for normal regression of patients successfully treated with first-line single-agent chemotherapy. If before the fourth course of single-agent chemotherapy the serum hCG concentration exceeds the P97.5 of this normal regression curve, it can be concluded with 50% sensitivity that a change of treatment is mandatory to gain cure (Fig 2, lightly hatched area). In line with the internationally accepted criteria for treatment of PTD, the course of hCG (plateau or increase) should also be included in the decision to start alternative therapy. The choice of second-line chemotherapy could be multiagent chemotherapy with EMA-CO. In the United Kingdom, McNeish *et al.* (15) described a successful regimen in which patients with resistance to MTX-leucovorin were administered either Actinomycin-D if serum hCG was less than 100 IU/L or EMA-CO if serum hCG exceeded 100 IU/L. In this study, 58 of 67 patients treated secondarily with dactinomycin were cured by this drug.

In conclusion, in the largest study to date, we describe the regression of serum hCG levels in patients with low-risk PTD treated with MTX. From these data, it can be concluded that serum hCG levels before the

fourth course of MTX can identify half the number of patients who are highly likely to need alternative therapy to cure their disease and for whom further treatment with single-agent chemotherapy will be ineffective.

References

1. Bentley RC: Pathology of gestational trophoblastic disease. Clin Obstet Gynecol 2003; 46: 513-522
2. FIGO Oncology Committee: FIGO staging for gestational trophoblastic neoplasia 2000. Int J Gynaecol Obstet 2002; 77: 285-287
3. Hancock BW: Staging and classification of gestational trophoblastic disease. Best Pract Res Clin Obstet Gynaecol 2003;17: 869-883
4. Yedema KA, Verheijen RH, Kenemans P, et al: Identification of patients with persistent trophoblastic disease by means of a normal human chorionic gonadotropin regression curve. Am J Obstet Gynecol 1993; 168: 787-792
5. Dutch Society for Obsterics and Gynaecology: Richtlijn Molazwangerschap 1.1 (Guideline Mole Pregnancy 1.1). 2005. http://www.nvog.nl
6. Berkowitz RS and Goldstein DP: Gestational trophoblastic disease. Cancer 76: 2079-2085, 1995
7. Bagshawe KD, Lawler SD, Paradinas FJ, et al: Gestational trophoblastic tumours following initial diagnosis of partial hydatidiform mole. Lancet 1990; 335: 1074-1076
8. Berkowitz RS, Goldstein DP, Bernstein MR: Natural history of partial molar pregnancy. Obstet Gynecol 1985; 66: 677-681
9. Goto S, Yamada A, Ishizuka T, et al: Development of postmolar trophoblastic disease after partial molar pregnancy. Gynecol Oncol 1993; 48: 165-170
10. Rice LW, Berkowitz RS, Lage JM, et al: Persistent gestational trophoblastic tumor after partial hydatidiform mole. Gynecol Oncol 1990; 36: 358-362
11. Dutch Society for Obsterics and Gynaecology: Richtlijn Persisterende Trophoblast Ziekte 1.1 (Guideline Persistent Trophoblastic Disease 1.1). 2005. . http://www.nvog.nl
12. Ng TY and Wong LC: Diagnosis and management of gestational trophoblastic neoplasia. Best Pract Res Clin Obstet Gynaecol 2003; 17: 893-903
13. Osborne R and Gerulath A: What is the best regimen for low-risk gestational trophoblastic neoplasia? A review. J Reprod Med 2004; 49: 602-616
14. Lurain JR: Treatment of Metastatic Gestational Trophoblastic Tumors, in: Hancock BW, Newlands ES, Berkowitz R (ed): Gestational Trophoblastic Disease. London, Chapman & Hall, 1997, pp 199-209.
15. McNeish IA, Strickland S, Holden L, et al: Low-risk persistent gestational trophoblastic disease: outcome after initial treatment with low-dose

methotrexate and folinic acid from 1992 to 2000. J Clin Oncol 2002; 20: 1838-1844
16. Matsui H, Suzuka K, Yamazawa K, et al: Relapse rate of patients with low-risk gestational trophoblastic tumor initially treated with single-agent chemotherapy. Gynecol Oncol 2005; 96: 616-620
17. Sobrero A, Aschele C, Rosso R, et al: Rapid development of resistance to antifolates in vitro: possible clinical implication. J Natl Cancer Inst 1991; 83: 24-28
18. Moscow JA, Gong M, He R, et al: Isolation of a gene encoding a human reduced folate carrier (RFC1) and analysis of its expression in transport-deficient, methotrexate-resistant human breast cancer cells. Cancer Res 1995; 55: 3790-3794
19. Gorlick R, Goker E, Trippett T, et al: Intrinsic and acquired resistance to methotrexate in acute leukemia. N Engl J Med 1996; 335: 1041-1048
20. Rotmensch J, Rosenshein NB, Block BS: Comparison of human chorionic gonadotropin regression in molar pregnancies and post-molar nonmetastatic gestational trophoblastic neoplasia. Gynecol Oncol 1988; 29: 82-86
21. Shigematsu T, Kamura T, Saito T, et al: Identification of persistent trophoblastic diseases based on a human chorionic gonadotropin regression curve by means of a stepwise piecewise linear regression analysis after the evacuation of uneventful moles. Gynecol Oncol 1998; 71: 376-380
22. McNeish IA, Strickland S, Holden L, et al: Low-risk persistent gestational trophoblastic disease: outcome after initial treatment with low-dose methotrexate and folinic acid from 1992 to 2000. J Clin Oncol 2002; 20: 1838-1844
23. Seckl MJ and Rustin GJ: Late Toxicity after Therapy of Gestational Trophoblastic Tumors, in: Hancock BW, Newlands ES, Berkowitz R, Cole L (ed): Gestational Trophoblastic Disease, online book. 2003, pp 367-379. http://www.isstd.org/gtd/20%20word%20for%20print.pdf
24. Rustin GJ, Newlands ES, Lutz JM, et al: Combination but not single-agent methotrexate chemotherapy for gestational trophoblastic tumors increases the incidence of second tumors. J Clin Oncol 1996; 14: 2769-2773
25. Bower M, Rustin GJ, Newlands ES, et al: Chemotherapy for gestational trophoblastic tumours hastens menopause by 3 years. Eur J Cancer 1998; 34: 1204-1207
26. Thomas CM, Segers MF, Houx PC: Comparison of the analytical characteristics and clinical usefulness in tumour monitoring of fifteen hCG(-beta) immunoassay kits. Ann Clin Biochem 1985; 22: 236-246

27. Schlaerth JB, Morrow CP, Kletzky OA, et al: Prognostic characteristics of serum human chorionic gonadotropin titer regression following molar pregnancy. Obstet Gynecol 1981; 58: 478-482
28. Behtash N, Ghaemmaghami F, Honar H, et al: Is normal beta-hCG regression curve helpful in the diagnosis of persistent trophoblastic disease? Int J Gynecol Cancer 2004; 14: 980-983
29. Steigrad SJ: Epidemiology of gestational trophoblastic diseases. Best Pract Res Clin Obstet Gynaecol 2003; 17: 837-847

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CHAPTER 8

General Discussion

This thesis describes our findings on the diagnostic utility of hCG measurements in gestational trophoblastic disease (GTD) and persistent trophoblastic disease (PTD) to distinguish biochemically hydatidiform mole from uneventful normal pregnancy, to make the distinction between complete and partial hydatidiform mole, and to investigate the significance of several serum hCG analytes and parameters to predict PTD after molar pregnancy (which might be beneficial since prophylactic chemotherapy reduces the incidence of PTD) (Chapters 2 and 5). We also explored the diagnostic potential of serum hCG ratios calculated from hCG concentrations in blood samples taken after mole evacuation to identify development of PTD at the most early stage (Chapter 3) and we compared the disappearance rates of two hCG analytes (hCG + hCGβ and hyperglycosylated hCG) in uneventful regression, as well as during administration of single-agent and multiagent chemotherapy in order to assess the clinical value of these two analytes in the follow-up of PTD (Chapter 4).

Regarding the issue of therapeutic options in PTD we studied the curative effect of a second curettage in patients with low-risk PTD after molar pregnancy (Chapter 6), and, to identify patients with PTD not responding to single-agent methotrexate chemotherapy in an early stage and, at the same time, to prevent unnecessary multiagent chemotherapy, we constructed a normal serum regression corridor for patients successfully treated with single-agent chemotherapy (Chapter 7).

We found that hCGβ and hCG + hCGβ concentrations, and the ratio hCGβ/hCG + hCGβ are mostly above the corresponding P95 level of normal pregnancy; hCGα concentrations in serum in many molar pregnancies are below the P50 level, while the majority of the hCGα/hCG + hCGβ ratios and almost all hCGα/hCGβ ratios in molar pregnancy are below P5 of normal pregnancy. Thus, our study based on a large patient sample showed that hCGβ in serum, and the ratios of hCGβ/hCG + hCGβ and hCGα/hCGβ are excellent diagnostic tests and parameters to make the biochemical distinction between hydatidiform mole and normal pregnancy at the 100% specificity level with more than 90% sensitivity. For practical use, the hCGβ assay is recommended. Our study further revealed that the distinction between complete and partial hydatidiform mole cannot reliably be made with any of the 9 tested hCG analytes or parameters including hyperglycosylated hCG because diagnostic accuracy of these tests is at best qualified as moderate. Finally, we found

that none of the 9 investigated hCG analytes or parameters had adequate diagnostic accuracy to permit the clinician to advise patients to undertake prophylactic chemotherapy after evacuation of a hydatidiform mole.

Why hCGβ is synthesized more abundantly in complete hydatidiform moles than in partial moles or in normal pregnancy, is unknown. However, the mechanism for transcriptional and translational regulation of hCGα and hCGβ subunit synthesis indicates that both hCG α and β-subunits are synthesized in cytotrophoblasts and syncytiotrophoblasts in normal pregnancy, but more abundantly in syncytiotrophoblasts (1). After subsequent posttranscriptional modification, subunits combine to form intact hCG (2-4). hCGα is encoded by a single gene on chromosome 6 (4), whereas the β-subunit of hCG is encoded by four genes: hCGβ-3, -5, -7, and -8, located on chromosome 19q13.3 (5). In normal pregnancy, hCG synthesis is regulated by an autocrine feedback loop whereby hCG down-regulates hCG/LH receptor gene expression (6). In choriocarcinoma cells, self-regulation of hCG synthesis is lost due to a defect in the hCG/LH receptor which resulted in truncated receptors located predominantly inside the cells. Besides the rise in number of hCG producing cells in hydatidiform mole, the truncation of the hCG/LH receptor could also explain how hCG reaches such very high levels in GTD (7). Recently, transcriptional regulation of the hCGα- and hCGβ genes has been elucidated (8-10). For hCGβ5, the most abundantly transcribed hCGβ gene in placentas of early and late gestational age and in choriocarcinoma, selective promoter factors 1 and 3 (SP1 and 3) and activating protein 2 (AP2) regulatory promoter sites were identified (10). The hCGα subunit promoter was found to contain cAMP-response elements (CREs) (9,11). With the promoter for hCGα synthesis being cAMP dependent and the promoter for hCGβ not being cAMP dependent, it might be explicable why in hydatidiform mole hCGβ is synthesized more abundantly than hCGα. Loss of, or an increase in, particular transcription factors during neoplastic transformation could lead to dissimilar amounts of hCG subunits being produced by these cells. In the same line, it is explicable that in partial mole, which contains both normal trophoblast and hyperplastic trophoblasts, less free hCGβ is synthesized than in complete moles.

It may be difficult to distinguish a hydatidiform mole from an abortion with hydropic villi because hydropic swelling often occurs in either

entity. However, the marked trophoblastic hyperplasia is lacking in a hydropic abortion (12). Diagnostic tools to distinguish complete mole from partial mole or hydropic degeneration have been developed. One of these tests makes use of 'genomic imprinting' which refers to the phenomenon that for certain traits, either the paternal or the maternal copy of the gene is expressed (13). It is argued that besides pathological examination, other techniques are beneficial in the proper diagnosis of hydatidiform mole (12,14-16). As shown in our study described in chapter 2, the distinction between a complete hydatidiform mole and normal pregnancy can be made with a diagnostic accuracy of 0.99 with the use of a free hCGβ assay. It would be interesting to perform an experiment which attempts to distinguish hydatidiform mole from hydropic degeneration with the same set of hCG parameters used in chapter 2 and to compare the diagnostic power between the hCG parameters and for instance the P^{57kip2}genomic imprinting test, which identifies the paternally not expressed and maternally expressed CDKN1C gene (16).

In our search for reliable biochemical predictors for PTD *before* evacuation of molar pregnancy (Chapter 5) we also examined hyperglycosylated hCG because it has been put forward that high levels of hyperglycosylated hCG are associated with aggressive trophoblast invasion in early pregnancy, at 3-4 weeks gestation (17,18) and in trophoblastic disease (19). It was also reported that this marker could distinguish quiescent GTD from invasive mole in cases of persistent low levels of hCG after mole evacuation (20). Our results indicate that none of the 9 hCG analytes or parameters tested including hyperglycosylated hCG had adequate diagnostic accuracy to permit the clinician to advise patients to undertake prophylactic chemotherapy after evacuation of a hydatidiform mole. This leads to the hypothesis that malignant transformation of a hydatidiform mole into PTD occurs after evacuation (Chapters 2 and 5). To further confirm or reject the diagnosis of PTD we explored the diagnostic potential of calculated serum hCG ratios from blood samples taken after mole evacuation to identify development of PTD at the most early stage (Chapter 3). Our retrospective study revealed that a ratio of two hCG concentrations measured in 2 serum samples obtained at different time points after evacuation identifies the majority of patients with PTD approximately 2 weeks earlier (i.e., by the seventh week after evacuation) than the internationally accepted FIGO 2000 criteria. Calculation of such a ratio from 2 already available serum hCG concentra-

tions after evacuation may provide a quantitative addition to the qualitative FIGO 2000 'increase' and 'plateau' criteria. This kind of ratio apparently reflects the residual activity of the hydatidiform mole after evacuation (and supports the abovementioned hypothesis that malignant transformation of a hydatidiform mole into PTD occurs after evacuation), whereas a ratio calculated from an hCG concentration measured in a serum sample taken before divided by an hCG concentration obtained after evacuation would mirror the total tumour activity present before evacuation. Several normograms for spontaneous regression of serum hCG have been developed with 50 % of patients having normalized serum hCG measurements between 6 and 14 weeks after evacuation (21-25). To our knowledge no studies have been performed on the diagnostic capacity of hCG-ratios in the diagnosis of PTD. In this study we analyzed at which time point after evacuation the criteria for PTD were met according to the current guidelines. We found that the median time to reach this diagnosis in the PTD group was at 4.7 weeks after evacuation when using the current FIGO 2000 criteria, and 4.4 weeks after evacuation if the Dutch guideline for PTD were applied. However, the median time to initiate therapy was 7.2 weeks. Possibly, this delay occurred because treating physicians were in doubt whether the criteria to diagnose PTD were truly met. Importantly, the median time to diagnose PTD by using the hCG-ratio was 3.0 weeks. Thus, the hCG-ratio identified patients earlier and possibly more accurate than in the case that the criteria as provided by FIGO 2000 were used.

The qualitative terms 'increase' and 'plateau' in serum hCG to confirm or reject the diagnosis PTD after evacuation of a hydatidiform mole may be subject to intra- and inter observer bias. Kohorn proposed to add to the definition of PTD according the FIGO 2000 criteria that an 'increase' should be defined as an hCG rise of 10 % or more for 3 values or more over a period of at least 2 weeks (26). However, no universal agreement has been reached on this proposal yet (27). The disadvantage of incorporating the premises of a 10 % rise for 2 weeks in the FIGO 2000 criteria for the diagnosis of PTD is that a rise within the range of normal serum hCG regression will lead to the diagnosis of PTD. Although we used these FIGO 2000 criteria for PTD as our 'gold standard' in our study, it does not implicitly mean that FIGO 2000 provides the best diagnostic criteria to diagnose PTD; it is very well possible that less patients are diagnosed with PTD while using normograms for normal hCG

regression, as compared to situations in which the concepts of 'increase' or 'plateau' are used without incorporating the premises that a serum measurement should exceed normal regression (21,24). However, because the development of normograms for every hCG assay does not seem feasible, the incorporation of a quantitative measure, for instance an hCG-ratio, in the criteria for PTD adds diagnostic accuracy to this definition.

Why is there such a large dispersion in the curative effect of a second curettage between the present and other studies (28,29)? The retrospective study by Schlearth *et al.* dealing with second curettage demonstrated a curative effect in approximately 16 % of patients and observed severe complications in 8.1 % of the second curettages (28). Our data are in line with the results of Schlearth *et al.*; we observed a curative effect in 9.4 % of the patients with low risk PTD, while 4.8 % of the patients had a severe complication due to second curettage (uterine perforation or hemorrhage> 1000 mL) (Chapter 6). Contrasting with the results of Schlaerth *et al.* and our data are the findings of Pezeshki *et al.* who reported the first prospective study on the curative effect of a second curettage in patients with PTD (29). The latter study showed a second curettage-only to be curative in 60 % of 282 patients with proven PTD. In this prospective study, criteria for the diagnosis of PTD are given as a plateauing or increasing serum hCG concentration without mentioning the period of plateauing. Upon request, the corresponding author of this study communicated that PTD was diagnosed if serum hCG was not normalized within 4 to 6 weeks after the first evacuation, or if serum hCG was increasing at any stage. Our previous study on normal serum hCG regression in patients with uneventful regression after evacuation revealed that serum hCG cumulatively normalizes in 5 % of patients within 6 weeks after first evacuation (and 50 % within 11 weeks and 95 % within 25 weeks) (24). Thus, it appears that patients are put at risk for complications if they are advised to undergo a second curettage 4-6 weeks after evacuation for 'persistent trophoblastic disease' according to the criteria of Pezeshki *et al.* because it is unnecessary in the majority of patients.

Another explanation for the large dispersion in the curative effect of a second curettage between our study and the study performed by Schlearth *et al.* on the one hand, and the study of Pezeshki *et al.* on the other hand may be that the Dutch Central Registry for Hydatidiform

Mole registers patients on a voluntary basis. From 1987 until 2003, 2122 patients with GTD were registered of which 422 developed PTD (20 %) (Chapter 6), 756 out of 2122 patients (36 %) had a spontaneous regression and clinical outcome was unknown in 944 out of 2122 patients (44 %). Due to 'reporting' bias, it may be possible that in the group of patients with an unknown clinical outcome, second curettages have been performed with possibly a curative effect.

Should patients with low-risk PTD be advised to undergo a second curettage? On should first realize that all evidence so far is derived from retrospective studies. To answer this question, the 'benefits' (cure, debulking effect and no need for chemotherapy) should outweigh the 'costs' (chance of major complications caused by the second curettage). The curative effect rates of a second curettage in PTD are reportedly 9.4 % (this thesis), 16 % (28) and 60 % (29). Major complications (uterine perforations) occurred in 3 patients (8.1 %, with 2 patients needing an hysterectomy) in one study (28), while our study reported a uterine perforation in 2 out of 85 patients (2.4 %, both managed conservatively) (Chapter 6). If chemotherapy will be necessary after the second curettage, it can be expected that one course less is needed. The 'costs' will be that there is a risk of approximately 5 % to 10 % that the second curettage will be complicated by uterine perforation for which a hysterectomy may be necessary, or hemorrhage>1000 mL. Overall, the 'benefits' and 'costs' of a second curettage seem to be at equilibrium. It can be imagined that some patients are reluctant to use chemotherapeutic agents, or do not wish to conceive anymore. These are the patients who may benefit from a second curettage. Patients with less reluctance towards chemotherapy, or those who definitely wish to conceive again should not be advised to have a second curettage for treatment of PTD to explicitly exclude the risk of uterine perforation. The true curative effect of a second curettage can only be investigated in a prospective randomized controlled trial.

It is remarkable that several different studies find that if single-agent chemotherapeutic treatment is successful, the serum hCG half-lives or median time to normalization is comparable with those of uneventful hCG regression. Not only were the results for the median time to normalization in the study of Rotmensch *et al.* comparable with our data (median time for normalization 11 weeks, comparable to the 5 two-weekly courses needed by 50 % of patients for normalization of serum hCG after successful single-agent chemotherapy (Chapter 7)), but we also

described the serum half-lives in three groups of patients (spontaneous regression of serum hCG, PTD cured by single-agent therapy and PTD in which single-agent therapy was followed by multiagent therapy) (Chapter 4). In this study, no significant differences were found between the half-live of hCG + hCGβ in spontaneous regression and in hyperglycosylated hCG in spontaneous regression as compared with the regression of these two hCG parameters in patients successfully treated with first-line single-agent therapy.

Regarding the pathogenesis of PTD development it is intriguing to consider why approximately 15 % to 20 % of complete hydatidiform moles and 0.5 % to 9.9 % of partial hydatidiform moles progress to PTD. Hypothetically, several mechanisms could be underlying these malignant sequelae of hydatidiform mole pregnancies:

1. Incomplete evacuation of trophoblastic tissue present in utero;
2. A defect in cell regulating mechanisms that is present at the moment of the first evacuation of the hydatidiform mole;
3. A defect in cell regulating mechanisms that develops in residual molar tissue after the evacuation of the hydatidiform mole.

Different from other solid malignancies, the diagnosis and prognosis of PTD is not solely based on histology of the primary tumour and its staging. HCG is a highly accurate tumor marker for trophoblast activity. If after a first evacuation hCG shows signs of residual trophoblastic activity (i.e. plateau or increase), PTD can be diagnosed and treatment can be established. The first hypothetical mechanism for the development of PTD is that this condition is the result of an incomplete evacuation. Normally, extra villous trophoblasts invade the myometrium up to a third of the entire myometrial wall (30). With the commonly used suction curettage for the evacuation of a hydatidiform mole, it is highly unlikely that the first third part of the myometrium, bearing the extra villous trophoblasts will be curetted as well. Therefore, this first hypothesis seems quite unlikely to be the cause for the persistence of some tumors as most patients will have residual tissue. Another finding that refutes the possibility that residual trophoblast after the evacuation of a hydatidiform mole is the cause of PTD, is presented in a study by Lao *et al.*(31). These authors performed a study in which the 'yield' (trophoblastic tissue or no trophoblastic tissue) of a routinely performed second curettage in patients with a hydatidiform mole was compared with the subsequent

need for chemotherapy for PTD; no such correlation was reported. Interestingly, the authors described that 76.9 % of patients with trophoblastic tissue detected in the second curetting showed no signs of PTD as derived from abnormal serum hCG regression and that 14.1 % of patients with no trophoblastic tissue detected with the second curetting, had signs of PTD for which chemotherapeutic treatment was established. This study indirectly contradicts our own finding (Chapter 6) and two other studies (28,29), i.e. that a second curettage has a curative effect in a small amount of patients with PTD. Possibly, the majority of PTD have other causes than residual tissue, but a small proportion of PTD seems to be caused by the remaining trophoblastic tissue in utero.

The second and third hypotheses for the development of PTD specifically address possible defects in cell-growth regulation as the mechanism. However, these regulatory mechanisms are beyond the scope of the present investigations and, for this reason, will not further be discussed. As already stated, the majority of PTD have possibly other causes than residual tissue, and a small proportion of PTD seems to be caused by the remaining trophoblastic tissue in utero. Thus, it appears reasonable to further investigate the hypothesis that postmolar hCG ratios (Chapter 3) reflect the *residual* tissue while pre-postmolar serum hCG ratios (that is, an hCG-concentration in a serum specimen taken before evacuation of the mole divided by an hCG concentration of a serum sample taken after evacuation) mirror the properties and potentials of *all tissue* present before evacuation of the mole. It would be interesting to compare the available results of the proportion of PTD predicted by postmolar hCG ratios (Chapter 3) with the proportion of PTD predicted by the pre-postmolar serum hCG ratios that still have to be calculated from available data. In order to optimise the detection of PTD, another suggestion is to assess the predictive potential of the two types of hCG ratios with the results of the FIGO 2000 and the NVOG scoring systems for detection of postmolar PTD.

Finally, recommendations for the Dutch Registry for Hydatidiform Mole (CMRN) are:

1. *Encouragement of registration*

As stated in the introduction of the present thesis, probably only half the total number of hydatidiform moles in the Netherlands are registered with the CMRN. Since hydatidiform mole is a rare entity, central regis-

tration is important to combine data and knowledge. In order to facilitate this registration, an online advice and registration service should be established.

2. Linking of the diagnoses registered with the Dutch Network and National Database for Pathology (PALGA), with the CMRN concerning GTD

In order to gather reliable data on incidence of GTD, automatic output from the PALGA system (registering all the histological diagnosis in The Netherlands) towards the CMRN should be established.

3. Scoring for high/low risk PTD according to the FIGO 2000 classification

To facilitate and improve the comparison of data on PTD obtained in the Netherlands with international data, gynaecologists and clinical oncologists should not only classify patients for high/low-risk PTD according to the Dutch guidelines but also according to the internationally accepted FIGO criteria. This will enable to compare both classifications, and may facilitate the comparison between Dutch patient groups and other patient groups in future research.

References

1. Shi QJ, Lei ZM, Rao CV, Lin J. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. Endocrinology 1993; 132:1387-95.
2. Boime I, Boothby M, Hoshina M, Daniels-McQueen S, Darnell R. Expression and structures of human placental hormone genes as a function of placental development. Biol Reprod 1982; 26:73-91.
3. Hoshina M, Boothby M, Boime I. Cytological localization of chorionic gonadotropin alpha and placental lactogen mRNAs during development of the human placenta. J Cell Biol 1982; 93:190-8.
4. Naylor SL, Chin WW, Goodman HM, Lalley PA, Grzeschik KH, Sakaguchi AY. Chromosome assignment of genes encoding the alpha and beta subunits of glycoprotein hormones in man and mouse. Somatic Cell Genet 1983; 9:757-70.
5. Berkowitz RS, Ozturk M, Goldstein DP, Bernstein MR, Hill L, Wands JR. Human chorionic gonadotropin and free subunits' serum levels in patients with partial and complete hydatidiform moles. Obstet Gynecol 1989; 74:212-6.
6. Licht P, Cao H, Lei ZM, Rao CV, Merz WE. Novel self-regulation of human chorionic gonadotropin biosynthesis in term pregnancy human placenta. Endocrinology 1993; 133:3014-25.
7. Licht P, Cao H, Zuo J, Lei ZM, Rao V, Merz WE, et al. Lack of self-regulation of human chorionic gonadotropin biosynthesis in human choriocarcinoma cells. J Clin Endocrinol Metab 1994; 78:1188-94.
8. Knofler M, Saleh L, Strohmer H, Husslein P, Wolschek MF. Cyclic AMP- and differentiation-dependent regulation of the proximal alphaHCG gene promoter in term villous trophoblasts. Mol Hum Reprod 1999; 5:573-80.
9. Knofler M, Saleh L, Bauer S, Vasicek R, Griesinger G, Strohmer H, et al. Promoter elements and transcription factors involved in differentiation-dependent human chorionic gonadotrophin-alpha messenger ribonucleic acid expression of term villous trophoblasts. Endocrinology 2000; 141:3737-48.
10. Knofler M, Saleh L, Bauer S, Galos B, Rotheneder H, Husslein P, et al. Transcriptional regulation of the human chorionic gonadotropin beta gene during villous trophoblast differentiation. Endocrinology 2004; 145:1685-94.
11. Knofler M, Saleh L, Strohmer H, Husslein P, Wolschek MF. Cyclic AMP- and differentiation-dependent regulation of the proximal alphaHCG gene promoter in term villous trophoblasts. Mol Hum Reprod 1999; 5:573-80.

12. Cheung AN. Pathology of gestational trophoblastic diseases. Best Pract Res Clin Obstet Gynaecol 2003; 17:849-68.
13. Li HW, Tsao SW, Cheung AN. Current understandings of the molecular genetics of gestational trophoblastic diseases. Placenta 2002; 23:20-31.
14. Bentley RC. Pathology of gestational trophoblastic disease. Clin Obstet Gynecol 2003; 46:513-22.
15. Hulsbergen-van der Kaa CA. DNA analysis of hydatidiform mole. PhD thesis, Catholic University Nijmegen, 1997.
16. Petignat P, Billieux MH, Blouin JL, Dahoun S, Vassilakos P. Is genetic analysis useful in the routine management of hydatidiform mole? Hum Reprod 2003; 18:243-9.
17. Kovalevskaya G, Birken S, Kakuma T, Ozaki N, Sauer M, Lindheim S, et al. Differential expression of human chorionic gonadotropin (hCG) glycosylation isoforms in failing and continuing pregnancies: preliminary characterization of the hyperglycosylated hCG epitope. J Endocrinol 2002; 172:497-506.
18. O'Connor JF, Ellish N, Kakuma T, Schlatterer J, Kovalevskaya G. Differential urinary gonadotrophin profiles in early pregnancy and early pregnancy loss. Prenat Diagn 1998; 18:1232-40.
19. Cole LA, Shahabi S, Oz UA, Bahado-Singh RO, Mahoney MJ. Hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen) immunoassay: A new basis for gestational Down syndrome screening. Clin Chem 1999; 45:2109-19.
20. Khanlian SA, Smith HO, Cole LA. Persistent low levels of human chorionic gonadotropin: A premalignant gestational trophoblastic disease. Am J Obstet Gynecol 2003; 188:1254-9.
21. Rotmensch J, Rosenshein NB, Block BS. Comparison of human chorionic gonadotropin regression in molar pregnancies and post-molar nonmetastatic gestational trophoblastic neoplasia. Gynecol Oncol 1988; 29:82-6.
22. Schlaerth JB, Morrow CP, Kletzky OA, Nalick RH, D'Ablaing GA. Prognostic characteristics of serum human chorionic gonadotropin titer regression following molar pregnancy. Obstet Gynecol 1981; 58:478-82.
23. Shigematsu T, Kamura T, Saito T, Kaku T, Nakano H, Kinugawa N. Identification of persistent trophoblastic diseases based on a human chorionic gonadotropin regression curve by means of a stepwise piecewise linear regression analysis after the evacuation of uneventful moles. Gynecol Oncol 1998; 71:376-80.
24. Yedema KA, Verheijen RH, Kenemans P, Schijf CP, Borm GF, Segers MF, et al. Identification of patients with persistent trophoblastic disease by means of a

normal human chorionic gonadotropin regression curve. Am J Obstet Gynecol 1993; 168:787-92.
25. Behtash N, Ghaemmaghami F, Honar H, Riazi K, Nori A, Modares M, et al. Is normal beta-hCG regression curve helpful in the diagnosis of persistent trophoblastic disease? Int J Gynecol Cancer 2004; 14:980-3.
26. Kohorn EI. The new FIGO 2000 staging and risk factor scoring system for gestational trophoblastic disease: description and critical assessment. Int J Gynecol Cancer 2001; 11:73-7.
27. Ng TY, Wong LC. Diagnosis and management of gestational trophoblastic neoplasia. Best Pract Res Clin Obstet Gynaecol 2003; 17:893-903.
28. Schlaerth JB, Morrow CP, Rodriguez M. Diagnostic and therapeutic curettage in gestational trophoblastic disease. Am J Obstet Gynecol 1990; 162:1465-70.
29. Pezeshki M, Hancock BW, Silcocks P, Everard JE, Coleman J, Gillespie AM, et al. The role of repeat uterine evacuation in the management of persistent gestational trophoblastic disease. Gynecol Oncol 2004; 95:423-9.
30. Huppertz B, Hemmings D, Renaud SJ, Bulmer JN, Dash P, Chamley LW. Extravillous trophoblast apoptosis--a workshop report. Placenta 2005; 26 Suppl A:S46-S48.
31. Lao TT, Lee FH, Yeung SS. Repeat curettage after evacuation of hydatidiform mole. An appraisal. Acta Obstet Gynecol Scand 1987; 66:305-7.

CHAPTER 9

Summary

Chapter 1 introduces the different entities in Gestational Trophoblastic Disease (GTD), Persistent Trophoblastic Disease (PTD) and human Chorionic Gonadotropin (hCG).

Mola hydatidosa are villous malformations of trophoblast, which are characterized by edematous villi covered with trophoblastic hyperplasia. Both mola hydatidosa and the non-villous counterparts choriocarcinoma and placental site trophoblastic tumor are classified as GTD. Genetically, mola hydatidosa can be subdivided into complete and partial hydatidiform mole. Distinction between a complete and a partial hydatidiform mole is important since they have a different propensity to have malignant sequelae.

In PTD, trophoblastic activity of the hydatidiform mole remains after termination of pregnancy that results in a plateau or increase of the serum concentrations of the pregnancy hormone, hCG. To date, an internationally accepted definition for diagnosis of PTD as proposed by FIGO (International Federation of Gynaecology and Obstetrics) is used in which PTD is defined as a plateau of serum hCG concentrations for three weeks, an increase of hCG for two consecutive weeks, the detection of hCG six months after evacuation, or the histological finding of a choriocarcinoma. In the Netherlands, the Dutch Society for Obstetrics and Gynaecology defines PTD as a plateau or increase of serum hCG concentrations for three consecutive weekly measurements, with at least one measurement exceeding the 95th percentile of a serum hCG regression curve established for normal uneventful post-molar regression. The reported frequency of PTD is 20 % in complete hydatidiform mole and 0.5 to 9.9 % in partial hydatidiform mole.

In normal pregnancy, intact hCG (i.e. a hCG α- attached to a hCG β-subunit), represents the majority of hCG, but other variants like free hCG α-subunit, free hCG β-subunits, nicked intact or nicked hCG-subunits and hyperglycosylated hCG are present as well. The production of subunits of hCG is under stringent physiological control in normal pregnancy, and is reported to be different in pathological states such as hydatidiform mole.

The present thesis studied the ability of several hCG parameters to distinguish hydatidiform mole from normal pregnancy and to predict the development of PTD. Regarding treatment of PTD, we explored the curative effect of a second curettage and we constructed a 'normal' regression curve for the early detection of resistance to first-line single-agent therapy in patients with low-risk PTD.

Chapter 2 explored the diagnostic accuracy of 'total' hCG (intact hCG+free hCG β-subunit measured together (hCG + hCGβ)), free hCG α-subunits (hCGα) and hCG β-subunits (hCGβ) obtained prior to evacuation, and the calculated ratios between these analytes to make the distinction between hydatidiform mole and normal pregnancy, between complete and partial and hydatidiform mole and between spontaneous regression and PTD.

Blood specimens collected in the Dutch Central Registry for Hydatidiform Moles were used to measure hCGα, hCGβ and hCG + hCGβ concentrations by radioimmunoassays. A group of 165 patients with complete (of which 43 with PTD) and a group of 39 patients with partial moles (of which 7 with PTD) were compared with 27 pregnant women with uneventful pregnancy. In addition, we calculated the ratios hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ, and hCGα/hCGβ. Receiver Operating Curve (ROC) analysis revealed that the distinction between hydatidiform mole and normal pregnancy is best possible on a single blood specimen with hCGβ, but also, excellent results are obtained with hCGβ/hCG + hCGβ and hCGα/hCGβ , with Areas-Under-the-Curve (AUCs) ranging between 0.922 and 0.999. To distinguish complete from partial hydatidiform mole, the six tested hCG analytes and parameters showed AUCs between 0.7-0.8. In predicting PTD, the three analytes and three calculated parameters showed limited diagnostic significance with AUCs<0.7

Chapter 3 assessed the diagnostic potential of serum hCG concentration ratios obtained after evacuation of hydatidiform mole to diagnose PTD and compared its diagnostic accuracy with the current FIGO 2000 standard.

A retrospective cohort survey was performed on patients registered with the Dutch Central Registry for Hydatidiform Moles between 1977-2004. After exclusion for various reasons, serum hCG concentrations were available for 278 patients. Of these, 87 patients developed PTD (study group) and 191 had a spontaneous regression of serum hCG (control group). Serum hCG ratios were calculated of two concentrations obtained after evacuation. Specificity and sensitivity were calculated and paired in ROC curve analysis resulting in calculation of AUCs.

Serum hCG concentration ratios showed an increasing diagnostic potential; the ratio obtained in week 1 and 5 after evacuation showed an

AUC of 0.568 and 0.935, respectively, and identified 20 % and 79 % of patients with PTD at the 95 % specificity level. The median time to diagnose PTD with the use of a ratio was 3.0 weeks versus 4.7 weeks if the criteria for PTD according to FIGO 2000 were applied.

In this retrospective study we found that a ratio of two serum hCG concentrations obtained after evacuation identifies patients with PTD approximately 2 weeks earlier than the internationally accepted FIGO 2000 criteria. This ratio makes it possible to identify the majority of patients who develop PTD by the seventh week after evacuation. The additional calculation of a ratio from two already available serum hCG concentrations obtained after evacuation may provide a quantitative addition to the qualitative FIGO 2000 'increase' and 'plateau' criteria.

It remains to be investigated prospectively, however, whether such an addition leads to a gain in diagnostic accuracy of the present FIGO 2000 criteria.

Chapter 4 explored the disappearance rates of 'total' hCG and hyperglycosylated hCG in serum after evacuation of a molar pregnancy.

We investigated the relationship between hyperglycosylated hCG and 'total' hCG concentrations after evacuation of the mole by comparing the time-course and half-life in three groups of 7 patients each. The patients of the first group had uneventful regression of their serum hCG after evacuation of hydatidiform mole, those of the second group needed single-agent chemotherapy (Methotrexate) for treatment of PTD and the patients of the third group had PTD that failed to respond to single-agent chemotherapy. Significantly longer mean values of serum half-life for 'total' hCG and hyperglycosylated hCG (Invasive Trophoblast Antigen, ITA) were found in the group not responding to single agent chemotherapy (Group 3: 3.02 and 2.51 weeks) as compared to the group that did respond to single agent chemotherapy (Group 2: 0.96 and 0.90 weeks) and the group that had uneventful regressions (Group 1: 0.81 and 0.66 weeks) (all p=0.003), but no differences were observed between the groups responding to single-agent chemotherapy and with uneventful regression. Significantly shorter mean half-life values for hyperglycosylated hCG than those calculated for hCG + hCGβ were observed in all three groups of patients. It is concluded that the possible clinical value of faster regression of hyperglycosylated hCG to baseline levels as compared with hCG + hCGβ remains to be investigated prospectively.

Chapter 5 assessed the diagnostic potential of ITA, hCGβ and hCG + hCGβ in serum samples obtained before evacuation and the calculated ratios of hCGβ/hCG + hCGβ, hCGβ/ITA and hCG + hCGβ/ITA, to predict the later development of PTD.

The study group comprised 97 patients with hydatidiform mole who did not develop PTD after mole evacuation, whereas 33 other patients developed PTD. Serum samples from 130 patients with hydatidiform mole with or without PTD were assayed using specific (radio)immunoassays for hCGβ, hCG + hCGβ and ITA. From these analytes we also calculated the ratios hCGβ/hCG + hCGβ, hCGβ/ITA, and hCG + hCGβ/ITA. To predict development of PTD from these analytes and parameters we performed ROC curve analysis resulting in AUCs which were expressed as diagnostic accuracy ranging from excellent (AUC>0.9 or <0.1) to poor (AUC 0.4-0.6). The diagnostic accuracy of ITA is moderate (0.618) and not different from that of free hCGβ (0.610) and hCG + hCGβ (0.622). It was concluded that ITA as well as the other analytes and parameters in serum taken prior to evacuation from patients with molar pregnancies cannot be used to predict the subsequent development of persistent trophoblastic disease.

Chapter 6 explored the curative effect of a second curettage in patients with low risk PTD in a retrospective cohort survey based on patients registered with the Dutch Central Registry for Hydatidiform Moles between 1987 and 2003.

The study group comprised 85 patients with low-risk PTD according to the Dutch guidelines who underwent a second therapeutic curettage as part of the treatment for PTD. The control group consisted of 209 patients with low-risk PTD who did not undergo a second curettage.

Primary outcome measures were the need for chemotherapy and if applicable, the number of chemotherapy courses. After second curettage, eight out of 85 patients (9.4 %) did not need additional chemotherapy which significantly differs from the patients in the control group who all needed chemotherapy (p<0.001). A debulking effect of the second curettage was observed: a median of 6 chemotherapy courses (interquartile range 3 courses) in the control group versus 5 courses (interquartile range 3 courses) in the study group (p=0.036). Four out of the 85 (4.8 %) patients with a second curettage had a major complication (uterine perforation or haemorrhage > 1000 mL). Since a second curettage cured

9.4% of patients with PTD in this historical cohort and reduced the number of courses of chemotherapy, we concluded that a second curettage seems to benefit a limited number of patients with PTD.

Chapter 7 explored the possibility to identify patients not responding to single-agent chemotherapy in an early stage and to prevent unnecessary multiagent chemotherapy at the same time.

We constructed a normal serum hCG regression corridor for patients successfully treated with single- agent chemotherapy. We included data from patients registered at the Dutch Central Registry for Hydatidiform Moles between 1987 and 2004. A normal serum hCG regression corridor was constructed from data of 79 patients with low-risk PTD who were cured by single-agent (Methotrexate (MTX)) chemotherapy (control group). Another group of 29 patients with low-risk PTD needed additional multiagent chemotherapy because their serum hCG failed to normalize with single-agent chemotherapy (study group). We found that this normogram made it possible to identify 14 % of patients who will need multiagent chemotherapy before the start of first-line single-agent chemotherapy with 97.5 % specificity. Likewise, serum hCG measurements obtained just before the start of the fourth MTX course identified up to 50 % of patients (at 97.5 % specificity) who would not respond to single-agent chemotherapy. We concluded that in the first few courses of MTX we are able to identify at high specificity half the number of patients that are likely to need multiagent chemotherapy to cure their disease and for whom further treatment with single-agent chemotherapy will be ineffective.

Chapter 8 comprises the general discussion, in which the value of different hCG parameters is discussed in the diagnosis of complete and partial moles and in the prediction of PTD. Next to that, a hypothesis why hCGβ is synthesized more abundantly in GTD than hCGα, is put forward. We state in our general discussion that, while the most widely accepted definition of FIGO for the diagnosis of PTD which incorporates qualitative criteria like “increase” and “plateau” in its definition, a more quantitative criterion like a hCG ratio might be of more value in the definition of PTD. We also address the large dispersion which several studies have reported on the curative effect of a second curettage in PTD and we address the clinical benefits and drawbacks of this procedure. We

illuminate the clinical utility of serum hCG regression corridors in follow-up of uneventful hydatidiform mole and in patients treated with single-agent chemotherapy for low-risk PTD. Finally, based on these studies we provide some recommendations for the improvement of the Dutch Registry for Hydatidiform Mole (CMRN).

CHAPTER 10

Samenvatting

In **hoofdstuk 1** worden de verschillende entiteiten binnen de zwangerschapsgerelateerde trofoblastziekten (*Gestational Trophoblastic Disease*, GTD), persisterende trofoblastziekte (*Persistent Trophoblastic Disease*, PTD) en humaan choriogonadotropine (hCG), geïntroduceerd.

Typisch voor mola hydatidosa is de aanwezigheid van villeuze afwijkingen van de trofoblast, welke worden gekarakteriseerd door oedemateus gezwollen vlokken die bedekt zijn met hyperplastische trofoblastcellen. Zowel de mola hydatidosa als de niet-villeuze tegenhangers, choriocarcinoom en *Placental Site Trophoblastic Tumour,* behoren tot de GTD. Genetisch kan mola hydatidosa onderverdeeld worden in complete en partiële mola hydatidosa, hetgeen van belang is omdat beide een verschillende kans hebben op maligne ontaarding.

Bij PTD blijft de trofoblast van de mola hydatidosa actief na het beëindigen van de zwangerschap met een plateau of stijging van het zwangerschapshormoon hCG in het bloed tot gevolg. Momenteel wordt er een door de FIGO (Internationale Federatie voor Obstetrie en Gynaecologie) een internationaal geaccepteerde definitie gebruikt die PTD definieert als er sprake is van een plateau van het serum-hCG gedurende drie weken, een stijging van de hCG-concentratie gedurende twee weken, detecteerbaar hCG zes maanden na evacuatie van de mola, of de histologisch bevestigde diagnose van een choriocarcinoom. De Nederlandse Vereniging voor Obstetrie en Gynaecologie definieert de aanwezigheid van PTD als een plateau of stijging van het serum hCG gedurende drie opeenvolgende wekelijkse metingen, waarbij ten minste één hCG-meting het 95^{e} percentiel van een eerder opgestelde serum hCG-curve voor spontane regressie van het serum hCG dient te overschrijden. De gerapporteerde frequentie van PTD is 20 % na een complete mola hydatidosa en 0,5 % tot 9,9 % na een partiële mola hydatidosa.

In de normale zwangerschap bestaat het merendeel van het aanwezige hCG uit intact hCG (een hCG β-keten verbonden aan een hCG β-keten), naast een aantal andere varianten, waaronder N-terminaal heterogene vrije hCG α-ketens, vrije hCG β-ketens en (door ontbrekende verbindingen in de β-ketens) specifiek afwijkende vormen daarvan (*nicked hCG en nicked hCGβ*), naast hypergeglycosyleerd hCG. Tijdens de normale zwangerschap staat de productie van de hCG-ketens onder strenge fysiologische controle en is anders in pathologische situaties zoals bij mola hydatidosa.

In dit proefschrift hebben wij de mogelijkheden bestudeerd om met

behulp van verschillende hCG parameters mola hydatidosa van een normale zwangerschap te onderscheiden en om PTD in geval van mola te voorspellen. Met betrekking tot de behandeling van PTD hebben wij het curatieve effect van een tweede curettage bestudeerd en hebben wij een curve voor 'normale' hCG-regressie tijdens behandeling met eerstelijns monochemotherapie voor laagrisico PTD ontwikkeld om vroegtijdig resistentie daarvan op te sporen.

In **hoofdstuk 2** hebben wij de diagnostische accuratesse van vóór de evacuatie verkregen 'totaal' hCG (intact hCG en de vrije hCG β-keten samen gemeten: hCG + hCGβ), de vrije hCG α-keten (hCGα) en de vrije hCG β-keten (hCGβ) onderzocht en de daarvan afgeleide, berekende ratio's om het onderscheid te maken tussen mola hydatidosa en normale zwangerschap, tussen complete en partiële mola hydatidosa en tussen spontane remissie en PTD.

Bloedmonsters uit de Centrale Mola Registratie Nederland, verzameld voorafgaand aan mola-evacuatie, werden gebruikt voor het bepalen van de hCGα, hCGβ en hCG + hCGβ -concentraties met behulp van radioimmunoassays. Een groep van 165 patiënten met een complete mola hydatidosa (waarvan 43 met PTD) en een groep van 39 patiënten met een partiële mola hydatidosa (waarvan 7 met PTD) werden vergeleken met een groep van 27 vrouwen die van week 6 tot week 16 van hun ongecompliceerde zwangerschap longitudinaal werden vervolgd. Daarnaast werden uit deze metingen de ratio's van hCGα/hCG + hCGβ, hCGβ/hCG + hCGβ, en hCGα/hCGβ berekend. *Receiver Operating Characteristics* (ROC) analyse toonde aan dat het onderscheid tussen mola hydatidosa en normale zwangerschap vrijwel absoluut gemaakt kan worden op basis van een hCGβ meting in een enkel bloedmonster, maar er werden ook uitstekende resultaten behaald met hCGβ/hCG + hCGβ en hCGα/hCGβ, met *Areas-Under-Curv*e (AUC) variërend van 0,922 tot 0,999. Voor het onderscheiden van een complete mola hydatidosa van een partiële mola hydatidosa varieerden de AUC's van de zes geteste parameters AUCs tussen 0,7 en 0,8. Analoog hieraan was het voorspellen van PTD vrijwel niet mogelijk omdat de drie gemeten parameters en de afgeleide ratio's met AUC's<0,7 slechts een zeer matige diagnostische accuratesse vertoonden.

Hoofdstuk 3 onderzoekt de waarde van serum hCG-ratio's, berekend uit bloedwaarden verkregen na evacuatie van mola hydatidosa voor het diagnosticeren van PTD. De diagnostische accuratesse van deze serum hCG-ratio's werd vergeleken met de huidige FIGO 2000 standaard.

Een retrospectieve cohortstudie werd uitgevoerd met patiënten die tussen 1977-2004 geregistreerd waren bij de Centrale Mola Registratie Nederland. Na exclusie om verschillende redenen, waren bruikbare serum hCG-concentraties beschikbaar van 278 patiënten. Van hen ontwikkelden 87 patiënten PTD (studiegroep) terwijl 191 patiënten een spontane regressie van het serum hCG hadden (controlegroep). Serum hCG-ratio's werden berekend uit concentraties die verkregen zijn uit twee serummonsters na evacuatie. ROC-curve analyse resulteerde in de berekening van AUC's en gaf inzicht in specificiteit en sensitiviteit. Serum hCG-ratio's toonden een toenemende diagnostische potentie; voor ratio's verkregen in week 1 en 5 na evacuatie werden AUC's van respectievelijk 0,568 en 0,935 vastgesteld en identificeerden 20 % en 79 % van de patiënten met PTD bij 95 % specificiteit. De mediane tijd om PTD te diagnosticeren met behulp van een ratio was 3,0 weken versus 4,7 weken indien de criteria voor PTD volgens FIGO 2000 werden toegepast.

In deze retrospectieve studie hebben wij vastgesteld dat een ratio berekend uit twee hCG-concentraties gemeten in serum verkregen na evacuatie in staat is patiënten met PTD ongeveer 2 weken eerder te identificeren dan het geval is volgens de internationaal geaccepteerde FIGO 2000 criteria. Deze ratio maakt het mogelijk om de meerderheid van patiënten die PTD ontwikkelen in de zevende week na evacuatie te identificeren. Het berekenen van een ratio van twee toch al beschikbare serum hCG concentraties zou een kwantitatieve aanvulling op de kwalitatieve FIGO 2000 criteria voor 'plateau' en 'stijging' kunnen zijn.

Vooreerst dient nog prospectief onderzocht te worden of een dergelijke aanvulling ook leidt tot een betere diagnostische accuratesse van de huidige FIGO 2000 criteria.

Hoofdstuk 4 onderzoekt de verdwijningssnelheid van 'totaal' hCG en hypergeglycosyleerd hCG (*Invasive Trophoblast Antigen*, ITA) in serum na de evacuatie van een mola hydatidosa. Wij onderzochten de relatie tussen hypergeglycosyleerd hCG en 'totaal' hCG-concentraties na evacuatie van een mola door het tijdsverloop en de halfwaardetijd in drie

groepen van ieder 7 patiënten te onderzoeken. De patiënten in groep 1 hadden een ongecompliceerde remissie van hun serum hCG na de evacuatie van een mola hydatidosa, de patiënten in groep 2 hadden monochemotherapie (Methotrexaat) nodig voor de behandeling van hun PTD en de patiënten in groep 3 hadden PTD die niet reageerde op monochemotherapie.

Significant langere gemiddelde serum halfwaardetijden voor 'totaal' hCG en hypergeglycosyleerd hCG werden gevonden in de groep die niet reageerde op monochemotherapie (Groep 3; 3,02 en 2,51 weken) in vergelijking tot de groep die wel goed reageerde op monochemotherapie (Groep 2: 0,96 en 0,90 weken) en de groep met een ongecompliceerde remissie (Groep 1: 0,81 en 0,66 weken), maar geen verschillen werden gevonden tussen de monochemotherapie- en de spontane remissiegroep. Significant kortere gemiddelde halfwaardetijden van hypergeglycosyleerd hCG in vergelijking tot hCG + hCGβ werden in de drie patiëntengroepen waargenomen. Wij concluderen dat de mogelijke klinische waarde van de snellere verdwijning van hypergeglycosyleerd hCG ten opzichte van hCG + hCGβ prospectief verder onderzocht moet worden.

In **hoofdstuk 5** hebben wij, ter voorspelling van PTD, de diagnostische waarde van ITA, hCGβ en hCG + hCGβ in serum verkregen voor evacuatie en van de daaruit berekende ratio's van hCGβ/hCG + hCGβ, hCGβ/ITA en hCG + hCGβ /ITA, onderzocht.

In de studie werden 97 patiënten met een mola hydatidosa die geen PTD ontwikkelden na evacuatie vergeleken met 33 patiënten die dat wel deden. Serum bloedmonsters van deze 130 patiënten met mola hydatidosa met of zonder PTD werden geanalyseerd met specifieke (radio) immunoassays voor hCGβ, hCG + hCGβ en ITA. Wij berekenden ook de ratio's van hCGβ/hCG + hCGβ , hCGβ/ITA en hCG + hCGβ /ITA. Om het optreden van PTD te voorspellen met deze metingen en afgeleide parameters, voerden wij ROC-curve analyse uit hetgeen resulteerde in AUC's als maat voor de diagnostische accuratesse die varieerde van excellent (AUC> 0,9 of <0,1) tot matig (AUC 0,4-0,6). De diagnostische accuratesse van ITA is matig (0,618) en niet verschillend van die van hCGβ (0,610) en hCG + hCGβ (0,622). Wij concluderen dat ITA en de andere metingen en parameters die verkregen zijn uit serum dat afgenomen is bij patiënten voor evacuatie van de mola hydatidosa, niet gebruikt kunnen worden om PTD te voorspellen.

Hoofdstuk 6 onderzoekt het curatieve effect van een tweede curettage bij patiënten met laagrisico PTD in een retrospectieve studie die gebaseerd is op patiënten geregistreerd bij de Centrale Mola Registratie Nederland tussen 1987 en 2003. De studiegroep omvatte 85 patiënten met laagrisico PTD volgens de Nederlandse richtlijnen, die allen een therapeutische tweede curettage ondergingen als onderdeel van de behandeling van PTD. De controlegroep bestond uit 209 patiënten met laagrisico PTD die geen tweede curettage ondergingen. De primaire uitkomstmaten waren de noodzaak voor chemotherapie en, indien van toepassing, het aantal chemotherapiekuren. Na een tweede curettage hadden 8 van de 85 (9,4 %) patiënten geen aanvullende chemotherapie meer nodig, wat significant verschilde van de patiënten in de controlegroep die allemaal chemotherapie nodig hadden. Een *debulking* effect werd na de tweede curettage waargenomen: de controlegroep ontving een mediaan aantal van 6 chemotherapiekuren (interkwartiele spreiding 3 kuren) versus 5 chemotherapie kuren (interkwartiele spreiding 3 kuren) in de studiegroep (p=0.036). Vier van de 85 (4.8 %) patiënten in de tweede curettagegroep had een grote complicatie (uterusperforatie of bloedverlies >1000 mL). Omdat een tweede curettage 9,4 % van de patiënten met PTD in dit historische cohort genas en het aantal chemotherapiekuren reduceerde, concluderen wij dat een beperkt aantal patiënten met PTD baat lijkt te hebben bij een tweede curettage.

In **hoofdstuk 7** onderzochten wij de mogelijkheid patiënten die niet reageren op eerstelijns monochemotherapie in een vroeg stadium te identificeren om tijdig op polychemotherapiebehandeling over te gaan. We construeerden een normaal hCG-regressiecorridor voor patiënten die succesvol met monochemotherapie behandeld werden.

Wij includeerden gegevens van patiënten die tussen 1987 en 2004 geregistreerd zijn bij de Centrale Mola Registratie Nederland. Een normaal serum hCG-regressiecorridor werd geconstrueerd voor 79 patiënten met laagrisico PTD die genezen werden met monochemotherapie (MTX) (controlegroep). Een andere groep van 29 patiënten met laagrisico PTD had aanvullend polychemotherapie nodig (studiegroep). Met dit normogram bleek het mogelijk om 14 % van de patiënten die polychemotherapie nodig zouden hebben voor de start van de eerste monochemotherapiekuur met 97.5 % specificiteit te identificeren. Op dezelfde manier kan met serum hCG-metingen die vlak voor de start van de vier-

de monochemotherapiekuur verkregen zijn, 50 % van de patiënten die polychemotherapie nodig zullen hebben met 97.5 % specificiteit geïdentificeerd worden. Wij concluderen dat wij in de loop van de eerste monochemotherapiekuren in staat bleken met een hoge mate van specificiteit de helft van het aantal patiënten te identificeren die polychemotherapie nodig hebben voor genezing en voor wie verdere monochemotherapie niet meer effectief is.

Hoofdstuk 8 betreft de algemene discussie omtrent de waarde van verschillende hCG-parameters voor het stellen van de diagnose complete en partiële mola en bij het voorspellen van PTD. Daarnaast wordt ingegaan op de vraag waarom er meer hCGβ dan hCGα in GTD geproduceerd wordt. Wij stellen in de algemene discussie dat de gangbare definitie van de FIGO voor de diagnose PTD met daarin kwalitatieve begrippen als 'stijging' en 'plateau' kan worden opgewaardeerd met een kwantitatiever criterium zoals een hCG-ratio. Wij bespreken ook de grote variatie in het curatieve effect van een tweede curettage zoals genoemd in verschillende studies en gaan in op de klinische voor- en nadelen van deze ingreep. Daarnaast gaan wij in op de klinische waarde van een regressiecorridor van serum hCG bij de follow-up van patiënten met spontane regressie en bij patiënten met laagrisico PTD die met monochemotherapie behandeld worden. Tenslotte adviseren wij de Centrale Mola Registratie Nederland de registratie van patiënten met hydatidiforme mola te blijven aanmoedigen, zo mogelijk een koppeling aan te brengen met het Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA) en te bevorderen dat de classificatie van laag-/hoogrisico PTD tevens plaatsvindt volgens de FIGO 2000 classificatierichtlijnen.

Bibliography

Papers

1. Dobbe JG, **van Trommel NE**, de Freitas Baptista JE, Ritt MJ, Steenbeek A, Molenaar HA. A Portable device for finger tendon rehabilitation that provides an isotonic training force and records exercise behaviour after finger tendon surgery. Med Biol Eng Comput. 1999; 37: 396-399
2. Dobbe JG, **van Trommel NE**, Ritt MJ. Patient compliance with a rehabilitation program after flexor tendon repair of zone II of the hand. J Hand Ther. 2002; 15: 16-21
3. **van Trommel NE**, Massuger LFAG, Verheijen RHM, Sweep CGJ, Thomas CMG. The curative effect of a second curettage in persistent trophoblastic disease: A retrospective cohort survey. Gynecol Oncol. 2005; 99: 6 – 13
4. **van Trommel NE**, Massuger LFAG, Sweep CGJ, Thomas CMG. Second curettage in persistent trophoblastic disease (PTD): The need for univocal definition of PTD. Gynecol Oncol. 2005; 99: 250-251
5. **van Trommel NE**, Sweep CGJ, Schijf CPT, Massuger LFAG, Thomas CMG. Diagnosis of hydatidiform mole and persistent trophoblastic disease: diagnostic accuracy of total human chorionic gonadotropin (hCG), free hCG α- and β-subunits, and their ratios. Eur J Endocrinol. 2005; 153: 565-575
6. **van Trommel NE**, Massuger LFAG, Schijf CPT, ten Kate-Booij MJ, Sweep CGJ, Thomas CMG. Early Identification of Resistance to First-Line Single-Agent Methotrexate. in Patients With Persistent Trophoblastic Disease. J Clin Oncol. 2006; 24:52-58
7. Duc HN, **van Trommel NE**, Sweep CGJ, Massuger LFAG, Thomas CMG. Clinical utility of hyperglycosylated hCG in serum taken before hydatidiform mole evacuation to predict persistent trophoblastic disease. Int J Biol Markers. 2006; 21: 45-49
8. **van Trommel NE**, Sweep CGJ, Ross HA, Massuger LFAG, Thomas CMG. Comparison of hCG+hCGβ and ITA disappearance rates in serum after evacuation of molar pregnancy. Int J Mol Med *(in press)*
9. **van Trommel NE**, Duc HN, Massuger LFAG, Schijf PT, Sweep CGJ, Thomas CMG. Early identification of persistent trophoblastic disease with serum hCG concentration ratios *(submitted)*

Abstracts

1. **van Trommel NE**, Sweep CGJ, Massuger LFAG, Schijf PT, Thomas CMG. Diagnosis of Hydatidiform Mole and Persistent Trophoblastic Disease: Diagnostic Accuracy of total Human Chorionic Gonadotropin, free hCG α- and β-subunits and their ratios. Poster-abstract at the 10th biennial International Gynaecologic Cancer Society (IGCS) meeting. Edinburgh, Scotland, October 3-7, 2004.
2. **van Trommel NE**, Sweep CGJ, Massuger LFAG, Schijf PT, Thomas CMG. 6 hCG parameters in hydatidiform mole. Oral presentation at the 17th annual Symposium Experimental Research Surgical Specialties (SEOHS). Amsterdam, The Netherlands, November 19th, 2004.
3. **van Trommel NE**, Massuger LFAG, Schijf PT, ten Kate-Booij MJ, Sweep CGJ, Thomas CMG. Early Identification of Resistance to First Line Single Agent Chemotherapy in Patients with Persistent Trophoblastic Disease. Oral presentation at the 14th International Meeting of the European Society of Gynaecological Oncology (ESGO). Istanbul, Turkey, September 25-29, 2005.
4. **van Trommel NE**, Massuger LFAG, Schijf PT, Verheijen RHM, Sweep CGJ, Thomas CMG. The Curative Effect of Second Curettage in Persistent Trophoblastic Disease. Oral presentation at the 14th International Meeting of the European Society of Gynaecological Oncology (ESGO). Istanbul, Turkey, September 25-29, 2005.
5. **van Trommel NE**, Massuger LFAG, Schijf PT, ten Kate-Booij MJ, Sweep CGJ, Thomas CMG. Distinguishing "moderate PTD" from "severe PTD": Early Identification of Resistance to First Line Single Agent Chemotherapy. Oral presentation at the 13th World Congress on Gestational Trophoblastic Diseases. Hong Kong, China. October 23-26, 2005.
6. **van Trommel NE**, Massuger LFAG, Schijf PT, Verheijen RHM, Sweep CGJ, Thomas CMG. The Clinical Benefit of a Second Curettage in Low Risk Persistent Trophoblastic Disease: Seventeen Years Experience in The Netherlands. Oral presentation at the 13th World Congress on Gestational Trophoblastic Diseases. Hong Kong, China. October 23-26, 2005.

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waar ik de lekkerste koekjes, biertjes, kookspullen enz. enz. kan kopen! Echter, ik hoop dat jullie beseffen dat de poets die jullie me bakten met een hotelkamer switch, nog terug betaald gaat worden!

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Lieve Eveline en Doriene, lieve paranimfen. Het was even zoeken om twee paranimfen te vinden met een geringere lengte dan ik, maar het is uiteindelijk gelukt.

Lieve Eveline aka Eefje Peefje Paranimf. Vol goede moed sleurde jij me uit mijn donkere hol in het lab. Het monopolie op De Enige Vriendin in Nijmegen heb je misschien een heel klein beetje verloren. Maar de Knabbel&Babbel vriendschap die we bij de statistiekcursus ontwikkelden (niemand kan zo goed uitleggen wat jij bedoelt als ik), reikt verder dan Eindhoven en de Alpen!

Lieve Doriene. Ruim tien jaar geleden togen we samen voor het eerst naar Paramaribo voor onze stage "billenwassen". Door de jaren heen ben ik steeds meer je tukkerse, nuchtere maar warme blik op de wereld gaan waarderen. Je bent de spin in het web van veel verschillende vriendschappen. Ik vind het heel bijzonder dat je me uit Amsterdam nagereisd bent om nu in dezelfde straat in Nijmegen te wonen. Je bent mijn kleine grote vriendin!

Lieve Ineke, lieve mam. Hoe kan je iemand bedanken die negen maanden met je in haar buik heeft rondgesjouwd en vervolgens dertig jaar alles in het werk heeft gesteld om je gelukkig te maken? Jouw weigering om in te zien dat er een probleem zou zijn om een specialisatie en gezin te combineren, hielp me over de schreef. Dank je wel.

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Lieve Hielke en Klaar. Lieve broer, we kennen elkaar al een tijdje. Ik geloof dat we vroeger in dezelfde bar aan de toog gehangen hebben, al kwam ik daar 20 maanden eerder dan jij. Gek, als ik aan jullie denk, zie ik altijd twee malloten in een teletubbie pak voor me. Om het smartlapperig te zeggen: 'We hebben heel wat met elkaar mee gemaakt.' Ik kan alleen maar zeggen: ik hoop dat we nog veel meer met elkaar gaan meemaken!

Dear doctor Monica Glenshaw. In Murambinda Mission Hospital, Zimbabwe, you taught me obstetrics and how to set up preventive health programs. I will never forget the look in your eyes when I performed my first caesarean section with you and I said: "Oh no, I think I cut right to the placenta!" and you replied: "That is not placenta, that is the baby's hair!" Thank you for your inspiration and friendship. I hope that our daughter Pi Sarah Monica will be a strong and witty person like you.

Lieve familie en vrienden. Het is niet makkelijk om te beschrijven hoezeer ik geniet van de gezelligheid, vriendschap en humor die we met elkaar delen. Dank voor al het meeleven in voor- en tegenspoed!

Lieve kleine dochter Pi. In mijn buik heb je de laatste fase van dit proefschrift opgevrolijkt met intermezzo's waarin je me het nieuwe spel blaasje tik (jij won!), afstandbediening werpen (ik kom toch verder), en geef-je-vader-een-kontje, leerde. Nu ik je niet alleen kan voelen, maar ook kan aankijken en vasthouden, heb ik niet genoeg woorden om te vertellen hoe lief en leuk ik je vind. Ik hoop dat ik nog heel lang, heel veel spelletjes met jou en Adam mag spelen!

Lieve Adam, lief lief. Spontaan vertrokken we naar Cuba, om erachter te komen hoe leuk het is om samen op pad te zijn. Niet alleen je Swaans (Spaans op z'n Swets met als bekendste vertaling voor "gisteren": "yesterdado") maakte grote indruk, ook je robuuste liefde en standvastigheid. Na reizen volgden de compromissen en ergens tussen Amsterdam en Afrika, troffen we elkaar in Nijmegen. Van compromissen kwam een conceptie. En nu maar hopen dat ons meisje jouw slanke bouw zal hebben. Ik hoop dat ik nog heel lang en gelukkig met je mag reizen!

Curriculum Vitae

Nienke Elisabeth van Trommel zag in Rotterdam op 28 juni 1975 per keizersnede het eerste levenslicht. Tijdens het hechten dronk haar moeder een glaasje champagne en maakte haar vader foto's. Ze bracht haar jeugd door in Reeuwijk en ging naar de Vrije School te Gouda. Na het in 1994 behalen van het eindexamen aan het Coornhert Gymnasium te Gouda, startte zij met de studie geneeskunde aan de Universiteit van Amsterdam. In 1995 verbleef ze voor een verpleeghulpstage in het Diaconessenhuis te Paramaribo, Suriname. In 2002 werkte ze voor haar "oudste co-schap" in Murambinda Mission Hospital, Zimbabwe. Tijdens haar studie hield ze zich ook met extra-curriculaire activiteiten bezig zoals het organiseren van theaterproducties, de verkoop van bitterballen in het Holland Heineken Huis bij de olympische spelen in Sydney in 2000 en nam ze deel aan de Maccabia.

Na het behalen van het artsexamen in 2002, werkte ze ter voorbereiding van een verblijf in de tropen aansluitend als arts-assistent niet in opleiding (AGNIO) op de afdeling kindergeneeskunde in het Slotervaart Ziekenhuis te Amsterdam en op de afdeling chirurgie in het Spaarne Ziekenhuis te Haarlem.

Van september 2003 tot en met februari 2004 werkte ze als AGNIO obstetrie en gynaecologie in het UMC Utrecht. Van maart 2004 tot en met september 2005, werkte zij als arts-onderzoeker op het gebied van molazwangerschappen voor de afdelingen obstetrie en gynaecologie en chemische endocrinologie in het UMC St Radboud te Nijmegen (promotores prof. dr. CGJ Sweep en prof. dr. LFAG Massuger, co-promotor dr. CMG Thomas). In oktober 2005 startte zij binnen het Nijmeegse opleidingscluster (opleider prof. dr. DDM Braat) als arts-assistent in opleiding tot specialist (AIOS) op de afdeling gynaecologie en verloskunde in het Catharina Ziekenhuis te Eindhoven (opleider dr. THM Hasaart).

Samen met haar moeder Ineke Sipkema en Theo Dekker, vormt ze het bestuur van de stichting "Vrienden Murambinda Hospital" die zich tot doel heeft gesteld dit ziekenhuis in Zimbabwe en de lokale bevolking te ondersteunen. Sinds 1999 schrijft ze op freelance basis met name medische artikelen voor NRC Handelsblad.

Nienke is verloofd met Adam Swets. Samen hebben ze een dochter Pi.