



Review

The quagmire of hCG and hCG testing in gynecologic oncology

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Abstract

Few molecules have created so much confusion as the hCG series of molecules. Here we present a comprehensive review of hCG as a tumor marker, of hCG and cancer and modern perspectives on the multiplicity of hCG, and its appropriate use in the management of gynecological malignancies and gestational trophoblastic diseases.

The complexity of hCG is better understood. There is regular hCG produced by syncytiotrophoblast cells in pregnancy and by hydatidiform moles. This hormone functions to advance uterine angiogenesis and promote progesterone production by corpus luteal cells. Hyperglycosylated hCG is an independent molecule to regular hCG, it varies significantly from hCG in structure and is produced by cytotrophoblast cells. It is an autocrine or cytokine which functions to promote growth, invasion and malignancy. It is an absolute marker of invasive mole and invasive choriocarcinoma. Hyperglycosylated hCG is invaluable in the diagnosis and management of gestational trophoblastic diseases. The free β -subunit of hCG is also an autocrine or cytokine and is produced in most gynecologic malignancies. Serum free β -subunit or its urinary degradation product β -core fragment is produced by 68% of ovarian, 51% of endometrial and 46% of cervical malignancies. Free β -subunit enhances growth and invasion in all these malignancies leading to poor prognosis. Free β -subunit and β -core fragment are good tumor markers for these malignancies.

There are few circumstances that create more confusion than the patient presenting with persistent low positive hCG results in the absence of pregnancy and absence of obvious malignancies. The series of hCG molecules as tumor markers will be reviewed to help the clinician best diagnose these often difficult clinical problems.

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¹ The USA hCG Reference Service is primarily a testing and consulting service for GTD and problems with hCG testing. A fee is charged for the cost of the assays, review of the clinical scenario, interpretation of the provided data in relation to the results and clinical experience of the lab which is formalized in a report for the patient's record. Further information regarding this service can be found at www.hcglab.com.

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Introduction

The science of human chorionic gonadotropin (hCG) has advanced a long way in the last 10 years. We now understand that hCG is not one molecule, but rather a group of three different molecules with separate functions not just in pregnancy and gestational trophoblastic diseases, but in other aspects of gynecologic oncology [1–3]. Regular hCG is the hormone produced by syncytiotrophoblast cells during pregnancy and non-invasive hydatidiform mole promoting progesterone production by the corpus luteum [4], and angiogenesis in the spiral arteries of the myometrium during pregnancy [5–8]. Hyperglycosylated hCG (hCG-H) is a structural variant of hCG produced by cytotrophoblast cells during invasive disease, invasive mole or choriocarcinoma [1,3,9,10]. It is the principal form of hCG produced in the first 2 weeks of gestation [9,11–13]. While both hCG and hyperglycosylated hCG have 4 O-linked and 4 N-linked sugar side chains, hCG-H has 1.5-fold larger N-linked structures and 2-fold larger O-linked structures [14–17]. Xenograft studies using choriocarcinoma cell lines in nude mice show that hCG-H is essential for either invasion or malignancy to occur [1,18–22]. It is inferred that hCG is both a marker of trophoblast invasion and the signal for invasion, whether invasion as in implantation of pregnancy or invasive mole or choriocarcinoma [1,9–11,19,22]. hCG is also seen in advanced gynecologic malignancies which de-differentiate, leading to the expression of the β -subunit of hCG. The free β -subunit of hCG (free β) or its degradation product β -core fragment can be detected in the blood and urine of over 50% of gynecological malignancies [3,18,23,24]. All advanced malignancies when biopsied can be stained for free β [25,26]. Free β directly promotes growth and invasion in all non-gestational malignancies leading to poor prognosis [27–29].

Understanding the complexity of the three hCG molecules (regular hCG, hCG-H and hCG β) as well as the nuances of hCG testing is challenging yet critical to practice management in gynecologic oncology. Patients are referred with persistent low levels of hCG and no apparent gestational history [30]. The differential diagnosis of patients with positive hCG tests must include false positive hCG (phantom hCG) and pituitary hCG in the menopausal patient, a solid malignancy or a rare gestational trophoblastic neoplasm (GTN) occurring from a remote gestational event. This review article will comprehensively review the three hCG related molecules as markers of gestational and non-gestational malignancy, the biochemical

impact on growth and invasion and the quagmire of hCG testing in gynecologic oncology. Much of the clinical and biomarker data is based on the published experience of the USA hCG Reference Service encompassing over 500 referred cases of gestational trophoblastic disease and other causes of measurable hCG. The USA hCG Reference Service is a clinical consulting service dealing with interpreting difficult hCG results and the biochemical diagnosis of gestational trophoblastic disease diagnosis. Clinical outcomes of patients with persistent low levels of hCG, false positive hCG, pituitary hCG and quiescent gestational trophoblastic disease are presented.

Management and diagnosis of gestational trophoblastic diseases

The ability to correctly diagnose gestational trophoblastic disease (GTD) lies in the understanding of the hCG molecules and the respective role of these molecules in invasion and metastases. The ability to measure the pertinent molecules (hCG, hCG-H, free β) in blood and urine has led to a better understanding of the changing spectrum of GTDs. Knowledge of biomarkers allows the best clinical decisions of when to and when not to initiate therapeutic interventions.

Complete and partial hydatidiform mole

A complete hydatidiform mole is the product of an empty ovum with no female haploid set, a diandrogenous fertilization leading to a diploid gestation composed entirely of hyogromatous cysts of villous placental tissue [31,32]. A partial hydatidiform mole originates in an ovum with an inactive haploid set, a diandrogenous fertilization leads to a triploid gestation composed of villous trophoblastic and fetal elements [33]. The two types of molar gestations are best differentiated cytogenetically [34]. The occurrence of hydatidiform moles in the United States is approximately 1 in 900 pregnancies [35].

Patients with a hydatidiform mole commonly present with unusually high serum hCG results (Table 1), partly due to the mass of trophoblast tissue present. While a normal pregnancy averages a serum hCG concentration at the 8–11 week peak of 93,598 mIU/ml (range, 27,300 to 233,000) [36,37], a hydatidiform mole often presents with much higher hCG levels and can reach >3,000,000 mIU/ml [37,38]. Laboratory errors can occur in cases with levels of hCG above 500,000 mIU/ml due to the “Hook Effect” [37]. Since most hCG test are limited in sensitivity to the

Table 1
USA hCG Reference Service experience, concentration of hCG, hCG-H and hCG free β (hCG β) in serum samples in GTDs and non-gestational malignancies

Source	<i>n</i>	Total hCG mIU/ml median (range)	hCG-H mIU/ml molar ^a % of total hCG \pm SD	hCG β mIU/ml molar ^a % of total hCG \pm SD
Complete hydatidiform mole (prior to evacuation)	30	192,995 (24,160–3,704,084)	4.9 \pm 2.1%	7.1 \pm 20% ^b
Partial hydatidiform mole (prior to evacuation)	21	48,900 (11,600–220,114)	3.6 \pm 1.7%	5.8 \pm 22% ^b
Invasive mole, recurrent mole (at commencement of therapy)	72	869 (24–30,255)	30 \pm 35%	7.7 \pm 11%
Choriocarcinoma/GTN (at time of diagnosis)	102	16,298 (5.2–597,000)	61 \pm 41%	7.8 \pm 8.4%
Minimally invasive choriocarcinoma (at time of diagnosis) ^c	11	370 (13.1–2,364)	21 \pm 14%	8.3 \pm 6.2%
Quiescent GTD (at time of diagnosis) ^c	101	22 (1–212)	0.31% \pm 2.16%	2.7 \pm 8.4%
PSTT (at time of diagnosis) ^c	21	30 (1–231)	7.1 \pm 13%	61 \pm 21%
Other gynecologic malignancies (at time of diagnosis) ^c	14	33 (0.5–474)	0.55 \pm 1.3%	91 \pm 11%

SD is standard deviation.

^a Values are measured in molar units and converted to equivalents of hCG, assuming that 1 ng/ml hCG is 11 mIU/ml (using hCG 1st RR standard).

^b As published by Van Trommel et al. [43].

^c As measured by USA hCG Reference Service, indicated diagnosis confirmed at biopsy by referring center.

pregnancy hCG range (peak 93,598 mIU/ml) the “Hook Effect” shows very low hCG results (i.e. 1–100 mIU/ml) when an extremely high hCG concentration is present due to consumption of all antibody binding sites [37,38]. A suspected diagnosis of hydatidiform mole must be communicated to the lab with the request for a 1:1000 dilution. Generally, an hCG result of >100,000 is indicative of hydatidiform mole [39,40].

In our experience at the USA hCG Reference Service [10,41] with 30 complete mole cases pre-evacuation, the median hCG was 192,995 mIU/ml and the range was 24,161 to 3,704,084 mIU/ml, 67% of pre-evacuation results were >100,000 (Table 1). Regular hCG is the predominant measurable hCG secreted by the syncytiotrophoblasts in complete mole cases. hCG-H accounts for just 4.9 \pm 2.1% of the measured hCG in these cases. Partial moles produce less regular hCG than complete moles. In 21 partial moles the median hCG was 48,900 mIU/ml with a range of 11,600 to 220,114 mIU/ml, only 14% of cases exceeded 100,000 mIU/ml. Only 3.6 \pm 1.7% of the total hCG of partial moles were hCG-H.

After dilation and curettage of a hydatidiform mole, patients are followed weekly until hCG becomes undetectable (<1 mIU/ml), then monitored monthly for at least 6 months [39,40,42]. It is advised that these patients be placed on reliable contraceptives and avoid pregnancy for one year [42,43], in that a pregnancy may hide a recurrence of GTD or invasive hydatidiform mole.

Invasive mole

The biggest danger with hydatidiform mole is recurrence, persistence or invasion by molar tissue which can invade the uterus and other organs. This disease is driven by unregulated hCG-H, the pregnancy implantation signal. Invasive mole is a malignancy comprising villous trophoblast cells. Many physicians consider hysterectomy in women first presenting with hydatidiform mole not interested in having further children. This avoids all chances of invasive disease. Approximately 29% of complete hydatidiform mole cases [44] and 6.6% of partial hydatidiform mole cases [45] will need treatment for molar invasion. Approximately 3% of complete mole cases

and 0.1% of partial mole cases will develop choriocarcinoma [46,47].

Invasive mole is marked by the significant presence of hyperglycosylated hCG (Table 1). When invasive moles are detected, hCG-H comprises 30 \pm 35% of total hCG thus making them distinguishable from non-invasive moles. The increase in the proportion of hCG-H represents an increase in the proportion of invasive cytotrophoblast cells, the invasive cells of invasive mole. Clinically [32,33,39,40,42–45], an invasive mole is diagnosed by one of the following behaviors of regular hCG measurements: 1) by a plateau in the decline of regular hCG over three weeks following evacuation of a mole or 2) by an hCG decline that has not fallen below 20,000 mIU/ml over a period on four weeks, or 3) by three rising weekly hCG values after prior undetectable levels. An invasive mole is best marked by hCG-H, with hCG-H accounting for more than 20% of the total hCG. This correlates to the first day of a plateau in an hCG decline, or on the first day of newly increased hCG [10,30] permitting more rapid treatment of invasive disease. Detection of an invasive mole dictates the need for single agent chemotherapy (methotrexate or actinomycin-D) to treat malignant disease. Treatment response assessment requires weekly monitoring of hCG. An hCG decline to <1 mIU/ml represents the disappearance of all trophoblastic tissue. hCG is an optimal tumor marker with 100% sensitive and specificity, perfectly correlating to the mass of trophoblast cells. Recurrence surveillance is required for 6 months to 1 year after completion of therapy with recommended reliable contraception.

The absolute amount of regular hCG and the pattern of change in hCG can help to distinguish invasive mole from a recently described entity called quiescent GTD. Invasion most commonly occurs as a plateau in the hCG decline at a level greater than 10,000 mIU/ml. It can also be seen as an increase in hCG occurring above 200 mIU/ml, or by a new increase in hCG occurring in the months following regression of disease (hCG <1 mIU/ml). In the experience of the USA hCG Reference Service, when a plateau occurs between 1 and 200 mIU/ml quiescent GTD is an important diagnosis to consider that has significant impact on the decision whether to treat the disease.

Choriocarcinoma and GTN

Choriocarcinoma is a malignancy of transformed cytotrophoblast cells. The transformation limits cytotrophoblast differentiation to syncytiotrophoblast cells so that most cases involve predominantly malignant cytotrophoblast cells. Cytotrophoblast cells produce hCG-H which drives growth and invasion. As much as 100% of the hCG produced in choriocarcinoma can be hCG-H (Table 1) [1,3,10]. Choriocarcinoma rarely follows normal pregnancy (1 in 20,000 live births) [48], and occurs at a rate of 1 in 33 complete hydatidiform moles or 1 in 1000 partial moles [44–46]. Choriocarcinoma is much more common among Asian people, tribal people in the Philippines, Indonesia and Central Africa [33,49]. Choriocarcinoma cases commonly first present with lung and brain metastasis, with hCG levels extending to greater than 5,000,000 mIU/ml [50]. In the USA hCG Reference Service experience with 82 referred cases, a less dramatic range of hCG was observed with the highest hCG measuring 597,000 mIU/ml (Table 1).

Choriocarcinoma is unique and different from all other hCG producing diseases in that it is marked by a high percent hCG-H of total hCG ($61 \pm 41\%$, Table 1). The high concentrations of hCG-H in choriocarcinoma can make this the most aggressive and invasive of all malignancies. While extremely fast growing, it responds exceeding well to chemotherapy. Five year survival rates for choriocarcinoma with chemotherapy range from 81% to 91% [50,51]. There is no better example of a reliable tumor marker than hCG in choriocarcinoma, 100% sensitivity with 100% specificity with hCG exactly monitoring the trophoblast mass. It is important to follow a patient weekly for hCG during therapy, and then monitor a patient biweekly for 6 months and monthly for 2 years following achievement of a negative hCG test to survey for recurrence of disease. While some centers will

start with methotrexate and actinomycin D before reverting to multi-agent chemotherapy such as EMA-CO, others find going straight to EMA-CO preferable [50,51]. A multidisciplinary approach may be required when distant metastases occur as stabilization with radiation therapy or surgical intervention (such as neurosurgery) may help improve morbidity. Measurement of hCG in the cerebral spinal fluids is invaluable to assess brain metastases. It is important to consider that 1 in 20 spontaneous abortions may be a hydatidiform mole. Rising hCG in these cases in the months following spontaneous abortion may be an invasive mole (with villous histology), or may be a choriocarcinoma. Invasive GTD is primarily treated by chemotherapy, with little or no surgery. As such, pathology is rarely performed. The generic diagnosis GTN is used to describe what is likely choriocarcinoma without histology confirmation.

Quiescent GTD (qGTD)

Quiescent GTD (qGTD) is a syndrome involving inactive or benign GTD. It comprises predominantly highly differentiated syncytiotrophoblast cells. It has minimal stem cytotrophoblasts therefore it lacks hCG-H the invasive signal (Table 1). Quiescent GTD was described by the USA hCG Reference Service in 2002 [52–54], its existence and tumor marker parameters were later confirmed independently in the USA and UK [55,56]. Fundamentally, it is an inactive form of invasive mole, GTN or choriocarcinoma existing when regular hCG levels fall below 220 mIU/ml. It was discovered during the evaluation of what appeared to be false positive hCG cases in 2000 and 2001. Seventeen of these cases had a recent history of choriocarcinoma or hydatidiform mole which were reported resistant to multiple chemotherapy protocols given at the time of the hCG plateau. The hCG was confirmed to be real and not

Table 2
Summary of quiescent GTD cases ($n=101$) from USA hCG Reference Service

Diagnosis at recurrence	# cases	Mean hCG at referral mIU/ml (range)	hCG-H at referral (n)	Recurrence by hCG-H (%)	Mean %hCG-H at recurrence (range)	Therapy received for low level hCG (n)
SAB	25	22 (7–90)	<2% (25)	4 (16)	39 (14–63)	Mtx (1)
Partial mole	9	42 (10–102)	<2% (8)	2 (22)	33 (29–37)	Mtx (5) EMA-CO (1)
Complete mole	46	37 (2–212)	10% (1)	10 (22)	48 (15–100)	Mtx or ActD (15) Mtx; ActD±surg (2) Mtx or ActD+surg (2) EMA-CO (2) Mtx, ActD, EMACO (2) Mtx, EMACO, Hyst (1) EMA-EP (1)
GTN	11	46 (6–144)	<2% (11)	1 (9)	14	Mtx (3) Mtx; ActD (2) Mtx, ActD, Hyst (1) Hyst, Mtx, EMACO (1) EMA-CO (2) Hyst (1)
Choriocarcinoma	10	32 (1–117)	<2% (10)	6 (60)	37 (15–63)	Mtx (1) Taxol (1) EMA-CO, EMA-EP (1)

SAB is spontaneous abortion assumed to be partial hydatidiform mole. Therapy abbreviations: Mtx, methotrexate; ActD, actinomycin D; EMA-CO, etoposide, Mtx, ActD, cyclophosphamide, vincristine; EMA-EP, etoposide, Mtx, ActD, etoposide and cisplatin; hys, hysterectomy.

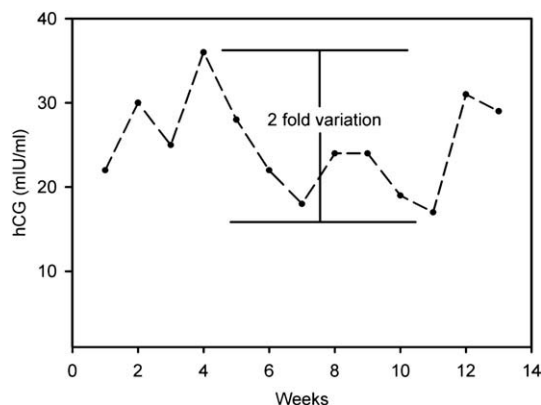


Fig. 1. Patterns of hCG in qGTD. Low levels of persistent hCG measurements are seen in the quiescent phase of GTD. Two-fold variations in lab values over time are not uncommon and do not signify activation of invasive disease.

false positive. No hCG-H was detected (<2%) in these cases indicating the absence of invasive disease. Histology and hCG staining following hysterectomy in two of these cases showed only differentiated syncytiotrophoblast cells. The lack of root cytotrophoblast cells explained the non-invasive status of this syndrome thus called qGTD. Chemotherapy is ineffective during this quiescent phase [10].

Table 2 summarizes the biochemical results of 101 qGTD cases evaluated by our group. qGTD can follow any gestational trophoblastic event from evacuation of complete or partial mole through to chemotherapy treated hydatidiform mole or choriocarcinoma. The majority of cases of qGTD cases in this series followed evacuation of complete hydatidiform mole (46%), but qGTD was also seen after chemotherapy treatment of GTN or choriocarcinoma (21%), evacuation of confirmed partial mole (9%) and spontaneous abortions assumed due to hydatidiform mole (25%). In these cases, the total regular hCG results at the time of persistence or plateau were less than 220 mIU/ml (range 1–212 mIU/ml). In the vast majority of cases, no hCG-H could be detected. In all cases, the persistent low hCG varied by as much as 2-fold over time, while still representing quiescent GTD (Fig. 1). For this reason it is essential to show multiple consecutive rising hCG values to illustrate the onset of invasive disease (or rising hCG-H >20%). It is noteworthy that some cases of qGTD persisted for as long as 2 years, although the majority resolved within 6 months. While 97 cases had no detectable hCG-H, 4 cases produced measurable hCG-H, accounting for 5.0%, 5.3%, 10%, and 10% or total hCG (Table 2). It is assumed that a minimal amount of cytotrophoblast cells was needed to replace syncytiotrophoblast cells producing this small amount of hCG-H like the $4.9 \pm 2.1\%$ hCG-H (Table 1) seen in non-invasive molar disease.

Many of the cases in this series reported prescribed chemotherapy for assumed invasive mole or persistent disease. Half of the 101 cases referred to the USA hCG Reference Service received needless chemotherapy or hysterectomy for assumed active disease (Table 2). There were no cases in which the therapy fully suppressed disease (hCG <1 mIU/L). The failure of chemotherapy to eradicate hCG producing trophoblasts is likely due to the lack of proliferation of the quiescent

cells. The failure of hysterectomy was attributed to the possible extra-uterine source of the cells. One patient died due to complications of the chemotherapy regimens. We conclude that chemotherapy and surgery should be avoided in cases of quiescent GTD. We also infer that it is important to consider quiescent GTD when a mole, GTN or choriocarcinoma case demonstrates an hCG plateau below 215 mIU/ml before initiating chemotherapy. If an hCG-H test is not available, then the persistence of total hCG results, permitting a 2 fold natural variation (Fig. 1), should be monitored and considered as evidence for inactive or static disease. It should be noted that <212 mIU/ml represents a miniscule trophoblast cell mass, and that >2000 mIU/ml of hCG in serum is required before a tumor could be seen by MRI [57].

Most cases of qGTD resolve with the spontaneous resolution of the cell mass [10]. As shown in Table 2, 22% of qGTD cases progressed to active disease, the majority of cases (60%) occurring in patients previously treated for choriocarcinoma. Rising hCG-H became evident prior to a significant rise in total hCG, which was the first evidence of active disease [10]. Considering the 22% risk of progression to active disease, it is critical to monitor hCG regularly in quiescent GTD cases with reliable contraception until hCG has been undetectable for at least 6 months.

Minimally invasive choriocarcinoma

The USA hCG Reference Service has collectively consulted on 11 unusual cases of invasive trophoblastic disease (Table 3).

Table 3
Minimally invasive choriocarcinoma cases referred to the USA hCG Reference Service

Case	hCG at referral mIU/ml	hCG-H %	History	Chemotherapy during minimally invasive disease
1	171	27	C	Mtx, ActD, EMA-CO
2	149	16	CM	None
3	305	35	GTN	None
4	157	18	GTN	None
5	626	12	C	Mtx, ActD, EMA-CO, EMA-EP, BEP, ICE, Xel
6	725	1.3	GTN	Mtx, ActD, EMA-CO
7	435	38	GTN	None
8	27	13	C	Mtx, ActD, EMA-CO, EMA-EP, Tax, VIP, BEP
9	2362	39	GTN	None
10	136	34	GTN	Mtx, ActD, EMA-CO, EMA-EP, Tax, VIP, BEP, Cyx
11	13	12	CM	None

In these 11 cases hCG rises slowly, requiring more than one month to double, indicating minimally progressive disease. Two types of case are described. New cases with no chemotherapy history, low proportion of hCG-H and very slowly progressive disease, and chemorefractory cases with history of failure to appropriately respond to any chemotherapy, with a low proportion of hCG-H and very slowly progressive disease. Mtx, methotrexate; ActD, actinomycin D; EMA-CO, etoposide-Mtx-ActD and cyclophosphamide-vincristine; EMA-EP, etoposide-Mtx-ActD and etoposide-cisplatin; Tax, taxol; BEP, bleomycin-ifosfamide-cisplatin; ICE, ifosfamide-carboplatin-etoposide; Xel, xelota; Cyx, cytoxan; VIP, vinblastine-ifosfamide-cisplatin. History abbreviations: C, choriocarcinoma; CM, complete mole; GTN (no histology).

These have been marked by a low proportion of hCG-H ($21 \pm 14\%$) concordant with extremely slow growth demonstrated over serial hCG measurements, with hCG taking more than one month to double in magnitude. Five of these cases were treated with up to 8 chemotherapy regimens (Table 3). These were considered by physicians as chemorefractory cases because they failed to respond appropriately to any of the chemotherapy regimens. Case 5 demonstrates an unusual pattern of intermittent response to chemotherapy in a patient with a history of choriocarcinoma (Table 3). Multi-agent chemotherapy suppressed hCG from 356 mIU/ml to 44 mIU/ml. Over the following 5 months, the hCG rose again to 1936 mIU/ml. The next regimen suppressed the hCG back down to 210 mIU/ml. Over the following 3 months the hCG again rose to 832 mIU/ml. The next regimen was given repeating the unsuccessful regression/progression pattern ultimately through 7 different regimens. We question whether the patient really is resistant to any chemotherapy regimen, or whether the trophoblastic malignancy is failing to respond appropriately to chemotherapy because of a low proportion of hCG-H made by low levels of malignant cytotrophoblasts with an inherent slow growth rate. These cases are, and will continue to be, difficult to treat. As it appears that much higher levels of hCG are needed before a tumor is evident [47], one must weigh the risk benefit ratio behind the toxicity of multi-drug chemotherapy versus the impact of slowly advancing small volume disease doing similar harm. We have seen 3 cases progress without chemotherapy to advanced disease with evident metastases at which time response to chemotherapy ensued. In these cases, cautious observation may be the best course of action until active disease is determined and then salvaged with chemotherapy. Further case experience will help better define this state of trophoblast disease.

Placental site trophoblastic disease (PSTT)

PSTT is a malignancy of extravillous cytotrophoblast cells. It is a very rare condition accounting for 1 in approximately 40,000 pregnancies. It also has a poorer prognosis than choriocarcinoma [57,58]. The five year survival rate for PSTT is 74% [57], compared with 91% for choriocarcinoma [51]. Classically it presents as a tumor in the uterus, which is readily removed by hysterectomy. Not all cases produce hCG [57,58]. Histologically the appearance of choriocarcinoma and PSTT cells are similar. The majority of the PSTT cases identified biochemically by our service were erroneously diagnosed as choriocarcinoma. Expert pathology review is critical to make certain the diagnosis is correct.

The range of regular hCG in PSTT cases, if present, varies substantially, reported between 6 to 107,000 mIU/ml. The USA hCG Reference Service consulted on 7 PSTT histologically proven cases. It was noted that all cases primarily produced the free β -subunit of hCG (hCG β) rather than regular hCG or hCG-H, even though these tumors involve cytotrophoblast cells. As discussed in the preceding section, hCG β acts like hCG-H as a promoter of cancer cell growth and invasion. It was suggested that the proportion of hCG β ($>30\%$ of total hCG) may be an absolute marker for discriminating choriocarcinoma and PSTT

[59]. Since this time the USA hCG Reference Service used the hCG β tests to identify 14 further cases of PSTT. hCG β and its urine degradation product β -core fragment successfully discriminated choriocarcinoma and PSTT [60,61]. Reviewing all 21 cases evaluated by our service to date, the median regular hCG was 30 mIU/ml (range 1–231 mIU/ml). The proportion of hCG β averaged 61% of total hCG (Table 1).

PSTT requires a different treatment protocol to GTN and choriocarcinoma so a correct diagnosis is paramount. The hCG β subunit test is widely available at clinical laboratories for Down syndrome screening (results presented as multiple of median). Special arrangements will need to be made to obtain a concentration for cancer assessment. Results are normally determined in nanograms per milliliter. One nanogram per milliliter is equivalent to 18 mIU/ml for conversion calculations.

Measuring hCG, hCG-H and free β -hCG in GTDs

Although hCG is the prototype biomarker for GTD, marketed hCG tests are designed for diagnosing pregnancy and its complications (such as Down syndrome) and not for diagnosing or managing cancer. Although regular hCG tests have been used in this manner for decades, there are subtle nuances to the tests that must be appreciated in order to best interpret them for cancer management. Our service has standardized assays for hCG, hCG-H and hCG β in serum and in urine and can serve as a point of consultation for any difficult hCG clinical problem.

The majority of modern clinical commercial hCG assays require an intact C-terminal peptide on the hCG molecule or its β -subunit for appropriate detection [62,63]. This is fine for pregnancy detection however the C-terminal peptide is often cleaved by macrophages in GTD and cancer [63]. The USA hCG Reference Service uses the Siemens Healthcare Diagnostics Immulite 1000 test to measure total hCG as its specificity does not involve the C-terminal peptide. This assay measures hCG, nicked hCG, hyperglycosylated hCG, hCG β , hyperglycosylated hCG β and hCG and hCG β missing the C-terminal peptide equally making it particular appropriate for serum and urine total hCG measurements for pregnancy and cancer applications. When monitoring a cancer or GTD, it is important to know which hCG test the lab is using. If they are not using the Immulite 1000 test it is suggested that the samples be evaluated at an external laboratory.

The hCG-H test discriminates invasive, minimally invasive and inactive (quiescent) disease, assessing the aggressiveness of the malignancy [10,64]. It is an absolute tumor marker with 100% sensitivity and 100% specificity. As shown in Table 1, hyperglycosylated hCG is $61 \pm 41\%$ of the hCG-related molecules produced in invasive choriocarcinoma (hCG and tumor mass doubling 2–5 days), $30 \pm 35\%$ of that produced in invasive moles, $21 \pm 14\%$ of that produced in minimally invasive choriocarcinoma (hCG and tumor mass doubling >20 days), and $0.31 \pm 2.16\%$ in quiescent or inactive GTD (hCG not doubling). Clearly, the proportion of hyperglycosylated hCG is a powerful indicator of aggression, and can direct the choice and urgency of chemotherapy. The hCG-H test is a

Table 4
Use of urine hCG β -core fragment as a tumor marker for detection of malignancies [67]

Malignancy	Patients	Sensitivity (>3 fmol/ml)
Healthy no history of malignancy	97	9.3%
Healthy benign disease	159	6.9%
Total	256	7.8%
Ovarian cancer	106	68%
Cervical cancer	89	46%
Endometrial cancer	110	51%
Total	305	57%

critical diagnostic assay used by our reference service. It is also available at Quest Diagnostics Inc., under order code 4823. A new hCG-H test will be released by Siemens Healthcare Diagnostics in 2009.

hCG β is an important diagnostic test for discriminating PSTT and choriocarcinoma. It is a critical test used by our service. The hCG β subunit tests is widely available at clinical laboratories as part of the Down syndrome triple screen which must be converted to mIU/ml needed to allow determination of percent against total hCG. Special arrangements will need to be made with the laboratory in order to obtain a concentration for cancer assessment.

Free β and β -core fragment tumor markers in gynecologic malignancies

It has long been known that most non-trophoblastic malignancies, particularly gynecologic malignancies, produce free β and its terminal urinary degradation product β -core fragment. Numerous reports note use of free β -subunit and β -core fragment as tumor markers in germ cell and other ovarian, cervical, vaginal, endometrial and uterine malignancies [29,59,65–80]. While free β is not a perfect or specific marker for these non-trophoblastic malignancies it is an excellent general gynecologic tumor marker, and a marker of poor prognosis [27,28,81]. Serum free β and urine β -core fragment have both proven useful in the detection and management of a wide range of gynecologic malignancies [29,59,65–80]. As shown in summary Tables 4 and 5, 57% of 305 gynecologic cancer cases tested for urine β -core fragment and 37% of 315 malignancy cases tested for serum free β were appropriately detected. The highest sensitivity for urine β -core fragment and serum free β is observed with ovarian malignancies

Table 5
Use of serum free β -subunit as a tumor marker for detection of malignancies [28]

Malignancy	Patients	Sensitivity (>3 fmol/ml)
Ovarian cancer	150	38% average of 3 reports
Cervical cancer	60	37% average of 2 reports
Endometrial cancer	55	33% average of 2 reports
Vulvar	50	38% single report
Total	315	37% average of 8 reports

Averages are determined from multiple reports by combining total cases positive for free β -subunit by total cases tested.

Table 6
Use of urine hCG β -core fragment as a tumor marker for detection of gynecological malignancies (52 cervical, 33 endometrial, 71 ovarian and 14 other gynecological malignancies), correlation of sensitivity with stage of malignancy [75]

Stage	Patients	Sensitivity (>3 fmol/ml)
New tumor, pretherapy, stage I	31	23%
New tumor, pretherapy, stage II	15	60%
New tumor, pretherapy, stage III	54	70%
New tumor, pretherapy, stage IV	24	100%
Recurrence of disease	46	74%

(sensitivity 68% and 38%, respectively), because of their more advanced stage, while lower sensitivity is recorded in cervical and endometrial malignancies (sensitivity 46 and 37%, and 51 and 33%, respectively). Measured free β -subunit or β -core fragment in a patient with malignancy correlates with grade and stage of tumor [27,67,80–82]. As shown in Table 6, β -core detects 100% of 24 stage IV gynecological malignancies, and 74% of disease recurrence. Both free β and urinary β -core fragment accurately record tumor mass, tumor regression and recurrence of disease. Fig. 2 shows the parallel use of CA125 and β -core fragment in monitoring progress in a case of serous cystadenocarcinoma of the ovary. Serum free β and urine β -core fragment work well as complementary markers with CA125 [27,67,80,81]. Serum free β and urine β -core fragment (or serum total hCG) are the markers of choice in managing patient with placental site trophoblastic disease (100% sensitivity) and with ovarian germ cell malignancies (70% sensitivity) [59,60].

Persistent low levels of hCG — a diagnostic dilemma

Perhaps the most difficult problem for clinicians to resolve is the meaning of a persistent low level hCG within the context of

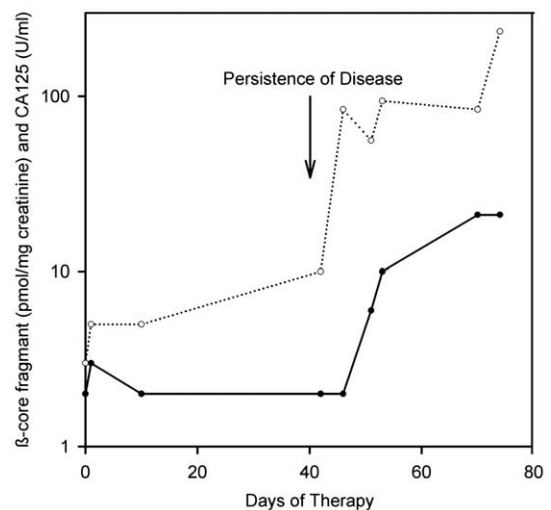


Fig. 2. Patient with serous cystadenocarcinoma of the ovary monitored by serum CA125 (---) and urine β -core fragment (—). Patient had been treated for ovarian cancer by surgery and chemotherapy, graph shows appearance or persistent disease.

the specific clinical scenario. A low level hCG in a patient with history of GTD or choriocarcinoma may lead to quite a different diagnosis than a low level hCG determined on preoperative screening in a 45 year old woman awaiting a kidney transplant. However, both can result in tragic consequences for the patient if the appropriate evaluation and diagnosis is not made. It is important to determine if the hCG measured is real or represents an actual pregnancy, gestational disease, or a normal pituitary hCG. This section will guide the clinician in the evaluation and interpretation of the low level hCG test result. False positive hCG test results and/or physiologic pituitary hCG is commonly found in women with a history of GTD or choriocarcinoma. Unless a tumor is evident, it is essential to exclude these possibilities before considering treatment for presumed disease.

False positive hCG

All modern hCG tests use two antibodies to distant sites on the hCG molecules. When hCG is present it is immobilized by one, the capture antibody, and labeled by the second, a tracer antibody with an enzyme, lanthanide or a radioactive label. The capture antibody–hCG–tracer antibody “sandwich” is formed, excess is washed and the amount of immobilized label is measured which is in direct proportion to the amount of hCG joining the sandwich together. The two antibodies can be mouse, goat, sheep or rabbit polyclonal or monoclonal antibodies. Humans exposed to animal parts or bites can develop human antibodies against animal antibodies (HAAA). Humans can naturally generate human anti-human immunoglobulin antibodies that can cross-react with and bind to animal antibodies. These are called heterophilic or cross-species antibodies. Humans with recent exposure to mononucleosis can be prone to develop HAAAs from digested meats; those with IgA deficiency syndrome have false positive assay problems due to heterophilic antibodies [83]. The hCG assay presents unique problems with false positive tests. Each human antibody is bivalent so if an HAAA or heterophilic antibody is present in a person’s serum it can bind and link together the capture and tracer antibodies in a test making a capture antibody-heterophilic antibody-tracer antibody sandwich of capture antibody HAAA-tracer antibody sandwich and cause an elevated test result or false positive hCG result. These false positive test results, in the absence of pregnancy, have led to many men and women wrongly being diagnosed with cancer resulting in needless surgery and chemotherapy. (Table 7) [84–93]. To avoid false positive tests, manufacturers incorporate animal serum and non-specific animal antibodies into all their test ingredients, with immobilized antibody, tracer antibody and other components. A big excess of non-specific antibodies overwhelmingly binds and eliminates the interference of heterophilic antibodies and HAAA in human serum samples.

False positive hCG tests were a big problem between 1999 and 2002. Hundreds of women were wrongly diagnosed with GTD because of false positive hCG tests [84–93]. Some had a history of GTD or cancer; others were simply diagnosed with cancer on the basis of a positive hCG test in the absence of pregnancy [30]. It was this false positive issue and the resulting

chaos that led to the need for a specialized hCG assay service and to the start of the USA hCG Reference Service [84]. The major problem at this time was the Abbott AxSym total hCG test. This test had serum in the diluent but not in the antibody components [93]. As such, when serum was tested undiluted (no diluent) a large number of false positive tests ensued. Legal action was taken against Abbott Diagnostics Inc. from these young women treated for suspected GTD. Abbott corrected the test in 2003. We have not heard about an Abbott AxSym hCG false positive patient since this time.

In recent years, we have seen just 5–6 false positive serum hCG cases annually stemming from a wide variety of different hCG tests. It appears that every test in extreme circumstances can give a false positive result. Most physicians are now very aware of the hCG false positive problem. Rarely are we referred a false positive hCG case undergoing therapy for GTD. More commonly the service is referred a case assumed to be false positive hCG which is later proven as pituitary hCG or qGTD.

Table 7 reviews the false positive cases examined by the USA hCG Reference Service. Most false positive cases follow an incidental pregnancy test during a physical examination or prior to surgery or other major medical procedures. Ten cases were false positive following a history of GTD wrongly predicted as recurrent disease. The average age of women with a false positive hCG was 34 ± 6.4 . The quantitative hCG in women with false positive hCG (as measured by the assay giving false positive results) ranged from 6.1 to 900 mIU/ml. At the USA hCG Reference Service we were never able to detect any hCG immunoreactivity in urine, since large molecules like antibodies fail to cross the glomerular basement membrane.

Pituitary hCG

The finding of normal physiologic pituitary secretion of hCG at or around the time of menopause is another confusing source of low hCG levels. Why is hCG produced by the pituitary at this time? The USA hCG Reference Service recently examined over 8300 urine samples from women with normal menstrual periods [94]. Low concentrations (> 1 mIU/ml) of hCG were detectable at the time of the luteinizing hormone (LH) peak in 232 of 277 (84%) of menstrual cycles [94]. It was inferred that low concentrations of hCG supplement LH during the menstrual cycle and are part of normal physiology. There is a single LH β -subunit gene buried among the 7 back-to-back hCG β -subunit genes [95,96]. hCG and LH share a common α -subunit. It is assumed that a small amount of hCG β is promoted by gonadotropin releasing hormone (GnRH) alongside LH β -subunit in pituitary gonadotrope cells during normal menstrual cycle physiology. During peri-menopause there is limited feedback inhibition leading to high LH and higher hCG production (serum levels of 1–32 mIU/ml). In patients with a history of oophorectomy and menopause, lack of estrogen production fails to inhibit GnRH, leading to high LH and hCG production. It is inferred that LH and hCG production [93,97] during these states, is completely normal physiology [94,97,98]. As such, a positive pregnancy test in these women is probably not cause for alarm.

Table 7

The USA hCG Reference Service experience with 170 cases with persistent low levels of hCG persisting for 3 months or longer, with no evidence of pregnancy or imaging evidence of tumor

<i>Diagnosis false positive hCG test, 92 cases</i>	
History	
Discovered by incidental pregnancy test	76 of 92
Discovered after treatment of hydatidiform mole	6 of 92
Discovered after treatment of choriocarcinoma/GTN	4 of 92
Cases receiving unnecessary chemotherapy	51 of 92
Cases receiving unnecessary hysterectomy	9 of 92
Duration of persistent hCG results before referral	3 months–2 years
Age (mean±standard deviation)	34±6.4
hCG and related test results at time of diagnosis	
Serum total hCG, mean±standard deviation	102±152 mIU/ml
Serum total hCG, range	6.1–900 mIU/ml
Urine total hCG, mean±standard deviation	<1 mIU/ml
Urine total hCG, range	<1 mIU/ml

Diagnosis pituitary hCG, peri-menopause, 28 cases

History	
Age range, patient has documented oligomenorrhea	33 to 53
Discovered by incidental pregnancy test	22 of 28
Discovered post-evacuation of hydatidiform mole	2 of 28
Discovered after treatment of choriocarcinoma/GTN	2 of 28
Critical surgery postponed due to positive hCG test	6 of 28
Cases receiving unnecessary chemotherapy	2 of 28
Cases receiving unnecessary hysterectomy	0 of 28
Pituitary hCG suppressed with high estrogen contraceptive pill	19 of 28
hCG and related test results at presentation	
Serum total hCG, mean±standard deviation	7.7±5.0 mIU/ml
Serum total hCG, range	2.0–25 mIU/ml
Urine total hCG, mean±standard deviation	4.5±1.9 mIU/ml
Urine total hCG, range	<1–12 mIU/ml

Diagnosis pituitary hCG, bilateral oophorectomy, 14 cases

History	
Age range	33 to 52
Discovered by incidental pregnancy test	10 of 14
Discovered post-evacuation of hydatidiform mole	0 of 14
Discovered after treatment of choriocarcinoma/GTN	3 of 14
Critical surgery postponed due to positive hCG test	3 of 14
Cases receiving unnecessary chemotherapy	1 of 14
Cases receiving unnecessary hysterectomy	0 of 14
Pituitary hCG suppressed with high estrogen contraceptive pill	7 of 14
hCG and related test results at presentation	
Serum total hCG, mean±standard deviation	9.1±5.5 mIU/ml
Serum total hCG, range	2.0–25 mIU/ml
Urine total hCG, mean±standard deviation	5.7±2.1 mIU/ml
Urine total hCG, range	2.2–30 mIU/ml

Diagnosis pituitary hCG, post-menopause, 36 cases

History	
Age range, patient has document amenorrhea	42 to 69
Discovered by incidental pregnancy test	28 of 36
Discovered post-evacuation of hydatidiform mole	1 of 36
Discovered after treatment of choriocarcinoma/GTN	0 of 36
Critical surgery postponed due to positive hCG test	9 of 36
Cases receiving unnecessary chemotherapy	3 of 36
Cases receiving unnecessary hysterectomy	1 of 36
Pituitary hCG suppressed with high estrogen contraceptive pill	21 of 36

Table 7 (continued)

Diagnosis pituitary hCG, post-menopause, 36 cases

hCG and related test results at presentation	
Serum total hCG, mean±standard deviation	11±8.9 mIU/ml
Serum total hCG, range	3.5–32 mIU/ml
Urine total hCG, mean±standard deviation	6.6±1.8 mIU/ml
Urine total hCG, range	2.0–24 mIU/ml

The 92 cases diagnosed as having false positive hCG were based on multiple observations by the USA hCG Reference Service. These are: 1. the presence of hCG immunoreactivity in serum but not urine; 2. negative results in 2 or more hCG tests; 3. the suppression of result by a heterophilic antibody blocking agent. The 78 pituitary hCG cases were defined as peri- or post-menopausal women or women with history of bilateral oophorectomy with low levels of hCG having excluded false positive hCG, non trophoblastic malignancy and choriocarcinoma/GTN or placental site trophoblastic malignancy. All serum and urine hCG values are those determined at the USA hCG Reference Service.

We have observed 2 rare cases [97,98] in which the persistent low level of hCG in a women over 40 came from a tumor rather than the pituitary. We have also observed numerous cases of pituitary hCG in women with a history of GTN, unfortunately persistence of disease was diagnosed or malignancy assumed leading to unnecessary chemotherapy treatment [97,98]. Numerous publications support the normal physiologic production of pituitary hCG hoping to prevent the false diagnosis and treatment of cancer [30,97–99]. Clearly, based on the large number of cases referred, the normality of hCG production in menopausal woman is still not well understood by the medical community.

There is one way to absolutely confirm pituitary hCG. This may be important in a cancer history case. If a patient is given a high estrogen oral contraceptive pill for 3 weeks it should completely suppress GnRH promotion of hCG [30,97–99]. Our reports suggest confirmation of pituitary hCG by high estrogen oral contraception if not medically contraindicated. Hormone suppression of pituitary hCG was confirmed historically in all cases with follow up: 19 of 28 peri-menopausal cases, 7 of 14 bilateral salphingo-oophorectomy cases and 21 of 37 menopausal cases.

Table 7 reviews the USA hCG Reference Service experience with peri-menopause, oophorectomized, and menopausal hCG cases. Peri-menopause was considered in patients having a continuous history of oligomenorrhea. While most women were over 40 years old, the extremes of age range were age 33 to age 53. A total of 28 cases were referred to the USA hCG Reference Service, of these 4 were confounded by a history of GTD. Six cases had to postpone critical surgery because of the positive hCG test and 2 had unnecessary chemotherapy for assumed malignancy. The average serum hCG test was 7.7±5.0 mIU with a range of 2.0 to 25 mIU/ml. Of the 14 oophorectomized women evaluated, three had a history of GTD, three had to postpone critical surgery and 1 received chemotherapy. The average serum hCG in this group was 9.1±5.5 mIU/ml with a range of 2.0 to 25 mIU/ml.

Thirty six women had confirmed menopause as defined by high follicle stimulating hormone levels and consistent amenorrhea, the age range was 42 to 69 years. Only one had a history of GTD. Yet nine of these women had to postpone critical surgery due to a positive pregnancy test, three were treated with chemotherapy and one underwent hysterectomy for

presumed malignancy. The average serum hCG was significantly higher in the menopausal group 11 ± 8.9 mIU/ml, with a range of 3.5 to 32 mIU/ml. Providers need to be aware of the natural physiology or normality of pituitary hCG.

Managing cases presenting with persistent low levels of hCG

The patient presenting with no obvious tumor by CT or MRI and low positive hCG results, persisting for 3 months or longer can be very troublesome. That hCG levels not rising in itself suggest that it is not an active malignancy. The composition of hCG is informative. Primarily hCG-H (can be measured by Quest Diagnostics Inc., 1 ng/ml is equivalent to approximately 11 mIU/ml hCG) suggests a trophoblastic malignancy, and primarily free β (component of the triple screen) suggests a non-gestational malignancy (1 ng/ml is equivalent to 18 mIU/ml hCG). Even though there is only a small chance that persistent low levels of hCG has its roots in a malignancy, it is important to first rule out a tumor.

Secondly, a false positive hCG test must be considered. Is the hCG positive in serum and urine? Ask the laboratory manager to test a urine sample on the same machine used for the serum samples. The laboratory director may frown on testing urine on the machine but research studies show that it is not an obstacle and can be done without a problem [11,53]. Avoid using point of care test, they have pregnancy only specificity and tend to be very insensitive. Are similar results obtained using different manufacturers hCG tests? Get your laboratory manager to send the serum to 2 outside laboratories. If the answer is no to these questions then the patient has false positive hCG due to interfering serum antibodies. The patient should be warned that other test like thyroid test and tumor markers may be unduly elevated due to interfering antibodies.

The other remaining options, and possibly most likely option, after all else has been excluded, are pituitary hCG and qGTD. These can be inferred from patient histories. If patient has history in the past 5 years of GTD or recent history of ectopic pregnancy or spontaneous abortion, qGTD is likely assuming that the pregnancy was an undiagnosed hydatidiform mole. If qGTD is the diagnosis then the serum hCG sample should contain no detectable hCG-H (<0.1 ng/ml of <1 mIU/ml). The other possibility is pituitary hCG. If the patient is older than 35 years and has oligomenorrhea or amenorrhea or had bilateral oophorectomy, then natural pituitary hCG can be assumed. Pituitary hCG can easily be confirmed by suppression with high estrogen oral contraceptives.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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