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Hyperglycosylated hCG in the management of quiescent and chemorefractory gestational trophoblastic diseases

Laurence A. Cole *, Carolyn Y. Muller

USA hCG Reference Service, Department of Obstetrics and Gynecology, University of New Mexico, Albuquerque, NM 87109, USA

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ABSTRACT

Introduction. The literature shows that hyperglycosylated hCG is the invasion stimulus in malignant gestational trophoblastic diseases. The USA hCG Reference Service has characterized 2 gestational trophoblastic disease conditions marked by low proportion of hyperglycosylated hCG. These are quiescent gestational trophoblastic disease, defined as inactive or benign invasive disease, and minimally invasive gestational trophoblastic disease, defined as slow growing or chemorefractory disease with hCG increasing very slowly (doubling rate 2–6 weeks). Here we examine the USA hCG Reference Service experience with both diseases.

Methods. Patient were referred to the USA hCG Reference Service, 133 cases shown to have quiescent gestational trophoblastic disease, 35 cases with aggressive and 30 with minimally invasive gestational trophoblastic disease.

Results. Quiescent or inactive gestational trophoblastic disease was demonstrated in 133 women. In 127 of these cases, no hyperglycosylated hCG was detected, and in 6 cases 4–27% hyperglycosylated hCG was detected. This is quiescent or inactive disease.
Only 1 of 35 cases with aggressive gestational trophoblastic disease (≥40% hyperglycosylated hCG) was chemorefractory. In contrast, 30 of 30 minimally invasive cases (≤40% hyperglycosylated hCG) were chemorefractory. In chemorefractory cases hyperglycosylated hCG ranged from ≤1% to 39% of total hCG. The USA hCG Reference Service showed that proportions hyperglycosylated hCG significantly increases as total hCG rises. They recommended in minimally invasive cases to wait to hCG was ≥3000 IU/L before commencing chemotherapy. This was successful in 10 of 10 minimally invasive cases.

Discussion. Measurement of hyperglycosylated hCG or invasiveness is a critical step in management of invasive gestational trophoblastic disease. Quiescent of inactive gestational trophoblastic disease requires no therapy. Minimally invasive disease in chemorefractory. The USA hCG Reference Service experience suggests waiting until hCG exceeds 3000 IU/L before commencing any chemotherapy.

Introduction

Low level hCG plateaus outside of pregnancy can pose a difficult management dilemma for health care providers. As ELISA assays evolved in the late 1990s, false positive hCG test results due to interfering heterophile antibodies were described and became a common cause for referral to the USA hCG Reference Service. Between 1999 and 2003 we consulted and subsequently reported many false positive hCG cases [1–4]. As this phenomenon became better recognized, assays were corrected and practitioners became more confident in making the diagnosis. Today, we will consult on only 4 to 5 false positive cases each year. However, our group continues to receive many requests per year to evaluate persistent low level hCG cases. Between 2002 and 2003 we dealt with many cases of assumed false positive hCG tests by physicians. The problem with these cases was that they were truly positive for hCG, their only oddity was the absence of hyperglycosylated hCG [5,6]. This led to the discovery of quiescent gestational trophoblastic disease in 2003. Today we look carefully at the cases with very low hyperglycosylated hCG and observe two disorders, quiescent gestational trophoblastic disease and minimally invasive gestational trophoblastic disease [7]. The USA hCG Reference Service experience with these two disorders is presented here.

Quiescent GTD and minimally invasive GTD represent biologic phases of GTD diagnosed by clinical behavior and a biomarker signature. Critical to both disorders is the presence of minimal or absent hyperglycosylated hCG. As published by multiple authors, hyperglycosylated hCG is a variant of regular hCG with double size O-linked sugar units and larger triantennary N-linked sugar units

* Corresponding author. The Howard and Friedman Distinguished Professor of Obstetrics and Gynecology, The USA hCG Reference Service, Department of Obstetrics and Gynecology, MSC10-5580, 1 University of New Mexico, Albuquerque 87131, NM, USA.
E-mail address: larry@hcglab.com (L.A. Cole).

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boosting the size of hCG from 36,700 to >40,000 molecular weight. It acts as an autocrine growth factor or cytokine to promote cytotrophoblast cell invasion and malignancy as occurs in implantation of pregnancy and in all invasion cases by trophoblast cells [8–13]. Quiescent disease arises from highly differentiated trophoblast cells. Because of the minimal presence or absence of cytotrophoblast cells it does not produce hyperglycosylated hCG and is a stable non-malignant non-invasive condition.

We have noticed though the years that choriocarcinoma, GTN (gestational trophoblastic neoplasm, choriocarcinoma without pathology) and invasive mole can all be conditions that present with low proportions of hyperglycosylated hCG and commonly slow growing chemorefractory conditions. Based on follow-up data, we have discovered that avoiding therapy in these cases and allowing patients to advance to an hCG levels of approximately 3000 IU/L permits the advancement of the cytotrophoblast proportions of trophoblasts which elevate the percent of hyperglycosylated hCG [7]. Patients then have a better likelihood of complete response from chemotherapy [7]. We call this group of conditions minimally invasive gestational trophoblastic diseases. As the spectrum of GTD expands, the biomarker evaluation becomes more difficult to interpret. The problem now is that low concentrations of hyperglycosylated hCG overlap in quiescent and minimally invasive conditions. We present both disorders and their overlapping hyperglycosylated hCG results here.

Methods

The USA hCG Reference Service is a unique one-of-a-kind referral service specializing in gestational trophoblastic diseases and cases of positive hCG outside of pregnancy. They examine patients records and serum and urine samples, measuring specifically total hCG, hyperglycosylated hCG, free β-subunit, β-core fragment, nicked hCG, intact hCG with the β-subunit C-terminal peptide and intact hCG without the C-terminal peptide, luteinizing hormone and follicle stimulating hormone. From the patient records and the test results a formal clinical report with suggested diagnoses and management is prepared. The USA hCG Reference Service is certified by the CLIA (32D0972561) and constancy is monitored by the College of American Pathologists (7176750-01).

We measure total hCG in serum and urine using the Siemens Immulite 1000 automated platform immunometric assay. This assay detects regular hCG, hyperglycosylated hCG, free β-subunit and nicked hCG equally on an equimolar basis. Most laboratory assays used by different laboratories throughout the world measure these three forms of hCG. The Siemens Immulite 1000 also detects β-core fragment at one quarter of the molar concentration than it detects regular hCG [14]. As demonstrated, this assay detects serum and urine hCG equally, with similar sensitivity (1.0–2000 mIU/ml), with no requirement for making any adjustments in the assay [15].

We measure hyperglycosylated hCG using a microtiter plate assay. Plates are coated with monoclonal antibody B152 for capturing hyperglycosylated hCG only. After a 4-h incubation at room temperature, the plate is washed and the tracer antibody monoclonal antibody 2009 labeled with peroxidase (Medix Inc., Division of Genzyme Inc., Norwalk CT) is added. After a further 2 h incubation, enzyme is reacted with the substrate and the concentrations of hCG are measured from a quadruplicate standard curve. We use the C5 standard for hyperglycosylated hCG [14]. An internal control is added to the hyperglycosylated hCG assay which is assessed daily and recorded according to CLIA regulations. The Service has consulted on 584 cases over 10 years. The Service has followed the guidelines from the University of New Mexico Human Research Review Committee the Internal Review Board (protocols 99-349, 02-548 and 04-132), concerning obtainment of data, patient confidentiality and reporting data.

All USA hCG Reference Service data are stored in a Microsoft Excel 2007 spreadsheet where it analyzed for mean, median, standard deviation and t test. This spreadsheet, as it is updated, is shared with the review board each year.

Results

From 1999 to the present, the USA hCG Reference Service has observed 133 cases diagnosed as quiescent or benign/inactive gestational trophoblastic disease (Fig. 1). All cases seemingly had low levels of hCG persisting for 3 months or longer and a history of documented gestational trophoblastic disease, 7 cases followed chemotherapy for choriocarcinoma, 62 cases followed evacuation or chemotherapy for complete hydatidiform mole, 9 cases followed the disappearance of hCG after treatment for ectopic pregnancy (assumed hydatidiform mole), 11 cases followed chemotherapy for GTN, 31 cases followed disappearance of hCG after miscarriage or spontaneous abortion (assumed hydatidiform mole), 8 cases followed evacuation or chemotherapy for partial mole, and 5 cases followed termination of pregnancy (assumed hydatidiform mole) (Fig. 1). In all cases of quiescent gestational trophoblastic disease diagnosed by the USA hCG Reference Service, all therapy was subsequently halted. Up until the referral to the USA hCG Reference Service, 55 of the 133 cases (41%) received ineffective chemotherapy with some patients receiving multiple regimens, including methotrexate ($n=44$), actinomycin D ($n=13$), EMA-CO ($n=8$), EMA-EP ($n=2$) or carboplatin, taxol and BEP combination therapy ($n=1$). No chemotherapy proved effective for this slow growing inactive disease.

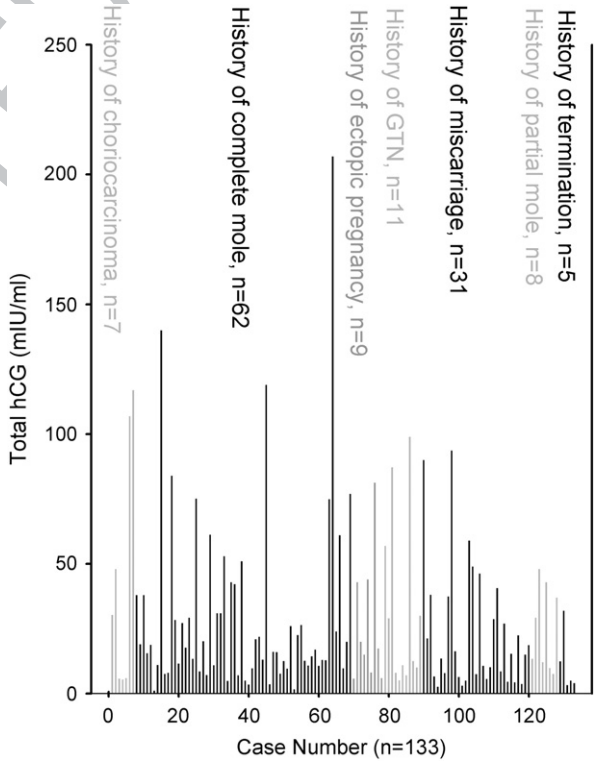

Fig. 1. Quiescent gestational trophoblastic disease as diagnosed by the USA hCG Reference Service, 133 cases. All cases seemingly had low levels of hCG persisting for 3 months or longer and a history of documented or presumed gestational trophoblastic disease, 7 cases followed chemotherapy for choriocarcinoma, 62 cases followed evacuation or chemotherapy for complete hydatidiform mole, 9 cases followed the disappearance of hCG after treatment for ectopic pregnancy (assumed hydatidiform mole), 11 cases followed chemotherapy for GTN, 31 cases followed disappearance of hCG after miscarriage or spontaneous abortion (assumed hydatidiform mole), 8 cases followed evacuation or chemotherapy for partial mole, and 5 cases followed termination of pregnancy (assumed hydatidiform mole).

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Of the 133 cases of quiescent gestational trophoblastic disease total tested by the Siemen's Immulite assay, total hCG accounted for 1.1 to 207 IU/L, median 16 IU/L. In 127 of 133 cases, there was no detectable hyperglycosylated hCG, level ≤0.05 ng/ml or equivalence of ≤0.55 IU/L (Fig. 1). In 6 cases hyperglycosylated hCG levels were low positive, accounting for 4.0%, 11%, 14%, 18%, 26% and 27% of serum total hCG concentration. These 6 cases were only considered quiescent gestational trophoblastic disease after demonstration of a history of gestational trophoblastic disease and weekly consistency in plateau for at least 3 months with hCG levels ≤200 IU/L. Interestingly, none of these cases originated from an ectopic pregnancy, miscarriage or pregnancy termination. We note that according to reported clinical follow-up to the USA hCG Reference Service, received in 92 of the 133 cases (69%), in most cases, quiescent gestational trophoblastic disease spontaneously resolves within 6 months as total hCG disappears. In the occasional case, quiescent gestational trophoblastic disease persisted for 1 year or longer. In 15% of cases, quiescent gestational trophoblastic disease preceded recurrence of invasive disease with the appearance of hyperglycosylated hCG and a continuous rise in hCG levels. Persistence of invasive disease was much more common in cases with a history of choriocarcinoma and GTN (~25% of cases).

The USA hCG Reference Service has also consulted on 35 cases with aggressive gestational trophoblastic disease as indicated by high hyperglycosylated hCG, ≥40% of total hCG, and low total hCG ≤2000 IU/L (Table 1A). The median total hCG was 341 IU/L and the mean proportion of hyperglycosylated hCG among these cases was 75 ± 22% (mean ± standard deviation). It was notable that only one of these 35 cases was resistant to chemotherapy (case 542 resistant to methotrexate and actinomycin D). We have heard that 27 of these 35 cases (77%) were successfully treated with chemotherapy, 1 continues chemotherapy and on 7 we have received no follow-up.

We have also consulted on 30 similar cases with low total hCG<2000 IU/L with low hyperglycosylated hCG, ≤40% of total hCG (Table 1B). In this group the median total hCG was 254 IU/L and the mean proportion of hyperglycosylated hCG was 13 ± 11%. These cases we classify as minimally invasive gestational trophoblastic disease. (*t*-test compared to aggressive gestational trophoblastic disease cases 75 ± 22% vs. 13 ± 11%, $p = 5 \times 10^{-19}$). As shown (Table 1B), 30 of 30 cases (100%) were chemorefractory, with lack of therapeutic response observed with as many as 9 different chemotherapy regimens administered to an individual before referral to the USA hCG Reference Service. Characteristically in these cases hCG increased very slowly with a doubling time of 3.4 ± 2.3 weeks. It seemingly is this slow doubling rate of growth and low percent invasion as indicated by hyperglycosylated hCG that makes these cases chemorefractory. Figs. 2 and 3 illustrate the biochemical and clinical course of 2 of the 30 cases, illustrating their ineffective chemotherapy treatments and slow growth rate. We have heard that 3 of these cases eventually died, independent of USA hCG Reference Service recommendations, 2 continue chemotherapy and just 3 of 30 (10%) were treated by chemotherapy successfully. A total of 12 (10>3000 IU/L hCG, 1>10,000 IU/L hCG and 1>100,000 IU/L hCG) were successfully treated after total hCG levels exceeded 3,000 IU/L (Table 1), 3 newer cases await reaching 3000 IU/L hCG.

Patients will often decide to avoid chemotherapy. Three cases referred to the USA hCG Reference Service rejected further chemotherapy after failing different chemotherapy regimens. The first case (case 198, Table 1) was from a Mormon family. She had undergone 7 different chemotherapy regimens for what the USA hCG Reference Service had diagnosed as quiescent gestational trophoblastic disease, none worked, the physician chose to disregard the USA hCG Reference Service report to suggest stopping therapy. At the point that she developed minimally invasive disease she chose to resort to her family's advice and God's help refusing further therapy. The second and third cases of minimally invasive disease had failed methotrexate chemotherapy, had low hCG concentration 331 and 20 IU/L, and argued against the need for chemotherapy for a disease without evidence of tumor. All 3 women, however, were willing to cooperate with outcomes research and supply the USA hCG Reference Service with monthly serum and urine samples. The biochemical and clinical course of these 3 women are illustrated in Fig. 4. As observed, the proportion of hyperglycosylated hCG steeply increased in line with the increasing hCG levels, so that the proportion of hyperglycosylated hCG was higher when total hCG entered the thousands of IU/L, and higher still as it entered the tens of thousands and hundreds of thousands of IU/L. All 3 women sought help after lung and brain metastases showed symptoms in 2 patients and lung metastases in one patient. Two were treated by their gynecologic oncologists with EMA-CO and survived. One patient, with a total hCG of 964,000 was treated by a general oncologist (case 198, Table 1), The oncologist concluded that the patient had become resistant to all chemotherapy agents during the time of quiescent gestational trophoblastic disease and minimally invasive disease. He treated her with Xeloda in place of EMA-CO or EMA-EP as recommended by the USA hCG Reference Service and she died from progressive disease (did not respond to Xeloda).

The USA hCG Reference Service noted that the proportion of hyperglycosylated hCG increased with advancing disease. They cautiously proposed in all subsequent cases of minimally invasive disease that treatment should be withheld until the total hCG exceeded 3000 IU/L. It was noted that patients should not be considered resistant to EMA-CO and EMA-EP if used during the chemorefractory slow growth phase of disease. It should be considered as the chemotherapy of choice once hCG exceeded 3,000 mIU/ml and hyperglycosylated hCG increased accordingly. This approach was proven in 10 of 10 (100%) cases. Two examples are shown in Figs. 3 and 5. Case 490 (Fig. 3) showed an hCG response from 26,099 IU/L to 22 IU/L by EMA-EP. The background 22 IU/L was shown to be pituitary hCG, suppressed by high estrogen oral contraceptives. Case 483 (Fig. 5) and cases 464,491,496,516,551,553,554 and 568 (Table 1B) all showed a complete response of hCG to undetectable hCG levels in response to EMA-CO or EMA-EP.

Both the quiescent gestational trophoblastic disease and the minimally invasive disease are marked by hyperglycosylated hCG proportions of 0–40% or by minimal concentrations of hyperglycosylated hCG, the aggressive signal (Table 1). How can they be differentiated? Statistically there is a big difference; the total hCG median is 16 IU/L in quiescent cases and 254 IU/L or more than 10-fold higher in the minimally invasive cases (*t* test, $P = 1.4 \times 10^{-15}$), clearly most of the lower hCG cases are quiescent. All but 6 of 133 quiescent cases were marked by non-detectable level of hyperglycosylated hCG. By *t*-test, hyperglycosylated hCG was much more evident in minimally invasive cases ($P = 2.8 \times 10^{-18}$) suggesting that non-detectable hyperglycosylated hCG cases are quiescent. All 133 quiescent cases were marked by a plateau in hCG levels below 207 IU/L. It is noteworthy that serum hCG as much as doubles or halves over 3 months due to variation in liver and kidney functions, but on average the plateaus are detectable with compliant follow-up. We never observed an increase beyond a doubling from the average level. In the 30 minimally invasive cases we observed hCG levels rise consistently with a doubling time of 2–6 weeks. We observed hCG in each case double at least twice before concluding it was minimally invasive disease.

Discussion

The USA hCG Reference Service is a unique service that has been consulting on difficult cases of hCG interpretations by physicians responding to non-pregnant gestational events. Multiple cases have involved refractory gestational trophoblastic disease, invasive mole or choriocarcinoma, many of which demonstrate initial responsive

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Table 1
USA hCG Reference Service early (<2000 IU/L) gestational trophoblastic disease 1 cases.

Code	Age	Total hCG (IU/L)	hCG-H (μg/L)	%	Ineffective chemotherapy	History	Outcome
(A) Aggressive (hyperglycosylated hCG >40%) cases							
64	25	62	2.5	45%		Choriocarcinoma	Treated successfully
92	53	863	83	>100%		Choriocarcinoma	Treated successfully
140	37	4.7	0.43	>100%		Choriocarcinoma	Treated successfully
186	22	31.9	1.22	42%		Partial Mole	Treated successfully
197	20	52.5	2.9	61%		Choriocarcinoma	No follow-up information
211	23	1993	170	94%		Complete Mole	Treated successfully
214	29	1504	140	>100%		Choriocarcinoma	Treated successfully
258	35	330	25	83%		GTN	Treated successfully
259	21	670	75	>100%		Complete Mole	Treated successfully
271	42	542	34	69%		Choriocarcinoma	No follow-up information
298	31	305	12	43%		GTN	Treated successfully
302	34	98.6	9.2	>100%		Choriocarcinoma	Treated successfully
313	35	341	15.2	49%		GTN	No follow-up information
314	35	600	44	81%		Choriocarcinoma	No follow-up information
347	36	521	53	>100%		GTN	Treated successfully
359	26	38.1	2.1	61%		Choriocarcinoma	Treated successfully
371	39	1929	181	>100%		Complete Mole	Treated successfully
395	31	639	71	>100%		GTN	Treated successfully
408	34	901	49	60%		Complete Mole	Treated successfully
409	31	60	16	>100%		Complete Mole	Treated successfully
417	30	143	8.1	62%		Choriocarcinoma	No follow-up information
420	34	36	1.9	58%		Complete Mole	No follow-up information
424	29	454	19.2	47%		Partial Mole	Treated successfully
441	28	1926	105	60%		GTN	Treated successfully
492	29	214	7.6	39%		Choriocarcinoma	Treated successfully
493	43	1208	66	60%		Partial Mole	Treated successfully
494	27	220	19	95%		GTD	Treated successfully
495	26	415	54	>100%		Complete Mole	No follow-up information
521	39	1113	86	85%		Complete Mole	Treated successfully
522	31	18.1	0.67	41%		Complete Mole	Treated successfully
542	33	869	36	46%	Mtx ActD	Complete Mole	Continues chemotherapy
544	N/A	11.1	2.54	>100%		Complete Mole	Treated successfully
545	37	9.03	0.98	>100%		Choriocarcinoma	Treated successfully
552	20	821	58	74%	Choriocarcinoma	Treated successfully	
557	34	48	8.7	>100%	GTD	Treated successfully	
Median		341 IU/L	75 ± 22%				
(B) Minimally invasive (hyperglycosylated hCG <40%) cases							
198	20	53	0.92	19%	ActD EMA-CO EP TAH Taxol-Ifosamide BEP Xeloda	Choriocarcinoma	Died
200	21	113	2.7	26%	ActD EMA-CO EMA-EP	Complete mole	Continues chemotherapy
204	24	23	0.32	15%	Mtx ActD EMA-CO EMA-EP Taxol-Ifosamide BEP Xeloda	GTN	No follow-up information
267	27	42	1.32	35%	Mtx ActD EMA-CO EMA-EP EMA-CO EMA-EP Taxol-Ifoseamide	GTN	No follow-up information
271	35	600	30	5.0%	Gecitabin-Cisplatinin BEP Xeloda ICE	Choriocarcinoma	Treated successfully
301	40	42.2	0.22	5.7%	Mtx ActD EMA-CO EMA-EP Taxol-Ifosamide BEP Xeloda	Complete mole	No follow-up information
370	26	1220	1.36	1.2%	Mtx TAH ActD	Complete mole	No follow-up information
380	46	238	2	9.2%	TAH ActD Etoposide	Complete mole	No follow-up information
395	31	639	11.7	20%	Mtx ActD EMA-CO EMA-EP	Choriocarcinoma	Treated successfully. Treated post >10,000
402	36	331	0.75	2.5%	Mtx	GTN	IU/L hCG successfully
423	30	28.4	0.29	11%	Mtx	Partial Mole	Treated successfully
431	38	663	2.5	4.1%	Mtx ActD EMA-CO EMA-EP Taxol BEP ICE	Choriocarcinoma	Died
436	24	725	0.25	0.38%	Mtx ActD EMA-CO EMA-EP Taxol-Carboplatin Gencitabine-Cisplatin Doxil-bleomycin	GTN	No follow-up information
447	38	1067	17.5	18%	Genctabine Xeloda-Toptecan Avastin ICE	Choriocarcinoma	Died treated post >100,000
459	24	20	0.23	13%	Mtx	Complete mole	IU/L hCG successfully
464	34	320	11	38%	Mtx ActD	Complete mole	Treated post >3000 IU/L hCG successfully
475	24	147	23.1	16%	TAH ActD EMA-CO EMA-EP Taxol	Choriocarcinoma	Continues chemotherapy
481	31	18.2	0.37	22%	Mtx	GTN	No follow-up information
483	27	445	15.3	38%	Mtx-Folinic acid	Choriocarcinoma	Treated post >3000 IU/L hCG successfully
490	50	1596	433	27%	Taxol-Ifosamide Xeloda Cytoxan	Choriocarcinoma	Treated post >3000 IU/L hCG successfully
491	34	2362	91	3.9%	Mtx	Choriocarcinoma	Treated post >3000 IU/L hCG successfully
496	27	60	2.2	3.8%	Mtx ActD	Complete mole	Treated post >3000 IU/L hCG successfully
516	46	71	0.09	1.4%	Mtx EMA-CO EMA-EP Taxol-Ifosamide BEP	Choriocarcinoma	Treated post >3000 IU/L hCG successfully
551	37	735	85.8	12%	Xeloda	Complete mole	Treated post >3000 IU/L hCG successfully

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Table 1 *(continued)*

Code	Age	Total hCG (IU/L)	hCG-H (μg/L)	%	Ineffective chemotherapy	History	Outcome
(B) Minimally invasive (hyperglycosylated hCG <40%) cases							
553	26	270	17.4	6.4%	Mtx	GTN	Treated post >3000 IU/L hCG successfully
554	33	26	4.18	16%	Mtx ActD	Complete mole	Treated post >3000 IU/L hCG successfully
568	29	208	0.36	0.17%	Mtx Mtx	Complete mole	Treated post >3000 IU/L hCG successfully
572	19	824	3.8	5.1%	TAH Mtx ActD	Choriocarcinoma	hCG not yet >3000 IU/L
574	33	97	0.41	4.7%	Mtx EMA-CO EMA-EP BEP Carboplatin-Paditaxel	Choriocarcinoma	hCG not yet >3000 IU/L
580	19	341	0.06	0.2%	EMA-CO TAH	Complete mole	hCG not yet >3000 IU/L
Median		254 IU/L	13 ± 11%				

These are divided into (A) 2 aggressive (hyperglycosylated hCG >40%) cases, 35 total and (B) minimally invasive cases, 30 cases, (hyperglycosylated hCG 3 <40%). hCG-H is hyperglycosylated hCG, GTN is gestational trophoblastic neoplasm. Mtx is methotrexate, and ActD is actinomycin 4 D, TAH is trans-abdominal hysterectomy.

disease but then showed low level plateaus using the commercially available total hCG tests. Our group has had a unique opportunity to study the majority of these cases over time, both biochemically and via provided clinical updates from the referring providers. Based on this experience, we have been able to define biochemical parameters for this important group of trophoblastic diseases.

Chemoresistant and refractory disease has been a challenge to understand in many solid tumors. Trophoblastic disease may be the first solid tumor system that demonstrates a biochemical marker of quiescence and extent of invasion which defines chemoresistant behavior. Our experience demonstrates that quiescent disease is 100% chemoresistant and can be defined biochemically. Our group also has proposed the definition of minimally invasive disease, (7) and cautiously have recommended halting treatment until the trophoblastic disease meets biochemical criteria of growth and invasion. We have shown in a small number of cases that cautious observation and patience will result in successful treatment of patients once the disease becomes active or aggressive.

We make multiple important conclusions in the management of gestational trophoblastic diseases:

(1) Measurement of hyperglycosylated hCG is invaluable, permitting the division of invasive gestational trophoblastic disease into quiescent, minimally invasive and aggressive disease and adjustment of therapy accordingly. Measurement of the proportion of hyperglycosylated hCG should replace the WHO rating system. The hyperglycosylated hCG assay is now as a kit from Focus Diagnostics or can be performed by the USA hCG Reference Service and should be used by all centers managing gestational trophoblastic disease cases.
(2) Quiescent gestational trophoblastic disease is an inactive or benign phase of trophoblastic disease with no invasion signal.

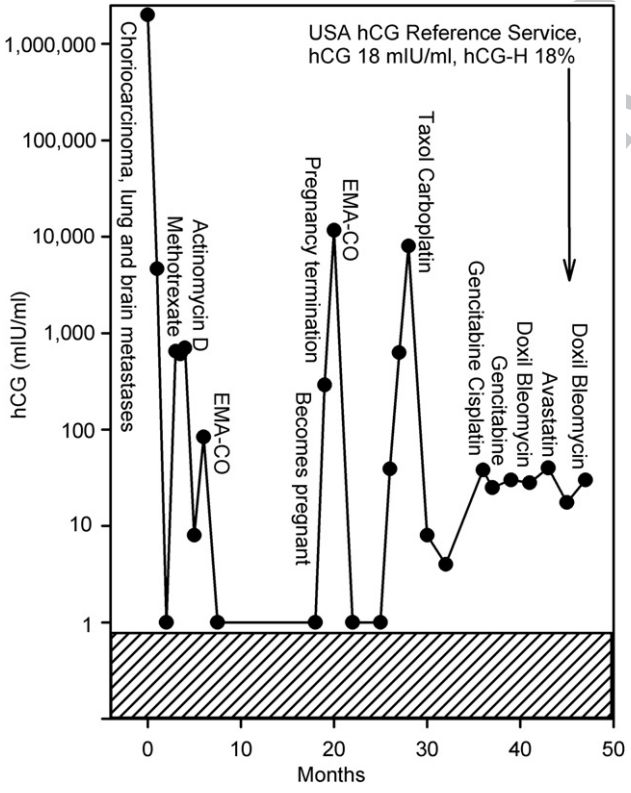

Fig. 2. Example of minimally invasive gestational trophoblastic disease, case 447 (Table 1).

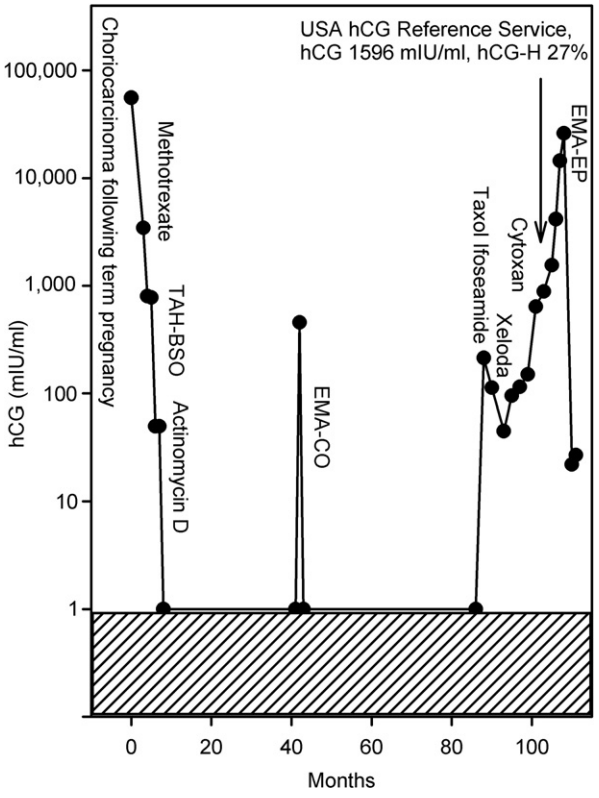

Fig. 3. Example of minimally invasive gestational trophoblastic disease, case 490 (Table 1).

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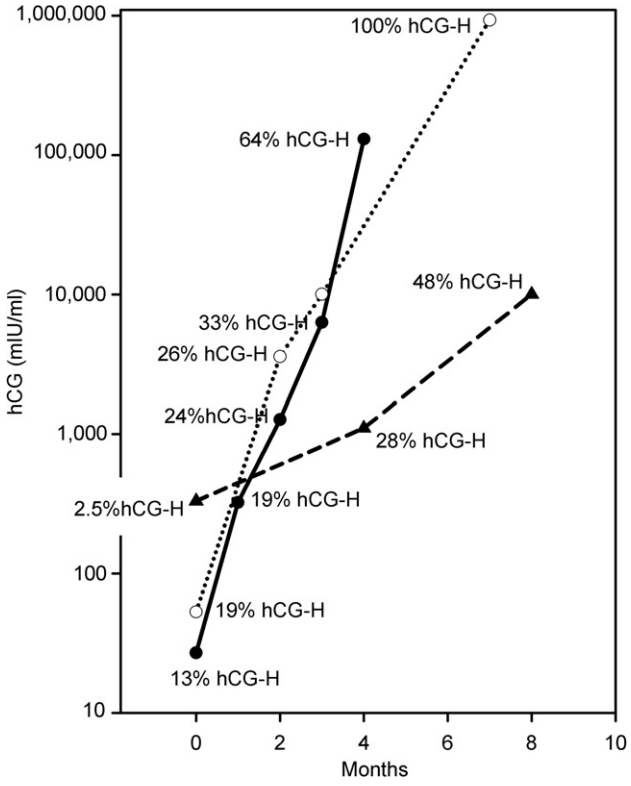

Fig. 4. Three cases refusing therapy for invasive disease, cases 198, 402 and 459 (Table 1). Relationship between rising total hCG concentration and proportion hyperglycosylated hCG.

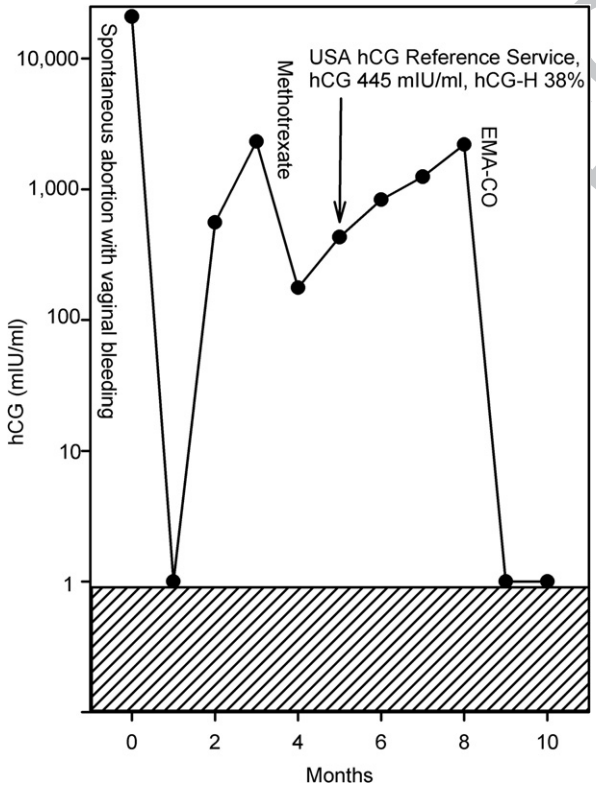

Fig. 5. Example of minimally invasive gestational trophoblastic disease waiting until hCG exceed 3000 IU/L before therapy (Case 483).

It can be diagnosed as a plateau in total hCG concentration in women being treated for gestational trophoblastic disease for 3 months or longer demonstrating total hCG levels below 207 IU/L, or by the demonstration of the absence of hyperglycosylated hCG.

(3) Chemorefractory gestational trophoblastic disease is seemingly a result of slow growing disease and not biological resistance to chemotherapy agents. It is probably due to the lack of growth signal due limited hyperglycosylated hCG, <40% of total hCG, as demonstrated here. Hyperglycosylated hCG is not only a measure of the type of trophoblast disease but also defines trophoblast disease biology at certain time points in the disease spectrum which can be used to define the best time for chemotherapy response to treatment. We recommend allowing patients to advance to a total hCG of >3,000 IU/L, then treating with proven combination therapy with either EMA-CO or EMA-EP. This is now the recommendation of the USA hCG Reference Service (All recent cases, numbers 464, and 483–580), and has worked in 10 of 10 selected case.

(4) Minimally invasive gestational trophoblastic disease is a common condition accounting for approximately, according to the USA hCG Reference Service experience 44% of cases whether part of recurrence of choriocarcinoma, GTN or hydatidiform mole with hCG <2000 IU/L. Minimally invasive gestational trophoblastic disease can be identified by slow rising total hCG with a doubling rate of 2–6 weeks, by <40% hyperglycosylated hCG of total hCG, and by resistance to chemotherapy regimens. We recommend allowing patients to advance to a total hCG of >3000 IU/L before treating with EMA-CO or EMA-EP.

We note that these recommendations go against the dogma of treating gestational trophoblastic disease by WHO recommendations, however further experience and validation of these strategies will likely change the practice of treating this disease. Large multi-center international trials are needed to confirm these results and the experience presented here. This year, for the first time, hyperglycosylated hCG is available for all laboratories to test, confirmation of these findings with quiescent and minimally invasive cases at other centers is now very important to confirm these findings.

Conflict of interest statement

The authors declare that there are no conflicts of interest. The data presented in this manuscript present no conflict of interest with any company of other agency, no funds were received to promote this publication, and no company or agency has modified it in any way.

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Precis

Hyperglycosylated hCG is a marker of gestational trophoblastic disease invasion. The absence of hyperglycosylated hCG or low presence marks quiescent disease or minimally invasive disease.

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