

Inappropriate Management of Women with Persistent Low hCG Results

Laurence A. Cole, Ph.D., and Sarah A. Khanlian

The USA hCG Reference Service is a consulting service with a specialized clinical laboratory aiding physicians in the interpretation of conflicting or nonrepresentative human chorionic gonadotropin (hCG) results. We have consulted on 189 cases with persistent low levels of hCG but no evidence of pregnancy or tumor. Quiescent gestational trophoblastic disease (GTD) was identified in 121 cases by the absence of invasive trophoblast antigen and nonresponse to chemotherapy (64 cases with a history of hydatidiform mole or gestational trophoblastic neoplasia (GTN) and 57 cases following antecedent pregnancy). Another 61 Reference Service cases had false positive hCG, and we observed 7 cases with low levels of hCG of pituitary origin (hCG subsequently suppressed by estrogen-progesterone medication). Most disturbing is that the majority of these cases (68%) received needless therapy for assumed GTN/choriocarcinoma/placental site trophoblastic tumor before consultation with the Reference Service. One hundred twenty-eight of the 189 patients (77 of 121 with quiescent GTD, 48 of 61 with false positive hCG and 3 of 7 with pituitary hCG) underwent therapy ranging from

Too many women are being needlessly harmed by chemotherapy, hysterectomy and other surgery because of misdiagnosis of persistent mole or GTN.

single-agent chemotherapy (117 cases), to EMA-CO combination chemotherapy (etoposide, methotrexate, actinomycin D alternating with cyclophosphamide and vincristine) (16 cases), to hysterectomy and/or bilateral salpingo-oophorectomy (31 cases). False positive hCG and pituitary hCG would obviously not respond to these treatments, and no treated cases of quiescent GTD responded to chemotherapy or fully responded to hysterectomy. The continued

needless treatment of patients with quiescent GTD, even after multiple publications, is entirely avoidable. Unfortunately, the number of needlessly treated cases referred to the Reference Service is increasing. (J Reprod Med 2004;49:423-432)

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Human chorionic gonadotropin (hCG) is a glycoprotein composed of 2 dissimilar subunits, α and β , held together by charge interactions. As little as

From USA hCG Reference Service, Department of Obstetrics and Gynecology, University of New Mexico Health Sciences Center, Albuquerque.

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Address reprint requests to: Laurence A. Cole, Ph.D., USA hCG Reference Service, Department of Obstetrics and Gynecology, University of New Mexico Health Sciences Center, MSC10 5580, 1 University of New Mexico, Albuquerque, NM 87131.

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65% of the molecular weight of hCG is due to amino acids or protein. The balance is due to sugar side chains. hCG is sometimes considered a muco-

Care is urgently needed in excluding false positive hCG results, quiescent GTD and pituitary hCG before diagnosing persistent mole or GTN requiring cancer therapy.

polysaccharide, like collagen, because of the large carbohydrate component. Free hCG, α and β subunits; degraded hCG molecules; forms of hCG with irregular sugar side chains; and hCG and free subunit fragments are present in serum and/or urine in cases with gestational trophoblastic disease (GTD).¹⁻⁵

The USA hCG Reference Service is a consulting service and specialized clinical laboratory aiding physicians throughout North America in the interpretation of conflicting or nonrepresentative hCG results. Using a combination of urine and serum assays, the presence of hCG is confirmed and the nature of hCG-related molecule immunoreactivity determined. From published findings, inferences are made about the structure and source of the hCG immunoreactivity and the patient's diagnosis.^{1,6-25}

The USA hCG Reference Service measures total hCG (all hCG forms) in parallel urine and serum samples using DPC Immulite (DPC, Los Angeles, California), which detects all forms of hCG found in serum and urine in GTD cases (hCG, hyperglycosylated hCG, sialic acid-deficient hCG, nicked hCG, free β subunit, hCG missing β subunit C-terminal peptide and β -subunit core fragment).^{1,6,7} Total hCG is also measured after treatment of serum with Scantibodies Inc. (San Diego, California) HBT heterophilic antibody-blocking agent. Serum and urine are also tested with the DPC Immulite free β -subunit test and in enzyme-immunometric assays specific for nicked hCG, intact hCG, hyperglycosylated hCG and β -subunit core fragment.^{1,7} Hyperglycosylated hCG (invasive trophoblast antigen [ITA]) is measured using the Nichols Advantage ITA test (San Clemente, California). Most tests are run at multiple dilutions to confirm the results. This combination of urine and serum assays confirms the presence of hCG and the nature of hCG-related molecule immunoreactivity determined. From pub-

lished findings, inferences are made about the structure and source of the hCG immunoreactivity and the patient's diagnosis.^{1,6-25}

Commonly, the USA hCG Reference Service investigates cases with persistent low levels of hCG in the absence of pregnancy and of imageable tumor. Two histories are common in these cases.^{6,7} First is a patient who has been treated in the past for hydatidiform mole or gestational trophoblastic neoplasm (GTN) (choriocarcinoma, placental site trophoblastic tumor [PSTT] or trophoblastic malignancy without pathology). Persistent low, fluctuating hCG results are observed, yet there is no evidence of pregnancy or imageable tumor. In these cases the nature of the hCG test result is questioned. The second type of history is a patient who has had an incidental pregnancy test that is positive. After excluding intrauterine and ectopic pregnancy, GTD or a tumor is considered. After exclusion of these sources of hCG, the nature and source of hCG are questioned.

In both scenarios, hCG levels in the range of 7–900 mIU/mL have been observed. Commonly, hCG results fluctuate monthly as much as 2-fold. In both scenarios chemotherapy or surgery is commonly implemented, in most cases without reducing the hCG result.

As shown here and in earlier publications,^{6,7} hCG immunoreactivity, regardless of the patient's clinical history, is either false positive, derives from dormant or quiescent trophoblast cells, or is of pituitary origin. All 3 of these diagnoses involve nonmalignant or noninvasive disease. It is the general observation of the USA hCG Reference Service that none of these cases is aided by chemotherapy or fully suppressed by hysterectomy or other surgery.^{6,7} Publications from the USA hCG Reference Service and numerous other centers clearly show that therapy should be avoided in these cases.⁶⁻²⁵ Unfortunately, patients continue to be harmed with needless chemotherapy and surgery.

This article describes the USA hCG Reference Service experience with cases of persistent low hCG results and the resulting diagnoses. As documented below, 128 of 189 women with persistent low hCG results received needless single-agent or multiagent chemotherapy, hysterectomy or bilateral salpingo-oophorectomy (BSO). None of the patients needed the therapy. This report describes a crisis in patient care. It should be read and carefully considered before treating any further patients with persistent low levels of hCG, regardless of their histories.

This study of 189 cases of persistent low levels of hCG coming from numerous medical centers throughout the United States was monitored and approved by the University of New Mexico Human Research Review Committee (HRRC) for the protection of human subjects (HRRC #02-548).

Persistent Low Levels of hCG

The USA hCG Reference Service has now consulted on, evaluated records of and performed serum and urine tests on 189 cases with persistent low levels of hCG. Patients were referred to the USA hCG Reference Service by >100 centers throughout Canada and the United States. In each case, according to clinical records provided, there was no evidence of intrauterine or extrauterine pregnancy or imaging evidence of tumor. These included 121 cases in which noninvasive or dormant (quiescent) GTD, was identified. We also observed 61 cases, considered by their physician as having or possibly having GTN but shown to have false positive or erroneously detected hCG (no disease). We additionally observed 7 cases considered to have GTN with persistent low levels of hCG later proven to be of pituitary origin, 4 with a history of trophoblastic disease and 3 with no such history.

Examining the 189 cases together (Table I), 128 (68%) received needless therapy for assumed GTN or persistent or invasive mole after evacuation of a hydatidiform mole. Of these, 117 received single-agent chemotherapy, 16 received EMA-CO (etoposide, methotrexate, actinomycin D alternating with cyclophosphamide and vincristine), and 31 had hysterectomy and/or BSO. In all cases, this was later shown to have been needless by the diagnosis of false positive hCG, quiescent GTD or pituitary hCG.

False Positive hCG

In 1998, in the first few months of operation, the USA hCG Reference Service investigated 3 unusual cases.¹⁰ In all 3 cases the women had an incidental pregnancy test that was positive, followed by persistent positive hCG values with small changes. Ultrasound, dilatation and curettage and laparoscopy ruled out intrauterine and ectopic pregnancy. Imaging techniques showed no tumor. The diagnosis of GTN or choriocarcinoma was made even though there was no previous history of trophoblastic disease or physical evidence of tumor. In 2 of the 3 cases chemotherapy was started, and in 1 case a hysterectomy was carried out. All 3 cases were then

referred to the USA hCG Reference Service. At that time the reported hCG concentrations were 17, 53 and 110 mIU/mL. It was a surprise for us when false positive hCG results were demonstrated in these individuals using modern immunometric assays. False positive assays were a problem with old-technology radioimmunoassays^{13,26} but were supposedly solved with the introduction of immunometric tests.²⁶

Immunoglobulins are large molecules and do not cross glomerular basement membrane. The findings of hCG in serum and not urine, a specific heterophilic antibody-blocking agent preventing false positive results and published data all clearly indicated that false positive results were due to interfering heterophilic antibodies in the immunometric assays.^{8,11-15,20} Heterophilic antibodies are human antiimmunoglobulin antibodies that bind animal immunoglobulins. These are present in the blood of a significant proportion of individuals.^{22,27}

Now, after more than 5 years of operation and multiple publications from the USA hCG Reference Service to increase awareness of the false positive problem,^{1,7-12,18} we have identified 61 false positive cases. In most cases GTN or choriocarcinoma was diagnosed, and in most cases major chemotherapy protocols, hysterectomy or other surgery were needlessly implemented. Cases continue to be identified with no decline in incidence, and physicians are continuing to treat cases without first confirming the real hCG level.

There have been 8 other publications in the last 2 years describing similar false positive hCG problems.^{14-17,19,21-23} The time has come to start listening. To the best of our knowledge at least 2 physicians have already been successfully sued in the United States, and an unknown number abroad, for not considering possible false positives. It is relatively easy to identify a false positive result, and the procedure can be implemented by any clinical laboratory. First, if the hCG is real, it will be present in serum and urine. (Urine tests in the United States are limited to 25 mIU/mL sensitivity and thus are meaningful only if the serum hCG is >100 mIU/mL.) Second, if the hCG is real, it should give similar results with other commercial hCG tests. Request that the laboratory arrange for the serum samples to be tested in 2 further independent assays.

As shown in Table I, of the 61 false positive cases, 49 (80%) received needless therapy for a disease that did not exist: 42 received chemotherapy, 4 had

Table 1 USA hCG Reference Service Experience with 189 Cases of Persistent Low Levels of hCG with No Evidence of Pregnancy or Imaging Evidence of Tumor

Variable	Finding
<i>Quiescent GTD</i>	
No. of cases	121 Total: 64 with history of hydatidiform mole or GTN + 57 with no history
Cases receiving therapy ¹	77 Total: 43 with history of hydatidiform mole or GTN + 34 with no history
Single-agent chemotherapy	71
EMA-CO combination therapy	12
Hysterectomy and/or BSO	25
Median hCG at time of referral (mIU/mL)	22
95th Percentile	226
Range	<2–773
Median percentage of ITA at time of referral ²	Not detected (ITA less than molar equivalent of 2 mIU/mL hCG)
95th Percentile	6%
Range	Not detected to 21%
Cases in which GTN later was indicated	9: 5 with history of hydatidiform mole or GTN, 4 with no history
Median time at which GTN was indicated (yr)	2
Range of time	0.25–4
Median hCG at time GTN was indicated (mIU/mL)	489
Range	190–6,000
Median percentage of ITA when GTN was indicated ²	86
Range	57–100
<i>False Positive hCG</i>	
No. of cases	61 Total: 10 with history of hydatidiform mole or GTN + 51 with no history
Cases receiving therapy ¹	48 Total: 9 with history of hydatidiform mole or GTN + 39 with no history
Single-agent chemotherapy	42
EMA-CO combination therapy	4
Hysterectomy and/or BSO	6
Median false hCG at time of referral (mIU/mL)	58
95th Percentile	300
Range	7–607
<i>Pituitary hCG</i>	
No. of cases	7 Total: 4 with history of hydatidiform mole
Cases receiving therapy ¹	3
Single-agent chemotherapy	3
EMA-CO combination therapy	0
Hysterectomy and/or BSO	0
Median false hCG at time of referral (mIU/mL)	4
95th Percentile	9
Range	2–11
<i>Total</i>	
No. of cases	189 Total
Cases receiving therapy ¹	128
Single-agent chemotherapy	117
EMA-CO combination therapy	16
Hysterectomy and/or BSO	31

Diagnoses are those reported to referring clinicians. The diagnoses of quiescent GTD were based on records of persistent low hCG results (<250 mIU/mL), confirmation of real hCG immunoreactivity and demonstration of absence of significant ITA immunoreactivity by the service. In some cases, GTN was indicated later by rapidly rising hCG results or the presence of a tumor. In these cases, GTN was confirmed by the presence of a significant proportion of ITA by the service. Diagnoses of false positive hCG were based on multiple observations by the service: (1) presence of hCG immunoreactivity in serum but not urine, (2) varying hCG results (>5-fold) or negative results in different hCG tests, (3) suppression by a heterophilic antibody blocking agent, and (4) detection of urine β -core fragment immunoreactivity in serum but not urine. The diagnoses of pituitary hCG were suggested by finding mostly hCG free β -subunit in serum, with total immunoreactivity <10 mIU/mL; it was verified when the physician showed that estrogen-progesterone medication suppressed the immunoreactivity.

¹Therapy once persistent low levels of hCG were observed; excludes previous therapy.

²ITA immunoreactivity as a percentage of total hCG immunoreactivity.

EMA-CO combination therapy, and 6 had hysterectomy or BSO.

In 51 cases the women had no history of GTD, and 18 of these 51 had no history of pregnancy.

These cases arose from incidental serum hCG tests prior to minor surgery or a radiographic procedure or as part of a block of tests upon visiting their practitioners. All the patients had clearly positive hCG

results, and after excluding intrauterine pregnancy by ultrasound and ectopic pregnancy by laparoscopy (most cases), the physicians were perplexed. When serum hCG levels persisted for months with minimal fluctuation, malignancy was considered even though imaging methods did not reveal a tumor. At that time, in most cases, GTN was assumed, and therapy commenced. After the patients did not respond to therapy, they were referred to the USA hCG Reference Service. After identification of false positive hCG, the therapy was either halted, or, in some cases where false positives were detected early, therapy was avoided entirely. An example of this kind of case is summarized in Table II.

In the remaining 10 of the 61 cases, the patient had a relatively recent history of hydatidiform mole or GTN. Persistent hCG levels were detected with month-to-month fluctuation. Persistent or invasive mole was considered, and in some cases chemotherapy commenced without success. The patient

Table II Example of a Patient with False Positive Persistent hCG Results

Time (d)	hCG (mIU/mL)	Notes
0	116	Incidental pregnancy test and ultrasound
8	126	Dilatation and curettage reveals no evidence of pregnancy
19	168	Laparoscopy reveals no ectopic pregnancy
22	150	Single-dose methotrexate for assumed ectopic pregnancy
36	116	
116	135	
117	110	Computed tomography and magnetic resonance imaging reveal no disease
128	134	
131	120	Four courses of methotrexate
147	122	
160	113	Computed tomography and magnetic resonance imaging indicate potential uterine irregularities
168	144	
178	120	Actinomycin D bolus chemotherapy
192	134	Magnetic resonance imaging indicates uterine irregularities
206	121	
237	124	Hysterectomy; pathology unremarkable
269	134	
276	142	
277	0	<i>hCG false positive; all therapy halted</i>

The initial diagnosis was ectopic pregnancy, then later (after 1 month) GTN was assumed on the basis of persistent positive hCG results in the absence of intrauterine or extrauterine pregnancy. USA hCG Reference Service results are shown in italics.

Table III Example of a Patient with False Positive Persistent hCG Results 122 Days After Evacuation of Complete Hydatidiform Mole

Time (d)	hCG (mIU/mL)	Notes
122	40	Elevated hCG detected in monthly serum samples
130		Methotrexate, 4 cycles
143	109	Actinomycin D, 2 cycles
157	41	
171	41	Urine hCG found negative (sensitivity, 25 mIU/mL)
174	100	Serum hCG negative in point-of-care test (sensitivity, 25 mIU/mL)
187	38	hCG results questioned
277	0	<i>hCG false positive, all therapy halted</i>

Persistent or invasive mole was assumed on the basis of persistent elevated hCG results and therapy commenced. USA hCG Reference Service results are shown in italics.

was then referred to the USA hCG Reference Service. An example of this kind of case is summarized in Table III.

The USA hCG Reference Service uses the following criteria to identify false positive results^{8-11,18}:

1. The finding of a >5-fold difference in serum hCG results or negative hCG results with an alternative professional laboratory serum hCG test (essential criterion).
2. The presence of hCG in serum and absence of detectable hCG or hCG-related molecule immunoreactivity in a parallel urine sample (essential criterion). *The quantitative measurement of urine hCG, sensitivity 2 mIU/mL, is an irregular, or "off label," use of a modern commercial laboratory hCG assay.*
3. The observation of false positive results in other tests for molecules not normally present in serum, such as urine β -core fragment (confirmatory criterion).
4. The finding that a heterophilic antibody-blocking agent prevented or limited false detection (confirmatory criterion).

Table I details the false positive hCG levels in the 61 false positive cases. The highest level observed, to the best of our knowledge was <1,000 mIU/mL. It is inferred that all false positive cases have results <1,000 mIU/mL.

In all 61 cases, false positive hCG was initially demonstrated at the USA hCG Reference Service. To the best of our knowledge, in all cases, after false positive hCG was identified, it was assumed that all the hCG results in recent history had been faulty,

and all treatment was halted, even though the physician's laboratory test, the problem test, remained positive. Women having false positive hCG results also commonly have falsely elevated results in other unrelated tests (carcinoembryonic antigen, CA-125, prostate-specific antigen, thyroid hormones, troponin, and other tumor and cardiac markers). It is important for physicians and patients to be aware of this to avoid future misdiagnoses.

In most cases, false positive hCG results (in the USA hCG Reference Service assays) were prevented by pretreatment of serum with Scantibodies Inc. HBT heterophilic antibody-blocking agent.¹⁶ Certain hCG assays have a propensity for giving false positive results. Forty-nine of the 61 false positive cases arose from physicians monitoring patients with the Abbott AxSym hCG β assay; 4 with the Bayer Centaur test; 2 each from the Dade Dimension RXL, Ortho Vitros Eci and Bayer ACS180; and 1 each from Tosoh Nexia and Bayer Immuno-1. It is highly recommended that physicians monitoring a patient with suspected GTD or other hCG-producing malignancies (testicular cancer and germ cell tumors) check with the laboratory regarding which test is being used and avoid the use of the Abbott AxSym hCG β and Bayer Centaur tests.

Many of the clinicians who managed the 61 false positive cases were deceived by transient decreases in the hCG values after chemotherapy or surgery. The decreases in hCG falsely suggested the presence of disease or indicated the success of therapy, making the case look like real GTN. These transient decreases in false hCG results were likely due to interim weakening of the immune system by chemotherapy or surgery, reducing the production of heterophilic antibodies and decreasing the extent of false positive hCG results.

ITA

Hyperglycosylated hCG is a variant of hCG with exceptionally large N- and O-linked oligosaccharide side chains.^{5,28} It is produced primarily in invasive trophoblast disease and in early pregnancy at the time of implantation.^{2,5-7,29} Cytotrophoblast, or primitive trophoblast, cells are phenotypically invasive cells. These are the principal cells in choriocarcinoma tumors and in blastocysts at the time of implantation.³⁰⁻³² While invasive cytotrophoblast cells produce hyperglycosylated hCG, differentiated syncytiotrophoblast cells produce regular hCG.^{32,33} A monoclonal antibody was generated against choriocarcinoma hyperglycosylated hCG³⁴

and a specific assay established. JAR and JEG-3 choriocarcinoma cells produce hyperglycosylated hCG rather than regular hCG.²

Lei and colleagues showed that JAR cells are tumorigenic in nude mice. As shown, the hyperglycosylated hCG produced by these cells is critical to tumorigenesis and cancer growth.³⁵ This finding was recently confirmed in our laboratory using JEG-3 choriocarcinoma cells and antibody against hyperglycosylated hCG (Cole et al, unpublished data). Quest Diagnostics and Nichols Institute Diagnostics have developed an automated immunoassay for detecting hyperglycosylated hCG. Considering that hyperglycosylated hCG is produced only by invasive cells and seemingly has a separate, direct role in the invasive process, the molecule was re-named ITA.

ITA accounted for no detectable percentage of total hCG in 49 of 53 cases of persistent real hCG or noninvasive GTD and <30% of hCG immunoreactivity in the remaining cases.⁶ In contrast, ITA accounted for 100% of the hCG immunoreactivity in 12 of 20 cases with a GTN or choriocarcinoma diagnosis and for >30% of the immunoreactivity in the remaining 8 cases.^{6,7} This indicates that the proportion of ITA is a nearly perfect marker of invasive disease.

The USA hCG Reference Service now routinely measures ITA as a marker of invasive GTD.^{1,6,7} ITA has now been measured in 94 of 121 persistent hCG cases with real hCG. ITA was undetectable (90 of 94 cases) or accounted for <30% of total hCG immunoreactivity (4 of 94 cases) in those with dormant or noninvasive GTD and that ITA values of 100% or >30% were recorded in 22 of 22 cases with GTN or choriocarcinoma. A commercial assay is now available for measuring ITA (Nichols Advantage ITA test). Alternatively, physicians can order the ITA/hCG combination through Quest Diagnostics (Nichols Advantage ITA test).

Quiescent GTD

Among other unexpected results recorded by the USA hCG Reference Service was the finding of persistent real hCG values in women with a history of hydatidiform mole or GTN. Sixty-eight cases have been evaluated. In 4 cases pituitary hCG was demonstrated (see below). In the remaining 64 cases relatively low hCG levels were detected, persisting with small fluctuations for 4 months to 12 years (not continuously rising, as would be expected in persistent or invasive mole). The median per-

sistent hCG levels at the time of USA hCG Reference Service referral was approximately 25 mIU/mL and the 95th percentile 200 mIU/mL. No tumor was shown by ultrasound, computed tomography or magnetic resonance imaging scans. In 43 of the 64 cases, persistent or invasive mole was diagnosed, and single-agent chemotherapy, multi-agent chemotherapy or surgery was prescribed; in all cases the therapy failed to completely suppress the hCG values. In all 64 cases, zero or minimal (<30% of total hCG) ITA immunoreactivity was detected, indicating the absence of invasive tissue.

In a forum at the XIth World Congress on Gestational Trophoblastic Diseases, it was agreed to call these dormant, noninvasive cases "quiescent GTD." A representative case is outlined in Table IV. In 5 cases, however, patients were referred to the USA hCG Reference Service a second time. After 0.25–3 years with proven quiescent GTD, hCG levels rapidly rose. (At the time of referral, hCG levels were 213–6,000 mIU/mL.) At that time the development of GTN was indicated, and the tumor was imaged. In all cases, significant ITA was detected: 57%, 65%, 81%, 100% and 100% of total hCG. To the best of our knowledge, in all 5 cases combination chemotherapy was started and successfully treated the GTN.

Table IV Example of a Patient with Persistent Low hCG Results or Quiescent Gestational GTD After a History of Complete Hydatidiform Mole

Time (d)	hCG (mIU/mL)	Notes
20	32	7 Mo after evacuation of complete mole
22	20	Computed tomography/magnetic resonance imaging reveals no disease
27	17	
41	21	Ultrasound reveals no abnormalities
43	33	
45	33	2 Courses methotrexate chemotherapy
60	29	
67	15	
78	31	Actinomycin D
80	20	
86	15	Dilatation and curettage reveals no trophoblastic tissue
103	20	
105	34	
105	34	<i>Real hCG confirmed, no ITA detected, quiescent GTD indicated</i>

Persistent low hCG results were detected 7 months after evacuation of the mole. While real hCG was demonstrated, no significant ITA was detected. USA hCG Reference Service results are shown in italics.

Table V Example of a Patient with Persistent Low hCG Results in the Absence of a History of Hydatidiform Mole or Evidence of Other Disease

Time (d)	hCG (mIU/mL)	Notes
0	44	Incidental pregnancy test
14	40	Dilatation and curettage reveals no evidence of pregnancy
21		Laparoscopy reveals no ectopic pregnancy
72	52	
222	30	Computed tomography/magnetic resonance imaging reveal no disease
240	32	
267	35	4 Courses methotrexate chemotherapy
301	29	
322	35	Actinomycin D
420	34	
627	60	Magnetic resonance imaging indicates uterine irregularities
632	60	Hysterectomy and BSO, pathology unremarkable
642	34	
668	20	3 Courses EMA-CO combination therapy
803	24	
808	24	<i>Real hCG confirmed, no ITA detected, quiescent GTD indicated</i>

No pregnancy was demonstrated. The patient was assumed to have GTN. USA hCG Reference Service results are shown in italics.

This indicated that in 5 of 64 cases (7.8%), after a considerable period with quiescent GTD, cells transformed, and invasive disease developed. Quiescent GTD appears to be a premalignant syndrome that progresses to GTN. For these reasons, cases need to be meticulously monitored for the needed number of years in which hCG is detectable and longer in case it recurs.

The USA hCG Reference Service also consulted on 57 further cases with persistent low hCG values and with a history of pregnancy but no history of GTD. In these cases, persistent low hCG levels were detected from 2 months to 6 years. A representative case is outlined in Table V. As in the cases with history of GTD, the median persistent hCG levels at the time of USA hCG Reference Service referral was 25 mIU/mL and the 95th percentile close to 200 mIU/mL. Also like the case with a history, no tumor was shown by ultrasound, computed tomography or magnetic resonance imaging and ITA was either not detected or accounted for <30% of total hCG. Like those with GTN history, after 1–4 years

with quiescent GTD, 4 of 57 cases (7.0%) were referred to the USA hCG Reference Service a second time for rising hCG results. At the time of referral, the hCG levels were 190–1,255 mIU/mL. In a forum at the XIth World Congress on Gestational Trophoblastic Diseases it was suggested that such cases be called “unexplained elevated hCG.” Considering the intense likeness between the cases with and without a history, however, we think that all these persistent low hCG cases are part of the same quiescent GTD syndrome. We have seen 121 cases with quiescent GTD, and 9 of them have progressed to GTN.

Other centers have reported similar quiescent GTD cases. Hancock and colleagues²⁴ observed 9 quiescent GTD cases. As observed in the USA hCG Reference Service analysis of the report, none responded to chemotherapy or surgery, and withholding therapy was recommended. As reported, in 2 of 9 cases choriocarcinoma later developed. Kohorn²⁵ observed 4 cases of quiescent GTD. In 1 case, rising hCG values were recorded, indicating GTN. In the USA hCG Reference Service experience, 7.4% (9 of 121) of cases progressed to GTN. The Hancock²⁴ and Kohorn²⁵ findings, with smaller numbers of cases, indicate that a higher proportion of quiescent GTD cases progress to GTN. The USA hCG Reference Service examines patient records on 1 and, occasionally 2 consultations. Rarely do we receive follow-up data on referred patients, and the actual number of subsequent malignant cases is probably higher. At this time we can only assume that the likelihood of developing GTN is somewhere between the extremes of our findings and those of others,^{24,25} or 7–25%.

What is quiescent GTD or the hCG source that does not respond to chemotherapy? In these cases, the hCG resembled the minimally hyperglycosylated forms produced by highly differentiated syncytiotrophoblast cells in the second or third trimester of pregnancy or in a 6–10-week gestational hydatidiform mole rather than that of invasive cytotrophoblast hCG. This observation, the lack of clear response to chemotherapy and the absence of an identifiable mass together suggested that the low levels of hCG might have arisen from spread-out noninvasive cells. It is likely that scattered highly differentiated syncytiotrophoblast cells produce pregnancylike hCG, remaining from evacuation of a hydatidiform mole or pregnancy. These would be noninvasive cells and, because they are slow growing, would respond less to chemotherapy.

The USA hCG Reference Service has now been consulted on 121 cases of quiescent GTD, whether arising from a history of hydatidiform mole, GTN or pregnancy. Table I details the nature of the hCG in these cases. According to records, quiescent GTD has been detectable in individuals for periods ranging from 2 months to as long as 12 years. In 77 of the 121 cases patients were given therapy for assumed GTN or persistent/invasive mole. In all cases the therapy was either ineffective or failed to completely suppress the hCG. Clearly, therapy is needless and potentially harmful to subjects in quiescent GTD cases. Seventy-one women received single-agent chemotherapy, 12 received combination therapy (EMA-CO), and 25 had seemingly needless hysterectomy or BSO.

In 9 cases the disease progressed to GTN or to pathology-proven choriocarcinoma or PSTT after 0.25–4 years (median, 2) with quiescent GTD. In these cases the median hCG result at the time of the second referral was 489 mIU/mL; the range was 190–6,000. Quiescent GTD is clearly a premalignant syndrome for GTN/choriocarcinoma/PSTT. At the time of the second referral, the median ITA result was 86% of total hCG; the range was 57–100% of total hCG.

Important Changes Needed in the Management of Hydatidiform Mole

Our quiescent GTD observations and the very large number of cases needlessly treated for GTN or persistent hydatidiform mole indicate an urgent need for changes in the management of all hydatidiform mole cases. After evacuation of hydatidiform mole, if the hCG results become undetectable and subsequently begin to increase, the case could be either persistent trophoblastic disease or quiescent GTD. They must be differentiated and can be using an ITA test (available at Quest Diagnostics Inc. and at USA hCG Reference Service) or by observing whether hCG results consistently or sharply rise (e.g., 5, 10, 20, 100, 600 mIU/mL), indicative of persistent or invasive disease requiring chemotherapy, or whether they rise and plateau (e.g., 5, 10, 20, 22, 18, 25, 24 mIU/mL), consistent with quiescent GTD. In cases that plateau, based upon the observations reported above and by others,^{24,25} therapy should be withheld. The median hCG level in true cases of quiescent GTD is 22 mIU/mL, and in 95% of quiescent GTD cases the hCG was <226 mIU/mL. Higher hCG levels (>226 mIU/mL) may be more indicative of persistent mole or GTN.

Table VI Example of a Patient with Pituitary hCG, 55 Years Old, with No Recent History of Gestation and Postmenopausal

Time (d)	hCG (mIU/mL): serum/urine	Notes
0	Positive	Symptoms of emesis, point-of-care urine hCG test performed
12	12	Serum hCG test performed
15	11	Repeat serum hCG considering postmenopause
15		Human antimouse antibody test performed for interfering antibodies or false positivity
45	<i>Total hCG 5.3/38.0</i>	<i>Reference service showed hCG and free β in serum and primarily β-core fragment in urine; considering postmenopause, low concentration and hCG fragmentation, pituitary hCG indicated</i>
62		Patient started on hormone replacement therapy
90	<i>Total hCG 1.8/1.3</i>	<i>Serum and urine hCG and related molecule production suppressed, pituitary hCG identified</i>

USA hCG Reference Service results are shown in italics.

Pituitary hCG

Seven cases presented with very low levels of persistent hCG, 4 following a history of hydatidiform mole and 3 postmenopausally, without a recent history of gestation. In most cases the USA hCG Reference Service detected very low levels of intact hCG and the clear presence of hCG free β -subunit and β -core fragment in serum and particularly urine. In all cases the hCG was eventually shown to be suppressed by hormone replacement therapy or estrogen-progesterone therapy. A representative case is presented in Table VI. In our experience (Table I) the median hCG levels detected are just 4 mIU/mL, the 95th percentile is 9 mIU/mL, and the range is 2–11 mIU/mL. Pituitary hCG is not the likely source of higher levels of hCG. It has been known for at least 20 years that the gonadotrope cells of the pituitary (which produce luteinizing hormone and follicle stimulating hormone) also produce a very small and variable amount of hCG, particularly in the absence of suppression in postmenopausal patients.^{36,37} Pituitary hCG is commonly detected today with modern, ultrasensitive hCG tests, particularly in postmenopausal women.²⁵ In 3 of 4 cases of pituitary hCG in the months following evacuation of hydatidiform mole, single-agent chemotherapy was given for assumed persistent mole. This unnecessary treatment emphasizes the need to place patients on estrogen-progesterone medication after evacuation of hydatidiform mole to prevent pituitary hCG interference in monitoring background hCG. This is standard practice at many centers, primarily to prevent pregnancy, for 1 year.³⁸

Conclusion

Too many women are being needlessly harmed by chemotherapy, hysterectomy and other surgery be-

cause of misdiagnosis of persistent mole or GTN. Care is urgently needed in excluding false positive hCG results, quiescent GTD and pituitary hCG before diagnosing persistent mole or GTN requiring cancer therapy.

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