Persistent Low Concentration of Human Chorionic Gonadotropin in a Nonpregnant Woman

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CASE
A 48-year-old woman presented for radio-iodine ablation therapy 3 months after undergoing a complete thyroidectomy performed for compressive goiter symptoms. The patient’s medical history included stage-3 follicular variant papillary thyroid cancer with no nodal involvement and no metastatic disease. A 6.5-cm papillary carcinoma had been identified and removed surgically. Despite this surgical treatment, 3 foci were detected by 125I uptake testing, a finding that prompted the use of therapeutic ablation.

Because the patient reported 5 months of amenorrhea, a quantitative serum human chorionic gonadotropin (hCG) test (hCG/H11001/H9252 assay, Roche Diagnostics) was performed to rule out pregnancy. The result was 7.0 IU/L (reference interval: <5.0) and the administration of 100 mCi I131 was cancelled owing to concern for a potential pregnancy. A repeat hCG test performed 4 days later was 7.0 IU/L. Follicle stimulating hormone (FSH) was determined to be 110 IU/L [reference intervals: 1.9 –11.6 (follicular phase), 1.4 –9.6 (luteal phase), and 21.5–131 IU/L (postmenopausal)].

DISCUSSION
hCG is produced by the trophoblastic tissue of the placenta and hence is a reliable marker for pregnancy. hCG also has clinical utility as a tumor marker because of its production by trophoblastic and nontrophoblastic neoplasms. Finally, hCG is also produced by the gonadotropic cells of the pituitary during the menopause (1, 2).

As demonstrated by this patient, a persistent low concentration of hCG is particularly perplexing. Such cases have 3 potential etiologies: (a) false-positive hCG due to interfering antibodies, (b) quiescent gestational trophoblastic disease, and (c) pituitary hCG.

FALSE-POSITIVE hCG DUE TO INTERFERING ANTIBODIES
There are several reports of falsely increased hCG due to endogenously produced interfering antibodies (3). Interfering antibodies can be of 2 types: human anti-animal antibodies (HAAA) or heterophile antibodies. HAAAs are directed toward a specific antigen and may be produced after treatment with therapeutic antibodies or exposure to animal antigens (4). Heterophile antibodies display nonspecific interaction with numerous different antigens and are believed to be caused by B cells that have not completed appropriate somatic mutation (5). These antibodies interfere with non-competitive immunometric assays by cross-linking the capture and signal antibodies, leading to falsely increased results (4).

Falsely increased hCG results have occasionally resulted in drastic and unnecessary treatment, and women have undergone hysterectomy and/or chemotherapy in an effort to treat presumed trophoblastic disease based solely on a false-positive finding of increased serum hCG (3).

Suspicion of interfering antibodies should be high when the results of any immunoassay are inconsistent with the clinical scenario. When alerted to the irregularity, the laboratory can investigate the possible presence of interfering antibodies. Common methods used to investigate possible interfering antibodies include the detection of hCG in urine, the use of a different hCG method, and the use of blocking agents. The simplest approach is to confirm the presence of hCG in a urine specimen, because the high molecular weight of interfering antibodies precludes their excretion in urine. This approach may not be practical, however, because when hCG is present at very low concentrations in serum the urine concentration may not reach the detection threshold of qualitative tests. Alternatively, testing can be performed using a different hCG method. Although some interfering antibodies cause problems with multiple assays, often the interference is absent in a method that uses different antibodies and blocking reagents. The effect of interfering antibodies can also be minimized by the addi-
tion of blocking agents. Most manufacturers include such blocking agents in their reagents, but such agents are not always 100% effective. Blocking reagents include purified, nonimmune animal immunoglobulin, and commercial sources are available (Scantibodies Laboratory).

QUIESCENT GESTATIONAL TROPHOBLASTIC DISEASE

Gestational trophoblastic disease (GTD) consists of a variety of disorders that arise from placental trophoblastic tissue. Several histologically distinct types of GTD exist, including hydatidiform mole, choriocarcinoma, and placental site trophoblastic tumor. In each, hCG can function as a marker of tumor burden and as a monitor for treatment response. Patients who have a history of GTD in conjunction with persistent low concentrations of hCG but who lack overt radiological evidence of GTD are considered to have quiescent GTD (Q-GTD) (6), although this classification is not widely accepted.

Based on the limited number of Q-GTD patients who have been described, it appears that the hCG concentration in these patients persists for ≥3 months at low concentrations [<100 IU/L, mean (SD) 34 (38) IU/L; n = 69] and remains stable over time (6). Not surprisingly, patients whose illness fulfills the definition of Q-GTD are often presumed to have persistent disease that requires additional interventions. Unfortunately, in patients in whom treatment has been attempted (57 of 69), 9% showed only a partial hCG decrease and 91% failed to respond (6).

In some Q-GTD patients, hCG concentrations increase months to years after the initial diagnosis, suggesting the reemergence of active GTD that is often responsive to chemotherapy. These findings have given rise to the hypothesis that the hCG of Q-GTD is due to the slow-growing, differentiated, and noninvasive syncytiotrophoblasts and suggests that Q-GTD is a premalignant syndrome that can progress to the metastatic state.

Although hCG is produced principally by syncytiotrophoblasts, hyperglycosylated hCG (HhCG) is predominantly synthesized by the more invasive cytotrophoblast cells (7). In cell culture and animal models, HhCG has been demonstrated to regulate cytotrophoblast invasion and the tumorigenesis of choriocarcinoma (7). Given these findings, HhCG might serve as an effective marker of invasive GTD. In 82 women with either choriocarcinoma or invasive GTD, HhCG accounted for 7%–100% of the total hCG. In contrast, in 69 women with Q-GTD, HhCG accounted for only 0.5%–2.1% of the total hCG (6).

Because they are at risk of developing invasive GTD, women with Q-GTD must be monitored by frequent hCG testing. Although the relative concentration of HhCG might suggest transformation to active

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**Fig. 1.** Pituitary hCG production.

The release of hCG from the pituitary gland may be due to declining ovarian function and the loss of negative feedback inhibition on the hypothalamic-pituitary-ovarian axis.
disease, increasing concentrations of hCG would clearly be an effective monitor.

**PITUITARY hCG**

A substance with hCG-like properties was first identified in the pituitary gland in 1976 and was subsequently localized to gonadotrophs (8). Later, the release of this substance was demonstrated to be stimulated by gonadotropin-releasing hormone (GnRH) and suppressed by administration of estrogen and progesterone (1). Pituitary hCG was not successfully purified until 1996 (2).

The reason for pituitary hCG production is unknown, but a possible explanation is illustrated in Fig. 1. During the perimenopause, the ovarian synthesis of steroids gradually declines, thereby releasing the negative feedback control on GnRH secretion, a process that leads to increased FSH and luteinizing hor-
Hormone (LH) production. Because GnRH can stimulate the release of pituitary hCG, it is likely that the peri- and postmenopausal increase in GnRH accounts for the production of hCG in some individuals.

hCG tests are commonly performed before procedures that might harm a fetus. As this case demonstrates, such screening tests are increasingly being used for older women in whom pregnancy is unlikely. Increased or positive hCG results occasionally occur, and these can unfortunately lead to delays in therapeutic procedures, undue worry, or unnecessary surgery or chemotherapy. The upper reference limits of hCG in older, nonpregnant women have been reported to be ≤8.0 IU/L during perimenopause (41–55 years) and ≤14.0 IU/L during menopause (>55 years) (9). This same study established the upper limit of hCG in nonpregnant premenopausal women (18–40 years) to be ≤5.0 IU/L. Although low concentrations of hCG in peri- and postmenopausal women are not unexpected, differentiating the hCG source is important because perimenopausal women may indeed be pregnant.

Two approaches for identifying the hCG source in perimenopausal women have been described. One advocates the measurement of FSH in perimenopausal women and suggests that a threshold concentration >20 IU/L be used to identify the pituitary as the likely hCG source when the hCG concentration is 5.0–14 IU/L (9). However, this threshold was established from a population that included only 3 perimenopausal women, and a larger study is needed to validate its clinical utility. The 2nd approach is based on the observation that pituitary hCG is suppressed by estrogen-progesterone replacement therapy and advocates its use to delineate pituitary secretion of hCG from trophoblastic disease in pre- or postmenopausal women (10).

DIAGNOSIS
Pituitary hCG.

RESOLUTION OF CASE
In the case patient the lack of an hCG increase and the increased FSH failed to support a diagnosis of pregnancy, and the patient was treated with I131. Nine days later, a body scan revealed the same 3 foci previously identified before the therapy, and another round of ablation was planned.

Although this case was readily resolved after several days by serial hCG measurements, the approach of Snyder et al. (9) would have addressed the physician’s concern regarding potential pregnancy from the outset. By using the serum hCG concentration in combination with the patient’s age and FSH result, a rapid determination of pregnancy status can be accomplished by use of the algorithm shown in Fig. 2. In premenopausal women, low hCG concentrations should be assumed to be pregnancy-related until proven otherwise. For women with uncertain menopause status and a low concentration of hCG, FSH can be clinically useful. Based on age alone, women >55 years old with an hCG concentration of 5.0–14 IU/L are unlikely to be pregnant. It is important to note that this approach only rules-out pregnancy as an hCG source. Other sources of hCG, such as germ cell or nontrophoblastic malignancies, must also be considered.

As demonstrated here, the incidental discovery of low-level hCG can be clinically challenging as the physician attempts to determine not only the cause but how to most effectively manage the patient’s care. Both clinicians and laboratorians must be aware of this issue to avoid unnecessary treatment delays or initiation of inappropriate therapies.

POINTS TO REMEMBER

- Patients with low serum hCG concentrations should have testing repeated in 2–3 days to determine if concentrations are increasing, decreasing, or unchanged.
- Evidence to support quiescent gestational trophoblastic disease includes a history of gestational trophoblastic disease and/or low relative percentage of HhCG.
- Evidence to support interfering antibody interference with the hCG assay includes absence of hCG in the urine, absence of serum hCG when tested is performed with a different method, and decreased serum hCG concentration after addition of blocking agents to the serum specimen.
- Evidence to support the pituitary as a source for the hCG include a decrease in hCG concentrations after 2 weeks of hormone replacement therapy and/or increased concentrations of FSH.

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References

Commentary
Carolyn Y. Muller

Human chorionic gonadotropin (hCG) is a near-perfect biochemical marker for pregnancy. Serial measurements of hCG assist in the differentiation of normal from abnormal gestational events, and sensitive home tests are easy for the lay public to perform and interpret. No test is perfect, however, and providers must understand how to interpret unexpected hCG results. Differential diagnosis must address problems inherent to the test itself (heterophile antibody reactions), abnormal hCG production (from neoplastic trophoblastic or nontrophoblastic tissues), and physiologic hCG (menopausal pituitary) production. Patient history of pregnancy, past gestations, menstrual cycles, and cancer can point to the most likely diagnostic category. Often more sophisticated testing of hCG variants will be required, such as determination of the percentage of hyperglycosylated hCG as a marker of invasive trophoblastic disease (choriocarcinoma) or measured ratio of the free β subunit as a marker of nongestational malignancy. Consultation with clinical and laboratory experts will ensure appropriate and safe clinical management. In the case presented, active papillary thyroid carcinoma was identified in a woman whose age was appropriate for perimenopause. Secretion of small amounts of hCG by the tumor itself was possible but statistically unlikely. Increased follicle-stimulating hormone concentration confirms menopause, and treatment of the cancer should not be delayed.

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Commentary
Ulf-Håkan Stenman

The article by McCudden et al. (1) published in this issue of Clinical Chemistry describes the clinical work-up of a patient with low-positive human chorionic gonadotropin (hCG) due to pituitary or heterophile antibody interference. In addressing this case it is also worthwhile to discuss additional confounding factors that affect hCG measurement.

ASSAY CALIBRATION
In previous studies cited by McCudden et al. (2, 3), hCG concentrations up to 16 IU/L were observed in postmenopausal women. When serum hCG is measured by a highly sensitive and specific method (PerkinElmer Wallac AutoDELFIA®), the upper reference limit for postmenopausal women is 5 IU/L (15.5 pmol/L), and the highest concentration that we have observed is 11 IU/L (4). These disparate findings are likely attributable to differences in assay calibration and the contribution of free hCGβ. Thus, hCG concentrations are assay dependent, necessitating the establishment of assay-specific reference intervals.

HCG ISOFORMS
Approximately 30%–70% of patients with various nontrophoblastic cancers produce hCGβ. The concentrations are usually low, but in 5%–10% of cases they are high enough to increase the concentration of total
hCG to above the upper reference limit. This hCGβ is distinguishable from pituitary hCG because hCGβ is not suppressed by estrogen-replacement therapy. A moderately increased hCG concentration in a cancer patient, however, may be caused by a combination of hCG and hCGβ, and in such cases the concentration of total hCG will be partially suppressed by estrogen-replacement, thereby confounding interpretation of the measured values. In these situations, specific determination of each form of hCG is very useful, but very few assays specific for hCGβ are available (5).

HYPOGONADISM

No systematic studies on hCG concentrations in hypogonadal men are available, but we recently observed hCG concentrations increasing from <0.5 to 4.5 IU/L after withdrawal of testosterone in a hypogonadal patient who had been treated for testicular cancer. This finding caused suspicion of a relapse, a diagnosis that was excluded on the basis of assay results for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and the suppression of hCG with testosterone replacement (6). This case shows that increased hCG associated with hypogonadism can also occur in men.

Hypogonadism can also be caused by intensive chemotherapy, which may lead to increased serum hCG similar to that seen in menopausal women. Such cases may cause confusion, especially when serum hCG is used to monitor the effectiveness of chemotherapy. It is important to differentiate increased hCG caused by a relapse from that induced by chemotherapy; this can be done by measurement of LH and FSH (5).

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References