False-Positive Serum Human Chorionic Gonadotropin (hCG) in a Male Patient with a Malignant Germ Cell Tumor of the Testis: A Case Report and Review of the Literature

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ABSTRACT

A 39-year-old male patient with a favorable prognosis stage IIB metastatic malignant germ cell tumor (GCT) and elevated pre- and postorchietomy serum human chorionic gonadotropin (hCG) was treated with three courses of combination chemotherapy resulting in a rapid normalization of his serum hCG.

Within 2 months after the cessation of chemotherapy, his serum hCG increased again, suggesting tumor recurrence. Pathological examination of the resected residual retroperitoneal lymph nodes revealed no vital tumor cells. Based on the further rise in his serum hCG and enlargement of mediastinal lymph nodes on computed tomography scan, the patient underwent second- and third-line chemotherapy, which did not result in normalization of his serum hCG. Reanalysis of stored serum samples with other immunoassays revealed that the elevated serum hCG levels collected before first-line chemotherapy were indeed elevated, but those collected after first-line chemotherapy were all falsely positive. Currently, the patient is still alive and disease free. This is the first report of a male cancer patient who received unneeded second- and third-line chemotherapy for relapse based on false-positive hCG results. We discuss the pitfalls of false-positive serum hCG measurements, including heterophilic antibodies, as in our IgA-deficient patient, and review the literature. The Oncologist 2008;13:000 – 000

INTRODUCTION

Malignant germ cell tumors (GCTs) are rare tumors, accounting for 1% of all malignancies, and the commonest type of cancer in young men aged 15–35 years. Patients with metastatic GCTs have an excellent prognosis because of the chemosensitivity of these tumors. The majority of patients present with elevated serum markers, for example, human chorionic gonadotropin (hCG) and/or α-fetoprotein (AFP), and their prechemotherapy levels have been integrated into the International Germ Cell Cancer Consensus Group consensus prognostic index for nonseminomatous GCT classification. Patients
are stratified into good-, intermediate-, and poor-prognosis subgroups based on the primary tumor site, levels of serum tumor markers, and whether extrapulmonary visceral metastases are present.

Serum hCG levels are thus essential in both the diagnosis and follow-up of hCG-producing GCTs. Staging procedures with chest and abdominal computed tomography (CT) and evaluation of serum markers are used to detect subclinical residual or recurrent tumor, and patient management includes treatment with chemotherapy in cases of a rise in a serum marker. After orchiectomy, an increased level of hCG indicates persistent disease, whereas after chemotherapy-induced complete remission of metastatic disease, reappearance of hCG indicates the presence of a relapse.

**CASE REPORT**

In February 2003, a 39-year-old male patient underwent a unilateral orchietomy because of a right testicular mass. He had an elevated serum hCG level of 30 U/l (upper normal value, 5 U/l), normal serum levels of AFP, and normal lactate dehydrogenase. Histological examination of the tumor showed an embryonal carcinoma. On an abdominal CT scan he had enlarged retroperitoneal lymph nodes of nearly 3 cm, and his postorchiectomy serum hCG level decreased but did not normalize, consistent with a stage IIB, favorable prognosis metastatic GCT. The patient was referred to our hospital, and systemic treatment consisted of three courses of chemotherapy with bleomycin, etoposide, and cisplatin (BEP) (March until April 2003). The prechemotherapy hCG of 13 U/l decreased to undetectable (<1 U/l) levels within 3 weeks after the start of chemotherapy (Fig. 1). Normalization of his serum hCG and the size of the enlarged retroperitoneal lymph nodes indicated that a complete remission was induced, requiring no further treatment.

Two months later, his serum hCG levels gradually increased to 55 U/l, suggesting an early marker-positive relapse. CT scans of the chest, abdomen, and cerebrum, an ultrasound of the contralateral testis, and a positron emission tomography scan did not reveal the site of relapse. Cerebrospinal fluid contained no malignant cells, and hCG was undetectable in the cerebrospinal fluid. Because the retroperitoneum was the most likely relapse site, a retroperitoneal lymph node dissection was performed on July 8, 2003, but microscopic examination of the resected lymph nodes showed no viable cancer cells.

Postoperatively, his hCG further increased to 280 U/l (Fig. 1). In August 2003, a CT scan of the chest showed two mediastinal lymph nodes of 11 mm diameter each, which were slightly larger than on previous CT scans. We diagnosed a relapse of the patient’s hCG-producing GCT and decided to treat him with second-line chemotherapy. From September to October 2003, three courses of chemotherapy with paclitaxel, ifosfamide, and cisplatin were administered, upon which his hCG decreased to 55 U/l. However, thereafter his hCG increased again to 341 U/l (Fig. 1). The enlarged mediastinal nodes did not change in size. We subsequently planned to treat the patient with high-dose chemotherapy and autologous stem cell rescue. He received two courses of ifosfamide plus etoposide followed by high-dose cyclophosphamide in November and December 2003, but stem cell mobilization failed, while his serum hCG values decreased to 145 U/l.

Surprisingly, in January 2004, a normal serum hCG level (<0.5 U/l) was reported, coinciding with a switch to measurements of serum hCG samples on a Modular Analytics E 170 system (Roche Diagnostics, Mannheim, Germany) by our clinical chemistry laboratory. In 2003, the AxSym analyzer total β-hCG method (Abbott Diagnostics, Abbott Park, IL) was used. In all subsequent serum hCG samples collected in 2004, the hCG levels measured using the Modular Analytics E 170 system were undetectable, whereas measurements with the AxSym analyzer resulted in hCG values of approximately 255 U/l. Retesting of the serum samples of March 2003, collected prior to the first BEP chemotherapy, on the Modular Analytics E 170 showed measurable, although somewhat lower, serum hCG values of 9 U/l (upper normal value, 5 U/l), which gradually decreased toward undetectable in April 2003, confirming the initially elevated hCG results found using the AxSym analyzer. Using the AxSym analyzer, hCG was also found in urine samples from January 2004 (7.8 U/l), in the presence of just a trace of albumin in the same urine sample. However, analyses of both serum and urine samples of December 2003, and January 2004, on an Immulite® 2000 (Diagnostic Products Company [DPC], now Siemens Healthcare Diagnostics, Deerfield, IL), an Abbott Architect (Abbott Diagnostics), and using an in-house radioimmunoassay method in the Dutch hCG Reference Laboratory in Nijmegen indicated that the hCG level was undetectable in all samples. Interestingly, AFP was measured in parallel with the hCG assay on the AxSym analyzer. AFP concentrations 7–10 μg/l before, during, and after chemotherapy (upper reference limit, 10 μg/l). The switch to the Roche system did not result in any shift. Other assays for protein hormones, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH), have shown no discrepant results.

Thus, our patient presented with a clinical stage IIB nonseminomatous GCT originating in the right testis with confirmed preorchiectomy as well as prechemotherapy elevated serum hCG levels. Following BEP chemotherapy, a pathologically confirmed complete remission was induced. However,
he was later misdiagnosed with a relapse of the disease as a result of falsely elevated serum hCG concentrations.

We considered that the reappearance and rise in serum hCG levels following the cessation of curative first-line chemotherapy in this good-prognosis male GCT patient could be ascribed to the presence of heterophilic antibodies, which may interfere with serum two-sided immunometric assays. Heterophilic antibodies have been reported in IgA-deficient individuals. Serum IgA levels measured in January 2004 were below the detection level of 0.14 g/l, no anti-IgA antibodies could be detected in the patient’s blood, whereas IgG levels were normal, consistent with an IgA deficiency. The medical history indicated that the patient indeed suffered from recurrent upper respiratory tract and middle ear infections during childhood, and IgA concentrations measured in 1993 were below the lower detection level of 0.1 g/l (normal value, 0.9–4.0 g/l). Various tests were subsequently performed by the clinical chemistry laboratory; the addition of a specific heterophilic antibody-blocking agent from Scantibodies Laboratory, Inc. (Santee, CA) to the AxSym analyzer hCG assay did not result in normal hCG measurements in the patient’s serum samples. Dilution of the patient’s serum with control serum or other diluents resulted in a nonlinear decrease in the patient’s serum hCG concentration. Serum levels of testosterone, LH, FSH, and sex hormone-binding protein were found to be within normal limits in all serum samples prior to the start of first-line (March 2003) as well as after the cessation of third-line chemotherapy (February 2004).

In this young male patient with testicular cancer, measurements of the patient’s serum samples resulted in false-positive hCG results as measured by the Abbott AxSym system, and the patient received treatment with high doses of chemotherapy for an erroneously diagnosed relapse of the disease. His history is remarkably misleading because of the initial presentation with an hCG-producing GCT, the prompt decline in serum hCG in parallel with a decrease in size of the enlarged lymph nodes following the institution of chemotherapy, the reappearance 2 months later consistent with an early and thus clinically unfavorable tumor relapse, followed again by a decline in his serum hCG during second-line and third-line treatment with cytotoxic agents. Currently, the patient is still alive and disease free.

**Figure 1.** hCG concentration (U/l) versus time. Serum hCG is plotted over the course of therapy. The BEP chemotherapy regimen was administered from March to April 2003. RPLND was performed on July 8, 2003. TIP chemotherapy was administered during September to October 2003. HDC, consisting of ifosfamide plus etoposide followed by high-dose cyclophosphamide, was administered during November to December 2003.

Abbreviations: BEP, bleomycin, etoposide, and cisplatin; hCG, human chorionic gonadotropin; HDC, high-dose chemotherapy; RPLND, retroperitoneal lymph node dissection; TIP, paclitaxel, ifosfamide, and cisplatin.
REVIEW OF THE LITERATURE

hCG is mainly used for the detection and monitoring of pregnancy and pregnancy-related disorders, but it is also an extremely sensitive and specific marker for trophoblastic tumors of placental and germ cell origin. Thus, treatment of metastatic or recurrent testicular GCTs is often initiated on the basis of rising hCG levels, even in the absence of clinical, radiological, or histological evidence of a relapse.

False-positive serum hCG levels have mainly been reported in women suspected of (ectopic) pregnancy, molar gravidity, or gestational trophoblastic neoplasm (GTN). During the last two decades Cole and his coworkers from the USA hCG Reference Service identified a total of 71 women with false-positive hCG results (also called “phantom hCG”), mean 102 ± 152 IU/l (range, 6.1–900) [1–6]. In most of these patients, extensive imaging procedures revealed no measurable abnormal lesion. Forty-seven patients received unneeded chemotherapy. Twelve patients even underwent surgery without finding a malignancy in the operation specimen. Sera of these women were referred to the USA hCG Reference Service, where different hCG assay methods were applied, and the high levels of hCG were found to be falsely elevated. Also, other authors have published case reports on women in whom false-positive hCG results led to a wrong diagnosis and often to invasive diagnostic procedures and harmful treatment [7, 8].

Only a few case reports have dealt with falsely elevated hCG results in male patients with GCTs, in some cases leading to unnecessary surgical procedures [9–13]. However, to our knowledge, our index patient is the first male case who has received unneeded systemic chemotherapy for falsely elevated hCG.

Several causes for false-positive serum hCG levels have been reported in the literature (Table 1). Heterophilic antibodies, low affinity antibodies frequently found in human serum, are mentioned as the most frequent cause of false-positive hCG results [1–6, 14–19]. These antibodies may develop after infections or contact with animal tissues or constituents. They crossreact with immunoglobulins from different species, but their titer is generally low. Heterophilic antibodies are notably known to interfere with two-sided immunometric assays, widely used for the detection of serum proteins. Two-sided assays typically use a mouse monoclonal capture antibody bound to a vessel or bead, which immobilizes the serum analytes (e.g., hCG) by binding one epitope on hCG. A tracer antibody, polyclonal or monoclonal IgG of animal origin, is labeled with an enzyme or chemiluminescent agent and thereby marks the immobilized antibody by attaching to a distant epitope on the antigen. Interference with heterophilic antibodies can occur because of linkage of the animal capture antibody to the tracer antibody in the absence of antigen, resulting in a false-positive assay outcome. Using modern two-sided assays, the estimated amount of interference is on the order of 1 in 10,000 samples assayed [3]. However, interference may occur more frequently when using certain commercially available assays. The estimated interference rate leading to false-positive results of the Abbott AxSym total beta-hCG test, which is used in 28% of laboratories in the U.S., is estimated to be 1 in 1,430 samples assayed [3]. Heterophile interference is also reported to lead to falsely elevated or decreased tumor markers, for example, AFP, cancer antigen 125, carcinoembryonic antigen, thyroglobulin, and other proteins, in assays frequently used in the clinical setting [20–28].

Several methods to reduce the incidence of false-positive hCG measurements resulting from heterophile interference are mentioned in the literature. Flooding the reaction mixture with nonspecific animal antibodies or specific antibodies against potential heterophilic antibodies is frequently used, promoting that heterophilic antibodies bind the excess added nontracer antibodies rather than the specific antibodies used in the immunometric assay. This method reduces the incidence of heterophile interference significantly but does not eliminate it completely, as could have been the case in our patient [3, 17]. Another method is to measure the hCG concentration in urine. Normally, antibodies are not present in urine, whereas serum hCG does appear in urine after excretion. Therefore, testing of urine with the same hCG assay might be informative as to whether the serum hCG is falsely positive. The presence of hCG in serum but not in urine is suggestive of heterophile interference because antibodies are normally not present in urine. However, urine testing is not discriminative in cases of relatively low (true) serum hCG values (<100 IU/l), because urine hCG levels may be too low to be detected. In addition, the common commercial kits are not validated to use urine as a matrix for detection of hCG [15]. Therefore, none of the manufacturers of commercial hCG kits claim that their assays should be used for detection of hCG in

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Abbreviation: hCG, human chorionic gonadotropin.
low-level production of hCG by the pituitary is known to occur in menopausal women because physiological blood tests [27, 33]. Immunometric assays, resulting in false test results of many collection tubes have been reported to interfere with hCG concentrations [32]. Also, additives to blood collection tubes containing hCG at a high concentration, collected from a poor-risk testicular cancer patient peripheral blood stem cells containing hCG at a high concentration, when his serum hCG levels were remarkably elevated, resulted in false hCG surges [32]. Also, additives to blood collection tubes have been reported to interfere with immunometric assays, resulting in false test results of many frequently performed blood tests [27, 33].

Unexpected but truly elevated serum hCG levels can be observed in menopausal women because physiological low-level production of hCG by the pituitary is known to occur. This may be confirmed by the resolution of hCG levels after the administration of hormone-replacement therapy for ≥2 weeks [5]. Similarly, a moderate increase in serum hCG concentration resulting from a physiological pituitary reaction can be detected in men with hypogonadism [34]. Although we did not measure testosterone levels in our patient’s serum during the course of chemotherapy, there were no clinical or biochemical signs of hypogonadism before and after the chemotherapy courses.

CONCLUSIONS

In conclusion, elevated hCG levels are an essential and integral part of the international staging systems of GCTs, and initiation of systemic treatment in a patient with a history of a marker-positive GCT with a rise in a serum marker in the absence of any radiological abnormal lesion is a commonly adopted strategy [35–37]. However, if hCG is the sole elevated marker, clinicians should be aware that kits for the assay of serum hCG have been licensed in the U.S. and many other countries only as an aid to the detection of pregnancy and not for monitoring of GCTs (which may even produce irregular forms of hCG), and this is clearly stated in the accompanying kit assay information brochures. Although hCG is a very useful marker for the diagnosis and staging of GCTs, and monitoring of the therapeutic response and detection of tumor recurrence, our case report emphasizes the potential pitfalls of using serum tumor markers in the absence of a clinical correlate as the sole reason to start chemotherapy in patients with GCTs. Confirmation of elevated serum hCG levels by alternative serum hCG measurement methods, exclusion of other non-malignant causes of elevated hCG levels, and optimal communication between clinical chemists and clinicians are essential to avoid unneeded medical interventions.

AUTHOR CONTRIBUTIONS

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REFERENCES


6 USA hCG Reference Service. Heterophilic Antibodies and False Positive


22 Kuroki M, Matsumoto Y, Arakawa F et al. Reducing interference from heterophilic antibodies in a two-site immunoassay for carcinoembryonic anti-