

PHANTOM hCG AND CLINICAL MANAGEMENT

To the Editor:

The measurement of apparent but spurious human chorionic gonadotropin (hCG) has been reported in the literature over the last three decades, and has recently been referred to as “phantom hCG”. Such false-positive results have led to some women being diagnosed with gestational trophoblastic disease (GTD) and undergoing a variety of diagnostic procedures, chemotherapy, hysterectomy, and other surgical procedures before it is recognized that the test is giving spurious results. It is relatively rare but immunoassay interference is still an important problem that can result in medical-legal or malpractice issues.

hCG is produced in pregnancy, in GTD, and in men with testicular germ cell malignancies. It is a perfect tumor marker for GTD with 100% sensitivity and specificity. Immunoassay is such a beautiful analytical technique. hCG assay has revolutionized the management of ectopic pregnancy and GTD and is now capable of detecting the presence of a pregnancy before a missed menstrual period. In most cases, false-positive hCG is discovered at the time of an incidental serum pregnancy test, as part of a check-up, before surgery, or before a diagnostic procedure. Persistent low hCG results in the range of 100–1000 mIU/mL over 2–6 weeks may be confused with an ectopic pregnancy and lead to methotrexate therapy or surgical intervention, including salpingectomy. It is vital to remember that a test is always a test. False-positive and false-negative results can occur with any specimen. Caution should be exercised when clinical findings and laboratory results are discordant. The ability of laboratory measurements to guide the clinician appropriately in every circumstance is limited [1]. Certain individuals have circulating factors in their serum that interact with the hCG antibody, the most common of which are hetrophile antibodies. These are human antibodies directed against animal-derived antigens used in immunoassays. People with hetrophile antibodies might have unusual results in a number of different kinds of assays.

False-positive serum hCG results are estimated to occur in 1/1,000 to 1/10,000 tests. Using two-site immunometric assays, the frequency with one such assay was reported to be as low as 1/1,000,000 tests [2]. Most false-positive results are due to interference by non-hCG substances (especially human luteinizing hormone, hLH, and anti-animal immunoglobulin antibodies) and the detection of pituitary hCG. Clinically significant false-positive results are rare. Misleading results are usually seen with values below 1,000 mIU/mL. Causes of false-positive hCG measurements have been summarized into four areas [3]: measurement of pituitary hCG-like substance; production of free hCG β -subunit; interference by non-hCG substances, including hLH or hLH β -subunit, both species-specific and heterophilic anti-animal immunoglobulin antibodies, rheumatoid factor, anti-hCG antibodies, and nonspecific serum factors; and assay issues such as carryover by positive displacement pipettes and contaminants that affect label detection (radioactive iodine or fluorophores).

To err is human, therefore an immunoassay may fail for the same reasons as any other laboratory test. Erroneous results for analytes may be reported due to sample mix-up, mislabeling, etc. Also, sample quality may affect some immunoassay results (e.g. presence of overt hemolysis, hyperbilirubinemia, lipemia).

Characteristics of false-positive hCG measurements include: low-level positive result (generally < 1,000 mIU/mL and usually < 150 mIU/mL); positive serum but negative urine; serial dilutions of serum that are not parallel to the hCG standard and yield higher or lower levels of hCG when multiplied by dilution factor; positive hCG results that are not consistent with clinical or surgical findings; no substantial changes in levels that were measured in serial blood samples, even after therapeutic procedures; and negative results in a different type of quantitative hCG assay [3]. Therefore, when there are discordant hCG results, the following steps are recommended [3]: measure urinary hCG, re-measure the hCG concentration using a different method, look for parallelism to the hCG standard in serial

dilutions of the serum, add normal mouse serum or other animal serum to the assay, test for anti-hCG antibodies, and measure hCG concentrations in several days or weeks.

Heterophile (or heterophilic) antibodies are present in human serum and recognize the animal immunoglobulins usually employed in commercial immunoassays (e.g. those derived from mice or rabbits). In sandwich-type immunoassay formats, these antibodies have the ability to link the capture and detection antibody without the presence of antigen, thus leading to false-positive results. In order to eliminate most of the interference, animal immunoglobulins such as rabbit, goat, or mouse immunoglobulin, or proprietary reagents developed by companies, are added to the reagent mixture.

The management of women with persistent low hCG results should be emphasized. The USA hCG Reference Service is a consulting service with a specialized clinical laboratory aiding physicians in the interpretation of conflicting or misrepresentative hCG results. Of 189 patients with persistent low levels of hCG but no evidence of pregnancy or tumor, quiescent GTD was identified in 121 cases. Another 61 cases had false-positive hCG and seven had low levels of hCG of pituitary origin. A total of 128 patients (77/121 with quiescent GTD, 48/61 with false-positive hCG, and 3/7 with pituitary hCG) had undergone various therapies ranging from chemotherapy to hysterectomy [4]. Unfortunately, the number of needlessly treated cases referred to the Reference Service is increasing. The observations of the Reference Service for 134 cases with persistent low hCG results have been reviewed, and the appropriate management of those presenting with persistent low hCG results reported. In a patient with a recent or remote history of hydatidiform mole or GTD, the first step is to confirm the diagnosis using a different laboratory and hCG test. If results are very different (more than twofold), then a false-positive hCG result is likely. If the patient is over 45 years old or post-oophorectomy, then pituitary hCG should be considered as the likely cause, regardless of history, and can be confirmed with 2 weeks or so of combination estrogen-progesterone therapy, which suppresses hCG in patients with this diagnosis. The diagnosis of quiescent disease can be confirmed by showing the absence of hyperglycosylated hCG (< 0.3 ng/mL). This test is approved by the US Food and Drug Administration and is readily available at national clinical laboratories (Nichols Institute Laboratories, invasive trophoblast antigen, ITA, test) [5].

hCG is a glycoprotein composed of two dissimilar subunits (α and β) joined non-covalently. It is not only heterogeneous in peptide structure but also in combination of subunits and in the structure of carbohydrate side chains. Common hCG-related molecules in serum samples include regular hCG, hyperglycosylated hCG (ITA), nicked hCG, nicked ITA, hCG missing the β -subunit C-terminal extension, free α -subunit, free β -subunit, free β -subunit missing the C-terminal extension, hyperglycosylated free β -subunit, and nicked free β -subunit. The same molecules plus β -core fragment are present in urine samples. While ITA and regular hCG predominate in pregnancy samples, one of these multiple hCG-related molecules may be the principal source of immunoreactivity in GTD, gestational trophoblastic neoplasm, choriocarcinoma, and placental site tumor cases as well as in testicular cancer and germ cell tumor. As such, it is critical to appropriately detect all these isoforms in the management of these diseases. Only two tests, the Immulite (DPC, Inc., Los Angeles, CA, USA) and UK radioimmunoassay (RIA; used at Charing Cross Hospital, London) appropriately detect all these hCG-related molecules. False-positive hCG results are a major problem in the management of GTD and cancer. A proportion of false-positive samples with the AxSym test (Abbott Laboratories, Inc., Chicago, IL, USA) can also be falsely positive with the UK RIA, but none are falsely positive with the Immulite test. Thus, the Immulite/Immuline 2000 is the only appropriate assay for monitoring patients with GTD or cancer [6]. In response to the false-positive hCG test problem, Abbott Laboratories added warning and limitation notices to its instruction manual in 2000. It is noted in these warnings that this test should only be used for pregnancy and not for GTD. In laboratories using the AxSym test, it is recommended that twofold dilution is a minimum to prevent a false-positive result [7].

In conclusion, if the hCG result is inconsistent with, or unsupported by, clinical evidence, results should be confirmed using an alternative hCG assay. This may include the qualitative hCG testing of urine.

Results may also be confirmed by performing serial dilution of the sample. In other words, for diagnostic purposes, results should be used in conjunction with other data, such as symptoms, results of other tests, and clinical impressions. Remember that an elevated serum hCG, for example, more than 25 mIU/mL, may represent a normal pregnancy and its complications such as abortion or ectopic pregnancy, a GTD or tumor, and a possibly false-positive or so-called phantom hCG.

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