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Phantom hCG

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A recent phenomenon of the false-positive “phantom” hCG has been noted in both laboratory and clinical medicine. These false-positive results have led to confusion and unwarranted treatment for suspected diagnoses of abnormal pregnancy and gestational trophoblastic disease. It is now known that the false-positive hCG is a consequence of interference of heterophil antibodies with standard assays for hCG.

Heterophil antibodies are human antibodies that have the capability to bind to other species’ immunoglobulins. Thus, they can cause significant interference in commercial kits that employ nonhuman immunoglobulins such as rabbit, mouse, goat, horse or sheep antibodies.[1-3] These antibodies can be categorized into three major groups.

The first category includes polyspecific/multispecific antibodies that have a weak to moderate affinity to various antigens. These antigens can be either auto-antigens or animal antigens, and can be detected in about 40% of the normal population.[2] Since they are detected in low titers and have very low affinity, most do not cause interference in immunoassays. The second category consists of anti-immunoglobulin antibodies that cross react with animal immunoglobulins. These are also weak in nature and have multispecific activities. Rheumatoid factor is the classic example in this category that may cause interference in immunoassays.[2,6] The third, and most important category, consists of high-affinity, high-specificity antibodies, which are produced against well-defined animal antigens. These are encountered in individuals who have had prior sensitization to an animal species. The sensitization may occur as a result of occupational animal exposure, animal products in diet or therapeutic intervention with animal proteins found in some therapeutic or imaging agents.[2,3,6] These antibodies are commonly referred to as human anti-animal antibodies (HAAA).[1-3,6] The commonly encountered human anti-mouse antibodies (HAMA) belong to this category.

The problem of “phantom” hCG was identified in the early 1970s when classic competitive immunoassays were in use.[1] Similar interference can be noted even in some older radioimmunoassays. In recent years, different hCG assays have been marketed to measure serum hCG, which vary in their response to human anti-animal antibodies and heterophil antibodies. Heterophil antibodies and, more

commonly, the HAMAs cause significant interference in tumor marker studies that employ immunometric techniques.[2,3] Circulating human heterophil antibodies bind to immunoglobulins of other species, particularly those used to generate immunoassay reagent antibodies (e.g., mouse monoclonal antibodies). The bridging heterophil antibodies can bind to both the capture and tracer antibodies, thus mimicking antigen-bridge capture and signal antibodies. This mode of interference is seen in 90% of cases of false-positive “phantom” hCG results.[1-3] In the remaining cases, the HAMAs bind to either the capture or the tracer antibody, resulting in falsely low or negative hCG results.

The false-positive “phantom” hCG can lead to a positive pregnancy test in patients who are not pregnant.[1,4,5] The problem is somewhat aggravated by the fact that human anti-animal antibodies and heterophil antibodies can remain in the blood for months or years.[1,4] A diagnosis of “phantom” hCG should be suspected in patients with a negative urine and a positive serum hCG. However, a negative urine test cannot be used to confirm a “phantom” hCG, if the patient’s quantitative serum hCG is below the cutoff level for positivity used in the urine qualitative hCG test.[4] Apart from pregnancy, heterophil bridging antibodies in immunometric hCG assays can cause falsely elevated levels in patients with gestational trophoblastic diseases, choriocarcinoma, testicular germ cell tumors and IgA deficiencies.[1,4] Studies have shown that “phantom” hCG results have had significant clinical impact and have resulted in therapeutic interventions such as unwarranted curettage, laparoscopy, chemotherapy and even hysterectomy for presumed gestational trophoblastic disease.[2,4]

Many techniques are being utilized by the manufacturers to minimize the interference of HAMAs. Addition of a diluent fluid to the assay in kits effectively neutralizes the HAMAs with serial dilutions of the test sample.[1,3] The other method involves the use of blocking agents that include preparations such as heterophil blocking tubes (HBT; scantibodies), that contain freeze-dried immunoglobulins that react with the patient’s specimen to inhibit binding of heterophil antibodies. However, the efficacy of these blocking agents is still debatable.[1-3]

With the increasing use of monoclonal mouse antibodies in diagnostic imaging studies and therapeutic interventions, there is a significant risk of sensitization, thus presenting another source of “phantom” hCG results. Therefore, due to a possible false-positive, increased awareness of this potential problem is essential and caution should be exercised in interpreting and reporting hCG results by the laboratory personnel.[2,3] It is necessary to interpret results in correlation with the clinical picture and other laboratory and imaging studies. If the problem of “phantom” hCG is suspected or identified, the test should be repeated by employing a different assay.[2,3] Alternatively, neutralization by serial dilutions or employing blocking agents can be employed. Some laboratories remove the immunoglobulins from hCG by chromatography before using the immunoassay.[2] Help from the USA hCG Reference Service can be obtained if the

aforementioned techniques are unavailable.[1] This is a specialized consulting service located at the Department of Obstetrics and Gynecology, University of New Mexico, that performs specialized tests as needed following careful review of the patient's history.

The American College of Obstetrician and Gynecologists recommend three procedures to rule out the presence of heterophil antibodies. The first is the urine test; the interference is confirmed if urine is negative and the serum value is at least 50 IU/L. Serial dilutions are the second way to check for linearity. Non-linearity suggests the presence of this interference. Lastly, pretreating the serum to remove heterophil antibodies may be used.[3] All of the above counteractive measures will help to alleviate the interference by "phantom" hCG and minimize unwarranted investigations and therapeutic interventions in individuals with suspected pregnancy or trophoblastic diseases.

References

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