Pathology Consultation on Human Chorionic Gonadotropin Testing for Pregnancy Assessment

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ABSTRACT

Objectives: To provide clarity on the use of qualitative and quantitative human chorionic gonadotropin (hCG) tests for the assessment of pregnancy.

Methods: A case scenario and a brief review of the relevant literature describing clinical and analytical considerations regarding hCG testing are presented.

Results: In pregnancy, hCG is nearly always detectable in serum and urine within 16 days after ovulation. Serial hCG testing is valuable in the evaluation of suspected ectopic pregnancy. hCG assays vary in their analytical specificity, and qualitative tests may be less analytically sensitive than claimed. Erroneous hCG test results can occur for several reasons.

Conclusions: hCG assays are reliable diagnostic tests for pregnancy assessment, but a clear understanding of their limitations is necessary for appropriate result interpretation.

Case Scenario

A 25-year-old woman contacted her physician to inform her of a positive home pregnancy test result. Her last menstrual period had been 36 days earlier. She was taking no medications, and her medical history was remarkable only for chronic fatigue syndrome. Five days later, she sought treatment from the hospital for vaginal bleeding. A quantitative serum human chorionic gonadotropin (hCG) test was 250 IU/L (reference interval, ≤5 IU/L). Transvaginal ultrasound (TVUS) revealed a 0.58-cm candidate for a gestational sac in the fundus of the uterus, but a pseudosac could not be ruled out; no other internal structures were distinguishable. Over the following 6 days, a quantitative serum hCG test was performed every 2 days and showed modest decreases in the hCG concentration (209, 232, and 171 IU/L, chronologically). A manual uterine aspiration was performed and the pathology report indicated a proliferative endometrium, but no villi
were seen. Two days later, the serum hCG concentration had decreased to 120 IU/L, and 2 days after that, paired urine/serum samples were obtained for additional hCG testing. The qualitative urine test was reported as positive, and the serum hCG concentration remained elevated at 148 IU/L. Methotrexate was administered 24 hours later, and serum hCG was measured twice over the next week with a result of 130 IU/L on both specimens **Table 1**. The physician phoned the laboratory to inquire about potential interferences. The most recent serum specimen was evaluated on two alternative quantitative hCG assays manufactured by different vendors, and all results agreed within 15%. Additional testing supported the presence of hCG in the specimens; nothing suggested that the results were falsely positive.

**Case Questions**

1. Why was serial hCG testing performed on this patient?
2. How does the concentration of serum hCG change over time in an intrauterine gestation?
3. Why was the physician concerned about possible interferences in the hCG tests?
4. What are some investigations the laboratory can consider when asked about interferences in hCG tests?

**Background**

hCG is a 38-kDa glycoprotein hormone normally synthesized by placental trophoblastic cells during pregnancy. It is composed of nonidentical α and β subunits that associate noncovalently to form a heterodimer. The same α subunit is identical in three additional heterodimeric glycoprotein hormones synthesized by the anterior pituitary gland: luteinizing hormone (LH), follicle-stimulating hormone, and thyroid-stimulating hormone. The unique β subunit of all four protein hormones provides biological specificity. The β subunit of hCG is closely related to the β subunit of LH, and both hormones bind to the LH receptor, which reflects the physiologic role of both hormones.

Following ovulation, LH initiates the conversion of the ruptured ovarian follicle into a corpus luteum that, in turn, produces progesterone to prepare the endometrium for possible implantation of a fertilized egg. LH is necessary to maintain luteal function for the last 2 weeks of the menstrual cycle and normally gradually declines as progesterone concentrations increase. If pregnancy occurs, hCG binds to LH receptors on the corpus luteum and stimulates progesterone secretion, allowing for maintenance of pregnancy.

The native, heterodimeric, conformation of hCG is considered “intact” hCG and is the biologically active molecule. hCG variants are defined as unpaired or “free” hCG subunits (α or β), differentially glycosylated forms of hCG, or hCG degradation products that accumulate in the serum and/or urine. There are six major hCG variants: free α subunit (hCGα), free β subunit (hCGβ), nicked (hCGn), nicked free β subunit (hCGβn), β core fragment (hCGβcf), and hyperglycosylated (hCGh). **Table 1**. Intact hCG, hCGα, hCGβ, hCGn, and hCGh are detectable in the placenta, blood, and urine. hCGα, hCGβn, and hCGβcf is detectable in the urine only.

**Clinical Utility of hCG in Pregnancy**

hCG is most commonly used as a biomarker of pregnancy because it is synthesized by trophoblast cells of the placenta. In fact, this is the only purpose for which hCG tests have been cleared by the US Food and Drug Administration (FDA). hCG also has established utility as a tumor marker for gestational trophoblastic disease and germ cell tumors and as a component of multimarker serum screening for fetal aneuploidies, but these topics are beyond the scope of this review.

**Intrauterine Pregnancy**

Pregnancy should be suspected whenever a woman of childbearing years experiences amenorrhea. A diagnosis of pregnancy has traditionally been made based on patient history and physical examination. Testing serum or urine for the presence of hCG is frequently used to assess a woman’s pregnancy status before interventions (eg, radiologic imaging, administration of teratogenic medications) that could potentially harm a fetus. Consequently, “How early in pregnancy can hCG be detected?” is a commonly asked question, and the answer depends on several variables. These include the length of the menstrual cycle phases and the analytical sensitivity of the hCG test used.

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**Table 1**

<table>
<thead>
<tr>
<th>Time, d</th>
<th>hCG, IU/L</th>
<th>hCG Change From Previous, %</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>250</td>
<td>NA</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>209</td>
<td>–16</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>232</td>
<td>+10</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>171</td>
<td>–26</td>
<td>Uterine aspiration</td>
</tr>
<tr>
<td>8</td>
<td>120</td>
<td>–30</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>148</td>
<td>+23</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>Not determined</td>
<td>NA</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>13</td>
<td>130</td>
<td>–12</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>130</td>
<td>0</td>
<td>None</td>
</tr>
</tbody>
</table>

hCG, human chorionic gonadotropin; NA, not applicable.
The mean ± SD menstrual cycle length is 28 ± 3.4 days, with the follicular phase accounting for the majority of the observed variation.8,9 This interindividual variability of the follicular phase confounds answering the question that asks how early in pregnancy hCG can be detected because the ability of hCG to be detected does not depend on menstrual cycle length but on the date of ovulation. Johnson et al10 demonstrated the impact that cycle variation has on the number of days before hCG can be detected following conception. In daily urine samples collected between 6 and 26 days after the LH surge (a biochemical predictor of ovulation) from 86 women achieving pregnancy, hCG was detected in 74.7% by the day of the expected period based on last menstrual period. However, when the day of the expected period was defined as 15 days after the LH surge, 100% of women had detectable hCG on that day. Similar results were reported in a study of 62 women achieving pregnancy by artificial insemination.10 When evaluated relative to the day of the LH surge, 100% of women had hCG detectable in the serum 13 days following the LH surge (2 days before the expected menses); urine hCG was detected in 100% by day 16 (1 day after the expected menses). Taken together, these studies demonstrate that hCG should nearly always be detectable in serum and urine within 16 days following the LH surge, which unfortunately cannot always be correlated to the date of the last menstrual period.

Ectopic Pregnancy

Quantitative measurements of serum hCG play an important role in the diagnosis and management of an ectopic pregnancy, which happens when a fertilized egg implants outside of the uterus. Ectopic pregnancy accounts for 1.5% to 2% of all pregnancies and most commonly occurs in the fallopian tube but has also been reported in the abdomen, cervix, or ovary.11 Women with an ectopic pregnancy usually have vaginal bleeding and/or abdominal pain, but some may also be asymptomatic until the fallopian tube ruptures. A ruptured ectopic pregnancy is a life-threatening situation and can lead to death from internal hemorrhaging. Thus, the accurate diagnosis of ectopic pregnancy is critical.

Both quantitative serum hCG measurements and TVUS are used to diagnose an ectopic pregnancy. The normal serum concentrations of hCG span a wide range and depend on gestational age, making a single hCG result insufficient to differentiate an intrauterine from an ectopic pregnancy or a spontaneous miscarriage. Serial hCG measurements are required to quantify the change in hCG over time and to determine if the changing concentration is consistent with normal gestation. In half of all intrauterine pregnancies, serum hCG concentrations increase by 125% or more, and 99% of intrauterine pregnancies have an hCG increase by at least 53% every 48 hours.12 An increase of less than 53% in 48 hours is an indicator that the embryo has an abnormal growth rate and may be ectopic. Indeed, in a study of women with an ectopic pregnancy, the hCG concentration increased by less than 53% in 2 days in 79% of women.13 Notably, an hCG increase of more than 53% in 2 days was observed in 21% of ectopic pregnancies.

Approximately 40% of women with an ectopic pregnancy will have a decreasing concentration of hCG. In 95% of spontaneous miscarriages, hCG will decline by 28% or more over 2 days,14 a decrease that is also observed in 8% of ectopic pregnancies.13 Thus, 29% of women with an ectopic pregnancy have serial hCG results that show increases or decreases not unlike those expected with viable intrauterine pregnancies or spontaneous miscarriages, respectively, a rather high false-negative rate. Therefore, results of hCG testing are frequently assessed in conjunction with TVUS to visualize a gestational sac.

If present, an intrauterine pregnancy should be evident by TVUS at 42 days or more of gestation.11 However, in the absence of such precise dating, the serum concentration of hCG is used as a surrogate marker for gestational age and is commonly interpreted against the “hCG discriminatory zone,” the concentration of hCG at which the sensitivity of TVUS for detecting an intrauterine gestation is nearly 100%. The absence of an intrauterine gestation when the hCG concentration exceeds the discriminatory zone suggests that the pregnancy is not viable but is not diagnostic of an ectopic pregnancy. The hCG discriminatory zone is commonly described as an hCG cutoff concentration between 1,500 and 2,000 IU/L,15 but it is not absolute and is influenced by several variables. Each institution should establish its own discriminatory zone based on TVUS instrumentation, expertise of sonographers, and the hCG assay used.16,17

If an initial TVUS is not diagnostic, follow-up of a suspected ectopic pregnancy is usually guided by algorithms involving serial hCG testing and repeat TVUS. An example of such an algorithm is shown in Figure 11. As with any algorithm, errors are possible and can result in false reassurance that an ectopic pregnancy is absent or medical intervention that can terminate a viable intrauterine pregnancy.

Analytical Considerations

Types of hCG Assays and Sample Matrices

Historically, hCG was detected using bioassays, which, after several decades, were replaced with radioimmunoassays.18 Today, most hCG assays detect and/or quantify the hormone using immunometric methods. Multiple qualitative point-of-care (POC) devices are available for detecting hCG in serum and/or urine; one quantitative POC assay is...
The complex and heterogeneous nature of the structural hCG variants present in serum and urine results in differential recognition of the variants by individual immunoassays. The analytical specificity for hCG variants is a function of the epitopes recognized by the assay’s antibodies. A considerable amount of work has been completed to define the specificity of the most commonly used qualitative and quantitative hCG assays. These studies used the highly purified first World Health Organization (WHO) international reference reagents (IRRs) for hCG and its variants to determine the variant recognition profiles of these hCG assays.\(^2\)\(^,\)\(^2\)\(^,\)\(^5\) In addition to determining which variants were recognized, the studies were able to determine interassay accuracy differences for the hCG variants.\(^2\)\(^6\)\(^,\)\(^2\)\(^7\)

hCG assays can be broadly grouped into three categories: those that recognize all major hCG variants, those that do not recognize hCGβc,f, and those that detect only dimeric hCG variants (intact hCG and hCGn). The hCG variant recognition profile of an individual immunoassay defines which clinical uses the assay is suited for. For example, assays that recognize only dimeric variants should not be used for oncology applications because some hCG-secreting tumors may produce only hCGβ.\(^5\) Differences in variant recognition are a major contributor to the inconsistencies observed when the same patient sample is quantified on different assays, requiring any serial hCG measurements to be performed on the same instrument/assay pair.\(^2\)\(^8\) In addition, antibody specificity is key to diagnostic sensitivity. False-negative results can occur on both qualitative and quantitative assays if there is a molar excess of an hCG variant recognized by one, but not both, of the solid-phase or detection antibodies.\(^2\)\(^9\)\(^,\)\(^3\)\(^0\) Devices that recognize all major variants are much less susceptible to these false-negative results.\(^3\)\(^1\)

**Analytical Sensitivity**

Most urine qualitative POC hCG assays have a claimed lower limit of detection (LOD) of 20 to 25 IU/L. In their product inserts, manufacturers often describe that this threshold was determined using purified intact hCG preparations and not using patient urine samples that contain a complex mixture of hCG and its associated variants. Recent publications using patient samples have found the analytical sensitivity of multiple POC devices to be higher than the claimed LOD, meaning that they have a propensity for false-negative results in very early pregnancy.\(^3\)\(^2\)\(^-\)\(^3\)\(^4\) Laboratories have documented that this can affect patient outcomes.\(^3\)\(^5\) One study concluded that the analytical sensitivity of POC hCG devices is a function of the relative abundance of hCG variants in urine and the device itself.\(^3\)\(^6\) In this study, Cervinski et al\(^1\)\(^6\) selected urine samples that had diverse expression of hCG variants and diluted them to the lowest concentration that would consistently produce a positive result. The LOD varied between patients for the same devices and between devices for the same patient, and this concentration was often more than 20 IU/L. Interestingly, the study also evaluated the same specimens using over-the-counter hCG devices and showed that their analytical sensitivity was much better than that of the POC devices.

The analytical sensitivity of automated hCG immunoassays is considerably lower (≤5 IU/L) than the POC devices. This is one reason why quantitative serum hCG assays should be considered the preferred method for evaluating a
woman’s pregnancy status. It should be noted that while the lower analytical sensitivity of quantitative assays improves the clinical sensitivity for the detection of pregnancy, it also decreases the clinical specificity. This is because a low concentration of hCG may be due to reasons other than a viable pregnancy, such as early miscarriage or pituitary secretion of hCG.37

As indicated above, one quantitative hCG immunoassay is FDA approved for measuring hCG in urine and reporting a qualitative result based on a cutoff of 30 IU/L. If this approach is used, some laboratories report interpretive results based on cutoff concentrations such as “negative” for samples less than 20 IU/L, “indeterminate” for samples 20 to 60 IU/L, and “positive” for samples more than 60 IU/L.32 However, the established reference interval for this urine assay using 120 women younger than 55 years was 2.1 IU/L or less,20 implying that the qualitative interpretations described above could be inappropriate. Some laboratories may have similar practices for serum hCG testing. These interpretive cutoffs are likely historical, rather than clinically or even empirically based, and could likely use revisiting.

Harmonization

Quantitative hCG assays are not harmonized, meaning that hCG results from different reagent platforms can yield different results when the same sample is tested. There are several sources of these discrepancies. As discussed, antibody specificity plays a key role in what molecular forms of hCG are recognized, which has a direct influence on the concentration of hCG ultimately determined by the assay. In addition, the secondary standards used by manufacturers to calibrate hCG assays may vary greatly in their purity. Furthermore, most commercially available hCG assays are calibrated against WHO third or fourth international standards (IS), which were prepared from pregnancy urine and assigned values by bioassay.

Recently, the highly purified intact hCG IRR was selected for use as the fifth IS for hCG, which has value assignment in both substance and activity concentrations.28,38 To date, one manufacturer has chosen to calibrate its hCG immunoassay against this new IS.39 Adoption of this material by subsequent vendors could help to improve comparability of hCG results, particularly if results are reported in molar units.28

Interferences

Erroneous hCG results (or erroneous interpretations based on true hCG results) can occur for a number of reasons. Mechanisms are usually applicable to both qualitative and quantitative assays, since they are specific to the biochemistry/physiology of hCG and/or the basic principles of immunoassays.

Sources of false-positive hCG results can be physiologic (pituitary hCG, malignancy, exogenous hCG) or analytical (interfering antibodies, elevated leukocytes). It is important to note that physiologic sources are not actually false-positive results because hCG is present and is appropriately detected. Rather, it is the incorrect interpretation of the positive hCG result as an indication of pregnancy.

Patients may have detectable hCG that is not indicative of pregnancy or residual products of conception. For example, nonpregnant peri- and postmenopausal women may be assumed to be pregnant due to hCG synthesis by the pituitary gland.37 hCG may also be produced by some malignancies, most commonly gestational trophoblastic disease and testicular germ cell tumors, but cases have been reported for many other cancer types.40 Exogenous hCG is legitimately used to treat infertility and may be used illicitly for performance enhancement. Both of these uses can lead to the detection of hCG in serum or urine. It is uncertain if the use of orally administered hCG as part of fad diets could also lead to its detection since over-the-counter hCG preparations used for this purpose are usually homeopathic and contain little, if any, actual hCG.

False-positive results can also occur when there is no hCG present in the specimen. The presence of interfering antibodies that crosslink the capture and signal reagent antibodies in the absence of hCG represent the most well-defined example.41 In these cases, a patient will often display persistent and minimally changing hCG concentrations. These false-positive results are most often discovered incidentally such as when screening for pregnancy. A sample can be evaluated for the presence of interfering antibodies using a number of techniques.

Interfering antibodies are reactive against the assay reagents rather than the measured analyte so specimens that contain them may fail to show the expected dose response when the sample is serially diluted.42 The presence of interfering antibodies can also be assessed by treating the sample with commercially available blocking agents that adsorb the interfering antibodies or by testing the specimen on alternative reagent platforms.43 Measuring the hCG concentration in urine may also help to distinguish an interfering antibody from endogenous hCG because antibodies are not found in the urine of healthy individuals. However, one case did report the presence of interfering antibodies in the urine of a patient with kidney disease that led to false-positive hCG results.44 A high number of leukocytes in urine and serum have also been described as the source of positive interference in both qualitative and quantitative hCG assays, respectively.45,46

Similarly, false-negative results may occur due to physiologic or analytical causes. Physiologically, testing for hCG in very early pregnancy can produce false-negative results if
testing is performed before implantation or if the concentration of hCG in either serum or urine is below the detection threshold.

The high-dose hook effect is a well-known phenomenon that can result in false-negative immunoassay results. This effect occurs when there is a very high concentration of antigen that saturates the capture and signal antibodies, preventing the formation of Ab/Ag/Ab complexes. The subsequent wash step will remove the signal antibody, which is bound to the antigen but not bound to the capture antibody, ultimately producing a falsely low result. The hook effect has been documented to occur most often in samples with extremely high concentrations of hCG that are associated with molar pregnancies and gestational trophoblastic diseases.47,48

The molecular complexity of hCG can result in a modification of this process that has been termed the “variant hook effect.”29,30,49 This occurs when only one of the antibodies, either the capture or the signal antibody, recognizes an hCG variant, but the other does not. A sample that contains a very high concentration of the variant, relative to hCG variants that are recognized by both antibodies, may produce a false-negative result when the variant saturates the binding sites of one of the reagent antibodies. Both the high-dose and the variant hook effect depend on antigen excess to inhibit Ab/Ag/Ab complex formation. Therefore, specimen dilution can identify the presence of either effect because a paradoxical increase in hCG concentration will be observed.

Reference Intervals

A serum hCG concentration of 5 IU/L or less has been the conventional reference limit for quantitative tests, but this cutoff has been shown to be influenced by age and/ or menopausal status. The 97.5th percentile for pre-, peri-, and postmenopausal women has been reported to be approximately 2, 6, and 8 IU/L, respectively.37,39 Sex-specific reference intervals may be useful if hCG testing is used as a tumor marker in oncology applications. Gestational age-specific reference intervals for hCG are sometimes reported with quantitative hCG test results. The clinical usefulness of such reference intervals is marginal because the interindividual variation in the hCG concentration is very high, even at the same gestational age.

Case Resolution

The gradually decreasing concentrations of hCG over time did not support a diagnosis of intrauterine pregnancy, leaving ectopic pregnancy and spontaneous miscarriage as the most likely causes of the clinical presentation. Although an ectopic pregnancy was considered unlikely, it could not be excluded, particularly given the pattern of hCG decrease before and following the uterine aspiration. Concerned that the patient may have had an ectopic pregnancy or that the manual uterine aspiration had not removed the small sac, methotrexate was administered and serial serum hCG concentrations were monitored. Detectable serum hCG was alarming to the physician, but so too was the fact that the interventions did not seem to influence the hCG concentration. The physician was encouraged to monitor the hCG concentration over the next few weeks. If hCG were to begin to rise, further interventions could be considered, whereas a continued decline would be reassuring. A week after the physician consulted the laboratory, the patient contacted the physician to inform her that she had begun menstruating. A serum hCG was performed and was undetectable.

References


