*Clinical Chemistry* Trainee Council
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**TITLE: Human Chorionic Gonadotropin**

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**Slide 1:**
Title Slide

**Slide 2:**
Human chorionic gonadotropin (hCG) is normally synthesized by placental trophoblasts during pregnancy. It is a dimeric glycoprotein hormone composed of non-covalently associated alpha and beta subunits. The alpha subunit of hCG is identical to the alpha subunit of 3 hormones synthesized by the anterior pituitary gland but the beta subunit of each of these hormones is unique and confers biological specificity. Among the beta subunits, hCGbeta and Lhbeta are vey homologous such that both hormones bind to the LH receptor.

The primary function of hCG is to maintain elevated concentrations of progesterone during early pregnancy by extending the functional life of the corpus luteum.

**Slide 3:**
hCG is a molecularly heterogenous hormone and numerous variants of hCG are usually present in the blood and urine. In addition to the intact, biologically active form of hCG, other hCG variants include degraded forms of the hormone. These include nicked hCG, produced from intact hCG by a protease that cleaves the beta subunit between amino acids 47 and 48, the free beta subunit of hCG, the free nicked beta subunit, and the beta core fragment.

All of these hCG variants can be detected in the serum and the urine. Only the beta core fragment, the terminal degradation product of hCG, is unique to the urine suggesting that it is produced by metabolic processes in the kidney.

 

**Slide 4:**
The detection and/or quantification of hCG is clinically useful for several reasons. hCG is primarily used for the diagnosis of pregnancy and this is the only purpose for which hCG tests are FDA approved. However, hCG is also used as a tumor marker, particularly for monitoring treatment of gestational trophoblastic disease and testicular germ cell tumors and it is also one of the markers used in multimarker biochemical screening for fetal aneuploidies.

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On average, hCG becomes detectable in the serum approximately 9 to 11 days after conception which corresponds to about 1 to 3 days before the day of expected menses. hCG appears in the urine a short while later but is highly variable.

During the first trimester of pregnancy, the hCG concentration in the serum doubles every 2 days with an intrauterine pregnancy but this doubling time is frequently prolonged with an ectopic pregnancy. As such, serial determinations of serum hCG are useful in the evaluation of a suspected ectopic pregnancy.

**Slide 6:**
hCG also has clinical utility as a tumor marker. All gestational trophoblastic diseases, including hydatidiform moles, placental site trophoblastic tumor, and choriocarcinoma, synthesize hCG although the relative abundance of the different hCG variants may differ from those observed in pregnancy.

Similarly, hCG can also be produced by testicular germ cell tumors. Approximately 20 percent of seminomas and 65 percent of nonseminomatous germ cell tumors produce hCG. Some testicular cancers, particularly seminomas, may produce only the free beta subunit of hCG. Therefore, to be useful as a tumor marker of testicular cancer, hCG immunoassays must be able to detect that variant.

As a tumor marker, hCG demonstrates its greatest utility in monitoring responses to therapy.

**Slide 7:**
Both qualitative and quantitative assays for detecting or quantitating hCG in serum and/or urine are in widespread use. While both serum and urine can be used for the qualitative detection of hCG, measuring the concentration of hCG is only clinically useful in serum. Although quantitative urine hCG testing has no established clinical utility, the use of a quantitative hCG assay using a urine sample may be useful for investigating discrepant point-of-care hCG test results (e.g. if a positive result is obtained in a urine sample yet no hCG is detected in the corresponding serum sample, or vice versa).

Like many clinical laboratory tests, hCG immunoassays are not harmonized. Reasons for this lack of harmonization include the use of different antibody pairs between assays as well as the use of calibrators that vary in relative concentrations of hCG variants. This results in wide variations in the measured concentration of hCG between assays as well as variation in the detection of the different hCG variants.

**Slide 8:**
The variation in hCG variant detection is clearly revealed with the use of purified hCG preparations. Of the 7 hCG assays shown here, only 2 (Immulite hCG and Elecsys hCG+β) were able to detect all 5 of the hCG variants tested. 5 assays detected all but beta core fragment, and 1 assay detected only the dimeric hCG variants (which was consistent with the assay's labeling).

Further, because the molar concentration of each hCG preparation has been assigned, the equimolar detection of each hCG variant for each assay could also be determined. If acceptable recovery of an hCG variant is defined to be 90-110 percent (shown by the dotted horizontal lines), then all but one hCG assay (DxI Total βhCG) demonstrated equimolar detection of intact hCG. The detection of the free beta subunit (hCGβ) was highly variable between assays as was the detection of the nicked free beta subunit (hCGβn).

**Slide 9:**
Analytical variation is not restricted to just quantitative hCG tests. Qualitative hCG tests also demonstrate variability regarding their ability to detect hCG variants. While all can detect dimeric hCG variants, intact hCG and nicked hCG (hCGn), other hCG variants are variably detected.

In this study, all but one of the qualitative devices evaluated detected the free beta subunit and the nicked free beta subunit and a few were also capable of detecting the beta core fragment. As might be expected, none of the devices detected the free alpha subunit of hCG.

**Slide 10:**
The clinical impact of variation between hCG assays is likely very minimal when only a single quantitative hCG measurement is required. For example, as would usually be the case in pregnancy testing or maternal serum screening for fetal aneuploidy. The clinical impact becomes more likely to be considerable when serial hCG testing is necessary as would be required for the evaluation of a suspected ectopic pregnancy or in oncology applications when hCG is used in treatment monitoring.

The clinical impact of the variation is less certain for quantitative urine investigations. Recall that, currently, there is no known clinical need to quantify hCG in urine yet some laboratorians find that practice useful when investigating discrepant point-of-care test results. In that case, the use of an hCG assay that detects the beta core fragment may be desirable because that variant is the predominant form of hCG in pregnancy urine.

Lastly, it is now appreciated that some qualitative urine hCG tests can produce falsely negative results due to the presence of large amounts of certain hCG variants (more on that later).

**Slide 11:**
Occasionally, hCG is detected in relatively low concentrations in the absence of pregnancy and may persist with little change in the concentration for several months to years. The identification of persistently low hCG is relatively uncommon. When it is discovered, it is typically attributed to one of only a few conditions: the presence of an interfering antibody, pituitary hCG, or exogenous hCG.

In many healthcare systems, standardized patient care protocols are resulting in an increasing number of women undergoing hCG testing to rule out a possible pregnancy prior to interventions that could harm a fetus, without regard to chronological age or the likelihood of pregnancy. Persistently low hCG often creates a clinical dilemma and may delay therapy or result in unnecessary treatments for presumed malignancies.

**Slide 12:**
Interfering antibodies cause interference by cross-linking the capture and signal antibodies used as reagents in immunometric assays. They have been documented as causing false-positive or falsely increased results in both qualitative and quantitative hCG assays.

Because they can cause false hCG results, clinical suspicion for the presence of interfering antibodies should be high when the result of an hCG test is inconsistent with the patient's clinical scenario. Unfortunately, the laboratory is infrequently aware of the patient's clinical status which means that these situations are essentially impossible for the laboratory to detect independently. When the laboratory is alerted to the possibility of an interfering antibody situation, there are several investigations that can be performed.

- Analyzing the urine for the presence of hCG is a logical first step. hCG is filtered into the urine and can be detected by testing a urine sample. Due to their large molecular mass, interfering antibodies are not excreted into the urine and so do not cause interference. In cases of truly elevated serum hCG, filtered hCG molecules would be detected in the urine so the absence of its detection suggests the presence of an interfering antibody.

- Serial dilution of the sample can also be useful. Because the interfering antibodies are reactive against the assay reagents and not hCG, samples that contain interfering antibodies may produce hCG results that deviate from the expected dose response.

- Blocking agents work by adsorbing the interfering antibodies from the sample thereby preventing their interference in immunoassays. However, their effectiveness depends on numerous variables including the interfering antibody class, specificity, and concentration.

- Repeating the test using a different hCG immunoassay, particularly one that utilizes antibodies produced from a different animal species than the assay in question can be helpful as well. Results that are very inconsistent (hCG detected vs. not detected, for example) offer supporting evidence of an interfering antibody. This investigation is not fool-proof, however, as some patients may have antibodies with broad specificity and can cross-react with multiple animal species and interfere with multiple assays.

**Slide 13:**
The pituitary gland as a source of hCG is not well known despite it being first identified more than 30 years ago. Why it produces hCG is not well understood but a quick review of the pituitary-ovarian axis can help explain the phenomenon.

Under the control of the hypothalamus, the gonadotrope cells of the anterior pituitary produce LH and FSH. These, in turn, stimulate the release of ovarian steroid hormones which themselves regulate FSH and LH through negative feedback mechanisms. As women enter perimenopause (at about the age of 40) ovarian steroid production decreases which releases the negative feedback inhibition. As a result, continuous stimulation of the gonadotrope cells of the pituitary gland leads to increased synthesis of LH and FSH. It is under this condition of hyperstimulation that the pituitary can secrete hCG and is one cause of elevated hCG in older, non-pregnant women.

Two approaches to identifying pituitary hCG have been proposed. Because FSH is elevated in menopause and suppressed in pregnancy, the concentration of serum FSH can ruleout pregnancy as a cause of the hCG. An FSH concentration less than 45 IU/L has been shown to be 100% sensitive for identifying hCG of placental origin, and a result greater than or equal to that threshold had a negative predictive value of 100% for ruling out hCG of placental origin. Alternatively, a 2-week course of estrogen replacement therapy has been shown to decrease the concentration of pituitary hCG.

**Slide 14:**

Like other immunoassays, hCG assays are susceptible to producing falsely negative or decreased results due to the high-dose hook effect. When hCG concentrations are pathologically increased, the molecules saturate both the capture and the signal antibodies preventing the formation of the "sandwich" and leading to inaccurate results.

A lesser known cause of falsely negative or decreased results is the hCG variant effect. This can occur when hCG concentrations are extremely elevated or even when they are within expected physiological limits. The effect is due to the recognition of an hCG variant by only one of the two reagent antibodies. If that specific hCG variant is present in a high enough concentration, it can saturate the antibody binding sites that recognize it, leading to the impaired detection of hCG variants that are recognized by both antibodies.

**Slide 15:**

The hCG variant effect has been described in both qualitative and quantitative hCG tests. Both reports have only identified this effect due to elevated concentrations of the beta core fragment of hCG. As mentioned earlier, this variant is only detected in the urine and so the variant effect is more of a clinical concern with qualitative urine tests. As quantitative hCG tests are typically not used for measuring hCG concentrations in urine, this phenomenon is less of a clinical concern for those types of tests but knowledge of the effect is useful nonetheless.

As shown on the left, the positive result of a qualitative urine hCG test was greatly diminished in the presence of elevated concentrations of the beta core fragment. The figure on the right shows the hCG variant effect in a quantitative hCG test that is very sensitive to it. As shown earlier, the DxI Total βhCG assay does not detect the beta core fragment yet the measured concentration of intact hCG is falsely decreased when the beta core fragment is elevated. Importantly, the concentration of the beta core fragment can be 0.06 to 1.0 umol/L by the fifth week of pregnancy and so the use of hCG tests with urine samples obtained near this time may produce misleading results.

**Slide 16:**
In summary, hCG is not a single molecule and is usually present in several molecular forms. Both quantitative and qualitative hCG tests show considerable variation in the recognition of hCG variants. Interfering antibodies and pituitary hCG can produce positive or increased hCG test results that may lead to clinical confusion. And lastly, falsely negative or decreased hCG test results can be caused by the high-dose hook effect or by the hCG variant effect.

**Slide 17:** References