Use of Serum FSH to Identify Perimenopausal Women with Pituitary hCG
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BACKGROUND: Human chorionic gonadotropin (hCG) tests are performed on many female patients before performing medical procedures or administering medications that may harm a fetus. hCG of pituitary origin has been shown to increase with age. Therefore, mild increases in serum hCG in an older patient can be of pituitary origin and does not necessarily indicate pregnancy. The inability to rule out pregnancy in perimenopausal women can create clinical confusion and may delay needed therapies. Our objective was to determine the diagnostic utility of serum follicle-stimulating hormone (FSH) concentrations to rule out hCG of placental origin in perimenopausal women with a low concentration of serum hCG (5.0–14.0 IU/L).

METHODS: Seven testing centers performed 39,742 physician-ordered serum quantitative hCG tests over a 15-month period. From these, 100 samples from women 41–55 years of age with serum hCG concentrations 5–14 IU/L were identified. We performed FSH testing and patient chart review for each sample.

RESULTS: Twenty-three patients were found to have hCG of placental origin (pregnancy, resolving abortion, or gestational trophoblastic disease), and in those cases serum FSH was 0.4–43.8 IU/L. An FSH cutoff of 45.0 IU/L identified hCG of placental origin with 100% sensitivity and 75% specificity. FSH >45 IU/L was never observed when hCG was of placental origin (negative predictive value).

CONCLUSIONS: These data indicate that quantitative serum FSH can be used to rule out pregnancy and hCG of placental origin in women 41–55 years of age with mild increase in serum hCG concentrations.

It has become standard practice at most institutions to determine the human chorionic gonadotropin (hCG)7 status (urine or serum) on all female patients before any medical intervention that could potentially harm a fetus, even in older patients who are likely peri- or postmenopausal. Although the majority of determinations on these older, nonpregnant patients are negative, numerous studies indicate that low concentrations of hCG can be present in these older cohorts of women even in the absence of pregnancy (1–3). Positive results from qualitative tests or quantitative serum results that exceed a threshold of 5.0 IU/L can create clinical confusion and delay needed therapies.

Aside from its synthesis during normal pregnancy, gestational trophoblastic disease, or some malignancies, a small amount of hCG is normally produced by the pituitary gland in conjunction with the structurally similar glycoprotein hormones luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (4, 5). Studies have demonstrated that pituitary production of hCG increases during menopause (2, 3). Recently, Cole et al. (2) reported that 2 weeks of treatment with estrogen-progesterone hormone-replacement therapy could be used to suppress serum hCG concentrations and confirm that it is of pituitary origin. However, this method is not useful in emergent situations when pregnancy must be ruled out quickly before diagnostic procedures or treatment.

Several years before menopause, there is an increase in circulating concentrations of FSH and a decrease in estradiol. LH concentrations generally remain similar to those in younger women. The use of FSH quantification to help identify menopause-associated low-positive hCG concentrations has been suggested (6). We previously suggested that women 41–55 years

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7 Nonstandard abbreviations: hCG, human chorionic gonadotropin; FSH, follicle-stimulating hormone.
| Table 1. Specimen collection period, number of specimens included, and testing methods for quantitative hCG and FSH measurement at each testing center. |
|---|---|---|---|---|---|---|---|
| **Center code** | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| **hCG method** | ADVIA Centaur® (Bayer Diagnostics) | Roche Elecsys® (Roche Diagnostics) | Access® DxI (Beckman Coulter) | Access® DxI (Beckman Coulter) | Dimension® (Dade Behring) | AIA®-1800 (Tosoh Bioscience) | AxSYM® (Abbott Laboratories) DxI (Beckman Coulter) |
| **FSH method** | ADVIA Centaur® (Bayer Diagnostics) | Vitros ECi® (Ortho-Clinical Diagnostics) | Access® DxI (Beckman Coulter) | Access® DxI (Beckman Coulter) | Access® DxI (Beckman Coulter) | Access® DxI (Beckman Coulter) | AIA®-1800 (Tosoh Bioscience) |
| **Specimen collection period** | 2/10/06 to 5/14/07 | 3/22/06 to 4/4/07 | 3/22/06 to 1/4/07 | 3/22/06 to 4/2/07 | 3/22/06 to 4/2/07 | 3/21/06 to 3/5/07 | 3/22/06 to 4/16/07 | 2/10/06 to 5/14/07 |
| **Quantitative serum hCG tests performed** | 5152 | 5755 | 3220 | 5226 | 3200 | 8428 | 8761 | 39 742 |
| **From women 41–55 years old** | 411 | 745 | 351 | 228 | 143 | 1108 | 1429 | 4415 |
| **From women 41–55 years old and hCG =5.0 IU/L** | 94 | 172 | 65 | 28 | 43 | 130 | 96 | 628 |
| **From women 41–55 years old and hCG 5.0–14.0 IU/L** | 16 | 38 | 20 | 10 | 5 | 32 | 38 | 159 |
| **Samples excluded** | 4 | 17 | 3 | 2 | 0 | 10 | 23 | 59 |
| **Insufficient sample volume** | 0 | 13 | 2 | 2 | 0 | 1 | 23 | 41 |
| **No chart** | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 6 |
| **Inconclusive pregnancy status** | 1 | 1 | 0 | 0 | 0 | 3 | 0 | 5 |
| **Patient duplicate** | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 7 |
| **Samples included** | 12 | 21 | 17 | 9 | 4 | 22 | 15 | 100 |
(perimenopausal) with a serum hCG between 5.0 and 14.0 IU/L have reflex FSH testing performed to help exclude a diagnosis of pregnancy (1). However, that study was limited because it included only 3 nonpregnant patients, 41–55 years of age, with serum hCG >5.0 IU/L. A larger cohort of perimenopausal women was needed to confirm a clinically useful diagnostic cutoff for FSH.

This study uses a population of women between the ages of 41 and 55 years with a low concentration of serum hCG (5.0–14.0 IU/L) to determine the diagnostic utility of serum FSH concentrations to rule out hCG of placental origin.

**Materials and Methods**

**STUDY POPULATION**

This multicenter, prospective, cohort study used leftover samples sent to the laboratory over an approximate 15-month period between February 10, 2006, and May 14, 2007, for physician-ordered hCG testing (study recruitment periods varied slightly between sites; see Table 1). The study period was defined as the time required to collect 100 specimens that met the inclusion criteria (see below). Testing centers included Washington University, St. Louis, MO; University of North Carolina, Chapel Hill, NC; Emory University, Atlanta, GA; Marshfield Clinic, Marshfield, WI; Marshfield Clinic Regional Center Clinics, rural Wisconsin; Johns Hopkins Medical Institutions, Baltimore, MD; and University of Washington, Seattle, WA (Table 1). The study design is shown in Fig. 1. During the study period, 39 742 specimens had quantitative hCG testing performed (excluding proficiency testing and quality control specimens). Specimens were included in the study if the serum hCG concentration from women 41–55 years of age was 5.0–14.0 IU/L. Specimens were excluded if from a male patient, if a patient chart was unavailable for review, if pregnancy status was uncertain and source of the hCG could not be determined, if there was insufficient sample to perform FSH testing, or if a sample from the patient was already included in the study (Fig. 1). Chart review was performed on all patients. The presence of hCG of placental origin was determined based on physician notes, subsequent hCG testing, and pathology reports. This study received approval from the Institutional Review Board of each institution.

**Fig. 1. Algorithm for specimen inclusion and exclusion.**
ANALYSIS OF COLLECTED SPECIMENS
All specimens used in the study had quantitative hCG and FSH testing performed. Because the goal was to develop guidelines that can be widely used, testing centers were selected to reflect a variety of quantitative hCG immunoassay methods currently in use (Table 1). All testing was performed in CLIA-approved laboratories according to manufacturer’s instructions. hCG testing was performed upon receipt of the specimen in the laboratory. Samples were refrigerated for up to 3 days or frozen for up to 3 weeks before FSH testing was performed.

STATISTICS
The study size of 100 patients was determined assuming that true sensitivity, specificity, and positive and negative predictive values were approximately 0.80 or above, which would produce confidence interval estimates that were expected to be approximately ±10% or less. An ROC curve was computed using Graph-Pad Prism, v. 4.00 (GraphPad Software). Exact 95% CIs for sensitivity, specificity, and positive and negative predictive values were computed using Stata, release 9 (StataCorp).

Results
In this study population, 11% (4415/39,742) of the serum hCG tests were performed on women 41–55 years of age (Fig. 1). In this subset of women, the prevalence of hCG concentrations <5.0 IU/L, 5.0–14.0 IU/L, and >14.0 IU/L was 85.8% (3787/4415), 3.6% (159/4415), and 10.6% (469/4415), respectively (Table 1). Of the 77 patients who had hCG of nonplacental origin, the vast majority (n = 68) were being tested because they had been or were going to be subject to agents that could harm a fetus (24 presurgical/medication/radiology; 20 cancer patients; 11 renal failure; 5 drug abuse; 4 congestive heart failure; 2 psychotic disorder or suicide; 1 stroke; 1 seizure). Four patients were evaluated for vomiting or abdominal pain and 3 for vaginal bleeding or missed menses. One patient was a bone marrow donor, and one was a healthy research study patient. Of the 23 patients with hCG of placental origin, 17 had resolving abortion/miscarriage, 4 had gestational trophoblastic disease, and two were early in pregnancy. These data are shown in Supplemental Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol54/issue4. The distribution of FSH concentrations in patients with hCG of placental origin and nonplacental origin is shown in Fig. 2. In contrast to the wide variation in the FSH concentrations in patients with hCG of nonplacental origin, FSH did not exceed 45 IU/L when hCG originated from placental tissue. An ROC curve for FSH to rule out hCG of placental origin is shown in Fig. 3. The area under the curve is 0.902 (95% CI 0.844–0.960). A cutoff of ≥45 IU/L was selected from the ROC curve to rule out hCG of placental origin. Serum FSH <45 IU/L has a sensitivity of 100% (95% CI 87.8%–100%) to identify specimens with hCG of placental origin and a specificity of 75.3% (95% CI 64.2%–84.4%). A value above the cutoff is also 100% predictive (95% CI 95.0%–100%) that the hCG is not of placental origin (i.e., negative predictive value 100% for FSH <45 IU/L), and a value below the cutoff is 54.8% predictive (95% CI 38.7%–70.2%) that hCG was of placental origin (positive predictive value).
Discussion

The results presented here support our previous findings and indicate that serum FSH has clinical utility to rule out pregnancy as the hCG source and can facilitate medically necessary treatment in women of perimenopausal age.

We suggest reflex FSH testing be considered in all patients between 41 and 55 years of age with serum hCG concentrations 5.0–14.0 IU/L if the possibility of pregnancy is being evaluated. It is not necessary in patients between 41–55 years of age with hCG < 5.0 IU/L, as pregnancy is unlikely. In patients between 41 and 55 years of age, an hCG > 14.0 IU/L should be considered consistent with pregnancy unless otherwise determined, as even postmenopausal women with hCG of proven pituitary origin uncommonly (17%) exceed 14 IU/L (2). If reflex FSH testing is performed after hCG test results of 5.0–14.0 IU/L in this age group, hCG results could be reported with the following interpretations. When FSH is ≥45 IU/L, “FSH was determined to be ≥45 IU/L. It is unlikely that the hCG is due to pregnancy.” When FSH is < 45 IU/L, “FSH was determined to be < 45 IU/L. The hCG may be due to pregnancy.” Note that this algorithm is intended exclusively for hCG tests that have been ordered to rule out pregnancy, and test results need to be interpreted with regard to clinical evaluation.

In our preliminary study, we had established 20 IU/L as a cutoff for FSH (1). In this larger study, use of this threshold resulted in 78% sensitivity and 84% specificity for ruling out pregnancy. Because FSH testing will be used to evaluate potential pregnancy, we believe it is essential that a cutoff with 100% sensitivity to rule out hCG of placental origin be used, as failure to do so may result in inappropriate treatments in pregnant patients. The difference between the FSH cutoff established in this study and our previous publication is likely due to the small number (n = 3) of nonpregnant, perimenopausal women with positive hCG in our previous publication.

Serum FSH is often used to help evaluate the menopausal status of women. Interestingly, FSH concentrations vary widely in postmenopausal women and therefore are not recommended for use in diagnosing menopause. Likewise here, FSH concentrations ≥ 45 IU/L should not be used to diagnose menopause, but rather these concentrations indicate that the presence of hCG is unlikely due to pregnancy or of placental origin.

In this study, the prevalence of women 41–55 years of age who had hCG testing performed and had hCG concentrations 5–14 IU/L was 0.4% (159/39 742). This is similar to the 0.2% prevalence estimated in our previous report (1). At this low prevalence, implementing reflex testing should not create economic or logistical burdens for the laboratory.

Although the exact source of hCG was not known for the women with hCG of nonplacental origin, the evidence suggests a pituitary origin. Concentrations of pituitary hCG have been shown to increase during perimenopause (2, 3), and as stated earlier, these hCG concentrations tend to be low (< 16 IU/L) (2). We suggest that our approach is most useful in situations where ruling out pregnancy is urgently needed. If the FSH concentration is ≥ 45 IU/L, then pregnancy is unlikely and the procedure or treatment should proceed. Later, if the hCG remains increased, and there is concern about the source of the hCG, then hormone replacement therapy can be initiated to identify a pituitary source as advocated by Cole et al. (2). If the hCG does not suppress, other sources of hCG should be investigated.

One potential caveat to this study is that the cutoff of 45 IU/L was established based on data from 5 different instruments. Because FSH assays are not well standardized, it is possible that use of the 45 IU/L cutoff may provide slightly different sensitivity and specificity for different assays. However, we should note that at the conclusion of this study all samples were performed using the Vitros ECI (except 7, which had insufficient quantity for retesting). Using the 45 IU/L cutoff, the sensitivity and specificity remained at 100% and 75%, respectively.

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References