Diagnostic Considerations in the Measurement of Human Chorionic Gonadotropin in Aging Women

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Background: Human chorionic gondodotropin (hCG) screening tests are frequently performed on female patients of childbearing age before any medical intervention that could potentially harm a fetus. Although older women usually have negative hCG test results, positive results do occur and may cause clinical confusion. We examined changes with age in serum hCG concentrations in nonpregnant women and investigated the use of serum follicle-stimulating hormone (FSH) measurements as an aid to interpreting higher than expected (“positive”) hCG results.

Methods: We used 240 serum specimens for each of 4 female cohorts: pregnant, ≥18 years; nonpregnant, 18–40 years (premenopausal); nonpregnant, 41–55 years (perimenopausal); and nonpregnant, >55 years (postmenopausal). Patients were excluded if they had an ectopic pregnancy, a history of trophoblastic disease, or a germ-cell tumor, or if no chart was available for review. Quantitative hCG and FSH tests were performed on each specimen.

Results: Serum hCG concentrations in nonpregnant women increased with the age of the women. hCG concentration results were higher and significantly different ($P<0.0001$) for nonpregnant women >55 years (<2.0 to 13.1 IU/L) compared with nonpregnant women 18–40 years (<2.0 to 4.6 IU/L) and 41–55 years (<2.0 to 7.7 IU/L). Nineteen nonpregnant women >40 years of age had hCG concentrations ≥5.0 IU/L, all with an FSH concentration >32.4 IU/L. The highest FSH concentration in pregnancy was 7.3 IU/L.

Conclusions: Serum hCG increases with age in non-pregnant women. A cutoff of 14.0 IU/L should be used when interpreting hCG results in women >55 years of age. Pregnancy is unlikely in perimenopausal women 41–55 years of age with an hCG between 5.0 and 14.0 IU/L if serum FSH is >20.0 IU/L.

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several instances have led to harmful therapeutic interventions (6).

In addition to its synthesis during normal pregnancy, trophoblastic disease, or cancer, a small amount of hCG is normally produced by the pituitary gland in conjunction with the structurally similar glycoprotein hormones luteinizing hormone, follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (3, 4).

An hCG cutoff concentration of <5.0 IU/L is generally used for the diagnosis of pregnancy; however, the upper limit of serum hCG in nonpregnant peri- and postmenopausal women has been reported in only a few studies (1–5). The primary objectives of this study were to determine this upper limit of serum hCG in these two populations of women and to evaluate the clinical use of a quantitative serum FSH measurement to assist in the interpretation of a positive hCG result.

Materials and Methods

Sample Collection and Exclusion of Patients
This collaborative retrospective cohort study used leftover serum samples sent to the laboratory between September 2003 and June 2004 for physician-ordered hCG, FSH, or thyroid-stimulating hormone tests. Samples sent to the laboratory for hCG tests were used first. To obtain sufficient numbers of specimens from female patients who were 41–55 and >55 years of age, samples were also randomly chosen from those sent for thyroid-stimulating hormone or FSH testing. Specimens were selected for the study if they met one of the following inclusion criteria: pregnant, ≥18 years; nonpregnant, 18–40 years (premenopausal); nonpregnant, 41–55 years (perimenopausal); or >55 years (postmenopausal). Patients were excluded if they had a history of trophoblastic tumor or ectopic pregnancy or if no chart was available for review. Menopausal status was defined by age alone, and no attempt was made to confirm menopausal status on the basis of additional clinical information. This study received approval from the Institutional Review Board of each institution.

Analysis of Collected Specimens
Quantitative analysis of hCG and FSH tests was performed on each specimen. At the University of North Carolina, quantitative hCG was measured with the Roche Elecsys® (Roche Diagnostics), and quantitative FSH was measured with the Vitros® ECi (Ortho-Clinical Diagnostics), both according to the manufacturer’s instructions. At Washington University, quantitative hCG and FSH were measured with the Advia Centaur® (Bayer Diagnostics) in accordance with the manufacturer’s instructions. The functional sensitivities (defined as the lowest concentration with a CV ≤20%) for hCG and FSH with either assay system were 2.0 and 1.0 IU/L, respectively.

Statistical Analyses
Statistical analysis of the data was performed with GraphPad Prism, Ver. 4.00 (GraphPad Software) and STATA release 8.2 (Stata Corporation). For data analyses, results that were below the assay functional sensitivity were used as reported by the instrument. A Bland–Altman analysis was performed to compare assay methods (9). Correlation was calculated with Spearman rank correlation, and differences between the groups were assessed by the Mann–Whitney U-test. Locally weighted regression (lowess smoothing) was used to fit smooth curves to hCG and FSH as a function of age (10).

Results

Data collected at the University of North Carolina and Washington University were combined to give a total of 240 values per study cohort (120 specimens from each institution).

Because distinct isoforms of hCG and FSH can be detected preferentially by different immunoassays (11), we sought to determine concordance between the two methods used in this study. Patient specimens for hCG (n = 23) and FSH (n = 25) were analyzed by both methods. Comparisons were accomplished by calculating the ratio of the Elecsys to the Centaur method for hCG and the ratio of the ECi to the Centaur method for FSH with a Bland–Altman plot to chart the ratio vs the mean hCG or FSH concentration (Fig. 1). Although a decreasing trend in the ratio with increasing concentrations of hCG was observed, concordance between the two methods for both hormones was clinically acceptable, and the data from both sites were combined.

We found a significant correlation between hCG concentrations and age for all 720 nonpregnant patients (r = 0.333; P <0.0001); however, when examined per cohort, correlation was significant only for hCG vs age in the nonpregnant perimenopausal (41–55 years) cohort (r = 0.156; P = 0.02). The correlation between hCG and age for the nonpregnant pre- and postmenopausal cohorts was r = 0.034 (P = 0.60) and r = 0.039 (P = 0.55), respectively (Fig. 2A). The correlation between hCG and FSH was examined in each cohort and was significant only in the nonpregnant perimenopausal (r = 0.286) and postmenopausal (r = 0.385) cohorts (P <0.0001).

Three (1.3%) women in the perimenopausal cohort and 16 (6.7%) women in the postmenopausal cohort had a serum hCG concentration that was greater than the conventional cutoff of 5.0 IU/L (12). None of these women had evidence of pregnancy or cancer. Table 1 shows the hCG concentration results and the 97.5 percentile concentration for each of the 3 nonpregnant cohorts. The postmenopausal (>55 years) cohort had an hCG concentration greater than and significantly different from the peri- and premenopausal cohorts (P <0.0001). The highest hCG concentrations seen in pre-, peri-, and postmenopausal women were 4.6, 7.7, and 13.1 IU/L, respectively (Table 1). These findings are similar to previous reports that cite increased hCG concentrations in postmenopausal women (Table 2).

Because FSH concentrations are increased in post-
menopausal women and suppressed during pregnancy, it was speculated that this hormone may help to evaluate perimenopausal patients with increased hCG concentrations. As expected, our data illustrate that FSH concentrations increase with age (Fig. 2B). When broken into cohorts, the postmenopausal cohort had the highest median concentration of FSH (46.9 IU/L), followed by the nonpregnant perimenopausal cohort (median, 23.5 IU/L), the nonpregnant premenopausal cohort (median, 4.7 IU/L), and finally the pregnant cohort (median, 0.6 IU/L). We divided the peri- and postmenopausal cohorts by hCG status, using the conventional 5.0 IU/L cutoff for a positive test (Fig. 3). The minimum FSH concentrations in peri- and postmenopausal patients with positive hCG tests were 51.5 and 32.4 IU/L, respectively. The highest FSH value determined in the pregnant cohort was 7.3 IU/L; therefore, an FSH cutoff between 7.3 and 32.4 IU/L could be selected that would adequately distinguish between positive hCG caused by pregnancy and positive hCG resulting from advancing age. A value of 20.0 IU/L, midway between these two points, was chosen as a conservative cutoff.

Discussion

Women are frequently given an hCG test to rule out pregnancy before any procedure that could harm a fetus, but positive pregnancy tests can lead to delays in the procedure. The results presented here, along with reports published previously, indicate that increased hCG concentrations >5.0 IU/L are not uncommon in peri- and postmenopausal women and should not be considered abnormal (1–5).

Our data clearly indicate that hCG and FSH concentrations increase with age, although both hCG and FSH are fairly constant during the stages of premenopause and postmenopause. Perimenopause is the start of the endocrine, biological, and clinical features of menopause. During this period of transition between the two stages, a significant correlation between hCG and age is observed. Herein lies the difficulty in interpreting the hCG test results.
of a woman 41–55 years of age who, hormonally, could be pre- or postmenopausal, or somewhere in between.

The highest hCG concentration in the nonpregnant premenopausal cohort was 4.6 IU/L, consistent with the conventional hCG cutoff of 5.0 IU/L. The highest hCG concentration in the nonpregnant peri- and postmenopausal cohorts was 7.7 and 13.1 IU/L, respectively; therefore, by rounding these values to the nearest whole number that would include all patients in the cohort, we recommend that the upper limits of normal serum hCG for nonpregnant peri- and postmenopausal women be 8.0 and 14.0 IU/L, respectively. In women >55 years of age, in whom pregnancy is unlikely, hCG concentrations <14.0 IU/L should be considered normal.

The use of FSH quantification to help identify menopause-associated low-positive hCG concentrations has been suggested previously (7). Similarly, we investigated the use of serum FSH to help interpret low-positive hCG concentrations in women 41–55 years of age in whom pregnancy is possible. On the basis of these data, we designed an algorithm for the interpretation of hCG results (Fig. 4). Pregnancy should be considered unlikely in all women with hCG concentrations <5.0 IU/L. hCG concentrations between 5.0 and 14.0 IU/L should be considered an indicator of possible pregnancy in premenopausal women. Pregnancy is unlikely in women >55 years of age with hCG concentrations between 5.0 and 14.0 IU/L, although additional testing is recommended if the concentration is >14.0 IU/L. FSH testing should be performed in women between 41 and 55 years of age with hCG concentrations between 5.0 and 14.0 IU/L. When FSH concentrations are >20 IU/L, pregnancy is unlikely. 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 together with clinical history.

Between August 1, 2004, and February 2, 2005, a total of 3751 physician-ordered hCG tests were performed at the University of North Carolina and Washington University hospitals. Of these, 547 (15%) were ordered for women 41–55 years, and 138 (4%) were ordered for women >55 years. In the 41–55 years group, 7 (0.2% of total) of the results were between 5.0 and 15.0 IU/L. Therefore, if reflex testing were established, it would not create a burden for the laboratory.

Although the exact source of hCG was not known for the peri- and postmenopausal women with increased serum hCG who were in our study, the evidence suggests a pituitary origin. Concentrations of pituitary FSH have been shown to increase during perimenopause (13). As expected, we noted a significant correlation between hCG and age and FSH and age beginning in the perimenopausal cohort. We also found a correlation between hCG and FSH beginning in the perimenopausal stage and persisting through menopause. This finding is indirect evidence to support a pituitary origin of hCG. More direct evidence was reported by Suginami and Kawaoi (14), who found enhanced immunohistochemical staining for hCG in the pituitary glands of postmenopausal women. Likewise, Hoermann et al. (15) found hCG immunoreactivity in a fractionated pituitary extract from a postmenopausal woman. Additionally, hCG of pituitary origin is typically found at low-positive concentrations, similar to those of the menopausal patients in our study (3, 4). To definitively test whether increased hCG originates from the pituitary, patients could be placed on estrogen–pro-

<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Definition of menopause</th>
<th>hCG, IU/L</th>
<th>Age range, years</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>High</td>
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<tr>
<td>Stenman et al. (4)</td>
<td>52</td>
<td>No menses for 3 years; not pregnant; FSH ≥30 IU/L</td>
<td>1.41</td>
<td>4.8</td>
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<tr>
<td>O’Dell et al. (3)</td>
<td>14</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.49</td>
<td>6.9</td>
</tr>
<tr>
<td>Vankrieken et al. (5)</td>
<td>56</td>
<td>ND</td>
<td>3.31</td>
<td>12.5</td>
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<tr>
<td>Borkowski et al. (2)</td>
<td>20</td>
<td>1 year after last menses; not on hormone replacement</td>
<td>0.619</td>
<td>3.87</td>
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<tr>
<td>Alfthan et al. (1)</td>
<td>433</td>
<td>&gt;50 years of age</td>
<td>1.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.9</td>
</tr>
<tr>
<td>This study</td>
<td>240</td>
<td>&gt;55 years of age</td>
<td>&lt;2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.1</td>
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<sup>a</sup> ND, not defined.<br/><sup>b</sup> Value represents the median hCG concentration.

**Table 1. hCG concentration ranges and the 97.5 percentile for the nonpregnant cohorts in the study.**

<table>
<thead>
<tr>
<th>Nonpregnant cohort</th>
<th>n</th>
<th>hCG range, IU/L</th>
<th>97.5 percentile, IU/L</th>
<th>P</th>
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<tbody>
<tr>
<td>Premenopausal, 18–40 years</td>
<td>240</td>
<td>&lt;2.0 to 4.6</td>
<td>2.5</td>
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<tr>
<td>Perimenopausal, 41–55 years</td>
<td>240</td>
<td>&lt;2.0 to 7.7</td>
<td>4.8</td>
<td>0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Postmenopausal, &gt;55 years</td>
<td>240</td>
<td>&lt;2.0 to 13.1</td>
<td>7.7</td>
<td>&lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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<sup>a</sup> Compared with the nonpregnant premenopausal cohort.<br/><sup>b</sup> Compared with the nonpregnant premenopausal and nonpregnant perimenopausal cohorts.
gestosterone therapy, which should suppress pituitary hCG (4). Although differentiating between the exact isoforms of hCG may aid in determining the source of increased hCG, insufficient evidence is available to support the use of hCG isoforms for this purpose.

Some limitations of our study need to be discussed. First, the molecular heterogeneity of hCG as well as the various antibodies used in clinical hCG assays are causes of interassay discordance (16). As such, the upper limits of hCG reported here may not be transferable to hCG assays different from the two that we used. Second, it is possible that the increased hCG in some of our postmenopausal women was caused by the interference of human anti-animal antibodies; however, we believe that this scenario is unlikely because interference by human anti-animal antibodies generally produces hCG concentrations higher than those observed in our cohorts (17). The third limitation is that in determining the upper hCG concentration in the different cohorts, we used age as the sole criterion for defining menopausal status. Other previous studies of hCG in postmenopausal women used age, FSH concentrations, and clinical features to define menopause (Table 2) (1–5). It is possible that some of our perimenopausal women were actually postmenopausal and that some postmenopausal women were actually perimenopausal; however, despite differences in the definition of menopause, the mean hCG concentrations for postmenopausal women (>55 years) in our study are similar to those of other published studies (Table 2) (1–5). In addition, age is often the only criterion available when hCG results are interpreted. Fourth, many nonpregnant peri- and postmenopausal patients in our study had FSH concentrations below the highest FSH concentration in pregnant patients. Some of the very low FSH concentrations can likely be explained by the use of estrogen–progestosterone replacement therapies that inhibit FSH (18). We did not exclude patients who were taking these medications from our study. Because estrogen–progestosterone medications also suppress hCG of pituitary origin (4), it is possible that the inclusion of patients on hormone therapy affected our final results. This may explain the absence of a significant correlation between hCG and age in postmenopausal women (>55 years); however, because patients on hormone replacement therapy may be a large subset of the postmenopausal population, we believe that inclusion of these patients has allowed us to define a more accurate hCG threshold for the postmenopausal population. Finally, our algorithm is intended for use in women 41–55 years of age; however, the FSH cutoff in the pregnant cohort contained only 5 pregnant perimenopausal women, and it is possible that the distribution of FSH was different in these women. We believe that this is unlikely because the state of pregnancy should inhibit FSH regardless of patient age. Nonetheless, additional studies are currently in progress to confirm an FSH reference interval in pregnant perimenopausal women.
In summary, concentrations of hCG in nonpregnant women increase with age and may produce false-positive pregnancy tests in nonpregnant peri- or postmenopausal women. In the future, widespread knowledge of this phenomenon coupled with expanded hCG thresholds may eliminate confusion regarding positive hCG tests. Finally, our demonstration that FSH concentrations may help determine pregnancy status in perimenopausal women with an hCG concentration ≥5.0 IU/L might reduce the clinical confusion caused by low-positive hCG test results.

We thank Roche Diagnostics for supplying hCG reagent and Bayer Diagnostics for supplying hCG and FSH reagents used in this study.

References