Expanded Hormone Exercise (ECE1)
The Phantom Pregnancy

Assume that a 45 year-old woman has been experiencing vague abdominal discomfort for several days and, this morning, developed chest pain and shortness of breath at work. Her supervisor called an ambulance which brought her to your healthcare center's Emergency Department. Electrocardiogram was normal. Initial laboratory results included an "undetectable" troponin level and mild leukocytosis. On physical exam, she appears to have ascites and the physician examining her asks you to perform human chorionic gonadotropin (hCG) on the specimen already in the laboratory to rule out pregnancy before she has a CT scan with contrast of her abdomen.

It is probably standard practice to request an hCG level (or to perform a qualitative pregnancy test) on all female patients before they undergo procedures which could potentially harm a fetus. Probably most of these tests produce a negative result, and it is also not clear that an important procedure would be significantly delayed by a positive result. The chance of an unknown pregnancy in older female patients who may be peri- or post-menopausal is probably especially low, and a positive pregnancy test would be problematic.

ECE1-01 was meant to represent such a specimen, and the target level of hCG was 15 IU/L. The exercise was inspired by a report published several years ago which attempted to validate a way of excluding pregnancy in older female patients with "borderline" levels of hCG. Although in the distant past, this was often attributed to cross-reactivity with luteotropin (luteinizing hormone, or LH) which, along with follitropin (follicle stimulating hormone, or FSH), is elevated in peri- or post-menopausal women. All modern immunoassays for hCG probably have very little cross-reactivity with these pituitary gonadotropins. However, we now appreciate that the pituitary also produces hCG as well. Low levels of hCG may be detected during the midcycle LH surge and, just as FSH and LH levels increase in menopause, so do pituitary hCG levels. The presence of an hCG level between 5-30 IU/L in a menopausal female patient is probably most consistent with menopause, not pregnancy or gestational tumor.

The hCG levels reported for this survey specimen ranged from 13-54 IU/L, with a mean of 30, somewhat higher than our target. The wide variability (34% c.v.) appears higher than that observed in the Ligand General K Survey, although in that Survey we have much larger peer groups and do not report all-method means. hCG exists in a number of different forms. Most immunoassays probably detect the native heterodimer reasonably well but commercially available assays vary in their ability to detect nicked heterodimer, free beta subunit, beta-core fragment, and other forms (1). If your laboratory's result on the specimen from the patient described above was the one that you reported for this Survey, how would you interpret it? How would you confirm that it represented pituitary hCG in a peri- or post-menopausal patient?

One way would be to demonstrate suppression of the hCG level after brief treatment with estrogen and progesterone hormone replacement (2). But thanks to Gronowski et al (3), the presence of an elevated FSH level should suffice. These investigators analyzed 100 samples from women 41-55 years of age with

serum hCG concentrations between 5-14 IU/L (see figure). After investigation of the clinical record, the hCG represented placental origin (mostly resolving spontaneous abortion) in 23 patients, and was attributed to pituitary origin in the remaining 77. In none of the patients with placental hCG was the FSH greater than 45 IU/L. In our exercise, as well, all of the participants reported FSH levels >45 IU/L (and LH levels >60 IU/L), confirming menopause.

Finally, the target level for thyrotropin (thyroid stimulating hormone, or TSH) in this specimen was 5-10 mIU/L. All participants reported values in this range, which would be considered mild subclinical hypothyroidism. If this patient was discovered to have an early pregnancy, this level of TSH would raise concern. If anything, TSH levels tend to be lower than normal in the first trimester because hCG has some thyrotropin-like activity. Pregnancy may be considered a stress test for the thyroid because of the rise in thyroxine-binding globulin levels and the need to produce more thyroid hormone for the developing fetus.

Although there is significant circumstantial evidence that subclinical maternal hypothyroidism is associated with adverse outcomes (including increased risk of spontaneous abortion), there is still controversy regarding whether all pregnant women should be screened for this condition and, as well, whether thyroid hormone treatment should be administered. Because of the lack of randomized controlled trials, the recently published recommendation from the American Thyroid Association's taskforce on thyroid disease during pregnancy is to treat pregnant women with subclinical thyroid disease only if they are positive for thyroid peroxidase (TPO) autoantibodies (4).

This case was based on a real patient. CT scan revealed an ovarian mass which turned out to be a papillary serous ovarian cancer. Exploratory laparotomy revealed early stage disease (no pelvic extension or positive lymph nodes) and she received postoperative chemotherapy. Although her anti-TPO antibody test was negative, the subclinical hypothyroidism was treated with thyroid hormone replacement.

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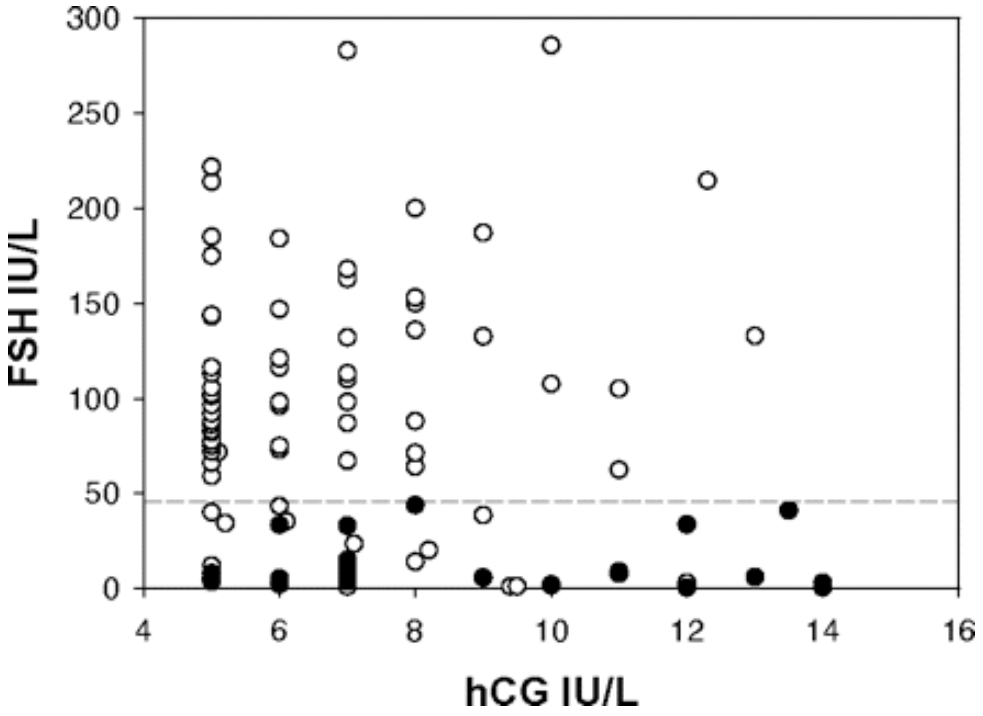

Caption for Figure

FSH levels in 100 potentially postmenopausal female patients with low level hCG levels. Closed circles are specimens from patients shown to have a placental source for the hCG. Open circles are specimens from patients with no clinical support for pregnancy or gestational tumor in whom the hCG is attributed to a pituitary source. (From reference 3, reproduced with permission.)

Expanded Lipid Exercise (ECE2)

The expanded lipid exercise was designed to represent a patient with hypertriglyceridemia, which often creates challenges in the accurate measurement of the other lipid and lipoprotein measurements. The all method median for triglycerides was 370 mg/dL. Because of the limited number of participants in the Survey and the lack of values from a reference method procedure, it is not possible to assess the various methods in terms of accuracy, but the two major methods for triglycerides did show relatively good correspondence. The National Cholesterol Education (NCEP) program recommendation for total error goal for triglycerides is $\pm$ 15%, and if one assumes that the correct value is the all method mean, all participants were within the total error goal. Similarly, all the assays used for total cholesterol showed relatively good correspondence, and again assuming that the all method median is correct, all results were within the NCEP total error goal of 9% for total cholesterol. Based on the CAP accuracy based surveys for total cholesterol and triglycerides, these assays are relatively robust and accurate, but assays for the specific lipoprotein fractions can be more variable (1), which was also found in this Survey.

Until the advent of direct tests for lipoproteins, cholesterol on LDL (LDL-C) was commonly determined after the physical separation of LDL and HDL. LDL was typically precipitated by a polyanion, and cholesterol on HDL in the supernatant was then measured, using a total cholesterol assay. Cholesterol on LDL could then be calculated from a fasting sample by the Friedewald equation (LDL-C= (total cholesterol) –(HDL-C) –(triglycerides/5)) when units are in mg/dL. The term triglycerides/5 provides an estimate of cholesterol on VLDL, the only other major lipoprotein fraction that contains cholesterol in a fasting sample. The Friedewald equation, however, is increasingly inaccurate for samples containing triglycerides over 200 mg/dL and is not recommended for samples with triglyceride values exceeding 400 mg/dL. In this Survey, the median calculated LDL-C was 149 mg/dL, which was considerably lower than all the direct LDL-C measurements, which had an all method median result of 169 mg/dL. This is consistent with previous studies that have shown that triglyceride enrichment of VLDL in dyslipidemia overestimates the amount of cholesterol on this lipoprotein fraction, leading to a lower calculated LDL-C value compared to its true value. Note, however, that the direct assays for LDL-C showed a relatively wide range in results from 152 mg/dL to 188 mg/dL. Although these are still within the total error goal

of 12% for LDL-C, if one assumes the median results is correct, there can be relatively large differences in the different direct LDL-C assays on some samples. The different direct assays for LDL-C depend on different physiochemical properties, and they can, therefore, yield quite different results, particularly on hypertriglyceridemic samples (1). Because of the limitations of the direct assays for LDL-C and the extra cost of performing the test, many labs still only determine LDL-C by calculation. Direct tests for LDL-C, however, can be performed on non-fasting samples and show closer correspondence to the reference method procedure for those sample with triglycerides greater than 200 mg/dL (2).

Overall, the methods for direct HDL-C showed relatively good correspondence, as has been previously described (1). The total error goal for HDL-C is $\pm$ 13%, but for those values less than 42 mg/dL, as in this sample, a more lenient criteria is used (precision SD$\leq$1.7: bias$\leq$5%). Because of the difficulties in accurately measuring HDL-C and LDL-C, apo A-I, which is the main protein on HDL, and apo B, the main protein on LDL, are often recommended as alternative tests. In some studies, these tests have been shown to be equivalent or even superior to HDL-C and LDL-C as cardiovascular risk biomarkers (3). There were only 2 participants in this study for these analytes, so it is difficult to make any conclusion from these results, but the two test results for both assays differed by more than 30%, which could potentially affect the clinical interpretation of the results, indicating perhaps a greater need for test standardization of these analytes. Four participants calculated non-HDL-C, which is simply done by subtracting HDL-C from total cholesterol, and they all showed relatively good agreement. This calculation obviates some of the problems due to errors in accurately calculating LDL-C when using the Friedewald equation. Non-HDL-C can also be used as cardiovascular risk biomarker (2) and is recommended to be used by the NCEP for those patients with triglycerides over 200 mg/dL.

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Expanded Hemoglobin A1c Exercise

The samples in this Survey are the expanded hemoglobin (Hb) A1c exercise (ECE3). This is a voluntary program that is not graded. The purpose is to determine whether the methods that laboratories use are subject to interference from hemoglobin variants. The ECE3-01 sample was prepared from whole blood obtained from a single healthy individual. The ECE3-02 sample was prepared from whole blood obtained from a single individual with hemoglobin AC. The target values were determined from the means of all results from two National Glycohemoglobin Standardization Program (NGSP) Secondary Reference Laboratories (SRLs). The SRLs used boronate affinity chromatography, which is not subject to interference from HbAC. Each laboratory analyzed each sample in triplicate on two separate days. These NGSP Network Laboratories use methods that are calibrated and traceable to the method used in the Diabetes Control and Complications Trial (DCCT). Comparison to the NGSP Network allows both manufacturers and clinical laboratories to trace their glycated hemoglobin results to the DCCT. The target HbA1c values for the survey are as follows: ECE3-01, 5.3% and ECE3-02, 4.8%.

There are over 1100 variant hemoglobins reported (1). The variant hemoglobin may interfere with some assays used to measure HbA1c and the nature of the interference depends on the variant and the specific method used (2). Nevertheless, HbA1c can be measured accurately in most samples with heterozygous variant hemoglobins if an appropriate assay is used. The NGSP website (www.ngsp.org) lists common variant hemoglobins and indicates whether these interfere with HbA1c measured by the 20 methods used most frequently to measure HbA1c.

HbC has Lys instead of Glu at position 6 in the beta chain of hemoglobin. Individuals with HbAC (also termed HbC trait) have one normal hemoglobin gene (HbA) and one HbC gene. HbAC has a prevalence

of 2-3% among African Americans and prevalence as high as 30% in parts of sub-Saharan Africa (3). Persons with HbAC have no symptoms.

Because the PT samples are prepared from human whole blood, the bias observed for the PT samples is expected to reliably reflect the bias that exists for patient samples analyzed with the same method. An acceptable limit is ± 7% of the target value. The percentage is a mathematical fraction, not the HbA1c reporting unit. The acceptable range for ECE3-01, which has a HbA1c value of 5.3%, would be HbA1c values between 4.9 and 5.7% and for ECE3-02, which has a HbA1c value of 4.8%, would be HbA1c values between 4.4 and 5.2%.

Thirty-two laboratories submitted results for ECE3-01 and results for ECE3-02 were obtained from 29 laboratories. Unexpectedly, high values (>5.7%) for ECE3-01 (no variant hemoglobin) were reported by two methods, namely BIO-RAD D10, which had HbA1c of 6.1%, and BECKMAN SYNCHRON LX, which had HbA1c of 6.0%. For ECE3-02, which has HbAC, high values (>5.2%) were reported by two methods, namely BECKMAN AU-BECKMAN RGT, which reported HbA1c of 6.2%, and BECKMAN SYNCHRON LX, which reported HbA1c of 5.5%. The BECKMAN AU is known to have interference from HbC (www.ngsp.org). By contrast, BECKMAN SYNCHRON is reported to have no interference from HbC (www.ngsp.org). Interestingly, HbA1c measurement on the Tosoh G7 has been reported by some to exhibit interference from HbC, while other publications indicate that HbC does not interfere (www.ngsp.org). The Tosoh G7 reported a result of 4.3% for ECE3-02, which is lower than the acceptable value and differs by >10% from the target. The reason for this is not known. Note that the small number of participants makes it difficult to draw conclusions regarding interference for a particular instrument as each instrument mentioned above was used by only one participant.

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