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Components of Biological Variation of Some Serum Analytes in Hospitalized Pregnant Women

To the Editor:

The importance of biological variation in setting analytical goals for precision of clinical biochemistry tests and in judging the usefulness of conventional population-based reference intervals is well established (1, 2). To what extent the existing data on components of biological variation can be used for hospitalized patients is less certain because most studies have dealt with ostensibly healthy adults.

We recently investigated the short-term biological variation of several analytes in sera from patients with myocardial infarction (3) and impaired renal function (4). Average individual biological variations were of the same magnitude as those in healthy individuals. We postulated that, in nonacute pathological processes where new homeostatic steady-states are reached, biological variations around the new means are of the same magnitude as in healthy individuals.

Raun et al. (5) reached similar conclusions about biological variations in plasma proteins and lipids in healthy subjects and in patients with multiple sclerosis and chronic inactive nephritis.

We formulated the present study (a) to further assess our hypothesis, (b) to add phosphate and alkaline phosphatase to the range of analytes investigated, (c) to study biological variation over a period of weeks rather than days, and (d) to assess biological variation in pregnancy. We investigated 12 serum analytes in 15 women hospitalized for various obstetric problems. The women, ages 19 to 38 years (mean 26 years), had been admitted during the third trimester of pregnancy, nine for intrauterine growth retardation without hypertension (plus one with hypertension), two for pre-eclampsia with hypertension, and one each for diabetes, diabetes with hypertension, and diabetes with pre-eclampsia. We collected 10-mL blood specimens by venipuncture from each subject at two- to three-day intervals during weeks 29–39 of pregnancy. Each woman supplied an average of 15 specimens (range: 11–21) over a six-week period (range: two to nine weeks). Specimens were transferred to plain 12-mL plastic containers, allowed to clot with minimal delay, and centrifuged: the serum was stored frozen at −20 °C until analysis. On the day of analysis, all specimens from a single patient were allowed to thaw at room temperature and each was mixed thoroughly. Sodium, potassium, chloride, bicarbonate, urea, creatinine, calcium, and albumin were determined with an Astra 8 analyzer (Beckman Instruments, Fullerton, CA 92634) with Beckman reagents and calibrators. Alanine aminotransferase (AST; EC 2.6.1.2), alkaline phosphatase (ALP; EC 3.1.3.1), and creatine kinase (CK; EC 2.7.3.2) activities were measured with a Cobas Bio centrifugal analyzer (Roche Diagnostics, Nutley, NJ 07110) with Beckman reagents. Inorganic phosphate was determined with the Cobas Bio and with Pierce auto/stat reagent (Pierce Chemical Co., Rockford, IL 61105); the standard was potassium dihydrogen phosphate (NBS Standard Reference Material 186-1-C; National Bureau of Standards, Washington, DC 20234). All analyses were performed in the format previously described (3, 4), which minimized analytical variation: all analyses were performed by a single operator, analyses for all samples from each subject were completed in a single working day, and the same lots of calibrators, reagents, and quality-control materials were used throughout the study. By analysis of variance techniques, the total variation was divided into analytical variance, intra-individual variance, and inter-individual variance; analytical variance was calculated from results of duplicate analyses for each sample. The mean, standard deviation, and coefficient of variation for each analyte were calculated. As expected for pregnant women (6), the means for concentrations of urea, creatinine, calcium, and albumin were lower than those for nonpregnant adults; the mean for ALP activity was higher. The overall means for other analytes fell within the relevant reference intervals except that the mean for the somewhat labile analyte bicarbonate was slightly lower.

The component variance and the percentages of each of the components of the total variance are shown in Table 1. Analytical variability was accounted for more than 25% of the intra-individual variance for sodium, chloride, bicarbonate, creatinine, calcium, albumin, and ALT. Given the widely accepted analytical goal that analytical variance should not exceed one-quarter of the biological variance (1), analytical precision for these analytes should be improved.

Estimates of average intra-individual biological variation were of the same magnitude as those documented in the many previous studies on healthy individuals (7), and in our

References

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studies of hospitalized patients (3, 4). This substantiates our previous hypothesis that, except in acute situations, average biological variations around the means are of the same order of magnitude as for healthy individuals, irrespective of whether analyte values are outside the conventional population-based reference intervals.

The square root of the ratio of intra- to interindividual biological variation provides information about the degree of individuality of an analyte (2). For all analytes except potassium, this ratio was <1.0. This suggests that, for potassium, the conventional population-based reference interval may be useful, particularly if the variation within the individual is at or near that of the population average. In contrast, all other analytes had a higher degree of individuality. Conventional population-based reference intervals are relatively insensitive to potentially important changes for these analytes; the best guide to possible pathology may be comparison with results of recent analyses.

We therefore conclude that, as advocated previously (3, 4), the wealth of previously published data on biological variation generated from healthy adults can and should be used more widely by clinical biochemists: (a) in daily interpretation of serial results from individual patients, (b) in setting analytical goals, and (c) in reinforcing the concept that, for most analytes in specimens from hospitalized patients, conventional population-based reference intervals have limitations in assessing the health of an individual.

References

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Bilirubin interference with uric acid assay in a Hycel: A look at azino uric acid

To the Editor:

Recently, BMD-Hycel (Boehringer Mannheim Diagnostics, Indianapolis, IN) modified their uric acid method from a carbonate–phosphotungstate-based reaction to an azino-based reaction. Since the modification we have participated in two CAP surveys. Both survey reports show uric acid recovery from the Hycel BMD SK580/K-II analyzer to be significantly lower than reported by most other participants using uricase methods. Results for the CAP specimens—C-07, C-08, and C-09—gave Student's t-test values greater than 10 when compared with all other uricase methods for uric acid.

This clearly is inconsistent with the assumption of one mean for each of these pools. The two survey series had bilirubin values of about 32 mg/dL for the first series and 56 mg/dL for the second series. When we compared results for icteric specimens, recovery values for uric acid were markedly lower with the SK580/K-II as compared with our Du Pont as III.

The BMD uric acid package insert states, "Bilirubin levels below 10 mg/dl have no effect on test results." Prompted by the CAP survey reports and our own observations, we tested the BMD uricase uric acid method for bilirubin interference over a wide range of bilirubin concentrations.

Because the three SK580/K-II calibrations sera contain various amounts of bilirubin, we had to prepare an aqueous uric acid solution (without preservative) to use for calibrating the analyzer with a bilirubin-free material.

Before our study we tested the linearity of the method's response vs uric acid concentration by calibrating the instrument with aqueous uric acid at 20, 80, and 160 mg/L, respectively, and assaying analytical recovery of aqueous solutions at multiple concentrations. For aqueous uric acid solutions of 20, 40, 70, 120, 160, and 200 mg/L, recovery with the SK580/K-II was 20, 40, 70, 119, 166, and 190 mg/L, respectively.

We measured the effect of bilirubin on uric acid concentrations of 200, 100, and 50 mg/L. These three uric acid solutions were used to reconstitute viable of bilirubin controls to make three pools of material, each with a bilirubin concentration of 200 mg/L. We diluted each bilirubin pool (using the same uric acid diluent as was used for reconstituting that pool) to provide a range of bilirubin concentrations for each of the three uric acid concentrations. These specimens were analyzed in the