Within- and Between-Subject Biological Variations of Follitropin, Lutropin, Testosterone, and Sex-Hormone-Binding Globulin in Men

José Valero-Politi and Xavier Fuentes-Arderiu

The within-subject and between-subject biological variation of the serum concentrations of follitropin, lutropin, sex-hormone-binding globulin, and testosterone; the ratio between the serum concentrations of testosterone and sex-hormone-binding globulin; and the concentration of testosterone in saliva have been studied in a group of 20 men during 12 months. The between-subject coefficients of variation (CVs) were 36.0% for follitropin, 37.0% for lutropin, 42.7% for sex-hormone-binding globulin, 21.3% for testosterone in serum, 28.8% for testosterone in saliva, and 51.6% for the ratio between serum concentrations of testosterone and sex-hormone-binding globulin. The medians of the within-subject CVs for the respective analyses and ratios were 17.3%, 24.0%, 12.1%, 10.9%, 17.3%, and 9.4%. These data were used to calculate the desirable imprecision, the critical difference for significant change detection, and the index of individuality.

Indexing Terms: variation, source of analytical goals, critical difference, steroid hormones, saliva

The applications in clinical biochemistry of the data on within-subject biological variation of biochemical quantities are well established (1). These applications include establishment of metrological or analytical goals, evaluation of the significance of a change between two successive results, assessment of the suitability of the population reference values, establishment of the number of specimens to be collected and analyzed for the estimation of the "homeostatic value" of a quantity, and selection between biochemical quantities with the same clinical utility. Here we provide data on within-subject and between-subject biological variation of the serum concentrations of follitropin, lutropin, sex-hormone-binding globulin, and testosterone; the ratio between the serum concentrations of testosterone and sex-hormone-binding globulin; and the concentration of testosterone in saliva in men. To our knowledge, there is only one publication on this topic, in which the biological variation of the serum concentration of follitropin, lutropin, and testosterone were studied over a period of 7 days (2).

Materials and Methods

Subjects and Specimens

The participants were 20 apparently healthy men, ages 26 to 467 years. These volunteers maintained their usual life styles, which involved no strenuous exercise, throughout the studied period; none of them was taking any medication.

During 12 months, at −1-month intervals, venous blood and salivaly (unstimulated) specimens were collected between 0800 and 0930 h. Venous blood specimens were drawn, with the volunteers in a sitting position, usually by a single phlebotomist and with minimal stasis, using the Venoeject® system (Terumo Europe, Leuven, Belgium), with 0.9 × 25 mm needles. Blood and saliva were centrifuged at 1400 × g for 10 min and the resulting serum and salivaly specimens were frozen at −80 °C until assayed. Using this protocol, the premetrological variation was considered negligible.

All procedures followed were in accordance with ethical standards of the hospital where the work was done.

Measurements

Concentrations of follitropin and lutropin were measured by fluoroenzymoimmunoassay (Stratus Immunoassay Systems; Baxter Diagnostics Inc., Miami, FL), and concentrations of testosterone and sex-hormone-binding globulin were measured in duplicate by radioimmunoassay (Extraction Testosterone [125I] radioimmunoassay kit, and Sex Hormone Binding Globulin [125I] immunoradiometric assay kit; both from Farmos Diagnostica, Oulunsalo, Finland). All specimens collected in the same month were analyzed within the same run.

1 Author for correspondence.
2 Ed. note: Follitropin is also known as follicle-stimulating hormone (FSH); lutropin is luteinizing hormone (LH).

Servei de Bioquímica Clínica, Hospital Princeps d'Espanya, 08907 L'Hospitalet de Llobregat, Barcelona, Spain.

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Each working day the quality of the measurements was controlled by using the following control materials with "physiological" hormone concentrations: Dade® Immunoassay Control Comprehensive Tri-Level (lot no. ACK-14; Baxter Diagnostics Inc.) for measurements of serum concentration of follitropin and lutropin; Lyphochek® Immunoassay Control Serum (lot no. 600002; Bio-Rad, Anaheim, CA) for measurements of serum concentration of testosterone and sex-hormone-binding globulin; and a pool of salivary specimens for measurement of salivary concentration of testosterone. We used these control data to estimate the between-day metrological variation. For the ratio between serum concentrations of testosterone and sex-hormone-binding globulin, we used the ratio between the control results from both methods in each run.

Statistical Treatment

For each quantity we estimated:

- the between-day metrological variance (S^2_{wb}) and between-day metrological coefficient of variation (CV_{wb}), using the results observed in the "physiological" control material included in every run;
- the between-subject biological variance (S^2_{sb}) and the between-subject coefficient of variation (CV_{sb}), using the mean ("homeostatic") value of each volunteer;
- the within-subject biological variance (S^2_{bw}) of each volunteer, using the equation

$$s^2_{bw} = s^2_{tw} - s^2_{mb}$$

where $S^2_{tw}$ is the overall within-subject variance (3);
- the within-subject biological coefficients of variation of each volunteer (with respect to the "homeostatic values") (CV_{bw}), as well as the median of all of them;
- the index of individuality (II) used to assess the utility of reference values, applying the formula (4)

$$II = \sqrt{(S^2_{mb} + \text{median } S^2_{bw})/S^2_{mb}}$$

- the minimal difference between two successive measurements of a quantity in the same patient to be considered significant (two-tailed test, $P \leq 0.05$), i.e., the critical difference ($d_c$) (5), using the formula

$$d_c(\%) = 2.77 \sqrt{S^2_{mb} + \text{median } S^2_{bw}}$$

- the desirable imprecision (CV_D) for each method of measurement, using the criterion (6)

$$CV_D \leq 1/2 CV_{bw}$$

Results

The results of the "homeostatic" mean, the between-day imprecision, the between-subject biological variation of the quantities considered here by using individual means ("homeostatic values") obtained from month-to-month data during one year. Nevertheless, data on the biological variation of concentrations of follitropin, lutropin, and testosterone in serum, among others, obtained from day-to-day data during 1 week, have been published (2). For all three biochemical quantities, the within-subject biological variation in men observed in the present study are higher than those observed by Risä and Arbö (2); this is not surprising, however, because our study covers a much longer time span. The between-subject biological variation is also higher in the present work, probably because of the greater number of volunteers involved. On the other hand, the within-subject biological variation of serum concentrations of follitropin and lutropin we obtained are lower than those observed in women (in the follicular phase) during a span of 6 months (7).

Critical differences. Not long ago, only the metrological variation was considered in evaluating the difference between two successive measurements of a quantity in a patient (8, 9). At present, it is well established that within-subject biological variation should also be considered (10). However, some controversy remains about the use of the mean, the median, or some other fractile, of the different CV_{bw} values. Moreover, because

| Table 1. "Homeostatic" Mean, Between-Day Imprecision (CV_{umb}), Median Within-Subject Biological Variation (Med CV_{bw}), Between-Subject Biological Variation (CV_{sb}), Desirable imprecision (CV_{D}), Critical Difference (d_{c}), and Index of Individuality (II) Observed in This Study |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Analyte | Mean | CV_{umb} | med CV_{bw} | CV_{sb} | CV_{D} | d_{c} | II |
| Follitropin, IU/L | 4.24 | 10.3 | 17.3 | 36.0 | ≤8.7 | 55.9 | 0.51 |
| Lutropin, IU/L | 4.03 | 8.8 | 24.0 | 37.0 | ≤12.0 | 69.2 | 0.68 |
| SHBG, nmol/L | 23.8 | 6.3 | 12.1 | 42.7 | ≤6.0 | 37.8 | 0.29 |
| Testosterone, nmol/L | 28.7 | 14.6 | 10.9 | 21.3 | ≤5.4 | 50.5 | 0.83 |
| Testosterone (saliva), nmol/L | 0.57 | 22.1 | 17.3 | 28.8 | ≤8.7 | 77.7 | 0.85 |
| Testosterone/SHBG | 1.35 | 21.6 | 9.4 | 51.6 | ≤4.7 | 65.3 | 0.39 |

* In serum except where noted.

SHBG, sex-hormone-binding globulin.

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the between-day metrological variance must be used for the calculation of the critical differences, each laboratory should make its own calculations for these values. Therefore, the critical differences shown in Table 1 are shown only for an example.

Desirable imprecision. Two proposals have been published for establishing the desirable impression of the methods of measurement of the biochemical quantities: a subjective approach based on the opinion of physicians (11), and an objective approach based on within-subject biological variation (6, 12). The latter is the approach used here. The desirable imprecisions we propose have been estimated from the within-subject biological variation data obtained in this study only, because, to our knowledge, only one study of the month-to-month within-subject biological variation of serum concentrations of follitropin and lutein have been published; however, in that work, which included only women, higher CV_bsw values were found. Nevertheless, the proposed desirable imprecisions probably are too large for measurements of hour-to-hour specimens obtained during functional tests.

Index of individuality. The index of individuality is used to assess objectively the utility of population reference values for diagnostic purposes. When the index of individuality is <0.6, population reference values are of very limited value for detecting unusual results for a particular individual; if the individuality index is >1.4, reference values are useful for diagnostic purposes (4).

The estimated index of individuality suggests that the quantities studied are more appropriate for longitudinal comparisons (monitoring or individual reference values) than for transversal comparisons (diagnostic-based or population reference values).

References


Early Diagnosis of Acute Myocardial Infarction with Use of a Rapid Immunochemical Assay of Creatine Kinase MB Isoenzyme

David R. Collins, 1,4 Dennis J. Wright, 1 Michael G. Rinsler, 1 Phillip Thomas, 2 Schoumo Bhattacharya, 3 and Edward B. Raftery 2

In 195 patients presenting with chest pain and referred acutely for cardiological assessment, blood was taken immediately for assay of creatine kinase (CK; EC 2.7.3.2) MB isoenzyme by an immunochemical method and results [mass units of enzyme per liter of plasma (µg/L)] were obtained within 30 min of sampling. Diagnosis of acute myocardial infarction in the patients was made independently, based on electrocardiograms and conventional cardiac enzyme profiles. The administration of any thrombolytic therapy in response to the CK-MB concentration result was also noted, allowing assessment of the assay’s potential influence on patient management in addition to the diagnostic efficiency evaluation. The study demonstrated that, when blood samples were collected on admission to hospital and the decision level suggested by the manufacturers was utilized, the assay had an immediate sensitivity of 52% and a specificity of 97%. Of the 81 patients who were shown by conventional means to have had acute myocardial infarction, 8 (10%) had equivocal electrocardiograms but positive CK-MB concentration results. In four of these patients (5%), thrombolytic therapy was given on the basis of the clinical features and a positive CK-MB concentration result alone.

Indexing Terms: immunoenzymometric assay · diagnostic efficiency · thrombolytic therapy

Since the advent of acute interventional therapy in

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