Biological Variation of Serum Amyloid A in Healthy Subjects

To the Editor:

Serum amyloid A (SAA) and C-reactive protein (CRP) are two commonly used acute-phase markers. Whereas data on the biological variability of CRP are widely available, no data are available for SAA. Here we report the results of a study to determine the biological variability of SAA; as a methodologic control, we also determined the biological variability for CRP. Five blood specimens were collected from each of 24 apparently healthy laboratory workers (12 men and 12 women; age range, 25–42 years) on the same day (a Wednesday) once a week over 5-week period. None of the women was pregnant or using oral contraceptives, and during the study, none of the subjects smoked, took any medication, or consumed substantial quantities of alcohol. The data from two male subjects were excluded from the statistical analysis for the following reasons: (*a*) one subject suddenly increased his physical activity in the third week of the study (i.e., intensive cycle training), which significantly increased his SAA (five times the baseline values); and (*b*) another subject became ill with the flu (a minor inflammatory process).

In accordance with the Helsinki II Declaration, the design and execution of the experiment were explained thoroughly to the subjects, and informed consent was obtained. Furthermore, the study was approved by the Ethics Committee of Azienda Ospedaliera Ospedale di Circolo (05/09/2000).

Blood was collected under standardized conditions to minimize sources of preanalytic variation. After an overnight fast, a blood specimen was taken by conventional venipuncture between 0800 and 0900 with the volunteers in the sitting position, avoiding venous stasis. All samples were drawn by the same phlebotomist, allowed to clot, and then centrifuged at 3000*g* for 15 min at room temperature within 1 h of collection. Sera were separated and stored at −70 °C until analysis. At the end of the 5 weeks, all frozen samples were thawed, mixed, and centrifuged for analysis in a single run in duplicate. SAA concentrations were determined by a latex-enhanced nephelometric immunoassay (N Latex SAA; Dade Behring Diagnostic) *(1)*, and CRP concentrations were determined by a nephelometric technique (N *High Sensitivity* CRP; Dade Behring Diagnostic) *(2)*. Both assays were performed on a Dade Behring Nephelometer II (BN II; Dade Behring Diagnostic) by the same analyst, according to the recommendations of the manufacturer.

After exclusion of outliers and logarithmic transformation of the data (required by the skewed distributions of CRP and SAA data), nested ANOVA was applied and the analytical (CV_A), within-subject (CV_I), and between-subject (CV_G) components were calculated *(3)*. In particular, we estimated (*a*) the index of individuality (CV_I/CV_G), which yields information about the utility of conventional population-based reference intervals; (*b*) the critical difference $[2.77(CV_A^2 + CV_I^2)^{1/2}]$, i.e., the minimal significant difference ($P < 0.05$) between consecutive measurements of the marker in the same patient. Desirable quality specifications for imprecision (I), bias (B), and total error (TE), calculated using the formulas $I < 0.5CV_I$, $B < 0.25(CV_I^2 + CV_G^2)^{1/2}$, and $TE < 1.65I + B$ ($\alpha < 0.05$), were also evaluated (Table 1) *(3)*.

The data for CRP (CV_A = 4.3%; CV_I = 32%; CV_G = 98%) were comparable to those in the literature *(4)*, thus confirming the reliability of the methodology.

The means and the intraindividual variances of SAA did not differ significantly between sexes, indicating that sex-partitioned reference values are not required for this protein. The desirable analytical imprecision for SAA, taken to be $\leq 0.5\ CV_I$, was 4%. Thus, in this limited assessment, the intrabatch imprecision (CV_A) of the BN II assay met this goal for the determination *(2)*. The index of individuality was <0.6, indicating that conventional population-based reference values are of very limited value in the detection of unusual results for a particular individual *(3)*. The value for the critical difference (69.6%) suggests that relatively large differences between the results of sequential specimens would be required for them to be significantly different.

To examine the classification accuracy of serial SAA samples within the reference interval, the values of SAA and CRP were divided into tertiles, given the small number of subjects of this study, using the first determination. For SAA, 18 of 22 (82%) of the second results were in the same tertile as the first results, and 19 of 22 (86%) of the third, fourth, and fifth results were also in the same tertile. For CRP, tertile agreement with the first measurement was observed for 20 of 22 (91%) results of the second measurement,

Table 1. Mean values; estimated average analytical (CV_A), intraindividual (CV_I), and interindividual (CV_G) variations; and derived indices for SAA.

Group	Mean concentration, mg/L	CV_A, %	CV_I, %	CV_G, %	II[a]	Imprecision, %	Bias, %	Total error, %	CD, %
Men	2.35	4.0	18	63	0.29	9.0	16.4	31.3	51.1
Women	2.37	4.0	27	56	0.48	13.5	15.6	37.7	75.3
All	2.36	4.0	25	61	0.40	12.4	16.5	37.0	69.6

[a] II, index of individuality; CD, critical difference.

18 of 22 (82%) of the third, 20 of 22 (91%) of the fourth, and 19 of 22 (86%) of the fifth measurement. Overall, the results obtained for 17 of 22 (77%) subjects for SAA and 18 of 22 (82%) subjects for CRP were always in agreement with the first results during the study. To measure the intrasubject agreement, the Cohen's *k* was used: values >0.80 indicate strong agreement *(5)*. For SAA, the *k* value was 0.818 between the first and second measurements, whereas between the first and third, the first and fourth, and the first and fifth measurements, the *k* value was always 0.863. For CRP, the *k* values were 0.912, 0.818, 0.912, and 0.863 between the first and second, the first and third, the first and fourth, and the first and fifth measurements, respectively. Thus, the variability of repeat SAA measurements was comparable to that for CRP.

Our data show that from the standpoint of analytical and biological variation, there is little to choose between SAA and CRP as markers for acute-phase response. Therefore, selection of the best test could be based on of other practicability characteristics and clinical performance.

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Heptaminol Interferes in the AxSYM FPIA Amphetamine/ Methamphetamine II Assay

To the Editor:

We report the observation of false-positive results obtained with the AxSYM® FPIA Amphetamine/Methamphetamine II assay (Abbott™), which is widely used to detect commonly abused amphetamine compounds in human urine. Two hospitalized patients treated with heptaminol chlorhydrate (1905 and 2413 mg daily oral doses, respectively) were positive for urine amphetamine (571 and 777 μg/L, respectively), based on a cutoff concentration of 500 μg/L. Heptaminol chlorhydrate, a cardiac stimulant and vasodilator, is widely used for the treatment of orthostatic hypotension. Recommended daily oral therapeutic doses for adults are usually 500-1500 mg. After oral administration, heptaminol is rapidly and completely absorbed. Metabolism includes hydroxylation, and nonconjugated forms are found in urine *(1)*. Because intake of amphetamine-related compounds was obviously unlikely and because heptaminol chlorhydrate is not listed as cross-reactant in the package information sheet *(2)*, the following investigations were performed because heptaminol (amino-6-methyl-2-heptanol-2 chlorhydrate) and methamphetamine (phenylisopropylmethylamine) have some structural similarity (methylamine group).

A previously published gas chromatography–mass spectrometry method *(3, 4)* was used to verify the absence of the following amphetamine-related compounds: amfepramone, amphetamine, benzodioxazoylbutanamine, benzphetamine, clobenzorex, dexfenfluramine, dimethoxybromoamphetamine, dimethoxymethylamphetamine, fencamfamin, fenproporex, mefenorex, methamphetamine, methoxyphenamine, methylbenzodioxazolylbutanamine, methylenedioxyamphetamine, methylenedioxyethamphetamine, methylenedioxymethamphetamine, methylphenidate, 4-methylthioamphetamine, norephedrine, norfenfluramine, phentermine, and pseudoephedrine. After alkalinization of the patients' urine samples and addition of three deuterated internal standards, the substances of interest were extracted with diethyl ether, derivatized with heptafluorobutyric anhydride, and then purified by successive washing with deionized water and 40 g/L NH_4OH. Chromatographic separation was performed on a Shimadzu™ GC-17A gas chromatograph equipped with a split/splitless injector and a Supelco™ PTE 5 capillary column [30 m × 0.32 mm (i.d.); 0.25-μm film thickness]. Detection was carried out with a Shimadzu QP 5000 mass spectrometric detector in the electron-impact mode, with acquisition in the selected-ion monitoring mode following three mass-to-charge ratios (one for quantification and two for confirmation). This method provided detection limits between 1 and 50 μg/L (i.e., low enough to verify positive results obtained with immunochemical assays) for the 23 compounds.

In a second step, heptaminol was measured in urine following precolumn derivatization with *o*-phthalaldehyde and reversed-phase HPLC with fluorescence detection *(5)*. The chromatography system consisted of a Waters™ M6000A pump and a Hitachi™ F1000 fluorescence spectrophotometer. Before *o*-phthalaldehyde derivatization, the patients'