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Letters to the Editor

Estimates of Within-Person Biological Variation and Reference Change Values of Serum S100B and NSE Proteins

To the Editor:

Biological variation (BV)[1] describes the natural fluctuation of body fluid constituents in vivo considered to be unaffected by other processes, e.g., disease. Estimates can be determined both within a single individual and across persons. Published BV values for several analytes are available in the literature *(1)*, but data remain incomplete for specialty test biomarkers. In this report, we provide new estimates of BV for S100-β protein (S100B) and neuron-specific enolase (NSE) proteins in a young cohort of healthy individuals (mean age, 25 years).

The β isoform of S100, S100B, is found in the highest concentration within astrocytes of the brain *(2)*. NSE is the γ isoform of the glycolytic enzyme enolase, which is highly concentrated within the brain and cells of neuroendocrine origin *(2)*. When increased in the blood, both markers have the potential to aid in early diagnosis of concussion injury *(3)*. This is especially important in contact sports that may put athletes at an increased risk of head injury.

Amateur mixed martial arts athletes in Upstate New York, US, were enrolled in the study and provided written consent before participating (Institutional Review Board # 633018). All subjects were deemed in good health to participate in the events after pre-fight evaluation by a physician. Blood was collected into standard serum-separator vacutainer collection tube, allowed to clot, centrifuged within 60 min of collection, and then frozen at −70 °C until analysis. Batch testing was conducted across 4 separate days in which a group of samples was immediately thawed and analyzed in a single run. S100B and NSE results were obtained by running these electrochemiluminescence immunoassay tests on a Roche Modular E170 instrument.

To calculate within-person BV (CV_I), we compared standard ANOVA and CV-ANOVA approaches. For the latter, each participant's raw data values are first normalized to that individual's mean value before performing ANOVA test, which has been reported to provide robust estimates of CV_I *(4)*. Homogeneity of variances on both data sets was verified using the Levene test before ANOVA test (Minitab version 18). We applied CV_I estimates from CV-ANOVA to calculate reference change values (RCV) using the standard calculation *(1)*. Our data set included 10 individuals sampled 4 times (n = 40 measurements), which provides the greatest power (0.94) and the narrowest CI width of ±55% on the basis of the available number of individuals we were able to test *(5)*. For S100B, we estimated CV_I = 18.9%, with RCV values equal to 44.1% and 52.4% for 1- and 2-sided distributions, respectively. For NSE, we estimated CV_I = 23.0%, with RCV values of 54.1% and 64.3% for 1- and 2-sided distributions, respectively (Table 1). CV_I estimates were similar for both analyses. Using the standard ANOVA, we estimated between-group variance (CV_G) = 42.4%. Normalizing test values for the CV-ANOVA approach ensures that CV_G = 0.0%, which removes any influence of CV_G on estimating CV_I *(4)*. We feel confident that these CV_I estimates closely reflect a true homeostatic set point for several reasons. First, samples collected immediately post-fight were excluded. Second, time course studies indicated that participants' test values returned to baseline within 48 h (data not shown). Lastly, we were able to model 1 event as case–control design because 4 subjects had their scheduled fights canceled on the day of the event but provided blood samples (i.e., controls). Compared with grand means (Table 1), the 4 control subjects had S100B mean values of 33, 42, 44, and 57 pg/L; for NSE, these subjects had means of 14.7, 15.1, 18.2, 16.3 ng/mL.

Analytical imprecision (CV_A) for both tests was determined during replicate experiments using manufacturer-supplied materials. We did not directly determine analytical imprecision using human samples; however, our results closely match manufacturer's serum sample data reported on the test data sheets (S100B: CV_A = 2.3%, mean 89 pg/L; NSE: CV_A = 3.1%, mean 11.4 ng/mL; Roche Diagnostics GmbH). Repeat analysis of a human sample (CV_A) was shown to have minimal effect on test power and CI estimate of CV_I when a test assay has good precision *(5)*. For this reason, and because of limited test supplies, we analyzed each sample once. The small contribution of CV_A to CV_I estimates was accounted for by subtracting analytical variance from within-person variance for the final results (Table 1). We believe this approach is valid because the RCV formula includes the contribution of CV_A to CV_I *(5)*.

The CV_I estimates provided in our report aim to improve the BV database of these 2 specialty tests. Using BV data aids the laboratory professional and clinician in several practical ways. It allows one to set analytical-quality goals for imprecision, bias, and total allowable error; determine an individual's RCV for interpreting serial testing results; or set internal rules (Δ check) to flag possible error in the preanalytical or analytical testing phases.

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[1] Nonstandard abbreviations: BV, biological variation; S100B, S100-β protein; NSE, neuron-specific enolase; CV_I, within-person BV; RCV, reference change value; CV_G, between-person BV; CV_A, analytical imprecision.

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Table 1. CV_I and RCV.[a]

Analyte	Grand mean (n = 40 subjects)	CV_I (%)[b]	CV_I (%)[c]	RCV %[d] (z = 1.65)	RCV %[c] (z = 1.96)	CV_A (%)
S100B	48 pg/L	19.8 (8.9–30.7)	18.9 (8.5–29.4)	44.1	52.4	1.0
NSE	14.2 ng/mL	22.1 (9.9–34.3)	23.0 (10.4–35.7)	54.1	64.3	2.9

[a] Blood was collected immediately prior to the fight at 7 independent events; at 2 events, additional collection times of 1 week prior, 48 or 72 h post-event, and 1 week post-event.
[b] CV_I ± 95% CI; Standard ANOVA (df = 30 within-group variance, df = 9 between group variance).
[c] CV_I ± 95% CI; CV-ANOVA (df = 30 within-group variance, df = 9 between-group variance).
[d] RCV = $\sqrt{2} \cdot z \cdot \sqrt{CV_I^2 + CV_A^2}$.

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