Letter to the Editor

Hemolysis correction factor in the measurement of serum neuron-specific enolase

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Keywords: data correction; hemolysis; neuron-specific enolase.

Neuron-specific enolase (NSE) is comprised of two isoforms, αγ and γγ, of the glycolytic enzyme enolase. It is present almost exclusively in neurons and neuroendocrine tissues (1–3). The protein is a valuable tumor marker for monitoring the outcome and disease course in patients with cancer of the neuroendocrine type, in particular small cell lung cancer and neuroblastoma (4, 5). In addition, the concentration of NSE in biological fluids might be a useful parameter for the assessment of neural brain damage as NSE was shown to be released into cerebrospinal fluid and blood following cerebral injury (5, 6). However, the αγ-isof orm was also reported to be present in circulating blood cells, especially in erythrocytes and platelets (3, 7). As a consequence, slight hemolysis – even undetectable to the naked eye (3) – can produce falsely increased degrees of immunoreactivity (1). Considering the role of NSE as an important biomarker, interpretation of NSE results is unreliable unless accompanied by an estimate of red cell disintegration (3).

In the case of hemolysis, it is common practice to include a comment on the laboratory report and not give the NSE value itself. We examined the possibility of using data correction to report a clinically more reliable result when measuring NSE in hemolysed serum, instead of reporting a comment that the assay could not be performed.

NSE was measured using a sandwich immunoassay with the electrochemiluminescence (ECL) technology installed on a Modular E170® (Roche Diagnostics, Mannheim, Germany). Evaluation of the magnitude of hemolysis was achieved using the hemolytic index (H). The serum index was routinely measured with a Cobas 6000® analyzer (Roche Diagnostics, Mannheim, Germany) using absorbance values of two bichromatic readings (570 and 600 nm, and 660 and 700 nm). The absorbance values are converted into semi-quantitative values corresponding to the hemoglobin (Hb) concentration in mg/dL (1 Hb = 1 mg/dL = 0.621 μmol/L Hb). The reported hemolysis units are linear up to 621 μmol/L free Hb. For H, a bias of –1.7% was calculated, within- and between-run CV values were 4% and 5%, respectively. For NSE, a bias of –5.8% was found, within- and between-run CV values were 1.6% and 4.9%, respectively.

At first, we assessed the correlation between increasing degrees of hemolysis and NSE by analyzing a dilution series of pure hemolysate in water with a hemolytic index from 0 to 100 (or 0–62.1 μmol/L Hb). Hemolysate was prepared from whole blood anticoagulated with lithium heparin. Following centrifugation for 10 min at 1885×g, cells were washed twice with saline and lysed by adding water (1 volume of cells: 1 volume of water). Completion of hemolysis was achieved by freezing for 24 h at –20°C, and then thawing the sample. Centrifugation (10 min; 1885×g) was used to remove cellular debris. To obtain a representative amount of data, 10 dilution series were analyzed. When performing the experiment using duplicate measurements in serum, no matrix effects were observed.

Next, the amount of NSE released as a function of the hemolytic index was evaluated by observing the NSE/H ratio. Whole blood, anticoagulated with lithium heparin or K3-EDTA from 100 samples with a normal red blood cell count and Hb concentration, was used to perform the experiment. In order to obtain a measurable H index and NSE concentration, 30 μL of saline-washed erythrocytes were lysed using 9.97 mL of water. Cellular remnants were removed using centrifugation for 10 min at 1885×g. Following centrifugation, the degree of hemolysis and the concentration of NSE were measured in the supernatant and the NSE/H ratio was calculated.

The results revealed a linear correlation between the concentration of NSE and the hemolytic index (r² = 0.9989). In addition, a mean value of 0.30 ± 0.08 μg/L NSE was found to be released by the red blood cells per unit of H (1 Hb = 0.621 μmol/L Hb). The data were normally distributed (Kolmogorov-Smirnov test) and ranged from 0.14 to 0.56 μg/L, with a double-sided 95% reference interval that extended from 0.17 to 0.50 μg/L (Figure 1).

Since we observed a consistent effect of hemolysis on the measurement of NSE, we propose using a compensating factor according to the degree of hemolysis present in the sample. To adjust the NSE concentration, a term equal to (H × 0.30 μg/L) should be subtracted from the measured NSE concentration. Application of this correction on 300
patient results (January 2008–March 2009), showed the presence of a positive interference up to 116%, with a hemolytic index ranging from 0 to 56. In addition, nearly 3% of the reported NSE results were falsely categorized as elevated (cut-off value: 12.5 µg/L). Since hemolysis becomes visually detectable when the concentration of free Hb exceeds 30 mg/dL (8), it was undetectable to the naked eye in these cases as the hemolytic index showed a maximum value of 12 (= 12 mg/dL or 7.5 µmol/L Hb). Despite the low degree of hemolysis, the interference ranged from 13% to 30%.

Plebani and Lippi (9) recently mentioned the role of serum indices in the evaluation of sample integrity and mathematical correction of the measured analyte concentrations. The observations concerning the mechanism and extent of interference proposed in this report can be converted into a rule-based algorithm, and subsequently incorporated in the laboratory information system (LIS). This approach of LIS integration was already suggested by Vermeer et al. (10) and allows complete automation of processing the results. As Lippi and colleagues observed satisfactory agreement between the hemolytic indices among various analytical platforms (11), we believe that our approach of data correction can promote interlaboratory harmonization when measuring NSE in hemolysed serum.

Conflict of interest statement

Authors’ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Ethical approval: The study was conducted according to the principles of the Helsinki declaration.

References


