Clin Chem Lab Med 2005;43(11):1215–1217 © 2005 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2005.210

Short Communication

Effects of hemolysis and storage condition on neuron-specific enolase (NSE) in cerebrospinal fluid and serum: implications in clinical practice

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Abstract

The concentration of neuron-specific enolase (NSE) in serum and cerebrospinal fluid (CSF) has been used as a biomarker in some cancers and, more recently, in neurodegenerative diseases. Pre-analytical conditions are very important for the quality of returned results. In this study, we evaluated the effects of storage conditions (temperature and duration of storage) and hemolysis on the concentration of NSE in serum and CSF. Our results demonstrate that samples for NSE measurement may be stored at −80°C for no more than 6 months in the case of CSF and 9 months in the case of serum samples. Even invisible hemolysis may increase NSE levels in samples. Consequently, an index of hemolysis should be determined before deciding whether or not to perform NSE measurement.

Keywords: cerebrospinal fluid; hemolysis; neuron-specific enolase; serum; storage conditions.

Enolase is a dimeric enzyme composed of α, β and γ subunits. Neuro-specific enolase (NSE) comprises two isoforms, αγ and γγ, which are synthesized by neurons and neuroendocrine tissues. On the other hand, an αγ isoform is abundant in erythrocytes (1, 2). The concentration of NSE in serum samples is increased in patients with small-cell lung cancer (3) and in children with neuroblastoma (4). More recently, the level of NSE in cerebrospinal fluid (CSF) was used as a biomarker in neurodegenerative diseases (5). NSE concentration in CSF from Creutzfeldt-Jakob patients was elevated, whereas it was not changed in Alzheimer's disease (6).

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Since pre-analytical conditions may strongly influence the data obtained in some analytical methods, we evaluated the effect of storage conditions (temperature and duration of storage) and of hemolysis on the concentration of NSE in serum and CSF.

NSE was measured by a non-radioactive automated immunoassay (Kryptor, Brahms France, Saint Ouen, France) using TRACE® (time-resolved amplified cryptate emission) technology (7). Hemolysis measurement was routinely evaluated with a Modular® analyzer (Roche Diagnostics, Meylan, France) using a kinetic colorimetric assay (340 nm) and samples were classified according to their hemolysis index: index 0, no hemoglobin (Hb) detectable; index 1, Hb <12 μmol/L; index 2, Hb <25 μmol/L; index 3, Hb <36 μmol/L; and index 4, Hb <48 μmol/L.

Blood specimens were obtained by venepuncture from patients with small-cell lung cancer or neuroblastoma suspicion. CSF was obtained for diagnosis purposes by lumbar puncture under strict aseptic conditions in patients suspected of neurodegenerative disease. CSF samples were immediately brought to the laboratory. For analysis, CSF and serum were centrifuged for 10 min at 4°C at 200×*g* and 2500×*g*, respectively, and analyzed immediately. To study the effects of sample storage, replicate aliquots of the samples were collected in separate polyethylene tubes and stored at different temperatures (−20°C or −80°C). After the desired storage time, aliquots were defrosted, centrifuged and NSE was measured. The range of NSE concentrations was 10–50 μg/L for serum and 10–70 μg/L for CSF. The influence of pre-analytical hemolysis was studied by adding increasing amounts of hemolysate to aliquots of a sample of hemolysis index 0. To preparing the hemolysate, whole blood anticoagulated with lithium heparinate was centrifuged to separate cells from plasma. One volume of cells was lysed with one volume of distilled water and 0.5 volumes of toluene. Complete lysis was obtained using a vortex mixer. Cellular debris was settled by centrifugation at 1000×*g* (8). After adding the hemolysate, we obtained samples with varying degrees of hemolysis, almost invisible (index 1) or visible (index 2, 3, 4).

The effects of storage conditions are shown in Table 1. The concentration of NSE in CSF decreased significantly (−27.6%) when stored at −20°C for 1 month. Similar data were previously reported by others in the case of plasma or serum (9, 10). At −80°C, the stability of NSE in CSF was better, but the NSE concentration still decreased progressively when

Table 1 Effects of duration and temperature of storage on NSE concentration.

Duration, months	Temperature, °C	NSE decrease, %	p
CSF			
1	−20	27.6±3.1	$p<0.0001$
1	−80		NS
3	−80		NS
6	−80	5.3±2.7	NS
8	−80	19.5±3.1	$p<0.001$
9	−80	21.9±4.7	$p<0.001$
Serum			
1	−80		NS
3	−80		NS
6	−80		NS
9	−80		NS

Statistical analyses were performed by Student's paired t-test with unstored samples (n=10). Results are expressed as mean decrease from baseline ±SD. NS, not significant compared to unstored sample.

samples were stored for more than 3 months. The decrease was 5% of the initial level after 6 months, 19.5% after 8 months and 21.9% after 9 months. On the other hand, the NSE concentration in serum was stable at −80°C for at least 9 months. These data show that NSE in CSF is very unstable. NSE stability might be influenced by the volume of the aliquot and by the protein concentration in the sample. In any case, the level of NSE measured depends on the temperature and duration of freezing. Our data indicate that all samples for NSE measurement may be stored at −80°C for at least 9 months in the case of serum and for 6 months in the case of CSF.

Figure 1 shows the influence of hemolysis on the concentration of NSE in serum or CSF. We observed a significant increase in NSE concentration in all hemolyzed samples (serum and CSF). A significant correlation between the increase in NSE concentration and the index of hemolysis, as determined by routine automated method (Modular®), was observed. Since red blood cells contain high amounts of NSE (2), NSE concentrations may only be measured adequately in samples with no hemolysis.

Knowledge of the effects of storage conditions and hemolysis on the concentration of NSE in serum or CSF is important, since these samples may frequently be stored and/or frozen before transport to a specialized laboratory. To the best of our knowledge, this is the first time that the influence of pre-analytical conditions on the measurement of NSE in CSF has been reported. Our data allow the conclusion that all samples for NSE measurement should be stored at −80°C and analyzed within 6 months after sampling in the case of CSF. Serum may be kept frozen for longer periods (up to 9 months). Moreover, it is necessary to systematically evaluate the index of hemolysis before deciding whether or not to perform NSE measurement in either serum or CSF.

Acknowledgements

We thank Brahms France Laboratory (Saint Ouen, France) for generously providing the NSE measurement reagent. We are grateful to our laboratory team members for technical assistance.

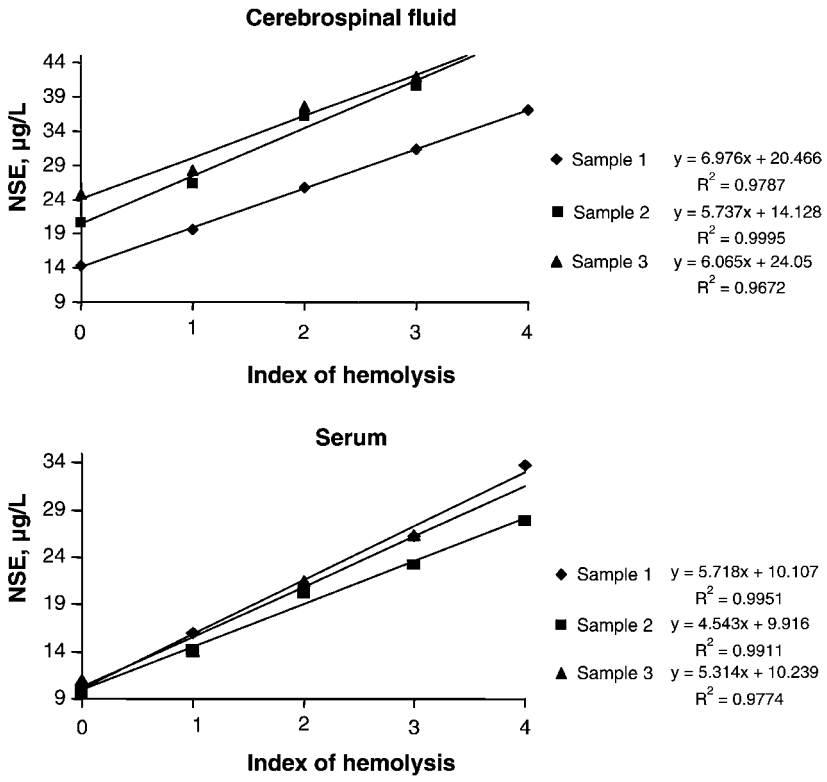

Figure 1 Correlation between NSE concentration and index of hemolysis. Preanalytical hemolysis was studied by adding aliquots of a hemolysate to serum or cerebrospinal fluid without hemolysis. Hemolysis index: index 0, no detectable hemoglobin (Hb); index 1, Hb <12 µmol/L; index 2, Hb <25 µmol/L; index 3, Hb <36 µmol/L; index 4, Hb <48 µmol/L.

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Received May 24, 2005, accepted August 3, 2005