



Neuron-Specific Enolase (NSE) analyte monograph

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1 Name and description of analyte

1.1 Name of analyte

Neuron-Specific Enolase (NSE)

1.2 Alternative names

Systematic name 2-phospho-D-glycerate hydrolase (EC 4.2.1.11); also phosphopyruvate hydratase, 2-phosphoglycerate dehydratase.

1.3 NMLC code: To follow

1.4 Description of analyte

Three tissue-specific isozymes of enolase exist and are expressed by different genes. Enolase α is ubiquitous, enolase β is muscle-specific and enolase γ is neuron-specific. These isozymes dimerise to form the three known isoforms of enolase: non-neuronal enolase (NNE) which is made up of $\alpha\alpha$ -enolase, muscle-specific enolase (MSE) which consists of $\beta\beta$ -enolase and NSE which exists as either $\gamma\gamma$ - or $\alpha\gamma$ -enolase. Although both $\gamma\gamma$ - and $\alpha\gamma$ -NSE are highly concentrated in mature neurones and cells of neuronal origin, $\alpha\gamma$ -NSE is also present in erythrocytes. The term 'neuron-specific' enolase is therefore somewhat inaccurate and misleading. The half-life of NSE is approximately 30 hours.

1.5 Function(s) of analyte

Catalyses the reversible dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate in the glycolytic pathway.

2 Sample requirements and precautions

2.1 Medium in which measured

1. Blood NSE is usually measured in serum.
2. NSE can be measured in CSF, but this is rarely indicated.

2.2 Precautions re sampling, handling etc.

1. Avoid haemolysis.
2. Serum should be separated ideally within 1 hour and analysed within 2 days if stored at 20-25°C, or within 5 days if stored at 2-8°C.

3. For long term storage, serum can be frozen for 3 months at -20°C or 9 months at -80°C. Once thawed, samples should not be refrozen.
4. CSF samples need to be analysed as soon as possible or stored at -80°C for a maximum of 6 months.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

1. Tumour marker in the diagnosis and monitoring of neuroendocrine tumours (NETs) such as small-cell lung cancer (SCLC), non-small-cell lung cancer (NSCLC) and neuroblastoma.
2. Prognosis determination in episodes of brain damage, particularly ischaemic damage after out-of-hospital cardiac arrest (OHCA).

3.2 Limitations

1. The stated specificity and sensitivity of NSE in the diagnosis of NETs and the recommended interpretation of results for use in neuroprognostication varies considerably in the literature. This may be (at least in part) due to the variable detection of isolated NSE release from erythrocytes and the use of different NSE methods between studies.
2. Even small degrees of haemolysis not visible to the eye can significantly increase [NSE].
3. Diagnostic cut-offs are difficult to standardise due to method variation.

4 Analytical considerations

4.1 Analytical methods

NSE is measured by immunoassay. The main methods utilise either chemiluminescence (Roche Elecsys and Diasorin Liaison) or time-resolved amplified cryptase emission (TRACE) (BRAHMS Kryptor).

4.2 Reference method: None.

4.3 Reference materials: None.

4.4 Interfering substances

1. Positive interference from haemolysis. This is because most immunoassays are specific to the γ subunit of NSE, which is also present in erythrocytes (as part of the $\alpha\gamma$ isoform). Even very slight haemolysis not visible to the eye can cause significant positive interference. The magnitude of interference is independent of [NSE] in serum and is linearly related to the degree of haemolysis. The use of generalised haemolysis correction factors should ideally be avoided as [NSE] within erythrocytes can vary significantly between individuals and hence the release of NSE relative to haemoglobin varies (between approximately 0.1 and 1.0 $\mu\text{g/L}$ NSE per mg/dL of free Hb). The haemolysis index threshold for sample

- acceptance should be set according to the intended use of the assay. Commonly used thresholds are 20 – 50 mg/dL free Hb.
2. The Roche NSE immunoassay makes use of biotin-streptavidin binding. High dose biotin therapy (>5 mg/day) causes negative interference. This can be avoided by taking samples from patients at least 8 hours after their last dose.
 3. As with most immunoassays, the presence of heterophilic antibodies can give rise to spurious results.

4.5 Sources of error

1. Falsely increased [NSE] due to delayed sample centrifugation.
2. Falsely decreased [NSE] upon delayed analysis of serum.
3. Free haemoglobin and NSE have an *in vivo* half-life of approximately 4 hours and 30 hours respectively, therefore *in vivo* haemolysis causing a false elevation of [NSE] may not be detectable via spectrophotometric 'haemolysis index' methods in the laboratory.
4. Different immunoassays can produce significantly different results; therefore, the same method must be used for treatment monitoring and serial measurements.

5 Reference intervals and variance

5.1.1. Reference interval (adults):

<16.3 µg/L (Roche Elecsys – based on 95th percentile of 547 healthy people)

<12.5 µg/L (BRAHMS Kryptor – based on 93% of 100 healthy males being below this concentration)

<18.3 µg/L (Diasorin Liaison – 95th percentile of 'healthy men and women')

5.1.2. Reference intervals (others):

The 2021 European Resuscitation Council guidelines state a threshold of [NSE] >60 µg/L at 48-72h post-ROSC to support a prediction of poor neurological outcome.

5.1.3 Extent of variation.

5.1.3.1 Interindividual CV: 16.6%

5.1.3.2 Intraindividual CV: 10.9%

5.1.3.3 Index of individuality: 0.67

5.1.3.4 CV of method:

1-5% at [NSE] >10 µg/L (Roche Elecsys and BRAHMS Kryptor methods)

5.1.3.5 Critical difference: –23%/+30%

5.1.4 Sources of variation:

No known age or sex related variation. No known diurnal variation.

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation

1. NSE correlates with tumour burden, number of metastatic sites and response to treatment in some NETs, therefore it can be used as a tumour marker. This is valuable in cases where histological biopsy is inconclusive or not possible.

- SCLC: Up to 75% of patients have an increased [NSE] at diagnosis. [NSE] is greater in patients with extensive disease (ED) SCLC than patients with limited disease (LD) SCLC, meaning that it can be used to help determine SCLC severity and prognosis. [NSE] also decreases following successful treatment and rises again after relapse, making it a valuable non-invasive treatment monitoring biomarker.
 - NSCLC: [NSE] is increased in 12-28% of patients at diagnosis, therefore it is less useful for diagnosis than SCLC. [NSE] is not useful for prognosis determination in NSCLC. [NSE] can be useful for SCLC and NSCLC differentiation when combined with pro-gastrin-releasing peptide (ProGRP).
 - Neuroblastoma: Children with stage I or II disease have much lower [NSE] and a better prognosis than children with stage III and IV disease, making NSE a useful staging and prognostic tool. In children with widespread metastatic (stage IV) disease, an [NSE] >100 µg/L is associated with much poorer outcome. [NSE] tends to decrease from pre-treatment levels upon remission and increase again upon relapse, making it a valuable monitoring biomarker.
2. As NSE is highly concentrated within neuronal cells, it leaks into the blood following neuronal damage. This means that high [NSE] can be a useful tool for predicting poor outcome after neurological damage.
- The 2021 European Resuscitation Council guidelines recommend measurement of serum NSE as part of multi-modal neuro-prognostication for comatose patients post-OHCA. These guidelines recommend serial measurement of NSE and state that increasing [NSE] in combination with high values at 48-72h post return of spontaneous recirculation (ROSC) support a prediction of poor neurological outcome.
 - A single [NSE] cut-off of >60 µg/L at 48 hours and/or 72 hours post-ROSC can be used to support a prediction of poor neurological outcome. This cut-off was chosen due to the low presumed chance of falsely predicting poor outcome (i.e. high specificity), particularly because poor outcome should not be predicted on [NSE] alone (at least one other predictor of poor outcome – e.g. absent pupillary and corneal reflexes, highly malignant EEG, anoxic injury on brain CT/MRI, etc. – should be positive as well to predict poor outcome).
 - Rising [NSE] (Δ NSE) may be a more reliable predictor of poor outcome than an isolated [NSE] at specific time points post-ROSC. This is because a rising NSE suggests ongoing neuronal damage, perhaps as a result of reperfusion injury. There is incomplete consensus as to the optimum time points for calculation of Δ NSE. However, it is recommended that the Δ NSE is assessed at 24-, 48- and 72-hours post-ROSC, with increasingly positive Δ NSE being more predictive of poor neurological outcome. Δ NSE assessment has the added benefit of not relying on one specific [NSE] cut-off across variable NSE analytical methods, as well as minimising the chance of isolated haemolysis leading to a false positive poor prediction.
 - Other clinical conditions in which [NSE] may aid neuro-prognostication include neonatal encephalopathy, stroke, haemorrhage, seizures and

traumatic brain injury. However, these indications do not yet appear in relevant guidelines as specific recommendations.

6.2 Confounding factors

1. [NSE] is only of use in predicting the neurological outcome post-OHCA and may be misleading in patients with poor prognosis secondary to other pathologies. This likely explains why multiple studies have reported that the power of [NSE] in predicting poor neurological outcome after OHCA is better in younger patients than older patients.
2. *In vivo* haemolysis gives a false elevation of [NSE] for the same reasons as *in vitro* haemolysis. Importantly however, the *in vivo* half-lives of free haemoglobin and NSE are approximately 4 and 30 hours respectively, therefore elevations of [NSE] due to *in vivo* haemolysis can occur beyond the time point that it can be detected in the laboratory using the haemolysis index.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

- Neuroendocrine tumours
- Neurological damage
- Haemolysis (*in vivo* and *in vitro*)
- Delayed sample centrifugation

7.1.2 Investigation

- Imaging and other biomarkers to investigate tumours
- Multimodal neuro-prognostication post-OHCA should include clinical assessment, electrophysiology and imaging, in addition to the NSE testing
- Free haemoglobin analysis to test for *in vitro* haemolysis (and *in vivo* haemolysis occurring within 4 hours of sample collection)

7.2 Low values

7.2.1 Causes

There is no lower reference limit for [NSE], but falsely low concentrations can occur after sample degradation.

7.2.2 Investigation: None.

7.3 Notes

[NSE] should not be used as the sole predictor of poor outcome after OHCA.

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions: See section 6.

9 Systematic Reviews and guidelines

9.1 Reviews

- Isgrò MA, Bottoni P, Scatena R. Neuron-Specific Enolase as a Biomarker: Biochemical and Clinical Aspects. *Adv Exp Med Biol.* 2015;867:125-43.
- Sharma K, John M, Gronseth G, et al. Serum Neuron-Specific Enolase Thresholds for Predicting Postcardiac Arrest Outcome: A Systematic Review and Meta-analysis. *Neurology* 2022;98(1):e62-e72.

9.2 Guidelines

- Nolan JP, Sandroni C, Soar J, et al. European Resuscitation Council and European Society of Intensive Care Medicine guidelines 2021: post-resuscitation care. *Intensive Care Med* 2021;47(4):369-421
- Wijdicks EF, Hijdra A, Wiebe S, et al. Practice parameter: prediction of outcome in comatose survivors after cardiopulmonary resuscitation (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2006;67:203-210.

9.3 Recommendations

Many studies have recommended [NSE] cut-offs for prediction of poor neurological outcome post-OHCA. A detailed bibliography is beyond the scope of this document, but a summary of some of the key studies can be found in the 2021 European Resuscitation Council and European Society of Intensive Care Medicine guidelines, as well as:

- Prediction of poor neurological outcome in comatose survivors of cardiac arrest: a systematic review. *Intensive Care Med* (2020) 46:1803–1851

10 Links

10.1 Related analytes: ProGRP, chromogranin A, S-100 β

10.2 Related tests: None

Additional References

1/ Iten M, Schild C, Haenggi M, et al. Different half-life of free haemoglobin and NSE may falsely predict poor prognosis after cardiac arrest. *Resuscitation* 2022;81:55 – 57.

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