Neuron-specific enolase in cerebrospinal fluid as a biomarker of brain damage in infants with hypoxic-ischemic encephalopathy

Alfredo Garcia-Alix*, Juan Arnaez

Neonatal encephalopathy resulting from an asphyxial episode occurring perinatally is a major cause of death and of permanent neurological disabilities worldwide. Therapeutic hypothermia (TH) started within 6 hours of life and maintained for 72 hours is now well established as standard treatment for infants with moderate-to-severe hypoxic-ischemic encephalopathy (HIE). Those infants have altered consciousness and other signs of neurological dysfunction. However, nearly half of infants with moderate-to-severe HIE treated with TH still die or survive with disability despite treatment. In addition, the globalization of TH is still pending, especially in low-to-middle income countries where prevalence rates of HIE are particularly high.

Early and accurate assessment of the severity of brain damage after a perinatal hypoxic-ischemic event remains one of the most difficult challenges in neonatal care. A variety of clinical, neuroimaging, and neurophysiological approaches, alone and in combination, have been used to detect brain dysfunction and predict neurological outcome (Shellhaas et al., 2015). However, current methods have inherent limitations in characterizing the complex nature of the ongoing brain damage during the first 72 hours of postnatal life. Importantly, these are golden hours to include potential neuroprotective therapies adjuvant to TH, provide crucial prognostic information for families, guide bedside management, and facilitate decisions about palliative care.

Thus, more information to better limit prognostic uncertainty during this window of opportunity is highly desirable. Neurobiochemical markers have gained momentum in the cooled infant with HIE to assess the severity of ongoing brain damage as well as the early and accurate identification of those infants at risk of neurodevelopmental disability. In addition, these markers can help identify those neonates who might benefit most from implementing other neuroprotective or brain repair interventions during the first days. Furthermore, neurobiochemical markers may help measure the effectiveness of these therapeutic interventions.

Although most research has focused on fluids such as blood and to a lesser extent urine, the results of these studies are inconsistent. Sampling neurobiochemical markers in cerebrospinal fluid (CSF) takes advantage of the fact that this fluid is in close contact with the brain, enabling detection of specific molecules that are released from the injured brain. Furthermore, unlike sampling in other biological fluids, brain-specific proteins (BSPs) are not affected by the severity of the blood-brain barrier disruption nor by the release of these biomarkers with multi-organ involvement, common in HIE (Garca-Alix and Quero, 2001). Consequently, analysis of this fluid offers an important window into the pathological underpinnings of hypoxic-ischemic brain injury. In summary, CSF sampling is likely to achieve the highest specificity for brain injury.

Several central nervous system-specific molecules such as brain-specific proteins, including neuron-specific enolase (NSE), S100B, glial fibrillary acidic protein, myelin basic protein, and activin A have been investigated in CSF as potential quantitative indexes of perinatal brain injury (Ramaswamy et al., 2009). These BSPs are measurable with commercially available, inexpensive kits with good reproducibility worldwide, and results are available within a few hours.

NSE: NSE is the CSF neurobiochemical marker that has been most studied in infants with HIE. NSE is a dimeric glycolytic enzyme concentrated in the cytoplasm of neurons and neuroendocrine cells and may represent up to 4% of the total soluble brain proteins. NSE is not normally secreted into extracellular fluid by intact neurons. However, when axons are damaged NSE is highly expressed in neuronal cytoplasm, and, given its location, it possesses relatively high specificity and sensitivity for detecting axonal damage and sensitivity for axonal injury and neuronal cell death. NSE is soluble and stable in biological fluids, and its measurement is not affected by bilirubin or lipemia. The main limitation of using NSE levels in CSF as a biomarker of neuronal injury is its high sensitivity to hemolysis, as NSE concentrations increase in hemolyzed samples. Since red blood cells contain large amounts of NSE, NSE concentrations should only be measured in samples with no hemolysis (Ramont et al., 2005).

Furthermore, for all biomarkers the storage conditions and method used for their determination could influence the values obtained, so these are key aspects that need to be considered. For NSE, storage in CSF appears to be unstable compared to that in serum. Ramont et al. (2005) evaluated the effects of storage conditions of NSE in CSF in a few samples and found that the concentration decreased significantly when stored at –20°C for 1 month, and though stability was better at –80°C, the NSE concentration still decreased progressively when samples were stored for more than 3 months.

In several prospective studies including small cohort sizes of infants with HIE in the pre-hypothermic era, CSF-NSE levels were shown to be related to the clinical severity of HIE and seemed to provide good prediction of outcome (**Figure 1**; Garcia-Alix et al., 1994; Tekgul et al., 2004). At present, only three studies have examined the predictive value of CSF-NSE concentrations in infants with HIE treated with TH. In one study fifty-one enrolled neonates with HIE were divided into two groups: hypothermia and control. CSF-NSE values were lower in those who underwent TH compared with normothermic infants, and CSF-NSE levels were higher in those who developed severe neurological impairment (Sun et al., 2012). This study did not examine the relation between CSF-NSE levels and other surrogates of the severity of hypoxic-ischemic injury including neurophysiological and neuroimaging studies.

In a recent study by our group which included 43 patients with different degrees of HIE, the sampling to measure CSF-NSE levels was carried out in the interval between 12 and 80 hours of postnatal age, with marked differences between those who had moderate-to-severe HIE and qualified for TH and those who did not. Furthermore, CSF-NSE levels were significantly higher in those infants with persistent abnormal amplitude integrated electroencephalogram background and with significant brain injury on magnetic resonance imaging. The age of sampling did not influence the association between CSF-NSE levels and the variables analyzed. Infants with adverse outcomes (Bayley-III test < 85, cerebral palsy or death) showed significantly higher CSF-NSE levels than those with normal outcome. The most accurate CSF-NSE cutoff level for predicting adverse outcome was 108 ng/mL, and 50 ng/mL when only surviving infants were included (Leon-Lozano et al., 2020).

A recent prospective study examined CSF-NSE levels in 26 infants who suffered a sudden and unexpected collapse – SUPC – at a median time of 60 minutes after birth (interquartile range, 55 to 90 minutes). SUPC is a rare but devastating event associated with a high risk of postnatal hypoxic-ischemic brain injury. In this study, CSF-NSE levels determined at a median of 48 hours of postnatal life (interquartile range, 39 to 65 hours) were correlated with the severity of early neurological dysfunction and with significant brain injury on magnetic resonance imaging. Infants who died or had adverse outcomes showed higher CSF-NSE levels than those with normal outcomes. The maximum predictive accuracy of adverse outcome (death, cerebral palsy, or

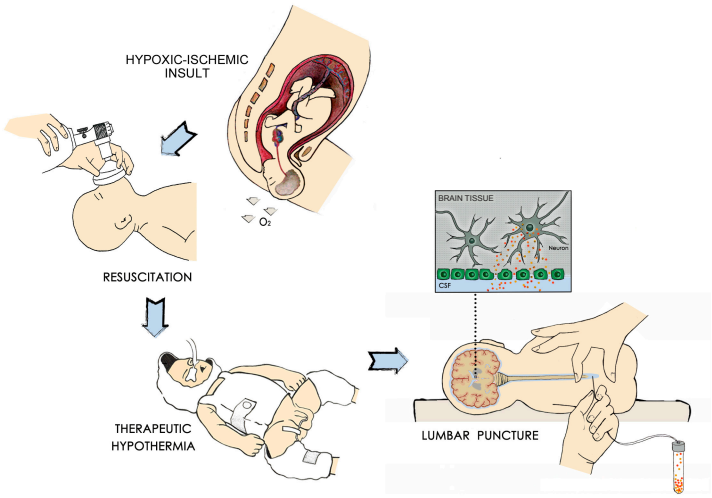

Figure 1 | **Neuron-specific enolase and hypoxic-ischemic brain injury.**
Neuron-specific enolase is released from the cytoplasm of neurons after hypoxic-ischemic brain injury. Infants diagnosed with perinatal hypoxic-ischemic encephalopathy after birth are candidates to receive hypothermia treatment. Neuron-specific enolase measured in cerebrospinal fluid (CSF) has emerged as a good biomarker of the severity of encephalopathy and subsequent neurological outcome.

developmental delay) was 61 ng/mL (area under the curve of 1.0, sensitivity 100%, specificity 100%) (Echeverria-Palacio et al., 2019).

In addition, the potential utility of CSF-NSE levels has also been examined in focal ischemic injuries such as neonatal cerebral arterial infarction (Arca et al., 2020). Arca et al. (2020) found that CSF-NSE levels were related to the extent and volume of the arterial infarction and to neurodevelopment at two years of age in the 38 enrolled neonates with this pathology.

Some considerations and caveats: NSE is released in a sequence that is temporally related with brain tissue damage. However, in newborns with HIE a well-defined intrapartum event is not always present, and surrogates for perinatal asphyxia in labor and delivery can also be the consequence of preexisting events before delivery (Garca-Alix and Quero, 2001). Also, several comorbid factors can influence the evolution of the lesion in the days following the initial insult.

Second, it is not known how soon NSE can be detected in CSF after a perinatal brain injury, nor the kinetics of release and clearance. Thus, the schedule of CSF sampling has been chosen arbitrarily, and the optimal time needs to be defined in accordance with the maximum predictive accuracy. Sampling within the first 72 hours of age has been used in most studies, but this time frame is wide and limits comparability of results. The CSF-NSE does not help to identify those infants who qualify for TH, since this therapy should be started within the first 6 hours of life. However, it can help to identify newborns who will benefit most from the implementation of new neuroprotective and neuroreparative interventions during the first days after the hypoxic-ischemic event.

Although NSE is measurable with inexpensive, commercially available kits with good reproducibility worldwide, and results are available within a few hours, the results obtained with different enzymatic methods and immunoassays cannot be compared directly. Differences may occur between suppliers.

Furthermore, although CSF is easily accessible by lumbar puncture, this is an interventional procedure, not free of risks and one that should not be performed when the infant is unstable or has significant coagulopathy. In addition, due to ethical restrictions, CSF presents limitations in the obtaining of serial samples unlike salive or urine, or even blood.

Future directions and summary: At present, there is growing evidence suggesting that determining NSE in CSF might offer an objective and quantitative assessment of the severity of brain damage after a perinatal or postnatal hypoxic-ischemic injury. CSF-NSE would be particularly important in those infants in whom neuroimaging cannot be obtained, or in those for whom discordant information between different evaluation tools arises.

Large-scale clinical studies are needed for robust validation of the utility of NSE in CSF in infants with HIE. Furthermore, we need to assess whether the simultaneous determination of NSE and other BSPs increases the precision of the neurodevelopmental prognosis. The development of improved techniques for rapid quantitative measurement at the same time of several biomarkers of hypoxic-ischemic brain injury would be a breakthrough in providing fast and accurate assessment of the severity of brain injury after a hypoxic-ischemic perinatal or postnatal event.

Alfredo Garcia-Alix*, Juan Arnaez
Neonatal Neurology, NeNe Foundation, Spain (Garcia-Alix A)
Neonatal Neurology, NeNe Foundation, Spain; Neonatology, Complejo Universitario de Burgos, Burgos, Spain (Arnaez J)
***Correspondence to:** Alfredo Garcia-Alix, MD, PhD, alfredoalix@gmail.com.
https://orcid.org/0000-0002-7972-8453 (Alfredo Garcia-Alix);
https://orcid.org/0000-0001-8883-5181 (Juan Arnaez)
Date of submission: November 5, 2020
Date of decision: January 13, 2021
Date of acceptance: April 14, 2021
Date of web publication: July 8, 2021

https://doi.org/10.4103/1673-5374.317972
How to cite this article: *Garcia-Alix A, Arnaez J (2022) Neuron-specific enolase in cerebrospinal fluid as a biomarker of brain damage in infants with hypoxic-ischemic encephalopathy. Neural Regen Res 17(2):318-319.*

References

Arca G, Arnaez J, Agut T, Nunez C, Stephan-Otto C, Valls A, Garcia-Alix A (2020) Neuron-specific enolase is correlated with lesion topology, relative infarct volume and outcome of symptomatic NAIS. Arch Dis Child Fetal Neonatal Ed 105:132-137.

Echeverria-Palacio CM, Agut T, Arnaez J, Valls A, Reyne M, Garcia-Alix A (2019) Neuron-specific enolase in cerebrospinal fluid predicts brain injury after sudden unexpected postnatal collapse. Pediatr Neurol 101:71-77.

Garca-Alix A, Quero J (2001) Brain-specific proteins as predictors of outcome in asphyxiated term infants. Acta Paediatr 90:1103-1105.

Garcia-Alix A, Cabanas F, Pellicer A, Hernanz A, Stiris TA, Quero J (1994) Neuron-specific enolase and myelin basic protein: relationship of cerebrospinal fluid concentrations to the neurologic condition of asphyxiated full-term infants. Pediatrics 93:234-240.

Leon-Lozano MZ, Arnaez J, Valls A, Arca G, Agut T, Alarcon A, Garcia-Alix A (2020) Cerebrospinal fluid levels of neuron-specific enolase predict the severity of brain damage in newborns with neonatal hypoxic-ischemic encephalopathy treated with hypothermia. PLoS One 15:e0234082.

Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J (2009) Systematic review of biomarkers of brain injury in term neonatal encephalopathy. Pediatr Neurol 40:215-226.

Ramont L, Thoannes H, Volondat A, Chastang F, Millet MC, Maquart FX (2005) Effects of hemolysis and storage condition on neuron-specific enolase (NSE) in cerebrospinal fluid and serum: implications in clinical practice. Clin Chem Lab Med 43:1215-1217.

Shellhaas RA, Kushwaha JS, Plegue MA, Selewski DT, Barks JD (2015) An evaluation of cerebral and systemic predictors of 18-month outcomes for neonates with hypoxic ischemic encephalopathy. J Child Neurol 30:1526-1531.

Sun J, Li J, Cheng G, Sha B, Zhou W (2012) Effects of hypothermia on NSE and S-100 protein levels in CSF in neonates following hypoxic/ischaemic brain damage. Acta Paediatr 101:e316-320.

Tekgul H, Yalaz M, Kutukculer N, Ozbek S, Kose T, Akisu M, Kultursay N, Gokben S (2004) Value of biochemical markers for outcome in term infants with asphyxia. Pediatr Neurol 31:326-332.

C-Editors: Zhao M, Qiu Y; T-Editor: Jia Y