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Original Article

Neuron-Specific Enolase in Cerebrospinal Fluid Predicts Brain Injury After Sudden Unexpected Postnatal Collapse

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ABSTRACT

Background: Biomarkers of brain injury with high predictive value in newborns in critical neurological status are increasingly required. Neuron-specific enolase in cerebrospinal fluid has been shown to be highly predictive in newborns with perinatal hypoxic-ischemic encephalopathy, but its utility has not been examined in sudden unexpected postnatal collapse.

Purpose: We analyzed whether the levels of neuron-specific enolase in cerebrospinal fluid can be a useful biomarker to estimate the severity of brain injury in neonates after a sudden unexpected postnatal collapse.

Methods: This is a prospective observational study of near-term infants who were consecutively admitted with sudden unexpected postnatal collapse in two neonatal intensive care units during a nine-year period. Variables were collected and analyzed regarding the perinatal period, clinical course, severity of encephalopathy, amplitude-integrated encephalography, magnetic resonance imaging findings, and outcome. Neuron-specific enolase in cerebrospinal fluid samples were obtained in 18 infants with sudden unexpected postnatal collapse between 12 and 72 hours after the collapse and compared with those of 29 controls.

Results: The levels of neuron-specific enolase in cerebrospinal fluid were higher in patients than in controls ($P < 0.001$). Levels of neuron-specific enolase in cerebrospinal fluid in infants with sudden unexpected postnatal collapse were significantly higher in patients who presented severe encephalopathy, seizures, abnormal amplitude-integrated encephalography background, or brain injury on magnetic resonance imaging. Receiver operator characteristic curve analysis revealed a neuron-specific enolase in cerebrospinal fluid cutoff value of maximum predictive accuracy of 61 ng/mL (area under the curve, 1.0; sensitivity, specificity, positive predictive value, and negative predictive value, 100%) for identifying infants who died or had adverse outcomes.

Conclusions: Levels of neuron-specific enolase in cerebrospinal fluid obtained between 12 and 72 hours after a sudden unexpected postnatal collapse event seem to be a useful biomarker for identifying newborns with severe brain injury and for predicting outcome.

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Introduction

Sudden unexpected postnatal collapse (SUPC) in healthy newborn infants is a rare but devastating event that carries a high risk of mortality and neurodevelopmental disability in survivors. The estimated incidence of SUPC differs widely, probably because of definition and inclusion and exclusion criteria, ranging from 2.6 to 133 per 100,000 live births.[1,2] SUPC has also been described as a severe acute life-threatening event or sudden unexplained early neonatal death. The definition of SUPC varies depending mainly on the time lapse between birth and the event occurrence. In general, it refers to a healthy term or near-term newborn, who is well at birth and in the first hours of life but suddenly collapses and requires resuscitation maneuvers.[1,3,4] SUPC has frequently been associated with primiparous mothers in unsupervised skin-to-skin contact or breastfeeding.[5,6]

Early assessment of brain injury after SUPC is challenging, and uncertainty regarding the eventual neurological outcome remains during the first hours after SUPC. In perinatal hypoxic-ischemic encephalopathy (HIE), a similar clinical setting, tools other than the amplitude-integrated electroencephalogram (aEEG),[7] such as clinical evaluation and neuroimaging studies, have a limited prognostic value in the earliest hours after injury.[8,9]

Brain damage induces the release of brain-specific proteins into the biological fluids.[10] Neuron-specific enolase (NSE) is a glycolytic enzyme concentrated in the cytoplasm of neurons and released in the setting of cell death. NSE concentrations may accurately reflect the degree of brain injury after perinatal hypoxic-ischemic injury, based on the hypothesis that during the insult this protein is released from the damaged neuron cells into the cerebrospinal fluid (CSF). CSF-NSE has been reported in neonates to be useful in identifying HIE and in correlation with outcome.[11-14] This promising biomarker has not yet been studied in newborns with SUPC.

We questioned whether CSF-NSE levels in newborns after a SUPC correlated with the severity of brain injury based on clinical assessment, aEEG, magnetic resonance imaging (MRI) findings, and neurological outcome.

Patients and methods

This is a prospective observational study of term infants who were consecutively admitted after a SUPC event to neonatal intensive care units (NICUs) in two university hospitals in Spain, from September 2009 to September 2018. Infants were included if they were born healthy but acutely required advance resuscitation (endotracheal intubation, chest compressions, or drugs) within the first 24 hours of life. Infants were excluded if they had a known or suspected genetic disease or major congenital malformations. Demographic data and prenatal and labor events were prospectively collected from medical records.

Like perinatal asphyxia, neonatal encephalopathy is considered when the infant presents a clinical syndrome of disturbed neurological function after SUPC. The severity of the encephalopathy was assessed at admission to the NICU and classified within six hours after the collapse in both centers using the same scheme—as mild, moderate, or severe—according to a previously described semi-quantitative score that included the aEEG findings.[15,16] All patients with moderate and severe HIE within six hours after SUPC received therapeutic hypothermia for 72 hours with a targeted central temperature of 33°C to 34°C through a servo-controlled whole-body cooling system. Continuous aEEG recordings for 72 hours were commenced immediately after NICU admission, and information, including worst background activity pattern and the presence of seizure, was collected. The following aEEG background patterns were considered abnormal: low voltage, burst suppression, and inactive or flat.[17] Clinical or electrical seizures on aEEG were also noted.

CSF-NSE samples were obtained by lumbar puncture performed as soon as the patients' clinical condition allowed, between 12 and 72 hours after the SUPC event. Aliquots of 0.4 mL were distributed in plastic tubes, which were immediately frozen and stored at −80°C until analyzed, to avoid leakage of erythrocyte NSE or leukocyte lysosomal proteases. All CSF samples were analyzed in the same laboratory, and aliquots grossly contaminated with blood or hemolyzed were discarded. NSE concentrations were determined by automated immunofluorescent assay (Thermo Scientific NSE Kryptor, BRAHMS GmbH, Germany). High, medium, and low controls provided by the manufacturer were assayed with each kit for quality assurance. The coefficient of variation for inter- and intra-assay variability was less than 10% for both measurements. The sensitivity of this method is 1 ng/mL. To compare CSF-NSE levels between patients who had an SUPC event and the control group, we included 29 newborns in whom a lumbar puncture was performed to rule out sepsis or meningitis and who did not present neurological symptoms nor confirmed meningitis.

Brain MRI was performed in the first two weeks after SUPC (median 5.5 days; interquartile range [IQR] 3, 9) using a 1.5-T unit (General Electric) with a specific neonatal head coil. The imaging protocol has been published in another site.[18] Minimally, axial and sagittal T1-weighted, axial and coronal T2-weighted, and axial diffusion-weighted images were available for all patients. Two researchers (T.A., A.G.-A.) masked to clinical data and CSF-NSE levels reviewed MRIs. Images were scored according to the schema previously reported by Rutherford et al.[19,20] This score graduates the severity of damage in four brain regions: the posterior limb of internal capsule (PLIC), basal ganglia and thalami (BGT), white matter, and cortex. According to the findings, each site is scored as normal, mild, moderate, or severely affected. Discrepancies in the scoring of the images were discussed and resolved by consensus.

All infants who survived were followed up at regular intervals by trained examiners who were unaware if the CSF-NSE concentrations. Follow-up evaluation included a structured neurological examination and serial head circumference measurement.[21] Microcephaly was considered when head circumference was more than two standard deviations below the mean for gender and age.[22] Cerebral palsy was defined and classified according to the Surveillance for Cerebral Palsy in Europe.[23] Developmental delay was considered if the child did not reach the developmental milestones as expected for his or her age. For data analysis, patients who, based on the last neurological examination, exhibited developmental delay, microcephaly, cerebral palsy, or epilepsy were defined as a group with adverse outcome.

Statistical analysis

Data analysis was carried out with the *Statistical Package for the Social Sciences* (SPSS), twenty-third version. Qualitative variables were summarized with absolute and relative frequencies, and with median and IQR for quantitative variables.

Mann-Whitney U test was used to analyze the differences in CSF-NSE levels between groups. This test also was used to estimate the correlation between CSF-NSE levels and clinical variables, biomarkers, and outcomes. Spearman coefficient (Rho) was calculated to estimate the correlation between quantitative variables. For significant difference, P value < 0.05 was established. Finally, to establish a cutoff, receiver operating characteristic curve analysis for CSF-NSE levels was performed, taking the area under the curve, sensitivity, specificity, positive predictive value, and negative predictive value.

Ethical considerations

Parents of eligible participants provided verbal agreement, and all of them signed a written informed consent. The institutional ethics review boards of both hospitals approved the study.

Results

During the period of study, 26 newborns had an SUPC event, but CSF-NSE was determined in 18 infants, which comprises the cohort of the study analysis. In three patients the CSF sampling was grossly hemorrhagic and therefore not analyzed, whereas in five, lumbar puncture could not be performed because of clinical instability. The control group (n = 29) showed no differences in gestational age, birth weight, or gender distribution compared with the SUPC group. Lumbar puncture was performed earlier in controls, at a median age of 26 hours (19, 52) compared with 48 hours (39.5, 65.5) in the SUPC cohort ($P = 0.032$) (Table 1).

Most infants (71%) were born from primiparous mothers, and 78% were transferred from other hospitals. Most infants were male (78%), and all the babies stayed with their parents with skin-to-skin care after birth (Tables 1 and 2).

Fourteen infants had moderate or severe HIE following the sudden unexpected postnatal event, and all were cooled. Of these, 93% had clinical or electrical seizures, and the same percentage had abnormal background aEEG patterns. MRI was performed in 13 of 14 infants with moderate-severe HIE, and abnormal findings were found in 79%, most of them with BGT and PLIC patterns of injury (Table 2).

All infants were followed up at a median age of 32 months (IQR 17, 39). Eight infants (44%) had an adverse outcome: five (28%) died during the neonatal period, all of them due to an end-of-life decision, and three (17%) showed significant neurological morbidity during follow-up. Two of the three infants had cerebral palsy with spastic tetraparesis, language and behavioral disorders, and need for gastrostomy feeding; one of them also had epilepsy and impaired vision and hearing. The third infant had language and behavioral disorders without motor impairment. Severity of HIE, abnormal background aEEG pattern, and significant MRI injury were associated with death or adverse outcome ($P < 0.05$) (Table 3). All six infants with moderate or severe BGT died or had abnormal outcome.

CSF-NSE levels were higher in the SUPC group compared with the control group ($P < 0.001$), and newborn infants with severe encephalopathy had significantly higher levels of CSF-NSE than those who had mild or moderate encephalopathy ($P = 0.010$) (Fig 1). Higher CSF-NSE levels were found in patients who had clinical or electrical seizures, abnormal background aEEG, or moderate to severe lesion of PLIC or BGT ($P < 0.05$) (Table 4 and Fig 2). The

TABLE 1.
Maternal and Perinatal Characteristics of the 18 Neonates With SUPC

	SUPC Cohort N = 18	Controls N = 29	*P* Value
Obstetric and maternal characteristics			
Familial SUP antecedents	0/18	0/29	-
Consanguinity	0/18	1/29 (3)	-
Caucasian origin	10/18 (56)	24/29 (83)	0.054
Maternal age	32 (28;34)	32 (29;35)	0.464
Diabetes or hypertension during pregnancy	2/18 (11)	2/29 (7)	0.631
Oligoamnios	1/18 (6)	0/29	-
Multiple pregnancy	0/18	2/29 (7)	-
Intrauterine growth restriction	2/18 (11)	0/29	-
Primiparity	12/17 (71)	12/29 (41)	0.056
Cephalic presentation	17/18 (94)	21/29 (72)	0.124
Eutocic delivery	12/18 (67)	19/21 (90)	0.936
Infant characteristics at birth			
Gestational age, weeks	40 (39; 41)	39 (38;40)	0.331
Male	14 (78)	19 (68)	0.372
Birth weight, g	3045 (2779;3275)	3245 (3038;3628)	0.069
Head circumference, cm	33.5 (33;34.5)*	34 (33;35)	0.263
Apgar 1 minute	9 (9; 9)	8 (8;9)	1.000
Apgar 5 minutes	10 (10;10)	10 (8;10)	0.020
Need for resuscitation	0/18	9/29 (31)	-

Abbreviations:
SUP = Sudden unexpected postnatal
SUPC = Sudden unexpected postnatal collapse
Quantitative variables are expressed as median(P_{25};P_{75}). Categorical values are expressed as n(%).
* Data were available for 13 of 18 infants.

TABLE 2.
SUPC Context and Neurological Findings of 18 Infants With SUPC

Aspects Related to SUPC	
Minutes of life to SUPC	60 (55;90)
SUPC context	
Bed-sharing	8/18 (44)
Breastfeeding	2/18 (11)
Skin-to-skin care (excluding feeding and bed-sharing)	5/18 (28)
Unspecified	3/18 (17)
First gasometry after SUPC event*	
pH	7.06 (6.80;7.19)
Bicarbonate, mEq/L	9.2 (5.5;16.5)
Base deficit, mEq/L	18.5 (14.5;24.5)
Encephalopathy	
No	1/18 (6)
Mild	3/18 (17)
Moderate	6/18 (33)
Severe	8/18 (44)
Active hypothermia protocol†	14/14 (100)
aEEG abnormal background	13/18 (72)
Seizures	14/18 (78)
Only clinical	1/14 (7)
Only electrical	6/14 (43)
Clinical-electrographically confirmed	7/14 (50)
MRI findings‡	
Rutherford score	2 (1;7)
Any injury on MRI	11/14 (79)
Any moderate to severe injury	8/14 (57)
PLIC moderate to severe injury	5/14 (36)
BGT moderate to severe injury	6/14 (43)
WM moderate to severe injury	5/14 (36)
Cortex moderate to severe injury	1/14 (7)
Outcome	
Death or adverse outcome	8/18 (44)
Death	5/18 (28)
Adverse outcome	3/18 (17)

Abbreviations:
aEEG = Amplitude-integrated electroencephalogram
BGT = Basal ganglia and thalami
HIE = Hypoxic-ischemic encephalopathy
MRI = Magnetic resonance imaging
PLIC = Posterior limb of internal capsule
SUPC = Sudden unexpected postnatal collapse
WM = White matter
Quantitative variables are expressed as median (P_{25};P_{75}). Categorical values are expressed as n/data available (%).
* pH values were available in 17 of 18 cases. In 15 of 17 cases, timing of first blood gas after SUPC was performed within one hour; in two patients timing of first gas was not available. First blood gas was extracted capillary in most patients.
† Active hypothermia was offered in all (and only) infants with moderate-severe HIE.
‡ MRI was performed in 14 of 18 infants: one patient with mild HIE and13 of 14 with moderate-severe HIE (one patient died before MRI could be carried out).

TABLE 3.
Findings of 18 Infants With SUPC According to Their Outcome

Variables	Death or Adverse Outcome (n = 8)	Nonadverse Outcome (n = 10)	*P* Value
pH	6.80 (6.80;7.08)	7.12 (6.89;7.21)	0.277
Bicarbonate, mEq/L*	7.75 (5.0;10.5)	11 (7.4;19.5)	0.121
Base deficit, mEq/L*	21.5 (24.0;18.8)	16.2 (20.2;7.9)	0.074
Severe encephalopathy	7/8 (88)	1/10 (10)	0.003
Clinical or electrical seizures	8/8 (100)	6/10 (60)	0.092
aEEG abnormal background	8/8 (100)	5/10 (50)	0.036
Moderate to severe lesion of PLIC or BGT	6/7 (88)	0/7 (0)	0.005

Abbreviations:
aEEG = Amplitude integrated electroencephalogram
BGT = Basal ganglia and thalami injury
PLIC = Posterior limb of internal capsule
SUPC = Sudden unexpected postnatal collapse
Continuous variables are expressed as median (P_{25};P_{75}), and categorical variables are expressed in n/data available (%).
* Data were available for 17 of 18 infants.

concentrations of CSF-NSE showed a positive correlation with the Rutherford score (Rho 0.732, $P = 0.003$).

Infants with death or adverse outcome showed higher CSF-NSE levels than those with normal outcome: 124.4 ng/mL (85, 244.8) versus 25.4 ng/mL (16.9, 31.1) ($P = 0.001$) (Fig 3). Receiver operator characteristic curve analysis revealed a CSF-NSE cutoff value of maximum predictive accuracy of 61 ng/mL (area under the curve, 1.0; sensitivity, 100%; specificity, 100%; positive predictive value, 100%; negative predictive value, 100%). All eight patients with death or severe outcome had CSF-NSE levels of 61 ng/mL or above (Supplementary Table 1).

Discussion

This study suggests that CSF-NSE concentrations are a valid surrogate for brain injury after SUPC. CSF-NSE concentrations are associated with clinical severity of acute encephalopathy, other markers of brain injury such as aEEG and MRI, and long-term outcome.

Early reliable markers of brain damage could help in the clinical setting by providing early and accurate identification of infants at risk of neurological sequelae after a hypoxic-ischemic event. These markers can be particularly useful owing to the inherent limitations of the currently available tools for assessing brain injury during the first 48 to 72 hours after injury. During this temporal window, clinical examination is limited by the use of complex life support devices and often confounded by sedatives and antiepileptic drugs. Furthermore, it seems that clinical assessment is less reliable early in the setting of hypothermia, the standard treatment used after hypoxic-ischemic insult.[9,24] aEEG background patterns correlate closely with neurological outcome in newborn infants with encephalopathy.[25] However, the predictive value of aEEG depends on how long the background patterns are severely altered in relation to the injury. In noncooled neonates with HIE, the predictive value for adverse outcome peaks at 24 hours of life, but in cooled infants the higher predictive values are delayed until 48 to 72 hours of life.[7] Qualitative evaluation of both conventional and diffusion-weighted MRI during the first 48 hours may underestimate hypoxic-ischemic lesions in the presence of significant brain injury.[9,26,27] Owing to these limitations, other quantitative techniques such as proton magnetic resonance spectroscopy and quantitative diffusion parameters have been developed.[28] However, in clinical practice, early imaging of sick newborns after hypoxic-ischemic injury is technically challenging. Such imaging usually requires transfer to radiology rooms, the use of specialized equipment, and interpretive expertise, and there is a risk of clinical instability during transport and study.

Despite the limitations inherent in the accurate assessment of brain injury during the first 48 to 72 hours after the injury, this challenge has to be addressed to provide prognostic information to families and to select patients for therapeutic interventions. In addition, it is crucial to identify accurately in this time frame those

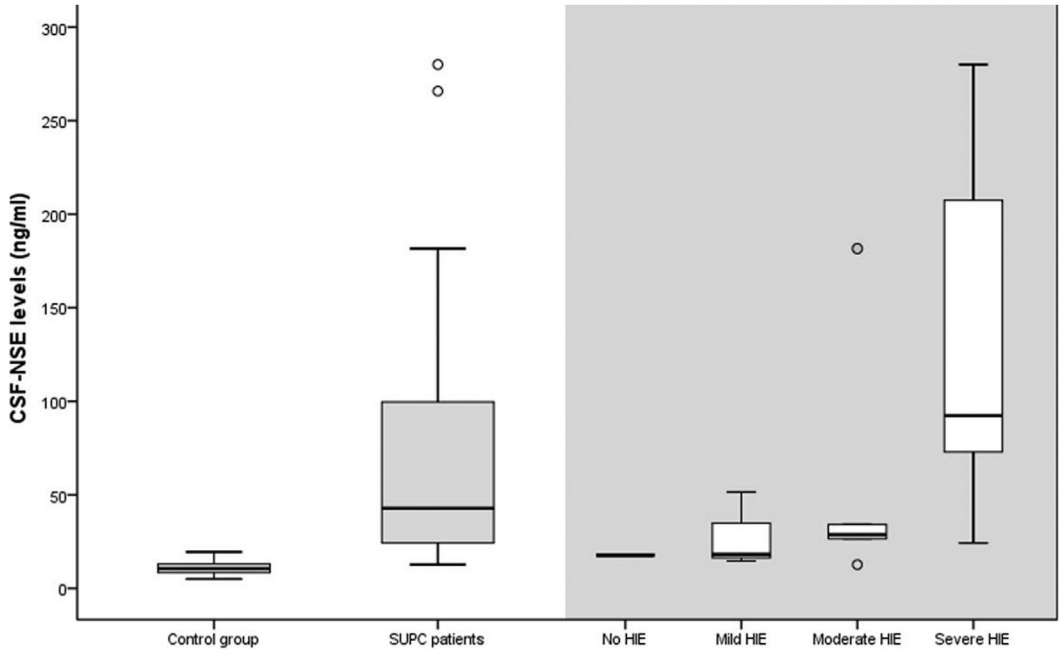

FIGURE 1. CSF-NSE levels in 29 controls and 18 infants with SUPC, according to the severity of the encephalopathy; CSF-NSE values are expressed in median (P_{25};P_{75}). CSF-NSE values were 10.6 ng/mL (8.1;13.5) and 42.9 ng/mL (22.8;157.2) in 29 controls and 18 patients with SUPC, respectively. Among patients with SUPC CSF-NSF values for no-HIE (one), mild HIE (three), moderate HIE (six), and severe HIE (eight) were 17.7 ng/mL, 18.2 ng/mL (14.6;-), 28.7 ng/mL (23.1;71.1), and 92.9 ng/mL (67;236.6), respectively. CSF-NSE, neuron-specific enolase in cerebrospinal fluid; HIE, hypoxic-ischemic encephalopathy; SUPC, sudden unexpected postnatal collapse.

TABLE 4.
CSF-NSE Values of 18 Infants With SUPC According to Their Neurological Findings and Outcome

Variables	N	Yes	N	Not	*P* Value
Severe encephalopathy	8	92.4 (67;236.6)	10	26.9 (16.9;38.5)	0.010
Seizures	14	73 (29.3;157.2)	4	16.2 (13.2;18.1)	0.003
aEEG abnormal background	13	85 (28.7;165.3)	5	18.2 (16.2;39.0)	0.026
Moderate to severe lesion of PLIC or BGT	6	133.3 (79;269.4)	8	28.7 (24.9;47.2)	0.017
Death or adverse outcome	8	124.4 (85;244.8)	10	25.4 (16.9;31.1)	0.001

Abbreviations:
aEEG = Amplitude integrated electroencephalogram
BGT = Basal ganglia and/or thalami injury
CSF-NSE = Neuron-specific enolase in cerebrospinal fluid
n = No. of patients
PLIC = Posterior limb of internal capsule
SUPC = Sudden unexpected postnatal collapse
CSF-NSE values (ng/mL) are expressed as median (P_{25};P_{75})

infants who will have a severe disability in case of survival. If end-of-life decisions are delayed, there is the possibility that the infant will survive but with severe impairment.[29]

Several central nervous system-specific molecules, particularly brain proteins, have been investigated in serum and in the CSF as possible quantitative indexes of perinatal brain injury.[30-33] NSE is a well-known biomarker of neuronal injury. NSE is a dimeric glycolytic enzyme that originates predominantly from the cytoplasm of neurons and neuroendocrine cells.[11-13,34] This enzyme is soluble and stable in biological fluids, and its determination is not affected by hyperbilirubinemia or lipemia.[35] Increased serum NSE after hypoxic-ischemic injury, however, is not necessarily of central nervous system origin: a hypoxic-ischemic event may result in multiple organ lesions with release of NSE from extraneural sources, such as endocrine glands, lymphocytes, red blood cells, platelets, and peripheral neurons.[35-37]

Several studies have examined the relationship between serum NSE concentrations and outcome of neonates with perinatal HIE. The inconsistency of the results, however, makes it difficult to reach any clear conclusions.[38-40] In contrast, the value of CSF-NSE to reflect brain injury based on clinical data, MRI, and outcome in infants with perinatal HIE has been more consistently clear.[10,11,41,42] In our study, like what has been reported in perinatal HIE, CSF-NSE levels correlated with the severity of neurological dysfunction in the early hours following the SUPC event.[11,41,43]

Patients with SUPC with severe encephalopathy had the highest CSF-NSE levels, similar to findings in other reports in perinatal HIE.[11,41]

Other well-known markers of ongoing brain damage and surrogate outcome measures such us persistent abnormal aEEG background and injury on MRI were related to higher CSF-NSE levels.[18,41,44] As far as we know, only two previous studies have analyzed the relation between CSF-NSE and aEEG patterns in perinatal HIE population. Thoremberg et al. found that infants with severe alteration of the aEEG background in the second day of life had higher CSF-NSE levels and poorer outcome.[41] Ezgu et al. reported an association between CSF-NSE and abnormal patterns in conventional EEG during the first 72 hours.[43]

Our results are consistent with previous studies using MRI that relate CSF-NSE to structural injury in infants with perinatal HIE. The predominant injury pattern in SUPC newborns was BGT and PLIC damage, which have also been described and related to adverse outcome in other SUPC case series.[45] This pattern of injury affecting the central grey matter has been noted after acute disruption of maternal-fetal gas exchange.[46,47] BGT also are more susceptible to severe hypotension, and their injury is related to a poor neurological outcome.[28,48,49]

Therapeutic hypothermia has been reported to reduce CSF-NSE levels,[12] but our study did not allow us to raise this question, because all patients with moderate or severe HIE with CSF-NSE levels were treated with hypothermia.

Our study has several limitations. Given the low incidence and prevalence of SUPC, even though our study included two centers during an eight-year period, it only managed to recruit 21 patients. Moreover, as described in other studies with neurobiological markers, the inability to obtain or analyze some CSF samples further reduced the number for analysis.

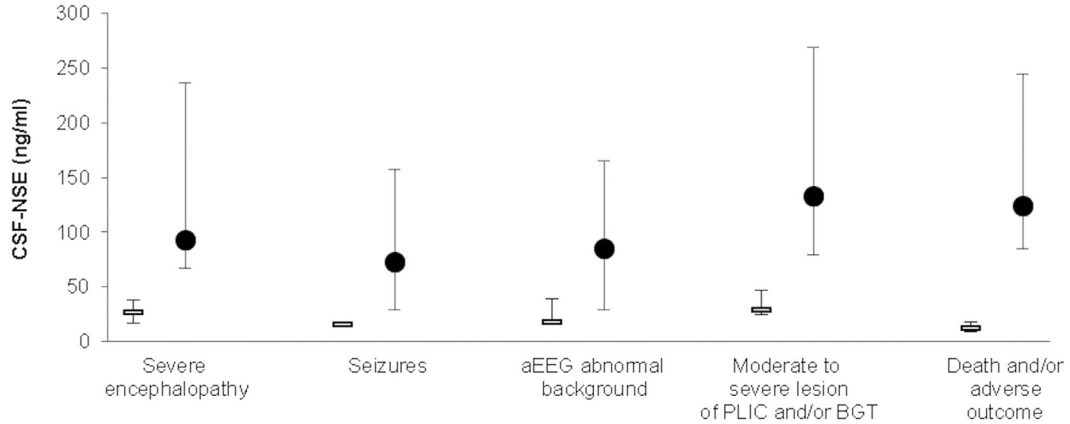

FIGURE 2. CSF-NSE values of 18 infants with SUPC according to their neurological findings and outcome; CSF-NSE (ng/mL) values are represented with median (P_{25};P_{75}). CSF-NSE values for infants with abnormal neurological findings are shown with a black circle, whereas those for infants with normal findings are shown with a square point. CSF-NSE, neuron-specific enolase in cerebrospinal fluid.

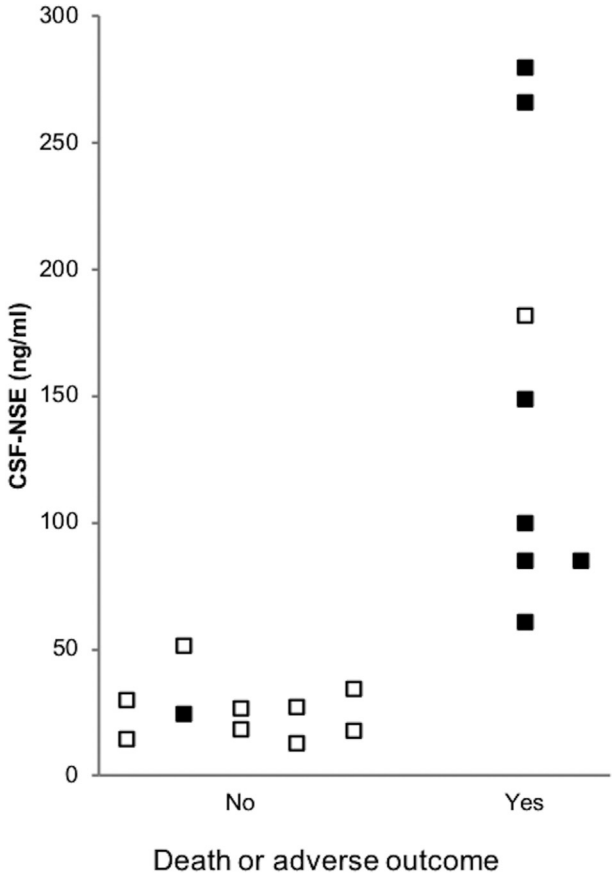

FIGURE 3. CSF-NSE values of 18 infants with SUPC according to their outcome and the severity of HIE; black squares represent infants with severe HIE (eight of 18), whereas square white points represent the rest of 10 infants without severe HIE. CSF-NSE, neuron-specific enolase in cerebrospinal fluid; HIE, hypoxic-ischemic encephalopathy.

Biochemical markers of brain injury, such as NSE, are released in a time-related sequence with brain damage. How soon NSE can be detected in CSF after a brain injury or the kinetics of its release and clearance are not known.[35] The scheduling of CSF sampling is always made arbitrarily, but in most studies sampling within the first 12 to 72 hours of life has been used.[11,41,43,50] Whether serial measurements might provide indirect information regarding ongoing neuronal injury after an SUPC event and might therefore help increase accuracy in predicting patient outcome is unknown. However, lumbar puncture is an interventional procedure, is uncomfortable, and is not free from inconvenience and risks, which makes it difficult to obtain serial samples. Nevertheless, in our study, in which lumbar puncture was performed when possible between 12 and 72 hours after the event, NSE concentrations in CSF were associated with the severity of injury after SUPC, as indicated by the degree of encephalopathy, clinical seizures, and presence of abnormal aEEG background and central grey matter injury in MRI.

In clinical practice, CSF-NSE levels may be particularly useful when additional information is needed about brain injury and prognosis, particularly if there are inconsistencies between prognostic tools such as neurological examination and neurophysiological studies, and if an end-of-life decision is considered during the first 72 hours. CSF-NSE levels $\geq$61 ng/mL show high specificity and positive predictive value for identifying infants at increased risk for adverse outcome. These high values of diagnostic efficacy compared with previously published data regarding perinatal HIE[10,11,42,43] may reflect the fact that the moment of the insult is precisely defined in the SUPC, whereas in the perinatal HIE it is not always clearly defined. In this latter condition, the insult may begin many hours before birth and injury may have been evolving at the time of birth with a different timing of clearance of CSF-NSE. Moreover, infants with perinatal HIE are highly heterogeneous not only at the time of the insult but also in the type and depth of the injury.

Conclusion

SUPC is a rare but potentially catastrophic entity associated with high morbidity and mortality. Our study suggests that NSE in CSF between 12 and 72 hours after SUPC can be a useful tool to identify those newborns under suspicion of severe brain injury based on clinical assessment, aEEG, and MRI. Furthermore, it appears to be able to distinguish infants who will fully recover from those who will present an adverse outcome.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pediatrneurol.2019.02.020.

References

1. Herlenius E, Kuhn P. Sudden unexpected postnatal collapse of newborn infants: a review of cases, definitions, risks, and preventive measures. Transl Stroke Res. 2013;4:236–247.
2. Lutz TL, Elliott EJ, Jeffery HE. Sudden unexplained early neonatal death or collapse: a national surveillance study. Pediatr Res. 2016;80:493–498.
3. Pejovic NJ, Herlenius E. Unexpected collapse of healthy newborn infants: risk factors, supervision and hypothermia treatment. Acta Paediatr. 2013;102:680–688.
4. Becher JC, Bhushan SS, Lyon AJ. Unexpected collapse in apparently healthy newborns–a prospective national study of a missing cohort of neonatal deaths and near-death events. Arch Dis Child Fetal Neonatal Ed. 2012;97:F30–F34.
5. Andres V, Garcia P, Rimet Y, Nicaise C, Simeoni U. Apparent life-threatening events in presumably healthy newborns during early skin-to-skin contact. Pediatrics. 2011;127:e1073–e1076.
6. Poets A, Steinfeldt R, Poets CF. Sudden deaths and severe apparent life-threatening events in term infants within 24 hours of birth. Pediatrics. 2011;127:e869–e873.
7. Del Rio R, Ochoa C, Alarcon A, Arnaez J, Blanco D, Garcia-Alix A. Amplitude integrated electroencephalogram as a prognostic tool in neonates with hypoxic-ischemic encephalopathy: a systematic review. PLoS One. 2016;11:e0165744.
8. McKinstry RC, Miller JH, Snyder AZ, et al. A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. Neurology. 2002;59:824–833.
9. Gunn AJ, Wyatt JS, Whitelaw A, et al. Therapeutic hypothermia changes the prognostic value of clinical evaluation of neonatal encephalopathy. J Pediatr. 2008;152:55–58, 58.e1.
10. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. Pediatr Neurol. 2009;40:215–226.
11. Garcia-Alix A, Cabanas F, Pellicer A, Hernanz A, Stiris TA, Quero J. Neuron-specific enolase and myelin basic protein: relationship of cerebrospinal fluid concentrations to the neurologic condition of asphyxiated full-term infants. Pediatrics. 1994;93:234–240.
12. Sun J, Li J, Cheng G, Sha B, Zhou W. Effects of hypothermia on NSE and S-100 protein levels in CSF in neonates following hypoxic/ischaemic brain damage. Acta Paediatr. 2012;101:e316–e320.
13. Merchant N, Azzopardi D. Early predictors of outcome in infants treated with hypothermia for hypoxic-ischaemic encephalopathy. Dev Med Child Neurol. 2015;57 Suppl 3:8–16.
14. Garca-Alix A, Quero J. Brain-specific proteins as predictors of outcome in asphyxiated term infants. Acta Paediatr. 2001;90:1103–1105.
15. Alsina M, Martin-Ancel A, Alarcon-Allen A, Arca G, Gaya F, Garcia-Alix A. The severity of hypoxic-ischemic encephalopathy correlates with multiple organ dysfunction in the hypothermia era. Pediatr Crit Care Med. 2017;18:234–240.
16. Carreras N, Alsina M, Alarcon A, Arca-Diaz G, Agut T, Garcia-Alix A. Efficacy of passive hypothermia and adverse events during transport of asphyxiated newborns according to the severity of hypoxic-ischemic encephalopathy. J Pediatr (Rio J). 2018;94:251–257.
17. Hellström-Westas L, Rosén I, de Vries LS, Greisen G. Amplitude-integrated EEG classification and interpretation in preterm and term infants. NeoReviews. 2006;7:e76–e87.

18. Agut T, Leon M, Rebollo M, Muchart J, Arca G, Garcia-Alix A. Early identification of brain injury in infants with hypoxic ischemic encephalopathy at high risk for severe impairments: accuracy of MRI performed in the first days of life. BMC Pediatr. 2014;14:177.
19. Martinez-Biarge M, Diez-Sebastian J, Kapellou O, et al. Predicting motor outcome and death in term hypoxic-ischemic encephalopathy. Neurology. 2011;76:2055–2061.
20. Rutherford MA. The asphyxiated term infant. In: Rutherford MA, ed. MRI of the Neonatal Brain. UK: Saunders; 2002.
21. Amiel-Tison C, Ellison P. Birth asphyxia in the fullterm newborn: early assessment and outcome. Dev Med child Neurol. 1986;28:671–682.
22. Fernández C, Lorenzo H, Vrotsou K, Aresti U, Rica I, Sánchez E. Estudio de Crecimiento de Bilbao. Curvas y tablas de crecimiento. Estudio Transversal. Bilbao, España: Fundación Faustino Orbegozo; 2011.
23. Surveillance of Cerebral Palsy in Europe. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. Surveillance of Cerebral Palsy in Europe (SCPE). Dev Med child Neurol. 2000;42:816–824.
24. Shankaran S, Laptook AR, Tyson JE, et al. Evolution of encephalopathy during whole body hypothermia for neonatal hypoxic-ischemic encephalopathy. J Pediatr. 2012;160:567–572.e3.
25. Weeke LC, Boylan GB, Pressler RM, et al. Role of EEG background activity, seizure burden and MRI in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischaemic encephalopathy in the era of therapeutic hypothermia. Eur J Paediatr Neurol. 2016;20:855–864.
26. Boudes E, Tan X, Saint-Martin C, Shevell M, Wintermark P. MRI obtained during versus after hypothermia in asphyxiated newborns. Arch Dis Child Fetal Neonatal Ed. 2015;100:F238–F242.
27. Bonifacio SL, deVries LS, Groenendaal F. Impact of hypothermia on predictors of poor outcome: how do we decide to redirect care? Semin Fetal Neonatal Med. 2015;20:122–127.
28. Charon V, Proisy M, Bretaudeau G, et al. Early MRI in neonatal hypoxic-ischaemic encephalopathy treated with hypothermia: Prognostic role at 2-year follow-up. Eur J Radiol. 2016;85:1366–1374.
29. Wilkinson D. The window of opportunity for treatment withdrawal. Arch Pediatr Adolesc Med. 2011;165:211–215.
30. Bennet L, Booth L, Gunn AJ. Potential biomarkers for hypoxic-ischemic encephalopathy. Semin Fetal Neonatal Med. 2010;15:253–260.
31. Lv H, Wang Q, Wu S, et al. Neonatal hypoxic ischemic encephalopathy-related biomarkers in serum and cerebrospinal fluid. Clin Chim Acta. 2015;450:282–297.
32. Douglas-Escobar M, Weiss MD. Hypoxic-ischemic encephalopathy: a review for the clinician. JAMA Pediatr. 2015;169:397–403.
33. Chalak LF, Sanchez PJ, Adams-Huet B, Laptook AR, Heyne RJ, Rosenfeld CR. Biomarkers for severity of neonatal hypoxic-ischemic encephalopathy and outcomes in newborns receiving hypothermia therapy. J Pediatr. 2014;164:468–474.e1.
34. Kelen D, Andorka C, Szabo M, Alafuzoff A, Kaila K, Summanen M. Serum copeptin and neuron specific enolase are markers of neonatal distress and long-term neurodevelopmental outcome. PLoS One. 2017;12:e0184593.
35. Isgro MA, Bottoni P, Scatena R. Neuron-specific enolase as a biomarker: biochemical and clinical aspects. Adv Exp Med Biol. 2015;867:125–143.
36. Polglase GR, Ong T, Hillman NH. Cardiovascular alterations and multiorgan dysfunction after birth asphyxia. Clin Perinatol. 2016;43:469–483.
37. Pelinka LE, Hertz H, Mauritz W, et al. Nonspecific increase of systemic neuron-specific enolase after trauma: clinical and experimental findings. Shock. 2005;24:119–123.
38. Massaro AN, Chang T, Baumgart S, McCarter R, Nelson KB, Glass P. Biomarkers S100B and neuron-specific enolase predict outcome in hypothermia-treated encephalopathic newborns*. Pediatr Crit Care Med. 2014;15:615–622.
39. Roka A, Kelen D, Halasz J, Beko G, Azzopardi D, Szabo M. Serum S100B and neuron-specific enolase levels in normothermic and hypothermic infants after perinatal asphyxia. Acta Paediatr. 2012;101:319–323.
40. Nagdyman N, Komen W, Ko HK, Muller C, Obladen M. Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. Pediatr Res. 2001;49:502–506.
41. Thornberg E, Thiringer K, Hagberg H, Kjellmer I. Neuron specific enolase in asphyxiated newborns: association with encephalopathy and cerebral function monitor trace. Arch Dis Child Fetal Neonatal Ed. 1995;72:F39–F42.
42. Tekgul H, Yalaz M, Kutukculer N, et al. Value of biochemical markers for outcome in term infants with asphyxia. Pediatr Neurol. 2004;31:326–332.
43. Ezgu FS, Atalay Y, Gucuyener K, et al. Neuron-specific enolase levels and neuroimaging in asphyxiated term newborns. J Child Neurol. 2002;17:824–829.
44. van Laerhoven H, de Haan TR, Offringa M, Post B, van der Lee JH. Prognostic tests in term neonates with hypoxic-ischemic encephalopathy: a systematic review. Pediatrics. 2013;131:88–98.
45. Foran A, Cinnante C, Groves A, Azzopardi DV, Rutherford MA, Cowan FM. Patterns of brain injury and outcome in term neonates presenting with post-natal collapse. Arch Dis Child Fetal Neonatal Ed. 2009;94:F168–F177.
46. Okereafor A, Allsop J, Counsell SJ, et al. Patterns of brain injury in neonates exposed to perinatal sentinel events. Pediatrics. 2008;121:906–914.
47. Bano S, Chaudhary V, Garga UC. Neonatal hypoxic-ischemic encephalopathy: a radiological review. J Pediatr Neurosci. 2017;12:1–6.
48. Chao CP, Zaleski CG, Patton AC. Neonatal hypoxic-ischemic encephalopathy: multimodality imaging findings. Radiographics. 2006;26 Suppl 1:S159–S172.
49. Gano D, Chau V, Poskitt KJ, et al. Evolution of pattern of injury and quantitative MRI on days 1 and 3 in term newborns with hypoxic-ischemic encephalopathy. Pediatr Res. 2013;74:82–87.
50. Garcia-Alix A, Arnaez J, Cortes V, Girabent-Farres M, Arca G, Balaguer A. Neonatal hypoxic-ischaemic encephalopathy: most deaths followed end-of-life decisions within three days of birth. Acta Paediatr. 2013;102:1137–1143.