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CLINICAL RESEARCH ARTICLE

CSF neopterin and beta-2-microglobulin as inflammation biomarkers in newborns with hypoxic–ischemic encephalopathy

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BACKGROUND: Inflammation plays a crucial role in the pathogenesis of hypoxic–ischemic encephalopathy (HIE). The aim of this study was to measure inflammation in HIE through an analysis of CSF neopterin and β2-microglobulin and to study the association with brain injury as shown by MRI findings and neurodevelopmental outcomes.
METHODS: CSF biomarkers were measured in study patients at 12 and 72 h. Brain injury was evaluated by MRI, and neurodevelopmental outcomes were assessed at 2–3 years of life. An adverse outcome was defined as the presence of motor or cognitive impairment.
RESULTS: Sixty-nine HIE infants were included. Median values of neopterin and β2-microglobulin paralleled the severity of HIE. Adverse outcomes were associated with early neopterin and β2-microglobulin values, late neopterin values, and the neopterin percentage change between the two samples. A cutoff value of 75% neopterin change predicted adverse outcomes with a specificity of 0.9 and a sensitivity of 0.75.
CONCLUSIONS: CSF neopterin and β2-microglobulin are elevated in HIE, indicating the activation of inflammation processes. Infants with adverse neurodevelopmental outcomes show higher levels of CSF neopterin and β2-microglobulin. The evolution of neopterin levels provides a better predictive capacity than a single determination.

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IMPACT:

- Brain inflammation in newborns with HIE could be measurable through the analysis of CSF neopterin and β2-microglobulin, both of which are associated with neurodevelopmental outcomes.
- Our study introduces two inflammatory biomarkers for infants with HIE that seem to show a more stable profile and are easier to interpret than cytokines.
- CSF neopterin and β2-m may become clinical tools to monitor inflammation in HIE and might eventually be helpful in measuring the response to emerging therapies.

INTRODUCTION

Hypoxic–ischemic encephalopathy (HIE) is a major cause of neonatal mortality and permanent disability. Therapeutic hypothermia (TH) is now the standard treatment, and it has significantly lowered mortality and improved neurodevelopment outcomes in infants with moderate and severe HIE.[1] Despite treatment with hypothermia, nearly half of neonates with HIE will have neurological disabilities.[2] There is an unmet medical need for biomarker tests, as an adjunct to neurophysiological and imaging studies, to accurately establish the severity of the injury, monitor ongoing damage, establish individual profiles, and improve outcome prediction.

Diverse mechanisms have been implicated to be involved in hypoxic–ischemic brain injury, including excitatory, oxidative, and inflammatory processes.[3–5] Nevertheless, despite our theoretical knowledge about the role of inflammation in brain damage in newborns with HIE, markers of the ongoing inflammatory response are lacking in clinical practice.

Previous studies confirmed the presence of elevated levels of interleukin (IL)-6, IL-8, IL-1β, or IL-10 in the serum[6–8] or cerebrospinal fluid (CSF) of neonates with HIE.[9,10] In addition, an abnormal neurodevelopmental outcome after hypoxia-ischemia has been associated with higher levels of IL-6 and other cytokines at birth.[6,7,11] However, cytokine levels show rapid dynamic changes within hours, making interpretation difficult in a clinical context. New surrogate biomarkers of inflammation in CSF, such as neopterin and beta2-microglobulin (β2-m), have the potential to be used in clinical practice to assess the inflammatory response.

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Neopterin is produced by monocyte-derived macrophages and dendritic cells secondary to stimulation by the proinflammatory cytokine interferon-gamma.[12] Neopterin is a pteridine derived from guanosine triphosphate and is formed in the synthetic pathway of tetrahydrobiopterine. Therefore, neopterin is an inflammatory factor and sensitive indicator for immune-mediated inflammatory disorders.[13] The neopterin concentration in blood and CSF has been used as a marker of the ongoing inflammatory process in a wide range of neurological diseases, infectious or not.[14–17]

Beta 2-microglobulin (β2-m) has been used as a marker of inflammatory injury in various central nervous system (CNS) diseases. β2-m is a low-molecular-weight (11,800 D) protein that constitutes the light chain of HLA class I antigens and is present on the surface of all nucleated cells.[18] The concentration of β2-m in biological fluids is related to the rate of cell membrane renewal, and high levels of this peptide reflect increased cellular turnover. High β2-m concentrations in CSF have been observed in newborns with infectious brain damage.[19–22] Furthermore, it has been shown that increased values of CSF β2-m reflect an immune system activation and that CSF β2-m is produced intrathecally.[23]

Given the pathogenic proinflammatory mechanism of brain injury in HIE, we hypothesize that the quantification of neopterin and β2-m in CSF may increase our understanding of the inflammatory response involved in ongoing brain injury in newborns with HIE treated with TH.

This study aimed to assess inflammation processes in newborns with HIE through an analysis of two surrogates, neopterin and β2-m, in CSF and to determine whether the concentrations of these biomarkers are associated with markers of brain damage, such as clinical grading of HIE and cerebral MRI findings, as well as with neurodevelopment at 2–3 years of age.

METHODS

This was a prospective cohort study in infants with HIE. We consecutively included infants with HIE born at ≥ 35 weeks gestational age and ≥1800 g admitted to (A) Sant Joan de Déu Hospital (Barcelona, Spain) and (B) Burgos University Hospital (Burgos, Spain) between April 2009 and August 2017. Infants were considered to have HIE if they met the following criteria: (1) at least one of the following clinical surrogates of hypoxic–ischemic insult: an altered (Category III) fetal heart rate pattern, sentinel event, and labor dystocia, understood as the use of forceps, vacuum or cesarian section; (2) an Apgar score ≤5 at 5 min or the need for resuscitation (tracheal intubation or mask ventilation for >10 min after birth, with or without chest compression and/or adrenaline) or acidosis (pH ≤ 7.0 and/or base deficit ≥16 mmol/L in umbilical cord blood or arterial, venous, or capillary blood within 60 min of birth; and (3) neonatal encephalopathy, defined as a syndrome of neurologic dysfunction manifested by a subnormal level of consciousness with or without seizures or palmary hyperexcitability.

The severity of HIE was assessed by one of the investigators in each center who were permanently on call (A.G.-A., J.A.) within the first 6 h after birth and before starting TH as mild, moderate, or severe HIE using a modified Sarnat scheme.[24] Hospital B did not include infants with mild HIE in the study. Infants with moderate or severe HIE received whole-body cooling (Techotherm TSmed 200N or Criticool, MTRE Ltd.) at 33.5 °C for 72 h and were monitored with amplitude-integrated electroencephalography (aEEG). All patients were evaluated and treated according to a strict clinical protocol common for the two centers for the management of HIE.

Biomarkers in CSF

The study protocol included 2 lumbar punctures: one at an 'early' time point (i.e., at 12 h of age) and one at a 'late' time point (i.e., at 72 h of age). The CSF β2-m and neopterin concentrations were measured by investigators who were blinded to the clinical data. CSF aliquots of 0.2 mL were distributed in plastic tubes, which were immediately frozen and stored at −80 °C until analysis. Samples were covered with aluminum foil to avoid the photodegradation of neopterin. Samples with signs of hemolysis were excluded.

Neopterin determination was performed by HPLC with electrochemical and fluorescence detection.[25] The quantification of β2-m was measured by turbidimetry using the commercial Quantia β2-microglobulin kit. The detection limit is 0.046 mg/L. The coefficient of variation for inter-assay and intra-assay variability for each determination method was <10% for each biomarker measured.

Neuroimaging studies

An MRI study using a 1.5 Tesla system (General Electric) was performed within the first two weeks of age. MRIs were reviewed by two investigators (T.A., A.G.-A.) blinded to clinical data and biomarker levels. Images were scored according to the scheme reported by Rutherford,[26,27] and moderate-to-severe injury was defined as a moderate–to-severe score in any of the regions analyzed (the posterior limb of the internal capsule (PLIC), the basal ganglia and thalami, the white matter, and the cortex). Discrepancies in the scoring of the images were discussed and resolved by consensus. The global injury pattern was defined as basal ganglia/thalami injury and white matter injury.

Neurodevelopmental outcomes

Neurodevelopmental assessments were performed in surviving infants between 24 and 36 months of age. The mental development index (MDI) and physical development index (PDI) were measured and calculated using the BSITD-III.[28] Cerebral palsy was defined and classified according to the Surveillance for Cerebral Palsy in Europe.[29] Motor functional impairment in children with cerebral palsy was scored according to the Gross Motor Function Classification System (GMFCS).[30]

Children who could not be assessed at the expected age (2–3 years) were contacted, and cognitive assessment was made using the Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III),[31] or the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV).[32] Motor function was assessed using the Movement Assessment Battery for Children–Second Edition (MABC-2),[33] and the total test score was standardized and converted to a percentile rank. For children whose parents refused the formal evaluation, questions focused on motor and cognitive skills were asked with a telephone survey. Based on this information, outcomes were assessed as normal or adverse, but developmental scores were not assigned to these children.

Adverse outcome was defined as the presence of any motor and/or cognitive impairment. Motor impairment was defined as GMFCS ≥level 1 and/or a composite score <85 on the Bayley-III motor area or a MABC-2 score ≤15th percentile. Cognitive impairment was defined as <85 on the cognitive composite score of the Bayley-III or a full-scale IQ <80 on the WPPSI-III or WISC-IV tests.

Statistical analysis

Descriptive statistics were used to summarize the overall information. Categorical variables were compared between groups using the chi-square or Fisher's exact test, when appropriate. Continuous variables were compared using the Mann–Whitney *U*-test or Kruskal–Wallis test. Adjustments were made for multiple comparisons using Scheffe post hoc pairwise analysis. The Spearman correlation coefficient (*rs*) was used to evaluate the correlation between quantitative variables. Sensitivity, specificity, and predictive values of different cutoff points and receiver operating characteristic (ROC) curves for neopterin and β2-m were estimated using the areas under the ROC curves as indices of performance. Confidence intervals were calculated with the exact method. Regression models were used to evaluate the influence of sex on the relationship between biomarkers and neurodevelopmental outcomes.

Statistical analysis was performed with SPSS version 17. All hypothesis comparisons were made bilaterally, and differences with a *P* level <0.05 were considered statistically significant.

Ethical considerations

Written information was given to the parents, and written consent was obtained from a parent available at the bedside upon the admission of each infant after an explanation of the study and before its onset. The study was approved by the human studies committees (CEIm "Comitè d'Ètica d'Investigaciò amb medicaments") of the participating hospitals.

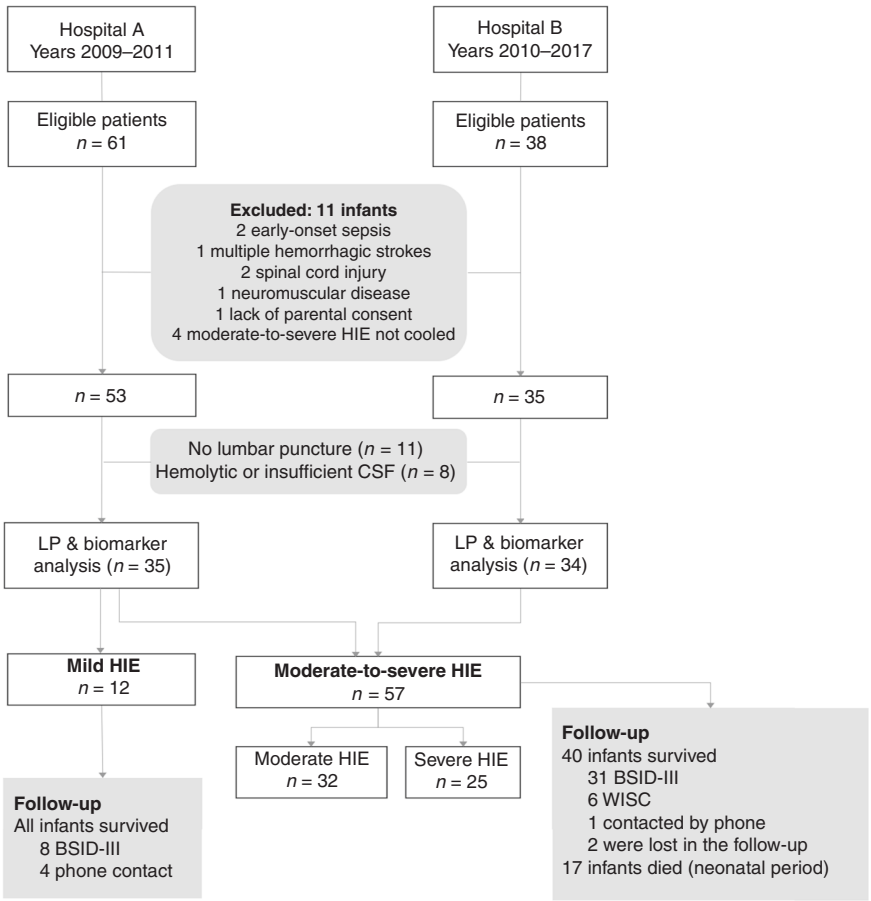

Fig. 1 Flowchart of patient inclusion. White boxes show the number of patients included according to the origin hospital and the severity of HIE. Grey round-edged boxes show the excluded patients and grey sharp-edged boxes show the follow-up data.

Table 1. Main perinatal characteristics of the population studied according to the severity of HIE.

	Mild HIE $n = 12$	Moderate HIE $n = 32$	Severe HIE $n = 25$	*P* value
Gestational age, weeks	39.5 (37, 40.5)	40 (38.5, 40.5)	38 (37, 39)	0.039
Birthweight, g	3205 (2895, 3600)	3283 (2890, 3510)	2553 (2450, 3080)	0.005
Sex (female)	5/12 (42)	14/32 (44)	13/25 (52)	0.77
Sentinel event	5/12 (42)	11/32 (34)	7/25 (28)	0.70
Eutocic delivery	1/12 (8)	2/32 (6)	2/25 (8)	1.00
Altered fetal heart rate	9/12 (75)	28/28 (100)	22/24 (92)	0.01
Advanced resuscitation[a]	8/12 (67)	24/32 (75)	22/25 (88)	0.28
Apgar score at 5 min	5.5 (4, 6.5)	4 (3, 6.5)	3 (0, 4)	0.001
Cord pH	7.02 (6.98, 7.05)	6.92 (6.8, 7.02)	6.8 (6.69, 6.95)	0.017
MRI score	0 (0, 1)	1 (0, 2)	9 (5, 11)	<0.001
Moderate-to-severe injury (MRI)	1/11 (9)	5/29 (17)	14/16 (88)	<0.001
Global injury pattern (MRI)	0/11 (0)	3/29 (10)	13/16 (81)	<0.001
Cerebral palsy	0/12 (0)	2/28 (7)	4/10 (40)	0.016
Adverse outcome[b]	0/12 (0)	7/28 (25)	6/10 (60)	0.004
Death	0/12 (0)	2/32 (6)	15/25 (60)	<0.001

Data are presented as median (interquartile range) or *n*/*N* (%). *P* value of <0.05 was considered as indicating statistical significance.
[a]Advanced resuscitation: tracheal intubation, chest compressions, and/or adrenaline.
[b]Adverse outcome is defined as MDI or PDI <85 at 24–36 months of age, IQ <80, MABC-2 ≤15, and/or cerebral palsy.

RESULTS

A flowchart depicting patient inclusion in the study is shown in Fig. 1. The main perinatal characteristics of the cohort are shown in Table 1. Seventeen infants died in the neonatal period, 15 of whom had severe HIE. The median age of death was 58 h (IQR 29, 90). In 14 infants, death occurred after an end-of-life decision that took into account a combination of persistent coma, a severely altered electroencephalographic pattern after 48 h of life, and

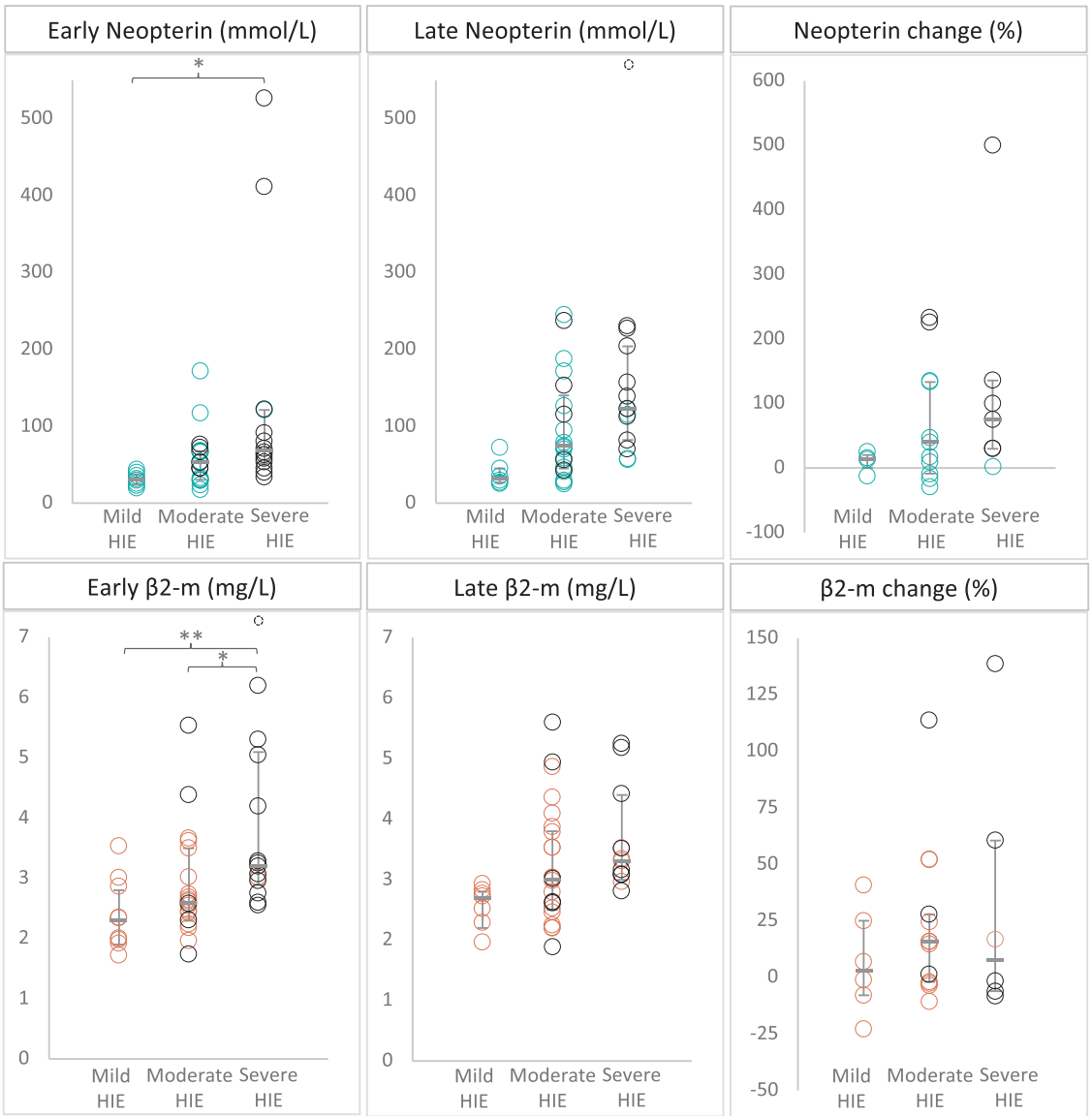

Fig. 2 Cerebrospinal fluid levels of neopterin and β2-m and the percentage change between samples in HIE infants according to the clinical severity of encephalopathy in the first 6 h of life. The dot plots show the values for both biomarkers (neopterin is represented in blue and β2-m in pink) at the two different time points and the percentage change between the two samples. Black circles represent infants with adverse outcome (MDI or PDI <85 at 24–36 months of age, IQ <80, MABC-2 ≤15, and/or cerebral palsy) or death. Dotted lined circles represent outliers (late neopterin: 1050 mmol/L, early β2-m: 9.8 mg/L, late β2-m: 12.6 mg/L). Median and IQR bars are shown. Significant differences between groups are indicated as follows: *$P < 0.05$; **$P \leq 0.01$.

neuroimaging findings (brain ultrasound and/or brain MRI) and was made through consensus between the medical team and the family. CSF inflammatory biomarker levels were not considered in end-of-life decisions.

CSF concentrations of inflammatory biomarkers and the timing of lumbar puncture

CSF analysis of inflammatory biomarkers was performed in 69 patients with HIE (Fig. 1). Fifty-two infants underwent LP at an early point, and 46 infants underwent LP at a late point; in 29 infants, lumbar puncture was performed at both the early and late time points (Fig. 1).

Early LP was performed at a median age of 13 h (IQR 12, 19), and late LP was performed at a median of 72 h (IQR 54, 76). Neopterin levels in HIE infants were 53.5 nmol/L (IQR 32, 71) at the early time point and 76.5 nmol/L (IQR 46, 146) at the late point. β2-m levels in HIE infants were 2.7 mg/L (IQR 2.4, 3.5) at the early time point and 3.03 mg/L (IQR 2.6, 3.7) at the late time point.

Correlation between early and late CSF concentrations of inflammatory biomarkers

There was a moderate correlation between the early neopterin sample and the β2-m level of 0.577 ($P = 0.001$) and a high correlation between the late neopterin sample and the β2-m level of 0.763 ($P < 0.001$). In infants with two LPs, the median percentage change was 29.8% (IQR 7.3, 133.7) for neopterin and 14.6% (IQR −3, 34.2) for β2-m levels between the two samples.

CSF concentrations of inflammatory biomarkers and HIE stage

CSF concentrations of neopterin and β2-m increased in parallel with the severity of the clinical grading of HIE. There were

Table 2. CSF levels of neopterin and β2-m in early and late CSF sample, and percentage change among samples, according to the severity of NE, MRI injury, and outcomes.

	Early CSF level		Late CSF level		CSF % change	
	n	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)
Neopterin (nmol/L)						
Severe HIE						
No	28	42 (30, 66.7)	26	64.5 (35, 115)	15	16.6 (−9, 133.3)
Yes	14	69 (52, 121.2)**	14	123 (82, 204)**	7	75 (29.5, 135.5)
Moderate-to-severe injury (MRI)						
No	21	40 (28, 66.5)	24	58.5 (32.5, 87)	14	16 (−9, 47)
Yes	14	56.5 (41.5, 121.2)	12	148.5 (98.5, 232.5)†	8	116.2 (29.8, 180.8)*
Global injury pattern (MRI)						
No	24	42 (30.2, 66.7)	26	64.5 (35, 115)	15	16.6 (−9, 75)
Yes	11	59 (34, 121)	10	131 (82, 204)**	7	133.3 (29.5, 226)
Adverse outcome[a]						
No	22	34.5 (29, 66.2)	24	64 (32.5, 105)	14	14.2 (−9, 39.7)
Yes	9	59 (43, 244)*	9	139 (70, 204)*	6	180.8 (99.2, 232.6)†
Adverse outcome or death						
No	22	34.5 (29, 66.2)	24	64 (32.5, 105)	14	14.2 (−9, 39.7)
Yes	19	67 (46, 81)**	15	139 (82, 227)**	8	117.4 (52.5, 229.3)**
Death						
No	32	42 (30, 67.7)	34	71 (43, 127)	20	32.3 (5.4, 134.2)
Yes	10	67.5 (52, 83.7)*	6	140.5 (113, 227)*	2	29.5 (29.5, 30.1)
β2-M (mg/L)						
Severe HIE						
No	28	2.5 (2.1, 3)	29	2.8 (2.5, 3.5)	19	14.6 (−2.5, 27.7)
Yes	14	3.2 (2.9, 5.1)**	13	3.3 (3, 4.4)**	6	7.6 (−6.1, 60.6)
Moderate-to-severe injury (MRI)						
No	24	2.6 (2.2, 3.2)	27	2.9 (2.5, 3.5)	18	4.25 (−3.5, 25)
Yes	9	3 (2.5, 5.1)	11	3.5 (2.9, 4.9)**	7	16.8 (−1.5, 113.8)
Global injury pattern (MRI)						
No	25	2.6 (2.2, 3.4)	29	2.9 (2.5, 3.5)	19	6.9 (−3.5, 27.7)
Yes	8	3 (2.5, 4)	9	3.3 (2.9, 4.4)	6	15.7 (−1.5, 60.6)
Adverse outcome						
No	24	2.5 (2.1, 3)	26	2.9 (2.5, 3.5)	17	14.6 (−2.5, 24.5)
Yes	7	3.2 (2.5, 5.2)*	9	3.02 (2.6, 4.9)	6	14.6 (−6.1, 113.8)
Adverse outcome or death						
No	24	2.5 (2.1, 3)	26	2.9 (2.5, 3.5)	17	14.6 (−2.5, 24.5)
Yes	18	3.2 (2.6, 5.1)**	15	3.1 (2.8, 5.1)	8	14.6 (−3.8, 87.2)
Death						
No	31	2.6 (2.3, 3.2)	36	2.9 (2.5, 3.5)	23	14.6 (−3.5, 27.7)
Yes	11	3.2 (2.6, 5)	6	3.9 (3.1, 5.1)*	2	29.5 (−1.5, 60.6)

Neopterin (nmol/L) and β2-m (mg/L) values are expressed as median (interquartile range). Neopterin and β2-m change (%) and B2-m change (%) values are expressed as median (interquartile range). Decreasing changes from early to late CSF samples are indicated as a negative percent.
[a]Adverse outcome is defined as MDI or PDI <85 at 24–36 months of age, IQ <80, MABC-2 ≤15, and/or cerebral palsy.
*$P < 0.05$, **$P \leq 0.01$, †$P \leq 0.001$.

statistically significant differences in neopterin values for CSF samples obtained at an early age between severe and mild HIE infants and in β2-m levels between severe and moderate HIE and severe and mild HIE infants (Fig. 2). Neopterin and β2-m changes between the early and late samples showed no significant differences in terms of the severity of HIE.

CSF concentrations of inflammatory biomarkers and MRI findings

MRI was performed in 56/69 infants at a median age of 10.8 days (IQR 7.9, 15.5); 9 infants with severe HIE died before MRI could be performed. Infants with moderate to severe injury showed higher β2-m and neopterin levels at the late LP and a higher percentage

Table 3. ROC curve analysis of CSF neopterin and β2-m level and percentage change and outcome.

	n	Cutoff	AUC (95% CI)	*S*	SP	PPV	NPV
Neopterin (nmol/L)							
Early CSF sample							
Adverse outcome	31	70	0.75 (0.58, 0.92)*	0.44	0.86	0.57	0.79
Adverse outcome or death	41	70	0.77 (0.62, 0.91)*	0.42	0.86	0.73	0.63
Late CSF sample							
Adverse outcome	33	133	0.74 (0.55, 0.93)*	0.56	0.88	0.63	0.84
Adverse outcome or death	39	133	0.78 (0.64, 0.93)*	0.53	0.88	0.73	0.75
% change							
Adverse outcome	20	75	0.95 (0.86, 1.00)**	0.75	1.00	0.75	1.00
Adverse outcome or death	22	75	0.89 (0.76, 1.00)**	0.75	0.86	0.75	0.86
β2-microglobulin (mg/L)							
Early CSF sample							
Adverse outcome	31	3	0.78 (0.57, 0.99)*	0.71	0.71	0.42	0.89
Adverse outcome or death	42	3	0.75 (0.59, 0.90)*	0.61	0.71	0.61	0.71
Late CSF sample							
Adverse outcome	35	3	0.56 (0.31, 0.81)	0.56	0.54	0.29	0.78
Adverse outcome or death	41	3	0.66 (0.48, 0.84)	0.73	0.54	0.48	0.78
% change							
Adverse outcome	23	60	0.58 (0.27, 0.90)	0.33	1.00	1.00	0.81
Adverse outcome or death	25	60	0.61 (0.34, 0.88)	0.38	1.00	1.00	0.77
Biomarkers combined							
Early CSF sample							
Adverse outcome	24	70/3	0.61 (0.33, 0.89)	0.33	0.88	0.50	0.80
Adverse outcome or death	32	70/3	0.58 (0.38, 0.79)	0.28	0.88	0.66	0.61
Late CSF sample							
Adverse outcome	30	133/3	0.64 (0.39, 0.90)	0.42	0.87	0.50	0.83
Adverse outcome or death	35	133/3	0.68 (0.48, 0.88)	0.50	0.87	0.66	0.76

AUC area under the curve, *NPV* negative predictive value, *PPV* positive predictive value, *S* sensitivity, *SP* specificity.
*$P < 0.05$, **$P \leq 0.01$.

of neopterin change between the early and late LPs. The global injury pattern on MRI was associated with late-sample neopterin values (Table 2).

CSF concentrations of inflammatory biomarkers and outcomes

The neurodevelopmental outcomes of 50/52 survivors were assessed at a median age of 30.8 months (IQR 24.3, 40.7). Thirteen infants developed adverse outcomes, and seventeen died in the neonatal period. Follow-up data are shown in Fig. 1 and Table 1.

Infants with adverse outcomes showed higher levels of neopterin ($P < 0.05$) and a greater percentage change between the early and late samples than those with normal outcomes: 180.1% (99.2, 232.6) vs. 14.2% (−9, 39.7); $P < 0.001$. β2-m concentrations were higher in infants with adverse outcomes only in the early sample ($P < 0.05$), while the percentage change between the early and late samples was not associated with adverse outcomes (Table 2 and Fig. 2).

The neopterin change between the two samples showed a high capacity for predicting adverse outcomes (AUC 0.95; 95% CI 0.86, 1). Optimal cutoff values for each biomarker and the combination of both are shown in Table 3.

Sex influence

There were no statistically significant differences between girls and boys in the values of early and late β2-m, early and late neopterin or the percentage change in either of them. Regression analysis showed that sex did not influence the association between the early sample, the late sample or the change in inflammatory biomarker levels and adverse outcomes or/and death, except for the early β2-m values. In this case, the OR changed from 4.3 (95% CI 1.1, 17.5) to 30.9 (1.2, 772.6) for adverse outcomes and from 3.3 (95% CI 1.3, 8.7) to 8.5 (95% CI 1.8, 39.9) for adverse outcomes or death when male sex was introduced in the model.

DISCUSSION

In this study, we examined the association between two surrogate inflammatory biomarkers in CSF and brain injury in infants with HIE. We have shown that neopterin and β2-m levels in CSF increase with the clinical severity of HIE, and they are associated with brain injury in neuroimaging and adverse outcomes. Our results indicate that the activation of inflammation processes in infants with HIE may be measurable.

Brain injury in HIE is the result of multiple pathogenic mechanisms. It is known that injury evolves within the first days of life, and the

precise duration of the therapeutic window is as yet unknown. The initial energetic failure is followed by a secondary phase characterized by apoptosis, oxidative stress, excitotoxicity, reperfusion, and inflammation.[3,4] Although this is widely understood by clinicians, the relative influence that each of these mechanisms has on brain injury may be different from patient to patient. At our disposal, we have multiple tools for assessing brain injury, such as MRI, aEEG, and plasma and CSF biomarker analysis.[34,35] However, specific tools targeted at the different pathogenic processes are needed as well. Understanding the pathological mechanisms underlying brain damage is essential to establishing individual profiles, monitoring processes underlying the evolving ongoing damage, choosing the most appropriate treatment, and establishing the prognosis. In the present study, we offered two candidate biomarkers for measuring inflammation processes in newborns with ongoing brain injury.

It is recognized that an inflammatory state may render the newborn brain more susceptible to hypoxic–ischemic damage.[4] Inflammatory histological findings of placentas from infants with neonatal encephalopathy[36,37] and a reduced response to TH in the presence of inflammation and infection in animal models with HIE support this theory.[38] An increased understanding of the role of inflammation in brain injury in HIE has given rise to multiple research studies that have correlated inflammatory biomarkers and HIE.[39] Nevertheless, most studies have focused on blood and CSF cytokine levels and their relation to HIE and outcomes. However, difficulties in cytokine interpretation have prevented the use of cytokines as biomarkers in clinical practice.[8,39–41]

Neopterin and β2-m have been used widely as inflammatory markers in the study of central nervous system infections and inflammatory processes.[13–17,20–22] In the present study, both biomarkers were elevated in infants with HIE, especially in those with severe HIE, indicating inflammatory processes in these patients. The association of CSF neopterin and β2-m with unfavorable outcomes reflects the influence of inflammation in brain injury in HIE.

An important question is whether the timing of the lumbar puncture influences the results. Interestingly, an increased percentage change in neopterin, but not in β2-m, between the early and late samples showed a much better predictive capacity than an isolated value of the first or late sample. In fact, we observed values to be fairly stable in most of the infants with good outcomes, so an increase over 75% in neopterin value serves as an alert to clinicians.

The late elevation of neopterin may be related to a reperfusion mechanism, as has been shown to be the case with other molecules such as excitatory glutamate.[42] Although we did not measure neopterin levels in plasma or blood serum, other studies have shown that CSF neopterin levels do not reflect systemic inflammation, as they appear to be produced intrathecally and are not related to blood–CSF barrier dysfunction.[14]

HIE has a convoluted nature due to uncertainty about the severity, timing and duration of the hypoxic–ischemic insult, the evolution of injury through several phases and the heterogeneity in the mechanisms of injury activated among patients. The uncertainty about the exact timing of injury constitutes an irremediable limitation for the predictive capacity of biomarkers at a given age-based timepoint, as well as for MRI or EEG findings. Nevertheless, biomarkers constitute a logical approach in the challenge of characterizing the underlying pathological processes in each individual that may allow clinicians to identify those patients with an increased risk of poor outcomes and to individualize care.

Consequently, the main role of inflammatory biomarkers should be to stratify patients according to the inflammatory response and monitor them. Although further studies are warranted, the increased neopterin value over time in patients with adverse outcomes shows that neopterin could be a suitable biomarker for monitoring damage and responding to a given treatment. This is congruent with the finding of Furukawa et al., who found neopterin to be a good inflammatory monitoring tool, as it normalizes with the remission of inflammatory activity.[13]

Although we are still searching for new therapeutic interventions in addition to TH, the multiple pathogenic mechanisms that lead to brain injury in HIE constitute different targets for possible synergistic treatments.[5] The use of melatonin and EPO are examples of neuroprotective strategies that act in the inflammatory cascade.[43,44] Further studies should be conceived to determine whether and which infants with HIE and inflammation biomarkers might benefit from additional treatments. Our data should serve to encourage researchers testing synergistic treatment with hypothermia in HIE to use CSF neopterin and β2-m as inflammatory biomarkers. The monitoring of CSF neopterin and β2-m levels could help us better understand the effect of neuroprotective therapies.

Our study has some limitations. Lumbar punctures were part of the research protocol for analyzing the temporal evolution of biomarkers; however, the analysis of CSF was performed in only 60% of eligible patients for a number of reasons, including clinical instability, severe coagulopathy and a low quantity or quality of CSF. These reasons, together with the low incidence of HIE in our setting, contributed to the moderate size of the sample. Despite our efforts to perform the different procedures at the protocol standard times, there was some variation in the early and late lumbar punctures, with IQRs of 12–24 and 54–82 h, respectively. Similarly, the MRI was performed at different times, with a difference of a week in some cases; however, we do not think that this factor would influence the capacity of MRI to characterize brain damage. A common limitation in studies analyzing predictive tools in HIE is the fact that a large proportion of infants with severe injury die, rendering it impossible to evaluate long-term sequelae in these infants. Larger studies powered to analyze death and disability in an independent way are needed. There were three infants with severe HIE who did not receive TH due to late transfer in two infants and a moribund state in one. As the goal of the study was to describe biomarker behavior in the hypothermia era, we excluded noncooled patients. A limitation in extending the measurement of CSF neopterin and β2-m across neonatal units is the need for one or two lumbar punctures. Some patients with HIE may be clinically unstable or present severe coagulopathy in the first hours of life, contraindicating lumbar puncture. Furthermore, some neonatologists may disagree about performing lumbar puncture in infants with HIE. However, CSF remains the essential biological fluid for understanding brain pathology, and complications related to lumbar puncture are rare.[45] Our study was focused on infants with HIE without infection in the hypothermia era. However, the presence of neonatal infection was judged on the basis of clinical and laboratory data, so we did not have maternal placentas for the assessment of chorioamnionitis in a more accurate manner. The strengths of our study include the prospective protocol, homogeneity in the timing of the lumbar puncture and the high rate of follow-up. Furthermore, neopterin and β2-m seem to present a more stable profile over time than cytokines.

CONCLUSION

Our study helps provide an improved understanding of the pathological processes of injury in HIE regarding the inflammatory pathway. Our study provides measurable indicators of CNS inflammation, which is an essential step in the testing of anti-inflammatory therapies. The evolution of CSF neopterin and β2-m may serve as an important adjunct to other tools for monitoring brain injury in HIE and eventually may prove helpful in measuring the response to emerging therapies.

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AUTHOR CONTRIBUTIONS

N.C. participated in the interpretation of data and the literature search and wrote the manuscript. A.G.-A. designed the study, participated in the acquisition and interpretation of data, and contributed to the drafting of the manuscript. J.A. participated in the acquisition and interpretation of data, reviewed the manuscript, and contributed to the drafting of the article. T.A., A.V., and C.S. participated in the acquisition of data and reviewed the manuscript. All authors approved the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Parental consent was obtained.

ADDITIONAL INFORMATION

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