Early predictors of abnormal MRI patterns in asphyxiated infants: S100B protein urine levels

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Abstract

Objectives: The early detection and stratification of asphyxiated infants at higher risk for impaired neurodevelopment is challenging. S100B protein is a well-established biomarker of brain damage, but lacks conclusive validation according to the "gold standard" methodology for hypoxic-ischemic encephalopathy (HIE) prognostication, i.e. brain MRI. The aim of the present study was to investigate the predictive role of urinary S100B concentrations, assessed in a cohort of HIE infants receiving therapeutic hypothermia (TH), compared to brain MRI.

Methods: Assessment of urine S100B concentrations was performed by immunoluminometric assay at first void and at 4, 8, 12, 16, 20, 24, 48, 72, 96, 108 and 120-h after birth. Neurologic evaluation, routine laboratory parameters, amplitude-integrated electroencephalography, and cerebral ultrasound were performed according to standard protocols. Brain MRI was performed at 7–10 days of life.

Results: Overall, 74 HIE neonates receiving TH were included in the study. S100B correlated, already at first void, with the MRI patterns with higher concentrations in infants with the most severe MRI lesions.

Conclusions: High S100B urine levels soon after birth constitute trustable predictors of brain injury as confirmed by MRI. Results support the reliability of S100B in clinical daily practice and open the way to its inclusion in the panel of parameters used for the selection of cases suitable for TH treatment.

Keywords: brain damage; hypothermia; hypoxic ischemic encephalopathy; perinatal asphyxia; S100B.

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Introduction

The Food and Drug Administration (FDA), the European Medicines Agency (EMA) and the National Institutes of Health (NIH) warmly support research projects on early neuro-biomarkers (NB) of perinatal brain injury as well as on the effectiveness of neuroprotective strategies.

Among several NB currently under investigation (glial fibrillary acid protein, GFAP; activin A, adrenomedullin, oxidative stress, neuron specific enolase), the S100B protein, mainly located in glial cells and in neuronal subpopulations of the central nervous system (CNS), has been suggested as a trustable marker in different biological fluids [1–10]. It's easy measurability, reproducibility, and assessment in invasive (cerebrospinal, CSF; blood) and non-invasive biological fluids (urine, saliva) support its usefulness as an early marker of brain development/injury in neonatal intensive care units (NICU) daily practice [10–12]. Before this can be done, a few issues still need to be

addressed. The main one regards the correlation between S100B and standard of care monitoring procedures.

Today, magnetic resonance imaging (MRI) is considered the gold standard for perinatal brain damage detection and prognosis in newborns complicated by intraventricular hemorrhage (IVH), perinatal asphyxia (PA) and hypoxic ischemic encephalopathy (HIE) [13, 14]. In this regard, it has been previously reported that MRI patterns correlated with long-term neurodevelopment in preterm and term infants [10, 11]. In the latter, the basal ganglia/watershed (BG/W) score was able to identify infants at risk for poor neuromotor and cognitive outcome at 3–12 months of age, respectively [10]. However, limitations of MRI monitoring regard its: (i) variability according to different assessment time-points, (ii) limited diagnostic value in the first hours from birth, and (iii) difficult performance in case of infant's unstable clinical conditions [13–16]. In this light, data on validation of S100B as a trustable NB of brain injury through MRI could be especially useful when standard monitoring procedures can be silent or unavailable.

Therefore, the aims of the present study were to investigate whether urinary S100B: (i) changed in PA HIE infants who had undergone therapeutic hypothermia (TH) strategy and later developed or not an abnormal MRI pattern, and (ii) correlated with MRI patterns.

Materials and methods

We conducted a prospective case-control study in 88 PA infants admitted to our third level referral centers for NICU between January 2014 and December 2019.

The Local Ethic Committees of the CoMBINe International Study (Alessandria, Catania, Rome, Italy; Cairo, Egypt; Maastricht, The Netherlands; Warsaw, Poland) approved the study protocol. Informed and signed consent was obtained from all parents of patients.

For sample size calculation, we choose, as the main parameter, S100B urinary levels changes in PA infants treated by TH at 72-h of life [9]. We assumed a decrease of 0.5 SD in S100B clinically significant. Considering an α=0.05 and using a two-sided test, we estimated a power of 0.95 including 68 PA TH-treated infants. We added further n=20 neonates to allow for dropouts (n=6), withdrawn consent (n=6), and mortality (n=2). Therefore, the study population consisted of 74 PA-HIE TH-treated (moderate HIE: n=44; severe HIE: n=30) infants. Forty-four out of 74 PA infants showed an MRI BG/W score 0; 14 out of 74 had an MRI BG/W score 1, 2; 16 out of 74 showed an MRI BG/W score 3, 4 (Figure 1). In light of this, the study population was corrected for the occurrence of an MRI BG/W score 0–2 (n=58; Group A) and MRI BG/W score 3–4 (n=16; Group B).

Sixty-nine out of 74 PA infants were delivered by emergency caesarean section (ECS) because of acute fetal distress classified according to the criteria of the American College of Obstetricians and Gynecologists [17].

Asphyxia was defined as Apgar score <3 at 5′, pH<7.0, base excess ≤–12 mmol/L in cord or venous blood taken within 60′ from

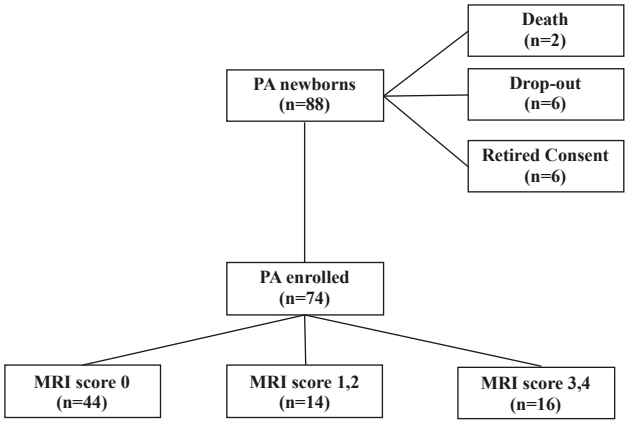

Figure 1: Flow chart describing the recruitment of patients.

birth, the need for resuscitation at birth and/or for positive pressure ventilation (>3′), and the occurrence of multiorgan failure [17]. Infants fulfilling three or more of the above criteria were included in the PA group, received mechanical ventilation, and were sedated by means of fentanyl citrate (Fentanest; Pharmacia & Upjohn International, Milan, Italy), 0.5–2.5 µg/kg/h, and midazolam hydrochloride (Ipnovel; Roche SA, Fontenay-sous-Bois, France), 50–400 µg/kg/h.

Clinical and laboratory parameters were recorded in all infants on admission to NICU and at 24-h, 72-h, and 120-h after birth. Cerebral ultrasound (CUS) and neurological patterns were assessed by a single examiner in each center, blinded to the urine test results.

Exclusion criteria were malformations of the CNS, chromosomal anomalies, major congenital heart disease malformations (CHD).

Standard monitoring procedures

Clinical and laboratory parameters (red blood cell count, RBC; hemoglobin blood concentrations, Hb; hematocrit rate, Ht; venous blood pH; partial venous oxygen and carbon dioxide pressures, pCO_2, pO_2; base excess, BE; blood ions, glucose, urea, and creatinine levels were recorded in all neonates before, during and after TH procedure.

TH treatment

In all infants passive cooling was started soon after PA diagnosis, and TH started within 6-h from birth. TH was performed by means of a total-body, servo-controlled, water-circulated device (Tecotherm iCool, Inspiration Healthcare Group, Crawley, UK), set to maintain a rectal temperature of 33.5 °C for 72-h [18, 19]. Rewarming phase was performed at a rate of ≤0.5 °C per hour, up to a rectal temperature of 37.0 °C. During the cooling and rewarming phases, the pCO_2 target range was set at 45–60 mmHg and hypotension was treated if mean blood pressure was persistently <40 mmHg for >20'.

Cerebral function monitoring (CFM)

CFM monitoring was performed from TH starting up to 72-h after the TH rewarming phase (CFM Olympic Brainz Monitor, Natus Medical Incorporated, CA, USA). EEG stick-on electrodes (Denis, Spes Medica, Genoa, Italy) were applied to neonatal scalps and used in a reduced

montage according to the International 10/20 system [20]. Amplitude-integrated electroencephalography (aEEG) background pattern classification was achieved by a single channel cross-brain aEEG trace (from central electrodes C3-P3), which also recorded EEG. aEEG results were defined as continuous or discontinuous normal voltage, burst suppression, low voltage, or flat trace. Electrographic seizures were diagnosed in the presence of repetitive, stereotyped waveforms with definite onset, peak and end, of 10" or longer of raw EEG, status epilepticus in case of continuous/repetitive ictal activity ≥30' [21]. The examiners who reviewed aEEG results were blinded to the urine test results.

CUS

CUS monitoring was performed by real-time ultrasound machine (Acuson 128SP5 Mountain View CA, USA), at 12-24-h from TH starting and at the discharge from hospital.

Neurological examination

Neurological assessment was performed daily, in line with Prechtl [22]. Based on the criteria of Jurgens–van der Zee [23], infants were classified as "abnormal" in the presence of one or more of the following symptoms: (i) increased/decreased excitability (hyperexcitability syndrome/convulsions/apathy syndrome/coma), (ii) increased/decreased motility (hyperkinesia/hypokinesia), (iii) increased/decreased tonus (hypertonia/hypotonia), (iv) asymmetries (peripheral/central), (v) CNS defects, and (vi) any combination of the above symptoms. An infant was considered "suspect" when only isolated symptoms were present [23].

HIE was classified in line with Sarnat [24]. It was defined as mild if hyperexcitability or hypotonia persisted without seizures for at least 72-h after birth; moderate if the infant was lethargic and had hypotonia, weak primitive reflexes, and seizures; and severe if the infant suffered frequent seizures, apnea, flaccid weakness, or coma.

MRI patterns

MRI was performed at 7–10 days from birth on a 1.5-T scanner, with infants wrapped into a vacuum cushion to minimize motion. The standard sequences were sagittal and axial spin echo T1, double-acquisition axial fast-spin echo T2 proton density, coronal fast-spin echo T2, and axial diffusion-weighted images. The images were analyzed by a pediatric neuroradiologist blinded to neonatal features, clinical grade of HIE and S100B concentrations. The pattern of brain injury was classified according to the BG/W scoring system by Barkovich [25, 26] and stratified according to severity as follows: normal (BG/W score 0); abnormal signal in basal ganglia or thalamus (BG/W score 1); abnormal signal in cortex (BG/W score 2); abnormal signal in cortex and basal nuclei (basal ganglia or thalami) (BG/W score 3); abnormal signal in entire cortex and basal nuclei (BG/W score 4). A score of ≥2 in the deep nuclear grey matter, or a score of ≥3 in a watershed pattern, was considered consistent with moderate-severe MRI injury [8, 27].

S100B measurement

Longitudinal collection of urine samples (100 μL) was achieved by a standard urine collector (Pennine Heathcare, London, UK) at first void (T0), 4 (T1), 8 (T2), 12 (T3), 16 (T4), 20 (T5), 24 (T6), 36 (T7), 48 (T8), 72 (T9), 96 (T10), 108 (T11), and 120 (T12) hours of life. A bladder catheter was inserted for urine sampling because of neonatal critical conditions. Urine samples were centrifuged immediately after collection (900 g for 10′) and stored at −70 °C until the assay. S100B concentrations were measured by an immunoluminometric assay (Liaison S100, Dietzenbach, Germany) according to the manufacturer's instructions. The detection limit of the assay was 0.02 μg/L, the coefficient of variation was ≤3.9% within-assay and ≤6.2% inter-assay for concentrations ranging between 0.12 and 17.5 μg/L.

Statistical analysis

When the Kolmogorov–Smirnov test showed that values were not normally distributed, S100B concentrations were expressed as medians and 5–95% confidence interval (CI) and statistical significance of differences evaluated using a non-parametric test. Data on neonatal outcomes, laboratory parameters and BG/W score were analyzed according to Tukey's one-way ANOVA and the Mann–Whitney U two-sided test. Comparison between proportions was performed using the chi-square exact test. Correlations between S100B at different monitoring time-points and BG/W score were calculated by linear regression analysis. Sensitivity, specificity and positive and negative predictive values (PPV, NPV) of S100B as diagnostic test for the detection of an abnormal MRI pattern in PA newborns were assessed using the receiver operating characteristic curve (ROC) test. The probability of developing brain damage by means of MRI when neither, one, or both tests were positive (higher than the cut-off point) was estimated and compared with the pre-test probability, defined as the prevalence of brain damage in the whole group of newborns. Statistical significance was set at $p<0.05$.

Results

Table 1 shows the perinatal characteristics of the studied populations. As expected, no significant differences ($p>0.05$, for both) were observed between the groups regarding weight and gestational age at birth. Gender, ECS rate, Apgar scores at 1–5′, the incidences of respiratory distress syndrome, need for mechanical ventilation and for inotropic therapy did not differ ($p>0.05$, for all) between the two groups. No significant differences ($p>0.05$, for all) in the aEEG patterns, Sarnat score and neurological examination were found between the groups as well as the timing of the beginning of TH ($p>0.05$).

In Table 2 standard laboratory parameters recorded in PA infants at admission into the study are reported. No significant differences ($p>0.05$, for all) were detectable between studied groups regarding RBC, Hb, Ht; venous blood pH, pCO_2, pO_2, BE; blood ion, glucose, urea and creatinine, concentrations at birth.

CUS patterns

Overall, 44 out of 74 PA infants had a normal CUS and 30 showed abnormal CUS patterns. Abnormalities included

Table 1: Perinatal characteristics of PA HIE treated by TH according to brain MRI patterns.

	Group A (n=58)	Group B (n=16)
Perinatal clinical characteristics		
Birth weight, g	3,648 ± 539	3,564 ± 451
Gestational age >36 weeks, n	58	16
Gender, (male/female)	22/36	6/10
Caesarean section, n/total	54/58	15/16
Factors associated with primary outcomes		
Apgar score, n/total		
at 1 min <3	48/58	12/16
at 5 min <3	38/58	9/16
Respiratory distress syndrome, n/total		
Mechanical ventilation support, no/total	44/58	11/16
Inotrope therapy, n/total aEEG	31/58	10/16
Normal, n/total	0/58	0/58
Moderate abnormality, n/total	48/58	14/16
Severe abnormality, n/total	10/58	2/16
Sarnat score		
Stage I, n/total	0/58	0/16
Stage II, n/total	42/58	12/16
Stage III, n/total	16/58	4/16
Neurological examination		
Normal/suspect/abnormal	0/42/16	0/13/3
Time to start cooling <6-h, n/total	58/58	16/16

PA, perinatal asphyxia; HIE, hypoxic ischemic encephalopathy; TH, hypothermia; MRI, magnetic resonance imaging; aEEG, amplitude-integrated electroencephalography.

Table 2: Laboratory parameters at birth of PA HIE treated by TH according to brain MRI patterns. Values are expressed as mean ± SD.

	Group A (n=58)	Group B (n=16)
RBC count, $10^6/mm^3$	4.0 ± 0.2	4.2 ± 0.2
Hb, g/dL	14.2 ± 0.2	14.2 ± 0.3
Ht rate, %	41.9 ± 0.4	41.5 ± 0.4
Venous blood pH	7.02 ± 0.1	7.00 ± 0.1
pCO_2 pressure, mmHg	67.8 ± 1.5	68.3 ± 1.5
pO_2 pressure, mmHg	25.2 ± 0.7	27.2 ± 0.8
BE	17.2 ± 2.8	−17.7 ± 2.3
Na^+, mmol/L	139 ± 4.1	140 ± 4.1
K^+, mmol/L	4.5 ± 0.4	4.6 ± 0.4
Ca^{++}, mmol/L	1.12 ± 0.07	1.12 ± 0.08
Plasma glucose, mmol/L	4.2 ± 0.2	4.2 ± 0.3
Urea, mg/dL	39.7 ± 3.2	40.2 ± 2.6
Creatinine, mg/dL	0.91 ± 0.13	0.89 ± 0.08

PA, perinatal asphyxia; HIE, hypoxic ischemic encephalopathy; TH, therapetic hypothermia; MRI, magnetic resonance imaging; RBC, red blood cell count; Hb, hemoglobin; Ht, hematocrit rate; pCO_2, partial venous carbon dioxide pressure; pO_2, partial venous oxygen pressure; BE, base excess.

IVH (n=3), hyperechogenenities at talami and basal ganglia (n=12), periventricular hyperechogenicities and generalized brain edema (n=14), and brain infarction (n=1).

MRI patterns

Overall, 44 out of 74 PA infants showed normal MRI imaging (BG/W score 0), whilst 30 out of 74 had abnormal (BG/W score 1–4) MRI patterns. BG/W score 1 occurred in 10 cases, BG/W score 2 in 4 cases; BG/W score 3 in 6 infants and finally BG/W score 4 in 10 cases.

S100B measurement

S100B was measurable in all samples collected. In Group A, the S100B pattern was characterized by a flat trend starting from T0 up to T12. No significant differences ($p>0.05$) were found among T0 and T1–T12 time-points (Figure 2).

In Group B, the S100B pattern of concentration was characterized by a progressive increase reaching the highest peak at T4 and starting to progressively decrease from T5 onwards reaching its lowest dip at T8. From T8 to T10, S100B increased again remaining higher than reference values at T12 (Figure 2).

When S100B levels at T0-T12 were compared between Group A and B we found significantly higher ($p<0.001$, for

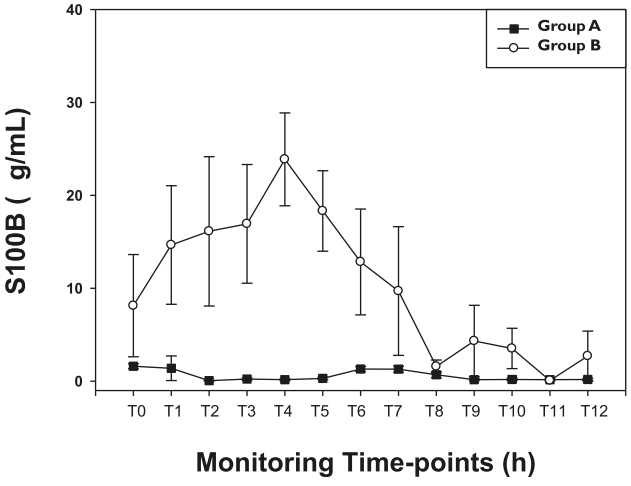

Figure 2: S100B urinary levels (mg/L) at different monitoring time-points in perinatal asphyxia infants with a basal ganglia/watershed areas score (BG/W) of 0–2 (Group A) and with a BG/W score of 3, 4 (Group B), respectively.
Data are given as mean and SD.

Table 3: S100B urine levels (µg/L) in perinatal asphyxia infants according to severity of magnetic resonance imaging findings by means of basal ganglia/watershed areas score (BG/W).

BG/W score	Median	25° centile	75° centile
B0	0.07	0.02	0.49
B1	1.50	0.34	1.72
B2	2.00	1.50	2.35
B3	3.00	2.93	3.55
B4	3.24	3.01	3.79

BG/W score, basal ganglia/watershed areas score.

all) protein levels in Group B from T0 to T7, at T9 and at T12, respectively. No differences (p>0.05, for both) were observed between groups at T10 and T11 (Figure 2).

In Table 3 S100B patterns in PA infants according to the severity of MRI findings (BW/G score 0–4) are reported. S100B was significantly (p<0.001) higher in PA infants with BG/W score 3, 4 than in those with MRI BG/W score 0–2. No differences (p>0.05) were observed in S100B levels between BW/G scores 3 and 4. Moreover, S100B was also significantly (p<0.001) different in PA infants with BG/W score 1, 2 than those with BGW score 0. No differences (p>0.05) were observed in S100B between BW/G scores 1 and 2.

At the cut-off >2.72 µg/L, chosen by the ROC curve, S100B at T0 achieved a sensitivity of 84.6% ($CI_{5-95\%}$: 54.6–98.1%), a specificity of 100% ($CI_{5-95\%}$: 93.7–100%) as a single marker for predicting the occurrence of an abnormal MRI pattern (area under the ROC curve 0.909, $CI_{5-95\%}$: 0.816–0.965). PPV was 100% and NPV was 96.6% ($CI_{5-95\%}$: 88.8–99.0) (Figure 3).

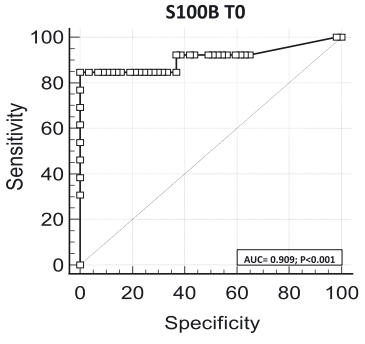

Figure 3: Receiver operating characteristic curve (ROC) of urine S100B concentrations for the early prediction of abnormal magnetic resonance imaging in perinatal asphyxia infants.
Urine S100B at the cut-off 2.72 µg/dL, achieved a sensitivity of 84.6% ($CI_{95\%}$: 54.6–98.1%) and a specificity of 100% ($CI_{95\%}$: 93.7–100%) as a single marker for prediction of occurrence of neurologic abnormalities (area under the ROC curve: 0.909).

Discussion

PA and HIE rates can vary between developed/developing countries (1.5 vs. 2.3–26.5/1,000 live-births) and constitute one of the major causes of mortality and morbidity [28]. Among a series of clinical, laboratory and radiological diagnostic parameters, MRI still represents the best standard-of-care tool to provide front-line physicians with useful information on CNS development/damage [13–16]. However, MRI cannot be always performed at the due time (within 5–7 days post-natal age) owing to infrastructural limitations and/or critical patient conditions [29–31]. In this time-period, no diagnostic parameter is able to detect brain damage as the symptoms are hidden by NICU neuroprotective strategies. Thus, the possibility to monitor CNS function/damage in NICU by NB could be especially useful once validated by means of MRI.

In the present study we found increased S100B urine levels in PA HIE infants. S100B correlated with the extent of CNS damage seen at MRI. Furthermore, ROC curve analysis results showed that S100B can be a trustable diagnostic test for the prediction of abnormal MRI patterns in PA-HIE infants.

The results of increased S100B in PA-HIE are not surprising and fit previous observations regarding protein assessment in biological fluids in infants complicated by IVH, stroke, meningitis and CHD [7–11, 31, 32]. Nonetheless, S100B pattern in TH-treated infants according to the severity of HIE and/or MRI findings warrants further consideration. In particular, S100B was significantly higher from T0 (<2-h from birth) up to the first 24-36-h in infants, showing at 5–7 days from birth an MRI BW/G score 3,4, compared to those with BW/G score 0–2. Bearing in mind that, at this stage, the output of standard of care monitoring procedures did not differ between groups, these results open the way to a series of possible explanations such as: (i) protein's role as early predictor of poor outcome in agreement with previous observations [7, 11, 33, 34], (ii) the severity of HIE, although 14 out of 74 PA infants showed a BW/G score of 0–2, (iii) TH procedure itself, particularly the cooling phase. In this respect, the protein's pattern confirms a previous observation suggesting that metabolic changes, typical of a prolonged low temperature, could somewhat delay S100B elimination by the kidney route [11], (iv) changes in brain-blood-barrier permeability known to interfere on systemic blood protein levels and on its urinary release [35], and finally (v) the starting point of TH efficacy suggesting that the longer the time needed for TH neuroprotective action the greater the entity of CNS damage [11]. This is suggested by a

delayed return of S100B within reference ranges taking into due account the short protein half-life (about 1-h) [4]. Altogether, it is possible to argue that the pattern of S100B in PA-HIE is supportive of: (i) a poor TH effect in severe HIE forms [36], and (ii) a role for the protein as useful predictor of brain damage at a stage when standard-of-care parameters are still silent or unavailable. In any case, further studies aimed at clarifying whether the time-window suitable for TH treatment and effectiveness could be postponed up to 12–24-h from hypoxic insult are awaited with great anticipation.

In the present study we also found that in infants with S100B above the thresholds defined by the ROC curve analysis (>2.72 μg/L), the PPV of an abnormal MRI pattern defined by a BW/G score >2 was as high as 100%, while it was 3.4% if these levels were below the threshold with PPV and NPV that differed from the overall prevalence of neonatal brain damage (18.6%) in the study population. Results support the possibility of identifying: (i) PA infants at higher risk of poor MRI repertoire and subsequent neurological outcome soon after birth, and (ii) cases suitable for TH treatment at a stage when standard of care procedures could be silent or unavailable. This relies on clinical, laboratory and EEG parameters today covering a key-role in clinical guidelines for TH patient selection. Even MRI has several limitations such as diagnostic accuracy in the first 24-h of life and the need to move critically ill infants for imaging performance [29–31]. Moreover, MRI and EEG data require experienced teams that are not always available at hub and spoke centers. Conversely, S100B measurement in urine allows the early detection of infants developing an abnormal MRI pattern and neurological outcome [9, 10]. Urine sample collection (100 μL) is much more feasible than blood, at low stress for the newborn and results can be obtained within 1-h at a time useful for inclusion or otherwise in TH treatment [37]. Additional advantages reside in the fact that altered kidney function does not constitute an adverse and/or confounding factor for S100B as a trustable predictor of CNS damage in PA-HIE infants [38].

The present study has some limitations: we did not stratify patients correcting for different therapeutic strategies (anesthesia, anticonvulsants) and although all the participant centers followed the same protocols for TH treatment and samples collection, the possibility of a potential bias should be taken into consideration (ii) we did not measure other trustable NB such as G-FAP (CSF, blood), although no data is available on its assessment in non-invasive biological fluids (urine, saliva) [39].

In conclusion, the present study provided evidence that early S100B urinary measurements are predictive of abnormal MRI findings in PA-HIE TH-treated infants soon after birth. Results represent the final step for inclusion of the protein in clinical guidelines as a trustable and early biomarker of brain damage in high-risk infants and open the way for investigations aimed at including S100B in a panel of parameters used for selection of cases suitable for TH treatment.

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Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all subjects' parents involved in the study. Written informed consent was obtained from all subjects' parents to publish the paper.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013). The Local Ethic Committees of the Cooperative Multitask against Brain Injury of Neonates (CoMBINe) International Network approved the study protocol.

Data availability: All data will be made available upon request. Please contact the corresponding author.

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