Original Research Paper

Volume - 12 | Issue - 09 | September - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Neonatology

ROLE OF SERUM S-100B PROTEIN AND MRI IN PREDICTING THE NEUROLOGICAL OUTCOME AND SEQUELAE IN NEONATES ≥ 36 WEEKS WITH BIRTH ASPHYXIA.

Dr Lathiesh Kumar Kambham MD (Pediatrics), DM (Neonatology).

ABSTRACT A prompt diagnosis of neonatal hypoxic-ischemic encephalopathy (HIE) remains a clinical challenge. This study aimed at exploring the potential of Serum protein S100B as a biomarker for evaluating neonatal HIE in newborns with moderate-to-severe hypoxic-ischemic encephalopathy. Blood samples were collected from neonates with mild, moderate, or severe HIE who were admitted to the Department of Neonatology, Madras Medical College (MMC), Chennai between September 2016 to March 2017. The plasma levels of S100 B protein were measured at different time points. Additionally, Neurodevelopmental outcomes were also studied using MRI in surviving infants (> 2 weeks). Eighty-four neonates enrolled in the study had moderate (n = 37), severe (n = 13) and mild HIE (n= 36). At birth, serum protein S100 B increased with the severity of HIE (P < .001), and remained elevated in neonates with moderate to severe HIE. Serum protein S100 B was greater up to 72 hours in moderate to severe vs mild HIE. The Elevated levels of S100B were associated with increased brain injury as studied by MRI. The study suggests S S100 B may serve as a potential biomarker for neonatal mild HIE (n=36), moderate (n=37) and severe (n=13) could be used for stratification at birth as elevated levels are correlated with the severity of HIE.

KEYWORDS : Hypoxic-Ischemic Encephalopathy, Serum protein S-100B, biomarker; neonate; MRI

INTRODUCTION

Neonatal HIE is a form of severe neonatal brain damage resulting from an asphyxia-related decrease in cerebral blood flow and hypoxia.[1] It comprises a constellation of symptoms in the neonates involving alteration in the level of consciousness, tone, and neonatal reflexes, like sucking and swallowing reflex, the presence of seizures in moderate and severe stages of HIE and cardiovascular (bradycardia, hypotension), and respiratory disorders (irregular respiration, apnea) in severe HIE. Furthermore, severe HIE has multisystem involvement ranging from neurological, renal, and liver damage to fluctuations in glycemic values.[2,3]

Globally, as cited in NCBI, an estimated 1.15 million babies develop hypoxic-ischemic encephalopathy every year. Up to 60% of infants with HIE would die or develop severe disabilities by the age of 2, encompassing mental retardation, epilepsy, and cerebral palsy. Assessment of the fetus during labor and the brain injury in neonates remains one of the biggest challenges in perinatal medicine.5 The study of meconium, non-reassuring fetal heart rate tracing, APGAR scores, umbilical artery blood gases, and physical exams, are commonly used tools to identify brain injury in the fetus. However, they lack precision.[4,5]

Clinical practices largely rely on ultrasound and/or MRI for diagnosis. Even though these methods have good sensitivity and selectivity; they are time-consuming and require transportation of the patient to the imaging facility. These aspects restrict routine use.[6] Additionally, in the initial stages when morphological changes are still happening, the exact extent of the lesion may be misjudged. It is therefore pertinent to use peripheral biomarkers to assess the extent of the damage.[6]

The extent of the biomarkers correlates with the size, location, and severity of the lesion, clinical outcome, and response to treatment.[7] Serum biomarkers should give evidence related to the pathophysiology of injury, improve stratification of patients by the severity of the injury, and contribute to the monitoring of the secondary insults and injury progression, response to treatment, and predicting functional outcome. The level of brain injury biomarker circulating in neonatal HIE would indicate brain injury and echo the extent of damage, resolving a clinical problem in the discrimination of mild, moderate, and severe injury.[8]

Serum protein S100B is a calcium-binding protein produced and secreted by astrocytes and Schwann cells. Although S100B is found mainly in glial cells, it is also present outside the central nervous system. In healthy individuals, a small amount is present in cerebrospinal fluid, blood, and urine.[9,10]

However, the levels of S100B rise in cerebrospinal fluid, blood, urine, and saliva during pathological conditions such as perinatal asphyxia, acute brain injury, and neuroinflammatory/neurodegenerative disorders. The levels of S100B also increase in cases of brain tissue damage. Hence, high S100B levels are considered to be an indicator of cellular damage.[11,12]

The aim of this study was to evaluate the utility of S100B as a potential biomarker of brain injury in babies with HIE. It was hypothesized that S100B levels would be higher in infants who had severe brain injury compared with infants with minimal or no injury.

Methods

Written consent was obtained from the parents or caregivers of the infant. This study was approved by the institutional Ethics Committee at Madras Medical College. Additionally, the study protocol was reviewed by the medical professionals who were handling the neonatal unit.

This observational correlational clinical study was carried out from September 2016 to March 2017 and included neonates with gestational age ≥36 weeks and birth weight ≥1,800g. The neonates have mild/moderate/severe HIE and were admitted to the Department of Neonatology, Madras Medical College (MMC), Chennai. The neonates who met the inclusion and exclusion criteria were enrolled in the study.

Inclusion Criteria

Neonates with perinatal asphyxia born at the study hospital or any other health facility were enrolled in the study. Perinatal asphyxia is defined as the need for resuscitation at birth, along with the presence of one or more of the following:

- Apgar score of <6 at 5 min after birth
- The continued need for resuscitation for more than 5 mins
- Umbilical cord pH or any arterial pH less than 7 within 60 min of birth and base deficit more than 16 mmol/L within 60 min of birth

Exclusion Criteria

Infants who met the following parameters are excluded from the study.

- Babies with major congenital anomalies
- Babies arriving after 24 hours of birth
- Babies enrolled in the therapeutic cooling study

Analysis

Neurological Examination

All neonates had a complete neurological examination and were classified as moderate or severe HIE as per the NICHD assessment. The examination was conducted by an onsite principal investigator. The scoring system consisted of six categories. The level of encephalopathy was assigned based on the severity of signs (moderate or severe) among the six categories. Seizures with normal, mild, or moderate encephalopathy were classified as "moderate encephalopathy", while seizures with severe encephalopathy were classified as "severe encephalopathy". All neonates had follow-up neurological examinations using the Hammersmith infant neurological examination until they were 3 months.

Sample Collection And Evaluation Of Serum S-100B

A venous blood sample (2 ml) was collected from all neonates at admission, 24 hours and 72 hours. Serum S-100B protein was

estimated using a commercially available kit (Serum S-100B – Calbiotech, USA) and values were recorded by Sandwich ELISA. Blood samples were collected again after 14 days and at 3 months of age and were evaluated for Serum S-100B protein. The mean serum concentrations (with standard deviation) were studied among neonates with normal and abnormal Serum S-100B protein values. The data were correlated with USG cranium findings, ECHO study, and MRI findings.

Magnetic Resonance Imaging

All neonates who survived beyond 2 weeks underwent an MRI at 14 days or later. MRI brain was correlated with Serum S-100B protein values and clinical examination by Hammersmith at 14 days and 3 months.

Pearson Correlation Coefficient

Pearson correlation coefficient was measured to understand the statistical relationship between the variables. The stronger the correlation, the greater the ability to influence the other biomarker with which there is a correlation (See table 1).

Table 1: Classification Of Correlation Co-efficient ®

Up to 0.1	Trivial Correlation
0.1-0.3	Small Correlation
0.3-0.5	Moderate Correlation
0.5-0.7	Large Correlation
0.7-0.9	Very Large Correlation
0.9- 1.0	Nearly Perfect correlation
1	Perfect correlation

ROC Curve Analysis

ROC curve analysis was performed to identify the predictability of study variables in the prognosis of the advanced HIE stage. The biomarkers with higher sensitivity and specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) suggest their correlation beyond their cut-off values to be prognostic markers for the neurological sequelae.

Table 2: Diagnostic Markers Based On Area Under The Receiver Operating Characteristic (AUROC)

0.9 - 1.0	Excellent test
0.8 - 0.9	Good test
0.7 - 0.8	Fair test
0.6 - 0.7	Poor test
0.5 - 0.6	Fair

Statistical Analysis

The predictive capacity of the biochemical indicators was evaluated by calculating sensitivity, specificity, positive and negative predictive values, and the area under the corresponding receiver operating characteristic curves. The area under the receiver operating characteristic curve quantifies the ability of the biochemical indicator to separate patients who experience adverse outcomes from patients who don't experience them. An AUROC value of 0.5 indicates that the test has no predictive power, whereas an area of 1.0 suggests that the test predicts the outcome perfectly every time.

The mean value was compared using the Mann-Whitney U test and Kruskal-Wallis one-way analysis of variance tests. Spearman's rank correlation coefficient was used to establish the correlation coefficient between biochemical indicators and stages of HIE. SPSS 10.0 software program was used for statistical analysis. Statistical significance was defined at P values of less than 0.05.

RESULTS:

Demographic Data Of The Patients

A total of 84 infants with encephalopathy were included in the study. The demographic and clinical characteristics of the study population are presented in Table 3. There were 35 male and 49 female neonates enrolled in the study. Out of the 84 neonates, 39 were of gestational age less than 38 weeks. Around 8 neonates had a birth weight of less than 2500 g and the other 76 neonates had birth weights between 2500 g and 3500 g. Only five neonates were small for gestational age.

Of the study population, 16 infants were delivered through the LSCS mode of delivery while the remaining infants were delivered through the normal route. Respiratory support was required in 79 neonates on admission. Oxygen saturation was less than 90 in 42 neonates upon admission and the mean arterial pressure was less than 30 mm in 76 neonates.

All three grades of encephalopathy were identified among the study population – 34 neonates with mild encephalopathy (HIE1), 37 neonates with moderate encephalopathy (HIE2), and 13 neonates with severe encephalopathy (HIE3).

All neonates had an APGAR score of less than 7 at 1 minute, which remained the same at 5 minutes in 28 patients. Around 54% of patients in both HIE2 and HIE3 groups had an APGAR score less than 7 at 5 min.

Table 3. Demographic Data

	Final Diagnosis			P value
	HIE 1 (n=34)	HIE 2 (n=37)	HIE 3 (n=13)	
Gender				
Female	24(70.6%)	19(51.4%)	6(46.2%)	0.162
Male	10(29.4%)	18(48.6%)	7(53.8%)	
Gestation age (weeks)				
36-37	19(55.9%)	12(32.4%)	8(61.5%)	0.070+
38-40	15(44.1%)	25(67.6%)	5(38.5%)	
Birth weight (g.)				
<2500	7(20.6%)	1(2.7%)	0(0%)	<0.001**
2500-3000	21(61.8%)	17(45.9%)	9(69.2%)	
3000-3500	6(17.6%)	19(51.4%)	4(30.8%)	
Mode of delivery				
LSCS	0(0%)	11(29.7%)	5(38.5%)	<0.001**
Vaginal	34(100%)	26(70.3%)	8(61.5%)	
APGAR score 1 min.				
<7	34(100%)	37(100%)	13(100%)	1.000
>7	0(0%)	0(0%)	0(0%)	
APGAR score 5 min.				
<7	1(2.9%)	20(54.1%)	7(53.8%)	<0.001**
>7	33(97.1%)	17(45.9%)	6(46.2%)	
Cord ABG (pH, Base deficit)				
<6.8	0(0%)	0(0%)	0(0%)	<0.001**
6.8-7.2	20(58.8%)	26(70.3%)	13(100%)	
>7.2	14(41.2%)	11(29.7%)	0(0%)	
Severity of encephalopathy @ admission (NICHD assessment)				
Mild	34(100%)	0(0%)	0(0%)	<0.001**
Moderate	0(0%)	37(100%)	0(0%)	
Severe	0(0%)	0(0%)	13(100%)	

** Chi-Square test/Fisher Exact test

Analysis of Serum S-100B protein

Baseline values of Serum S-100B protein are presented in table 4. Serum S-100B protein increase was correlated to an increase in the severity of HIE. There was an 80%-124% increase in mean concentrations of Serum S-100B protein as the severity progressed and a gradual decrease over 72 hours across the three grades of HIE patients.

Table 4. Baseline Investigations Of Serum Protein S-100B

	HIE 1	HIE 2	HIE 3
Upon admission**	11.50 ±1.50	19.57 ±5.24	23.69 ±1.03
24 hrs after admission**	10.09 ±1.22	17.07 ±2.57	22.62 ±2.33
72 hrs after admission**	9.35 ±1.74	11.57 ±2.10	16.77 ±2.17

P<0.001**, ANOVA test, P=0.03*

Correlation between Serum S-100B protein and MRI findings

The infants with abnormal MRI findings had a significant elevation of mean serum protein concentration of 62% upon admission, 54% after 24 hours, and 39% after 72 hours compared to infants with normal MRI findings (see Table 5). The serum protein concentrations decreased in the normal and abnormal MRI groups over 72 hours period.

Table 5. Serum Protein S-100B In Relation To MRI Changes

Serum Protein S-100B	MRI Changes		P value
	Normal	Abnormal	
Admission	13.08±4.40	21.20±4.28	<0.001**
24 hrs	12.01±3.81	18.51±3.84	<0.001**
72 hrs	9.68±1.78	13.45±3.20	<0.001**

Assessment of Serum S-100B protein in relation to Hammersmith

neurological examination.

Upon admission, it was seen that neonates with abnormal neurological findings had 81% higher mean serum protein concentration than normal neonates. These observations persisted at 24 hours and 72 hours. A 41% elevation was observed at 72 hours. At 3 months of age, there was a 57% elevation of Serum S-100B protein concentrations compared to neonates with normal findings.

The mean concentration of Serum S-100B protein decreased in infants with normal and abnormal neurological findings for 72 hours. However, a 51% elevation was observed in neonates with abnormal neurological findings (See table 6).

Table 6. Serum S-100B Protein In Relation To Hammersmith Neurological Examination

	Hammersmith Neurological Examination Of Neonates					
Serum protein S-100B	14 days			3 months		
	Normal	Abnormal	P value	Normal	Abnormal	P-value
Admission	11.57±1.49	20.98±4.69	<0.001**	14.84±5.26	23.24±2.30	<0.001**
24 hrs	10.26±1.41	18.73±3.38	<0.001**	13.18±3.96	20.86±3.02	<0.001**
72 hrs	9.31±1.70	13.10±3.04	<0.001**	10.13±2.00	15.52±2.52	<0.001**

ROC curve analysis

Serum S-100B protein exhibited high specificity and sensitivity. It was observed that Serum protein S-100 B has a cut-off value of >12 (See table 7) and the AUROC value was 0.958.

Table 7: ROC Curve Analysis

Variables	ROC Results To Predict Advanced Stage				Cut-off	AUROC	SE	P value
	Sensitivity	Specificity	LR+	LR-				
Serum protein S-100B	100.00	76.47	4.25	0.00	>12	0.958	0.018	<0.001**

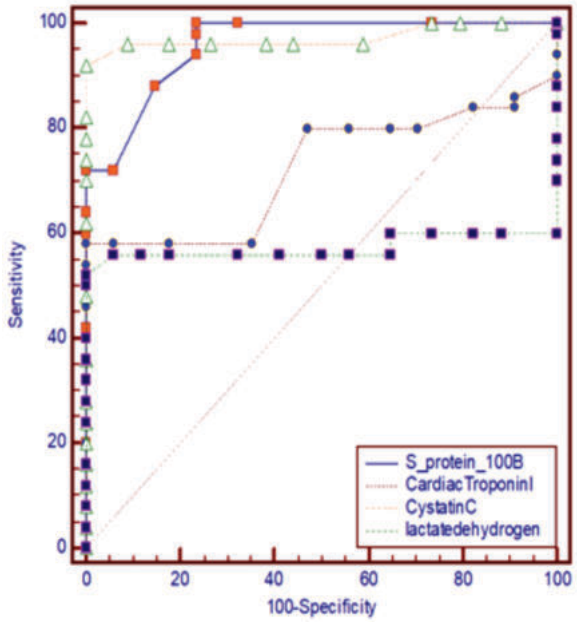

Figure 1: Sensitivity And Specificity Of Serum S-100B Protein

INFERENCE

Based on these observations it can be concluded that Serum S-100B protein could be a reliable biomarker in predicting neurological sequelae. Further investigations are needed to confirm these preliminary findings and evaluate if these biomarkers, alone or in combination with other candidate brain-specific proteins, offer predictive value. Additional covariables associated with secondary injuries, such as seizures, fever, hypotension, and hypoxia will also need to be considered as these will also have the potential to affect biomarker trajectory.

DISCUSSION

In the present study, Serum protein S-100 biomarker were shown to have high specificity and sensitivity values, making them more trustworthy biomarkers for the prognosis of neurological events. Peripheral blood will only recognize S100B due to its molecular weight.[22] After the brain has suffered hypoxic ischemia damage, it is mostly discharged from the astroglial cells, and the continued glial cell death will raise the blood concentrations of S100B protein.[23] Glia cells are also essential for the proper functioning of the central nervous system, as they control how the brain develops and maintain cellular homeostasis. According to studies, disturbed blood-brain barrier integrity, renal secretion, or brain volume redistribution all have an impact on serum S-100B concentration. As a result, its level will positively correlate with brain damage, including subarachnoid haemorrhage, intracerebral hematoma, subdural hematoma, and cerebral hypoxia.[22]

Neonatal hypoxia causes the blood barrier to break down, which causes the S-100B protein to continuously leak into the blood and urine and raise the amount of S-100B concentration. The extracerebral sources of S-100B do not lead to a rise in serum concentrations, despite the fact that this protein will originate from other organs, such as muscle, the heart, broken bones, and adipose tissue.[24] Thus, the serum S-100B protein is characterized as a particular protein of brain injury. Overproduction of S-100B can exacerbate neuroinflammation and neuronal dysfunction. It functions as a neurotrophic factor and a protein essential for the survival of neurons.

Majority of human investigations sought to identify a simple-to-measure biomarker of central nervous system impairment that might be used as an early detection tool. Studies on stroke have revealed that 2 to 3 days after an acute stroke, serum S100B levels peaked.[25] The S-100B peak was said to occur 24 hours after hypoxic brain injury brought on by cardiopulmonary bypass and extracorporeal circulation.[26] S-100B in urine, umbilical arterial blood, peripheral arterial blood, saliva, CSF, and serum have all been the subject of comparative research.[27] In the present study, the infants who showed abnormal on MRI had significant elevation of mean serum protein concentration of 62% compared to normal infants upon admission and 54% elevation after 24 hrs and 39% elevation is still evident after 72 hrs which is almost equivalent to normal serum protein concentrations upon admission. Additionally, abnormal neonates show 81% higher mean serum protein concentration than normal neonates which is persistent at 24 hrs but is still 41% elevation at 72 hrs in abnormal neonates. At 3 months of age, the higher mean serum protein concentrations of abnormal infants are around 57% compared to normal upon admission.

The present study based on ROC curve analysis demonstrated that Serum protein S-100 B has a cut off value of >12. On the other hand, AUROC value of 0.958 for Serum protein S-100B. The results suggest a high sensitivity and specificity values for these biomarkers. Similarly, in another study the sensitivity of S-100B 12 g/L was 50%, specificity was 85 per cent, positive predictive value (PPV) was 82 per cent, and the negative predictive value (NPV) was 55% for predicting newborn outcomes as measured as moderate or severe HIE.[28] A recent meta-analysis including six of the original 1620 studies mentioned that Serum S-100B had an overall diagnostic sensitivity of 0.80 and a specificity of 0.79. Although an elevated blood S-100B level at 24 hours after birth can indicate brain injury, it shouldn't be the only factor used to forecast the result of Perinatal Asphyxia.[27]

In a study, there was a significant increase in protein S100 levels in the serum of infants with birth asphyxia, which was most marked in infants with moderate and severe HIE. Neonatal death and cerebral palsy at 18 months were associated with increased S-100 levels, which were inversely correlated with the infants' perinatal pH and related with abnormal cardiotocography (CTG) at hospital admission.[29] Depending on blood samples taken from newborns in the first few days after their birth, this study was conducted. The affected infants' S100 readings were highest on day 1 and lowest on days 2 and 3, but still clearly higher than controls like the present study. This could be a sign of continued cell death, consistent with secondary damage, consistent with prolonged apoptosis and/or necrosis several hours after the initial brain injury.[30] Repeated values may therefore be helpful in predicting the subsequent brain injury.[28] Therefore, in the present study serum

biomarkers were assessed at 14 days or later after birth asphyxia and at three months of age to determine the neurological outcome.

In the present study, all patients were assessed with APGAR score at 1 minute, as <7 and at 5 minutes the score is <7 in 28 patients. Around 54% of patients in both HIE2 and HIE3 groups still have APGAR score <7 at 5 min. The newborn's physical health at birth is reflected by the Apgar score, a clinical indicator. Low Apgar scores and the requirement for cardiac resuscitation at birth can result from perinatal hypoxia, as well as other risk factors such as severe infections, premature birth, and maternal analgesia.[37] While the majority of infants respond to resuscitation techniques quickly and have excellent outcomes, newborns who do not respond within the first 10 minutes of life have a significant risk of surviving with serious problems.[37]

The present study MRI findings revealed that there is a significant incidence of oedema and ventricular dilatation in HIE2 group patients and haemorrhage (parasagittal and parenchymal also) owing to the elevated levels of biomarkers such as Serum protein S-100B. One of the best imaging techniques for detecting brain damage in asphyxiated term infants is magnetic resonance imaging (MRI). MRI has superior resolution, sensitivity, and specificity when detecting cerebral hypoxic-ischemic lesions compared to CT. It can identify between 75 and 100 percent of asphyxia-related cerebral lesions, especially those that involve the thalamus, basal ganglia, and white matter.[38] The benefit of MRI is that it shields newborns from ionizing radiation. On the other hand, because of the lengthy scanning period, sedation is frequently necessary while performing an MRI on a newborn. By the third day of life, brain lesions may be discovered using MRI, and over the next two weeks, they may grow more obvious and well-defined. It was discovered that an MRI from the second week after delivery might foretell how newborns with HIE will fare.[39] In neonates with HIE who were receiving therapeutic hypothermia, a recent cohort research discovered a significant association between initial (4th day of life) and later (second week of life) sequential MRI examinations. It has been demonstrated that there is a substantial correlation between the localization, extent, and intensity of hypoxic-ischemic brain injury in the two scans. The findings of this study indicate that MRI may be a helpful predictive tool in the early stages of life.[40] Documenting damage to the basal ganglia, internal capsule, white matter, brainstem, and cortex requires research lasting between 7 and 21 days. It can aid in the early detection of comorbidities or consequences including infarction, bleeding, and abnormalities when used in connection with cranial ultrasonography. Research has been done on the development of diffusion anomalies on MRI in term infants receiving therapeutic hypothermia (the current course of treatment) after HIE.[41] The aforementioned resulted in the creation of an MRI injury grading system. In the Bayley-linguistic, III's motor, and cognitive domains, worse outcomes were substantially correlated with higher MRI damage grades.[42] For prognostic value, the time period of imaging is crucial. In infants receiving TH treatment, late MRI scans performed at a median age of 8 days exhibited a 95% sensitivity, 94% specificity, and 91% positive predictive value for mortality or impairment at age 2.[43] Early brain scans can overestimate the seriousness of injury since, in comparison, cell death is still growing during the first few days of birth.[44] Other more sophisticated methods, such as tractography and diffusion tensor imaging, may detect brain lesions earlier.[45]

The goal of the early therapy of asphyxiated babies after arrival to the neonatal intensive care unit (NICU) is to prevent injury from secondary events associated with hypoxia ischemia rather than the pathophysiologic sequence that leads to hypoxic-ischemic brain injury. It has been demonstrated that in order to prevent or lessen the persistent brain injury in asphyxiated babies, early diagnosis and treatment of the most frequent events that could exacerbate brain damage, as well as effective and immediate supportive intensive care, are crucial. The management of this group of neonates must include ensuring regular sugar levels, haemoglobin, and electrolyte levels values, correcting blood gases, and addressing changes in acid-base status and lung and heart support.[46] There is consensus on the possibility of therapeutic neuroprotective options following birth asphyxia. There is a treatment window following the onset of the neurotoxicity cascade before secondary energy failure begins and more serious brain damage is developed.[30] Specific techniques for identifying the infants who are most at risk for poor neurologic outcomes are needed in order to choose the children for premature interventional cerebral protective treatment.

The present study has certain limitations. S-100B will be helpful as a biomarker in upcoming research due to its reliability of measurement, although there are methodological issues with measuring serum S-100B. The quantitative measuring approach for the S100B value is not yet developed enough, which could lead to measurement error. Studying the dynamic monitoring technique of S value in this area is both possible and important. Investigating if the patient's time spent in hypoxia has an impact on the S-100B value is also necessary. What time of day do the cytokines peak and when do they interact with the PA which we should discuss in the future? It is critical to recognize that the majority of biomarkers have undergone testing within clearly defined cohorts. They would probably lose some of their predictive power in more varied cohorts. It is not unexpected given these problems that just clinical evaluation and EEG monitoring are frequently utilized.

CONCLUSION

Biomarkers (BMs) can be useful in a variety of contexts, such as early treatment enrollment within 6 hours of birth, monitoring infant response to treatment, and assessing the prognosis following treatment termination for parental counselling and enrollment in developmental support services. Each objective has unique restrictions. Thus, in order to enhance the short- and long-term prognosis of HIE newborns, barriers for neonatologists, biochemists, and neuroscientists will require the adoption of additional treatment approaches in addition to HT. In this context, MRI and biomarkers (BM) will be the most effective instruments for long-term monitoring of treatment efficacy. Therefore, it is envisaged that BMs will provide additional information that will aid doctors in: i) predicting the course of the disease, ii) identifying injured brain regions, and iii) pinpointing the date of the injury, revealing insight on the likely pathogenesis and best course of treatment.

REFERENCES:

1. Thelin EP, Nelson DW, Bellander BM. Secondary peaks of S100B in serum relate to subsequent radiological pathology in traumatic brain injury. Neurocrit Care. 2014;20:217-229.
2. Nguyen DN, Huyghens L, Wellens F, Schiettecatte J, Smitz J, Vincent JL. Serum S100B protein could help to detect cerebral complications associated with extracorporeal membrane oxygenation (ECMO). Neurocrit Care. 2014;20:367-374.
3. Pham N, Fazio V, Cucullo L, et al. Extracranial sources of S100B do not affect serum levels. PLoS One. 2010:5:e12691
4. Elting JW, de Jager AEJ, Teelken AW, et al. Comparison of serum S-100 protein levels following stroke and traumatic brain injury. J Neurol Sci. 2000;181:104-110
5. Bötiger BW, Möbes S, Glätzer R, et al. Astroglial protein S-100 is an early and sensitive marker of hypoxic brain damage and outcome after cardiac arrest in humans. Circulation. 2001;103:2694-2698.
6. Luo et al., The Role of S100B Protein at 24 Hours of Postnatal Age as Early Indicator of Brain Damage and Prognostic Parameter of Perinatal Asphyxia, Global Pediatric Health, 2019, 6: 1–7
7. Thorngren-jerneck et al., S100 Protein in Serum as a Prognostic Marker for Cerebral Injury in Term Newborn Infants with Hypoxic Ischemic Encephalopathy, Vol. 55, No. 3, 2004
8. Rosen H, Rosengren L, Herlitz J, Blomstrand C 1998 Increased serum levels of the S-100 protein are associated with hypoxic brain damage after cardiac arrest. Stroke 29:473–477
9. Wyatt JS, Edwards AD, Azzopardi D, Reynolds EO 1989 Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury. Arch Dis Child 64(7 Spec No):953–963
10. Halvorsen CP, Olson L, Araújo AC, Karlsson M, Nguyễn TT, Khu DTK, Le HTT, Nguyễn HTB, Winbladh B and Russom A: A rapid smartphone-based lactate dehydrogenase test for neonatal diagnostics at the point of care. Sci Rep 9: 9301, 2019
11. Lv H, Wang Q, Wu S, Yang L, Ren P, Yang Y, Gao J and Li L: Neonatal hypoxic ischemic encephalopathy-related biomarkers in serum and cerebrospinal fluid. Clin Chim Acta 450: 282-297, 2015.
12. Turker G, Babaoglu K, Gökalp AS, Sarper N, Zengin E, Arisoy AE. Cord blood cardiac Troponin I as an early predictor of short term outcome in perinatal hypoxia. Biol Neonate. 2004;86:131-7.
13. Kanik E, Ozer EA, Bakiler AR, Aydinlioglu H, Dorak C, Dogrusoz B, Kanik A, Yaprak I. Assessment of myocardial dysfunction in neonates with hypoxic-ischemic encephalopathy: is it a significant predictor of mortality? J Matern Fetal Neonatal Med. 2009;22(3):239-42.
14. Agrawal J, Shah GS, Poudel P, Baral N, Agrawal A, Mishra OP. Electrocardiographic and enzymatic correlations with outcome in neonates with hypoxic-ischemic encephalopathy. Ital J Pediatr. 2012;38:33
15. Treiber M, Gorenjak M, Balon BP. Serum Cystatin-C as a Marker of Acute Kidney Injury in the Newborn After Perinatal Hypoxia/ Asphyxia. Ther Apher Dial. 2014;18(1):57-67.
16. Antonucci R, Porcella A and Pilloni MD: Perinatal asphyxia in the term newborn. J Pediatr Neonat Individual Med 3: e030269, 2014
17. Kudrevičienė A, Lukoševičius S, Laurynaitienė J, Marmienė V, Tamelienė R, Basevičius A. Ultrasonography and Magnetic Resonance Imaging of the Brain in Hypoxic Full-Term Newborns. Medicina (Kaunas). 2013;49(1):42-9
18. Rutherford M, Malamateniou C, McGuinness A, Allsop J, Biarge MM, Counsell S. Magnetic resonance imaging in hypoxic-ischemic encephalopathy. Early Hum Dev. 2010;86:351-60.
19. Agut T, León 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
20. Bednarek, N.; Mathur, A.; Inder, T.; Wilkinson, J.; Neil, J.; Shimony, J. Impact of therapeutic hypothermia on MRI diffusion changes in neonatal encephalopathy. Neurology 2012, 78, 1420–1427
21. Groenendaal, F.; De Vries, L.S. Fifty years of brain imaging in neonatal encephalopathy following perinatal asphyxia. Pediatr. Res. 2017, 81, 150–155.
22. Tharmapoopathy P, Chisholm P, Barlas A, Varsami M, Gupta N, Ekitzidou G, et al. In

clinical practice, cerebral MRI in newborns is highly predictive of neurodevelopmental outcome after therapeutic hypothermia. Eur J Paediatr Neurol. 2020;25:127–33
23. Imai K, de Vries LS, Alderliesten T, Wagenaar N, van der Aa NE, Lequin MH, et al. MRI changes in the thalamus and basal ganglia of full-term neonates with perinatal asphyxia. Neonatology. 2018;114(3):253–60.
24. 'Dea M, Sweetman D, Bonifacio SL, El-Dib M, Austin T, Molloy EJ. Management of multi organ dysfunction in neonatal encephalopathy. Front Pediatr. 2020;8:239.
25. Agarwal R, Jain A, Deorari AK, Paul VK. Post-resuscitation Management of Asphyxiated Neonates. Indian J Pediatr. 2008;75(2):175-80.
26. Zhu Y, Yun Y, Jin M, Li G, Li H, Miao P, Ding X, Feng X, Xu L, Sun B. Identification of novel biomarkers for neonatal hypoxic-ischemic encephalopathy using iTRAQ. Italian Journal of Pediatrics. 2020 Dec;46(1):1-9.
27. Auriti C, Prencipe G, Inglese R, Moriondo M, Nieddu F, Mondì V, Longo D, Bucci S, Del Pinto T, Timelli L, Di Ciommo VM. Mannose Binding Lectin, S100 B Protein, and Brain Injuries in Neonates With Perinatal Asphyxia. Frontiers in pediatrics. 2020:527.
28. Doandes, F.M., Manea, A.M., Lungu, N., Cioboata, D., Brandibur, T., Costescu, O., Hudisteanu, A., Boia, E.R. and Boia, M., 2021. Clinical, biological and electroencephalographic monitoring of newborns with neurological risk in the Neonatal Intensive Care Unit. *Experimental and therapeutic medicine*, *22*(1), pp.1-7.
29. Graham EM, Burd I, Everett AD, Northington FJ. Blood biomarkers for evaluation of perinatal encephalopathy. Frontiers in pharmacology. 2016 Jul 13;7:196.
30. Merchant N, Azzopardi D. Early predictors of outcome in infants treated with hypothermia for hypoxic–ischaemic encephalopathy. Developmental Medicine & Child Neurology. 2015 Apr;57:8-16.
31. Riljak V, Kraf J, Daryanani A, Jiruška P, Otáhal J. Pathophysiology of perinatal hypoxic-ischemic encephalopathy-biomarkers, animal models and treatment perspectives. Physiological research. 2016 Dec 2;65.
32. Guingab-Cagmat J, Cagmat E, Hayes RL, Anagli J. Integration of proteomics, bioinformatics, and systems biology in traumatic brain injury biomarker discovery. Frontiers in neurology. 2013 May 31;4:61.
33. Lv H, Wang Q, Wu S, Yang L, Ren P, Yang Y, Gao J, Li L. Neonatal hypoxic ischemic encephalopathy-related biomarkers in serum and cerebrospinal fluid. Clinica chimica acta. 2015 Oct 23;450:282-97.
34. Gazzolo D, Abella R, Marinoni E, Di Iorio R, Li Volti G, Galvano F, Frigiola A, Temporini F, Moresco L, Colivicchi M, Sabatini M. New markers of neonatal neurology. The Journal of Maternal-Fetal & Neonatal Medicine. 2009 Jan 1;22(sup3):57-61.
35. Michetti F, Corvino V, Geloso MC, Lattanzi W, Bernardini C, Serpero L, Gazzolo D. The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. Journal of neurochemistry. 2012 Mar;120(5):644-59.
36. 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 paediatrica. 2012 Mar;101(3):319-23.
37. Ishweki A, Pérez-Muñuzuri A, López-Suárez O, Baña A, Couce ML. Relevance of urinary S100B protein levels as a short-term prognostic biomarker in asphyxiated infants treated with hypothermia. Medicine. 2017 Nov;96(44).
38. Massaro AN, Jeromin A, Kadom N, Vezina G, Hayes RL, Wang KK, Streeter J, Johnston MV. Serum biomarkers of MRI brain injury in neonatal hypoxic ischemic encephalopathy treated with whole-body hypothermia: a pilot study. Pediatric critical care medicine: a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2013 Mar;14(3):310.