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Article

Serum Neuron-Specific Enolase and Lactate Predict Hypoxic-Ischemic Encephalopathy in Asphyxiated Neonates: A Retrospective Cohort Study

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Abstract: Background: Hypoxic-ischemic encephalopathy (HIE) is one of the most severe complications of perinatal asphyxia, which is related to high mortality and permanent neurological impairments. Current clinical tools for early identification of at-risk infants lack sensitivity and objectivity, underscoring the urgent need for accessible biomarkers that can guide timely intervention. Methods: This retrospective cohort study was conducted at the Tenth Affiliated Hospital of Southern Medical University from 2020 to 2025. The study cohort consisted of 57 neonates with perinatal asphyxia. 28 of the infants had the diagnosis of HIE and 29 of the infants didn't have the diagnosis of HIE. Clinical characteristics, laboratory parameters, and Weeke MRI scores were compared between the two groups, and multivariable logistic regression analysis identified independent risk factors for HIE. Results: Compared with controls, the HIE group showed higher elevations in serum NSE (median 73.15 vs 45.80 ng/mL, $P < 0.0001$) and blood lactate (mean 11.63 ± 2.702 vs 8.800 ± 3.453 mmol/L, $P = 0.0014$). Multivariable analysis confirmed both NSE (aOR = 1.047, 95% CI: 1.013–1.082, $P = 0.007$) and lactate (aOR = 1.280, 95% CI: 1.015–1.614, $P = 0.037$) as independent predictors of HIE, with a Nagelkerke R^2 of 0.486. Conclusions: Serum NSE measured at 24–72 hours and blood lactate measured within the first hour postpartum were independent factors associated with HIE in asphyxiated neonates. NSE, as a direct marker of neuronal injury, may serve as a valuable early biomarker for risk stratification.

Keywords: Perinatal Asphyxia; Hypoxic-ischemic encephalopathy; Neuron-specific enolase; Lactate; Neonate

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1. Introduction

The incidence of perinatal asphyxia of live infants in industrialized countries is expected to be in the range between 1% and 1.5%, while in developing areas the frequencies are substantially increased [1, 2]. The most severe complication of perinatal asphyxia is hypoxic-ischemic encephalopathy (HIE) with high morbidity and mortality [3]. Of the ones that survive, about one third suffer from permanent neurodevelopmental disorders, like for instance deficit in the cognition and motor function, cerebral palsy, epilepsy, also with sensory disorders [4-7]. Even if neonatal intensive care has progressed and therapeutic hypothermia is used for moderate to severe instances [8], reliable biomarkers for early and objective prediction of neurological outcomes in asphyxiated neonates remain unavailable in clinical practice [9].

The clinical diagnosis of HIE relies primarily on the modified Sarnat staging system, which incorporates the autonomic function, reflexes, the muscle tone and the level of consciousness [10]. Decades of use have established the modified Sarnat staging system as the standard clinical approach for HIE diagnosis. However, its limitations are known. As it is pointed out in a systematic review by Caramelo et al. the lack of the instruments

to measure objectively in the first important hours after the birth hampers the time of a diagnosis [11]. Besides, the neurological signs associated with HIE can be very faint, particularly in less severe cases and can be masked by the sedative agents or by the other medical treatment at the time [12]. Moreover, to do dependable neurological assessments one needs a seasoned practitioner and it is in itself subjective [13]. These are the reasons that make so important the search for objective, easily quantifiable biomarkers that can complement the clinical judgment and that can allow an earlier detection of the infant at risk.

At present, cranial magnetic resonance imaging (MRI) is the gold standard for the assessment of brain damage of HIE. Different MRI based scoring methodologies have been set up to measure the extent of such injuries. In particular, the Weeke scoring that was introduced in 2016, has better inter-rater consistency (reported coefficient of 0.9) and makes better predictions of neurodevelopmental outcomes than previous such models as the Barkovich score [14]. However, MRI is not universally available in the acute setting, and ischemic alterations may not be visible on the scans done in the first days after birth [15]. These practical limitations highlight the need for complementary tools that can be deployed earlier.

Neuron-specific enolase (NSE) is a glycolytic enzyme predominantly located in neurons and neuroendocrine cells [16]. Upon neuronal injury, NSE is released into the circulation, making it a potentially useful marker of brain damage. Caramelo et al. showed that NSE is among the most promising biomarkers associated with unfavorable outcomes in HIE neonates [11]. Similarly, blood lactate, a product of anaerobic metabolism, reflects the degree of tissue hypoxia and has been associated with adverse outcomes in neonates following perinatal asphyxia [17].

Most previous studies have compared HIE neonates with healthy controls, which does not adequately address risk stratification in asphyxiated neonates. The key clinical challenge is to distinguish, among asphyxiated infants, those who will develop HIE from those who will not. Predicting HIE remains difficult, and no single biomarker has proven reliable for early risk stratification [18]. Serum NSE combined with MRI and aEEG has been shown to improve prediction of neurodevelopmental outcomes [19], and blood lactate levels predict adverse outcomes in this population [20]. Furthermore, lactate remains a significant predictor of neurological outcomes even in the post-hypothermia era, whereas traditional HIE staging loses its predictive value in cooled infants [9]. Nevertheless, the independent predictive value of these markers in the specific context of asphyxiated neonates remains incompletely characterized. Therefore, further investigation is warranted to clarify the role of NSE and lactate in early risk stratification for this high-risk population.

This study therefore aimed to compare serum NSE and blood lactate levels between asphyxiated neonates with and without HIE, and to determine their independent association with HIE after adjusting for potential confounders.

2. Methods

2.1. Study Design and Participants

This retrospective cohort study was conducted at the Tenth Affiliated Hospital of Southern Medical University between January 2020 and December 2025. Consecutive surviving neonates with perinatal asphyxia and a gestational age ≥ 35 weeks were eligible for enrollment. The study protocol was approved by the Clinical Research and Ethics Committee of the Tenth Affiliated Hospital, Southern Medical University (approval IRBIITKY-2026-264-01), and waived the requirement for informed consent because of its retrospective nature.

Perinatal asphyxia was diagnosed when at least two of the following conditions were met: (1) an Apgar score below 5 at five minutes; (2) a base excess < -12 mmol/L during the first hour after birth; (3) the need for endotracheal intubation with intermittent positive-pressure ventilation that continued until ten minutes after birth; (4) the development of

multiorgan failure within the first 24 hours of life; or (5) signs of fetal compromise, including thick meconium in the amniotic fluid and/or abnormal cardiotocographic findings such as sustained fetal bradycardia below 100 beats per minute, late decelerations accompanied by reduced variability, or severe recurrent decelerations with diminished variability [21].

Inclusion criteria were: (1) diagnosis of perinatal asphyxia according to the above definition; (2) availability of complete clinical and laboratory data; and (3) interpretable cranial MRI images. Exclusion criteria were: (1) major congenital anomalies; (2) inherited metabolic disorders; (3) confirmed congenital infections; (4) intraventricular hemorrhage grade III or IV; and (5) incomplete data for key variables.

2.2. Group Assignment

Neonates meeting the ACOG-AAP criteria for HIE were assigned to the HIE group [22]. Asphyxiated neonates without HIE were assigned to the control group.

2.3. Data Collection

Two investigators independently extracted data from electronic medical records using a standardized case report form. Discrepancies were adjudicated by a third reviewer. The following variables were recorded: gestational age, birth weight, sex, mode of delivery, history of intrauterine distress, umbilical arterial pH and blood lactate within the first hour of life, 5-minute Apgar scores, HIE severity graded according to the modified Sarnat staging system [23], administration of therapeutic hypothermia, serum NSE levels measured at 24–72 hours of life via electrochemiluminescence immunoassay (Roche Diagnostics, Basel, Switzerland), and cranial MRI findings.

2.4. MRI Acquisition and Scoring

Cranial MRI was performed using a 1.5T scanner (Siemens Magnetom, Erlangen, Germany). MRI abnormalities were quantified using the Weeke scoring system, which comprises three subscores for gray matter (basal ganglia and thalamus), white matter, and cerebellum, with a total score ranging from 0 to 57[14]. All MRI scans were independently reviewed by two experienced pediatric radiologists who were blinded to clinical information, following the methodology of previous MRI scoring studies [14].

2.5. Statistical Analysis

All the statistical analyses were carried out using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). For the continuous variables, normality was tested by using the Shapiro-Wilk test. For data with a normal distribution, they are shown as mean ± standard deviation (SD). Differences between the groups were tested by the independent-samples t-test. For non-normally distributed variables, the results are reported as median and interquartile range (IQR), with the Mann–Whitney U test applied for comparisons. Categorical variables are described by frequencies and percentages, and were analyzed using either the chi-square test or Fisher's exact test, according to the appropriateness.

Variables with a univariate P value < 0.1 were considered for the multivariable binary logistic regression analysis. Because the sample size was limited, the final model included NSE and lactate only. The model fitness was checked with the Hosmer-Lemeshow test for goodness-of-fit and with the Nagelkerke R^2 statistic. Results are shown as adjusted odds ratios (aOR) and their corresponding 95% confidence intervals (CI).A two-tailed P value lower than 0.05 was considered as statistical significance. The study followed the STROBE guidelines for cohort studies [24].

3. Results

3.1. Baseline Characteristics

A total of 57 neonates with perinatal asphyxia were included in the final analysis: 28 in the HIE group and 29 in the control group. Baseline characteristics of both groups are shown in Table 1. The two groups were well matched regarding gestational age (P = 0.7542), birth weight (P = 0.6050), sex distribution (P = 0.5648), mode of delivery (P = 0.2924), and history of intrauterine distress (P > 0.9999). According to Sarnat staging, 24

HIE cases (85.7%) were classified as mild, 3 (10.7%) as moderate, and 1 (3.6%) as severe. Therapeutic hypothermia was administered to 25 (89.3%) neonates in the HIE group, with a median time to initiation of 4 hours (IQR: 3–6).

Table 1. Baseline Characteristics of HIE and Control Groups

Characteristic	HIE Group (n=28)	Control Group (n=29)	P Value
Perinatal factors			
Gestational age (weeks), median (IQR)	38.50 (37.08–39.95)	39.20(37.05–40.10)	0.7542
Birth weight (g), mean ± SD	2993 ± 552.2	2910 ± 641.0	0.6050
Male, n (%)	21 (75.0)	19 (65.5)	0.5648
Cesarean section, n (%)	11 (39.3)	16 (55.2)	0.2924
Intrauterine distress, n (%)	15 (53.6)	16 (55.2)	>0.9999
Umbilical arterial pH, median (IQR)	7.130 (6.980–7.160)	7.180 (7.135–7.235)	0.0005
5-minute Apgar score, median (IQR)	7.500 (7.000–8.000)	8.000 (7.000–9.000)	0.1070
Laboratory parameters			
Serum NSE (ng/mL), median (IQR)	73.15 (61.68–108.2)	45.80 (34.85–60.37)	<0.0001
Blood lactate (mmol/L), mean ± SD	11.63 ± 2.702	8.800 ± 3.453	0.0014
MRI findings			
Weeke total score, median (IQR)	1.000 (0.250–2.000)	1.000 (0.000–1.000)	0.1986
Clinical classification			
HIE severity (Sarnat), n (%)			
– Mild	24 (85.7)	N/A	-
– Moderate	3 (10.7)	N/A	-
– Severe	1 (3.6)	N/A	-
Therapeutic hypothermia, n (%)	25 (89.3)	N/A	-

IQR: interquartile range; SD: standard deviation; NSE: neuron-specific enolase; N/A: not applicable.Data are presented as median (IQR), mean ± SD, or n (%). P values were calculated using the Mann-Whitney U test, independent-samples t-test, or Fisher's exact test as appropriate. Bold P values indicate statistical significance ($P < 0.05$).

3.2. Laboratory and Imaging Findings

Table 1 also summarizes the laboratory and imaging parameters between the two groups. As shown in Figure 1A, serum NSE levels were markedly higher in the HIE group compared with the control group (median: 73.15 vs. 45.80 ng/mL; $P < 0.0001$). Similarly, as shown in Figure 1B, blood lactate levels were significantly elevated in the HIE group (mean: 11.63 ± 2.702 vs. 8.800 ± 3.453 mmol/L; $P = 0.0014$), while umbilical arterial pH was significantly lower (median: 7.130 vs. 7.180; $P = 0.0005$). No significant differences were observed between the groups for 5-minute Apgar scores (median: 7.500 vs. 8.000; $P = 0.1070$) or Weeke MRI scores (median: 1.000 vs. 1.000; $P = 0.1986$).

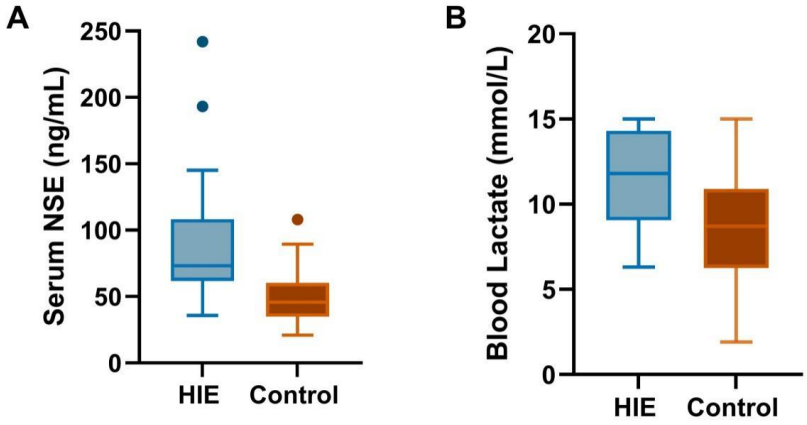


Figure 1. NSE and Lactate in HIE Versus Controls. (A) Serum NSE Was Higher in HIE Than Controls (Median 73.15 Vs 45.80 ng/mL; P < 0.0001, Mann-Whitney U Test). (B) Blood Lactate Was Higher in HIE Than Controls (Mean 11.63 ± 2.702 Vs 8.800 ± 3.453 mmol/L; P = 0.0014, *T*-Test). Boxes Show Interquartile Range; Horizontal Lines Show Medians; Whiskers Show 1.5×IQR. HIE, Hypoxic-Ischemic Encephalopathy; NSE, Neuron-Specific Enolase.

3.3. Multivariable Logistic Regression Analysis

Variables with a P value < 0.1 in the univariate analysis-NSE, blood lactate, and umbilical arterial pH-were considered for the multivariable model. Because the sample size was limited, the primary model included NSE and lactate only to maintain model parsimony and avoid overfitting. As shown in Table 2, multivariable logistic regression identified both serum NSE (aOR = 1.047, 95% CI: 1.013–1.082, P = 0.007) and blood lactate (aOR = 1.280, 95% CI: 1.015–1.614, P = 0.037) as independent risk factors for HIE. The Hosmer-Lemeshow test yielded a P value of 0.023, and the model had a Nagelkerke R^2 of 0.486 with an overall predictive accuracy of 77.2%.

Table 2. Multivariable Logistic Regression Analysis of Risk Factors for HIE

Variable	B	S.E.	Wald	df	P Value	aOR	95% CI
Serum NSE (ng/mL)	0.046	0.017	7.398	1	0.007	1.047	1.013–1.082
Blood lactate (mmol/L)	0.247	0.118	4.362	1	0.037	1.280	1.015–1.614
Constant	-5.487	1.573	12.166	1	<0.001	0.004	-

aOR, adjusted odds ratio; CI, confidence interval; NSE, neuron-specific enolase. Model summary: Nagelkerke R^2 = 0.486; Hosmer–Lemeshow test P = 0.023 (calibration imperfect); Overall predictive accuracy = 77.2%. Bold P values indicate statistical significance (P < 0.05).

4. Discussion

In this retrospective cohort study of 57 asphyxiated neonates, we found that both serum NSE and blood lactate were significantly elevated in those who developed HIE compared with those who did not. Multivariable analysis confirmed both markers as independent risk factors for HIE. NSE demonstrated a more precise effect estimate (aOR = 1.047, 95% CI: 1.013–1.082, P = 0.007) than lactate, suggesting it may be the more reliable marker for clinical use. The Hosmer-Lemeshow test yielded a P value of 0.023, indicating imperfect calibration of the model. In the context of a modest sample size (n=57), this finding may be unstable, and the result should be interpreted with caution. We therefore consider the model exploratory. Despite this calibration limitation, NSE and lactate remained independently associated with HIE, supporting their potential utility for early risk stratification.

4.1. NSE as a Predictor of HIE

The strong correlation found between the level of NSE and HIE in our study population should be studied in detail. One of the main advantages of this work is the composition of the control group: instead of using healthy newborns, we used asphyxiated infants who did not develop HIE for comparison. This way of methodological choice is more closely in the clinical reality because the thing that physicians have to do is to find which of the asphyxiated neonates are more likely to go to HIE. The fact that NSE is an independent predictor even in such a narrow comparative framework shows that it could be useful in clinical practice. Previously, Mooney et al. showed that the combination of various clinical and biochemical factors improves the prediction of the risk of HIE [18]. Our results add to this in the sense that we show that NSE is a very different and independent value as a direct indicator for the evaluation of the risk of HIE.

Another point that deserves to be particularly emphasized: although 85.7% of HIE cases presented with a mild clinical features, the difference of NSE in the groups was statistically significant ($P < 0.0001$). It means that the increases of the NSE can be before the clinical identification and the radiological visualization of the neural damages. As it has been suggested before, MRI anomalies are present in as many as 61% of the infants with the diagnosis of the mild HIE [25].But these alterations are often subtle and they may not produce in important variations in Weeke scores. In an evaluation of three different MRI scoring methodology for the mild HIE, Machie et al. reported the detection rates of 57% with the Weeke score, 7% with the Barkovich score and 24% via the NICHD NRN score [26]. In particular, even if using the more sensitive of these assessment tool, in 43% of the mild HIE no abnormalities were found [26]. The absence of a statistically significant difference in Weeke scores between the cohorts in our study ($P = 0.1986$) is compatible with this observation, it means that the MRI can bypass the subtle injuries that NSE which reflect the biochemical impairment before structural modification, is able to detect.

The 95% confidence interval for NSE is very narrow, from 1.013 to 1.082, which is a sign of a very stable effect estimate. In fact, every 1 ng/mL rise in NSE was associated with 4.7% rise in the odds of HIE. Although the sample was small, the relatively narrow confidence interval suggests a precise estimate in this cohort.But this finding requires validation in larger studies. In an analogous study, Huang et al. demonstrated that serum NSE levels at day 3 of life were significantly associated with neurodevelopmental outcomes at 18 months, with NSE levels of 20.52 ± 6.42 μg/L in the normal outcome group compared with 39.82 ± 5.92 μg/L in the moderate delay group and 44.60 ± 9.01 μg/L in the severe delay group ($P < 0.001$) [19].

Our findings are consistent with recent evidence. A 2023 systematic review by Caramelo et al. concluded that serum NSE levels correlate with unfavorable outcomes in HIE neonates, noting that elevated NSE was associated with worse prognosis following perinatal asphyxia [11]. Huang et al. reported that serum NSE, when combined with MRI and aEEG, significantly improved the prediction of neurodevelopmental outcomes, achieving a sensitivity of 100% and specificity of 97.70% for predicting developmental delay [19]. Our results align with these findings and extend them by using an asphyxiated control group rather than healthy controls, thereby addressing a more clinically relevant question.

4.2. Blood Lactate as a Complementary Marker

In the multivariable analysis, blood lactate was also an independent predictor (aOR = 1.280, $P = 0.037$). This agrees with previous studies that show that initial lactate is a prognostic marker for a poor neurological outcome in neonates with asphyxia [9]. Tu et al. did a prospective cohort investigation in the post-hypothermia period and showed that the lactate concentrations measured both in the first six hours of life and on the fourth day were an independent predictor of the neurological status [9]. Importantly, they found that while traditional HIE staging was not predictive of outcomes in cooled infants, lactate remained significant [9]. This is very relevant for our present study, because in our cohort, the therapeutic hypothermia was done in 89.3% of the HIE patients. However, the overall confidence interval that is seen for the lactate (1.015-1.614) in comparison with the NSE,

suggests that the lactate gives a less precise estimation of the risk. A possible explanation for this is that the lactate level is more influenced by the variations of the systemic perfusion and can be influenced by the things not directly related to the cerebral injury.

The increase in the lactate level within the HIE cohort is an index of the degree of intensity of the hypoxic damage, and this is in agreement with the reduced pH measurements we have seen for these patients. We did not include pH in the multivariable model because the sample size was limited and we aimed to avoid overfitting by restricting the number of predictors. But the clinical importance of pH is not lost. Because of the strong correlation of the acidosis and the HIE, the pH will continue to be a standard cutoff to start the therapeutic hypothermia. The specific contribution of the NSE is that it is specific, it gives direct evidence of the neuronal damage, so it gives data that are added to the data of the systemic hypoxia given by the lactate and the pH.

4.3. Reasons for the Lack of Difference in Weeke Scores Across Groups

No significant difference emerged in Weeke MRI scores between the HIE and control groups. Several considerations may account for this.

First, the Weeke scoring system was originally developed and validated in neonates with moderate-to-severe HIE [14]. Its sensitivity to very subtle injury-the type most likely present in mild cases-may be limited. Machie et al. found that 43% of mild HIE cases had no detectable abnormality on any MRI scoring system, including the Weeke score [26]. Even the most sensitive scoring systems thus miss a substantial fraction of subtle injuries, and in our predominantly mild cohort this limitation probably contributed to the absence of a group difference.

Second, the control group-asphyxiated neonates without clinical HIE-may have sustained some degree of subtle brain injury. Li et al. reported MRI abnormalities in up to 61% of neonates with mild HIE, and noted that some injury can occur even in infants who do not meet formal HIE criteria [25]. Walsh et al. likewise found MRI abnormalities in a considerable proportion of infants with mild neonatal encephalopathy [27]. If the control group was not truly "normal" in terms of brain structure, the contrast between groups would be attenuated.

Third, the timing of MRI acquisition may have influenced the results. Specific types of injury, in particular those concerning the white matter, could be not completely visible on the images done in the first seven days of life [28]. In the present population of study, the median age at the time of MRI was six days. Although this is the time that it is conventionally done, it is possible that some developing lesions were not seen.

The fact that there is no variation of Weeke scores does not contradict the importance of the results obtained for NSE and lactate. It rather suggests that biochemical indicators could detect the neuronal damage at a previous stage than the structural changes that can be detected by MRI. This gain in time could make NSE and lactate particularly useful for first screening and risk assessment, in situations where the MRI is not available or in case of unclear imaging results.

4.4. Comparison with Apgar Scores and Ph

There was no statistically significant difference in the two cohorts for the Apgar scores obtained at five minutes ($P = 0.1070$). It is interesting to discuss this because the Apgar assessment is still the main clinical tool to assess the status of the newborns at the moment of the delivery. In a study population where all the newborns had perinatal asphyxia, the variation of the Apgar values was limited, and so the metric may have less capacity to discriminate the levels of risk. These limitations are why we need more sensitive biomarkers that can detect small distinctions among the babies with asphyxia. In this line of thought, Mooney et al. reported that although the Apgar scores were strong individual predictors in their machine learning framework, the inclusion of these scores with the pH and lactate measures increased the total predictive precision of the model [18].

There was a robust univariate link with umbilical arterial pH and HIE ($P = 0.0005$), as is known for its use to assess hypoxia. However, because the sample size was limited

and we aimed to avoid overfitting, pH was not included in the primary multivariable model. This should not be misunderstood as a statistical limitation that reduces the clinical importance of acidosis for HIE. Indeed, pH remains a standard cutoff to start the therapeutic hypothermia. The strength of NSE and lactate is that they give additional information that goes beyond the one we can get from pH only: NSE is a marker of neuronal damage, while lactate is a sign of persistent tissue hypoxia.

4.5. Clinical Implications

The present results are also very relevant for the clinical practice. The measurement of the serum NSE has different practical advantages. First of all, this biomarker can be measured with the usual techniques of the laboratory and the data are available from the first three days of life. The inclusion of the measure of NSE in the first clinical evaluations could improve the ability to identify newborns who had the asphyxia and that could be at greatest risk of developing HIE. Moreover, NSE may provide a more precise risk estimate than lactate. Therefore,it could be a better stand-alone indicator for guiding the medical decisions. The detection of high concentrations of either NSE or lactate could produce a prompt evaluation of the newborns in a neurological way, for example with a continuous aEEG monitoring or with an earlier MRI scan, that could allow more immediate therapy. Huang et al. showed that the combination of NSE, aEEG and MRI almost perfectly predict the neurodevelopmental outcome (100 % sensitivity, 97.70 % specificity) [19]. This is evidence for the big potential of using a multimodal diagnostic strategy.

4.6. Limitations

It is important to mention here a number of limitations of this study. Firstly, given the retrospective nature of the study design there is the potential for a bias, since the information was obtained from pre-existent medical charts, in which there are always some gaps in the documentation. Secondly, as the research was carried out at one institution, it is not clear to what extent these results can be extrapolated to other kind of patients or to other clinical setting. Thirdly, the sample size of 57 individuals might have produced insufficient statistical power to carry out robust subgroup analyses. Moreover, the cohort with HIE was mostly mild (85.7%), which can limit the relevance of these results for the case of moderate or severe HIE. However, this prevalence probably reflects current clinical trends, for which with the better early detection and the timely intervention we can have a higher proportion of milder presentations. Additionally, as there is no long term neurodevelopmental follow up data we cannot evaluate the prognostic utility of these biomarkers for beyond the neonatal stage. Finally, the model showed only a moderate overall predictive accuracy (77.2%), that could be a limitation because it might come from the restricted number of predictors in the analysis. Mooney et al. demonstrated that the inclusion of additional clinical variables with the use of the machine learning methodologies can enhance predictive performance [18], suggesting avenues for future refinement.

4.7. Future Directions

It is essential to confirm these results with larger-scale prospective studies using the same protocols for the collection of the biomarkers. The best period to assess the levels of NSE and the clinically meaningful threshold values are still to be defined. The predictive power could be improved by using the NSE with other markers as, for example, S100B or GFAP [11]. As pointed out by previous papers, the efficacy of the biomarkers is different in infants submitted to the cooling therapies and not submitted to the cooling therapies [9], suggesting that future predictive models should account for therapeutic hypothermia. Most important is the need of a long neurodevelopmental monitoring to see if the early increases of the biomarkers are associated with the functional outcome going beyond the neonatal stage. As shown by Huang et al. the combination of the biochemical marker with the aEEG and the MRI is promising for the future research [19].

5. Conclusions

In this predominantly mild HIE cohort of 57 asphyxiated neonates, both NSE and blood lactate were found to be independent predictors of HIE. We found that the estimated effect of NSE is stable and well estimated, with an adjusted OR of 1.047 (95% CI: 1.013–1.082, P = 0.007), which suggests its possible use as an early biomarker for finding infants at risk. These results are in accordance with the evidence in recent prospective studies and systematic reviews. In contrast to previous studies that used healthy control groups, this study used as a comparison group an asphyxiated group of infants. Therefore, it is a context that is closer to the clinical practice for the assessment of the biomarker. The fact that NSE predicts in different kinds of study designs and in different kind of study populations is also strength for the potential of clinical translation of this. If we want to validate these results and to find clinically useful thresholds, especially in the context of the use of therapeutic hypothermia and multi-marker prediction models we need future prospective studies with larger cohorts and with a longer follow-up.

Authors' contributions: Conceptualization: Yincong Chen, Zhifeng Chen. Methodology: Yincong Chen, Yanling Lu, Huixue Lu. Formal analysis: Yincong Chen, Kuihuan Tan. Investigation: Yincong Chen, Yanling Lu, Yueqin Ding. Data curation: Yincong Chen, Huixue Lu. Writing – original draft: Yincong Chen. Writing – review & editing: Yincong Chen, Zhifeng Chen. Visualization: Yincong Chen, Kuihuan Tan. Supervision: Zhifeng Chen, Yanling Lu. Resources: Zhifeng Chen, Yanling Lu. All authors read and approved the final manuscript.

Institutional Review Board Statement: The study was approved by the Clinical Research and Ethics Committee of the Tenth Affiliated Hospital of Southern Medical University (approval IRBIITKY-2026-264-01) and performed in line with the principles of the Declaration of Helsinki.

Informed Consent Statement: The ethics committee waived the requirement for informed consent because of the retrospective nature of the study.

Data Availability Statement: The data supporting the findings of this study are not publicly accessible because they contain information that could compromise participants' privacy. Nevertheless, they can be obtained from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no competing interests.

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