A Pilot Study of Soluble Form of LOX-1 as a Novel Biomarker for Neonatal Hypoxic-Ischemic Encephalopathy

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Objective To evaluate the soluble form of lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) as a biomarker of severity staging and prognosis in neonatal hypoxic-ischemic encephalopathy (HIE).

Study design We performed an observational study enrolling 27 infants with HIE and 45 control infants of gestational age ≥36 weeks and birth weight ≥1800 g. The HIE criteria were pH ≤7.0 or a base deficit ≥16 mmol/L within 60 minutes after birth, and a 10-minute Apgar score ≤5 or resuscitation time ≥10 minutes. HIE severity was evaluated using modified Sarnat staging. We measured plasma sLOX-1 level and assessed general and neurologic signs at discharge, and classified infants with no neurosensory impairments as intact survival.

Results sLOX-1 level within 6 hours after birth was correlated with the severity of HIE. sLOX-1 differentiated moderate-severe HIE (median, 1017 pg/mL; IQR, 553-1890 pg/mL) from mild HIE (median, 339 pg/mL; IQR, 288-595 pg/mL; $P = .007$). The sensitivity and specificity of the differentiation with a cutoff value of ≥550 pg/mL were 80.0% and 83.3%, respectively. In 19 infants with therapeutic hypothermia, a sLOX-1 cutoff value of <1000 pg/mL differentiated intact survival (median, 761 pg/mL; IQR, 533-1610 pg/mL) from death or neurosensory impairment (median, 1947 pg/mL; IQR, 1325-2506 pg/mL; $P = .019$) with 100% specificity and a positive predictive value.

Conclusion sLOX-1 may be a useful biomarker of neonatal HIE for severity staging and outcome prediction. Further investigations will facilitate its clinical use. *(J Pediatr 2019;206:49-55).*

Neonatal hypoxic-ischemic encephalopathy (HIE) is a perinatal brain injury associated with death in 0.29 million infants per year worldwide and neurodevelopmental impairment in many survivors.[1] Although therapeutic hypothermia is effective for moderate and severe HIE, mortality and neurologic sequelae remain persistent problems.[2-5]

One method of staging HIE severity is used clinically but without good biological evidence.[2,6,7] An accurate biomarker that can be used within 6 hours after birth would be helpful in the diagnosis, treatment, and prognosis of HIE. Neuron-specific enolase,[8-12] S100B,[8-12] glial fibrillary acidic protein,[12-14] and ubiquitin carboxy-terminal hydrolase L1 protein[12,14,15] have been evaluated as possible biomarkers but have not been used clinically.[16,17] Magnetic resonance imaging (MRI) has been proposed as a biomarker of brain injury[18]; however, the use of MRI is limited during the progressive phase of HIE.

We have demonstrated that levels of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) increased in neural cells of a rat brain model of HIE, and that the administration of LOX-1-neutralizing antibody reduced brain injury.[19] A scavenger receptor of oxidized low-density lipoprotein, LOX-1 is induced by oxidant species, inflammatory cytokines, and ischemia-reperfusion in thrombocytes, endothelial cells, neurons-and macrophages.[20-23] The extracellular segment of LOX-1, soluble form of LOX-1 (sLOX-1)[24] appears in blood and has been used as a predictive factor for acute coronary syndrome and stroke.[25,26] However, sLOX-1 levels have never been measured in infants with and without HIE.

ABR	Auditory brainstem response
aEEG	Amplitude-integrated electroencephalography
AUC	Area under the curve
HIE	Hypoxic-ischemic encephalopathy
LOX-1	Lectin-like oxidized low-density lipoprotein receptor-1
MRI	Magnetic resonance imaging
NICHD	National Institute of Child Health and Human Development
NPV	Negative predictive value
PPV	Positive predictive value
ROC	Receiver operating characteristic
sLOX-1	Soluble form of lectin-like oxidized low-density lipoprotein receptor 1

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We speculated that blood sLOX-1 level would be a useful biomarker of brain injury in newborn infants and reflect the severity of HIE. Therefore, we investigated the use of sLOX-1 in the diagnosis and prognosis of HIE and evaluated the trajectory of sLOX-1 values in the neonatal period.

Methods

This multicenter prospective observational pilot study to evaluate the association between sLOX-1 and HIE severity and outcomes was approved by the Ethical Committees of the participating institutions and hospitals. We obtained participation consent from parents of all enrolled infants.

A total of 78 infants were enrolled in the study between June 2014 and May 2017. Inclusion criteria for the HIE and control groups were gestational age ≥36 weeks and birth weight ≥1800 g. Exclusion criteria were chromosomal abnormality, congenital infection, congenital heart disease, major malformation, and incomplete clinical information.

Infants with HIE qualified for the study based on the criteria published by the National Institute of Child Health and Human Development (NICHD)[2]: pH ≤7.0 or base deficit ≥16 mmol/L in any blood within 60 minutes after birth, and 10-minute Apgar score ≤5 or resuscitation with assisted ventilation for ≥10 minutes after birth. Infants meeting these criteria were classified as having HIE. Infants in the control group met the study's inclusion criteria but did not have HIE. We divided the infants with HIE into mild, moderate, and severe HIE groups using modified Sarnat staging. We examined neurologic condition in 6 categories: consciousness, spontaneous activity, posture, tone, primitive reflex (suck and Moro), and autonomic nervous system (pupils and heart rate). Infants with abnormalities in 3 or more categories were classified as having moderate or severe HIE, and infants with abnormalities in 2 or fewer categories were classified as having mild HIE. Moderate and severe HIE were distinguished by the number of moderate or severe signs. If the distribution of signs was equal, the distinction was made based on level of consciousness.[2] Therapeutic hypothermia was performed for infants with moderate and severe HIE without severe complications, such as disseminated intravascular coagulation and severe pulmonary hypertension.

Neurosensory impairments were defined as feeding disorders (necessitating tube feeding at discharge), hearing disorders, seizures, and severe hypotonia. Intact survival was defined as the absence of these abnormalities at discharge. We performed automated auditory brainstem response (ABR) testing (NatusALGO 3i; Atom Medical, Tokyo, Japan) at 35 dBnHL in all infants at discharge.[27,28] Infants with poor response underwent diagnostic ABR testing (Neuropack S1 MEB 9402; Nihon Kohden, Tokyo, Japan). An infant with prolonged latencies or indistinct waves at 80 dB SPL on ABR was classified as having a hearing disorder.

Plasma samples were obtained at ≤6 and >6 hours after birth as available. A 0.5-mL blood sample was collected from a vein or artery in an EDTA-2K (ethylenediaminetetraacetic acid dipotassium salt dihydrate) tube and immediately centrifuged for 15 minutes at 3000 rpm at 4°C. The supernatant was preserved as a plasma sample at -80°C until measurements were performed. Blood sampling was performed on days 0, 1, 2-4, and 5-9 after birth. sLOX-1 levels were measured by a sandwich enzyme-linked immunosorbent assay using 2 types of monoclonal antibodies against the extracellular domain of LOX-1.[26]

MRI scans were obtained in all surviving infants with moderate or severe HIE, 3 infants in the mild HIE group and 3 infants in the control group, at 3-4 weeks after birth. MRI conditions were T1-, T2-, and diffusion-weighted sequences with 1.5 T. Based on the image reading reports by radiologists, brain injury was assessed according to the NICHD Neonatal Research Network brain injury pattern as grade 0, 1A, 1B, 2A, 2B, or 3, with grade 0 indicating normal and increasing severity of injury with increasing number.[29]

Statistical Analyses

Statistical analyses were performed using SPSS Statistics version 24.0 (IBM, Armonk, New York). Clinical characteristics of infants and their mothers at hospitalization were described. sLOX-1 levels were compared among the severity groups using the Kruskal-Wallis test, followed by the Mann-Whitney *U* test with Bonferroni correction. To evaluate the performance of sLOX-1 as a biomarker for differentiating moderate-severe HIE from mild HIE and to set appropriate cutoff values, sLOX-1 levels were compared using the Mann-Whitney *U* test, a receiver operating characteristic (ROC) curve was constructed, and the area under the curve (AUC) was calculated. The positive predictive value (PPV) and negative predictive value (NPV) were calculated using the sensitivity and specificity extracted from the ROC and the prevalence of each group among all infants with HIE observed in this study.

To examine the temporal trend of sLOX-1 level at each HIE severity level, we assessed the median sLOX-1 levels stratified by the day after birth. For infants who had blood sampling done twice in a day, the average value was used in this temporal analysis. We also compared the infants' outcomes at discharge. We used the Spearman rank correlation coefficient to illustrate the relationships between individual sLOX-1 levels and clinical features. To test the performance of sLOX-1 as a prognostic biomarker for outcome at discharge, a ROC curve was created, and a pair of sensitivity and specificity values was calculated. The PPV and NPV were also calculated as above. We performed plasma sLOX-1 measurement and clinical analyses every 3 months. Participant enrollment was ended when we had obtained significant differences between the controls and each HIE group as well as significant cutoff values of severity and prognosis.

Results

A total of 78 infants were enrolled with informed parental consent. **Figure 1** (available at www.jpeds.com) shows the reasons for exclusion and HIE severity group classifications for the participants. Diagnoses for the 45 infants in the control group included low birth weight in 29%, suspicion of infectious

Table I. Maternal and neonatal characteristics

Characteristics	Control (n = 45)	Mild HIE (n = 6)	Moderate HIE (n = 16)	Severe HIE (n = 5)	P value All	P value Control vs HIE	P value Mild vs M-S HIE
Mothers							
Age, y, mean ± SD	31.8 ± 6.1	33.5 ± 2.1	32.2 ± 5.7	29.6 ± 4.1	.48	.86	.41
Gravida, median (IQR)	1 (0-2)	0 (0-1)	1 (0-2)	1 (0-2)	.19	.04	.29
Parity, median (IQR)	1 (0-2)	0 (0-1)	0.5 (0-1)	1 (0-1)	.62	.37	.38
Placental abruption, n (%)	0 (0)	2 (33)	4 (25)	2 (40)	<.001	<.001	.59
Nonreassuring fetal status, n (%)	9 (20)	4 (67)	14 (88)	4 (80)	<.001	<.001	.30
Emergency cesarean delivery, n (%)	6 (13)	4 (67)	8 (50)	4 (80)	<.001	<.001	.53
Neonates							
Birth weight, g, mean ± SD	2759 ± 528	2767 ± 792	3091 ± 315	3095 ± 558	.52	.04	.37
Gestational age, wk, median (IQR)	38 (37.1-39.3)	40.4 (37.1-40.4)	40.1 (39.4-40.6)	39.1 (38.4-40.4)	.001	<.001	.80
Male sex, n (%)	23 (51)	2 (40)	13 (81)	3 (60)	.10	.20	.07
Apgar score, n (%)							
1 min ≤5	1 (2)	6 (100)	15 (94)	5 (100)	<.001	<.001	.78
5 min ≤5	0 (0)	3 (50)	7 (44)	5 (100)	<.001	<.001	.56
10 min ≤5	0 (0)	2 (33)	4 (25)	3 (60)	.004	.006	.61
Intubation in delivery room, n (%)	1 (2)	3 (50)	12 (75)	5 (100)	<.001	<.001	.16
Resuscitation at 10 min, n (%)	3 (7)	4 (67)	14 (88)	5 (100)	<.001	<.001	.20
Blood gas values in first 60 min							
pH, median (IQR)	7.28 (7.24-7.33)	6.79 (6.95-7.15)	6.96 (6.77-6.99)	6.65 (6.63-6.98)	<.001	<001	.20
Base deficit, mm/L, median (IQR)	4.5 (3.1-7.3)	16.1 (12.0-19.6)	18.1 (15.4-22.0)	27 (19.0-27.5)	<.001	<.001	.45
Age at classification, hr, median (IQR)	NA	2.3 (1.5-3.0)	2 (1.7-2.4)	1 (1.0-3.0)	.34	NA	.63
Outborn, n (%)	9 (20)	5 (83)	16 (100)	5 (100)	<.001	<.001	.22
sLOX-1, pg/mL, median (IQR)	411 (229-595)	339 (288-595)	924 (543-1725)	1325 (1075-2506)	<.001	.002	.007

M-S, moderate and severe; *NA*, not available.
Comparisons were made using the χ^2 test, Fisher exact test, 1-way ANOVA, and Kruskal-Wallis test among 4 groups and the Student *t* test and Mann-Whitney *U* test between 2 groups.

disease in 18%, transient tachypnea of the newborn in 16%, and mild jaundice in 11%. Maternal and neonatal characteristics are presented in **Table I**, and supplemental perinatal characteristics are provided in **Table II** (available at www.jpeds.com). Placental abruption, nonreassuring fetal status, and emergency cesarean delivery were observed more frequently in the HIE group compared with the control group. The infants with HIE also had lower Apgar scores and higher rates of intubation, 10-minute resuscitation, and severe acidemia.

sLOX-1 level was measured within 6 hours after birth in 64 infants. The average time of first sampling was 2.5 ± 1.6 hours after birth in the control group and 3.3 ± 1.5 hours after birth in the HIE group. Differences in sLOX-1 levels were observed between the control and moderate HIE groups, between the control and severe HIE groups, and between the mild HIE and severe HIE groups (**Table I** and **Figure 2**, A). To distinguish moderate and severe HIE from mild HIE as an indication for initiation of therapeutic hypothermia, we compared sLOX-1 levels between the combined moderate and severe HIE group (median, 1017 pg/ml; IQR, 553-1890 pg/ml) and the mild HIE group (median, 339 pg/ml; IQR, 288-595 pg/ml; $P = .007$) and found significantly higher levels in the former group (**Figure 2**, B). ROC curve analysis showed an AUC of 0.854 (95% CI, 0.707-1.000) (**Figure 2**, C; available at

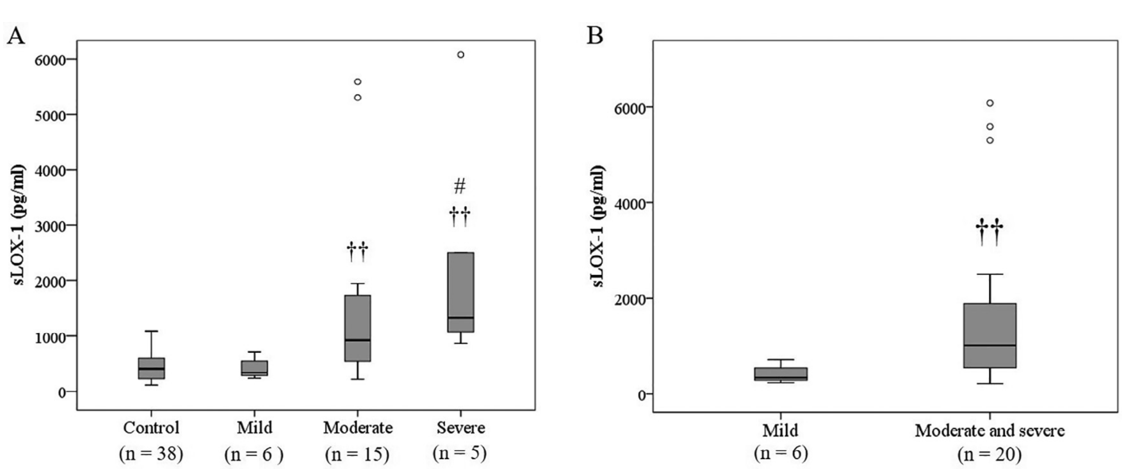

Figure 2. sLOX-1 levels in the first 6 hours after birth, and the clinical utility of sLOX-1 as a diagnostic biomarker. The circles denote samples with extremely high levels. **A,** $^{††}P < .01$ vs control; $^{\#}P < .05$ vs mild HIE. **B,** $^{††}P < .01$ vs mild HIE.

Table IV. Characteristics in-hospital and short-outcome of infants at discharge

Characteristics	Control (n = 45)	Mild HIE (n = 5)	Moderate HIE (n = 16)	Severe HIE (n = 5)	*P* value
Death or neurosensory impairment, n (%)	0 (0)	0 (0)	1 (6)	4 (80)	<.001
Death, n (%)	0 (0)	0 (0)	0 (0)	1 (20)	.07
Hypothermia, n (%)	0 (0)	0 (0)	15 (94)	5 (100)	<.001
Oxygen inhalation, n (%)	18 (40)	4 (80)	16 (100)	5 (100)	<.001
Mechanical ventilation, n (%)	4 (9)	4 (80)	16 (100)	5 (100)	<.001
Severe complications, n (%)	0 (0)	0 (0)	2 (13)	2 (40)	.005
Persistent pulmonary hypertension	0 (0)	0 (0)	1 (6)	2 (40)	.38
Disseminated intravascular coagulation	0 (0)	0 (0)	1 (6)	2 (40)	.005
Early-onset sepsis	0 (0)	0 (0)	0 (0)	0 (0)	NA
Multiorgan failure	0 (0)	0 (0)	0 (0)	1 (20)	.07
Length of hospitalization, d, median (IQR)*	11.5 (7.0-17.5)	15.5 (13.5-23.5)	32 (26.0-36.0)	72 (68.0-156.5)	<.001
Length of oxygen inhalation, d, median (IQR)*	0 (0-4.0)	2.5 (1.0-4.0)	13 (8.5-14.5)	17 (12.0-24.0)	<.001
Length of mechanical ventilation, d, median (IQR)*	0 (0-0)	0.5 (0-1.5)	6 (5.0-7.0)	9 (9.0-13.0)	<.001
Days to full feeding, median (IQR)*	4 (3.0-5.0)	5.5 (4.0-7.0)	8 (7.5-9.0)	12.5 (10.5-15.0)	<.001
Neurosensory impairments, n (%)*	0 (0)	0 (0)	1 (6)	3 (75)	<.001
Feeding disorder	0 (0)	0 (0)	1 (6)	3 (75)	<.001
Seizure requiring anticonvulsants	0 (0)	0 (0)	1 (6)	0 (0)	.37
Hearing disorder	0 (0)	0 (0)	0 (0)	1 (25)	.06
Severe hypotonia	0 (0)	0 (0)	0 (0)	3 (75)	<.001
Abnormal MRI, n (% of infants with MRI)	0/3 (0)	0/3 (0)	7/16 (44)	3/4 (75)	.11
Grade 0	3 (100)	3 (100)	9 (56)	1 (25)	.11
Grade 1A-1B	0 (0)	0 (0)	6 (38)	0 (0)	.21
Grade 2A	0 (0)	0 (0)	0 (0)	0 (0)	NA
Grade 2B-3	0 (0)	0 (0)	1 (6)	3 (75)	.03

Full feeding means enteral feeding >100 mL/kg/day. The Fisher exact test and Kruskal-Wallis test were used for comparisons among the 4 groups.
*Data presented for survivors.

www.jpeds.com). When the cutoff value was set to ≥550 pg/mL, the sensitivity and specificity were 80.0% and 83.3%, respectively, and the PPV and NPV of moderate and severe HIE were 94.1% and 55.6%, respectively.

At ≥6 hours after birth, a total of 60 blood samples were obtained to assess temporal changes in sLOX-1 levels in the study groups between day 0 and days 5-9 (**Table III** and **Figure 3**; available at www.jpeds.com). In the moderate HIE group, sLOX-1 levels decreased significantly from day 0 to days 2-4 and then to days 5-9 (**Figure 3**, C). Among the 4 groups, the severe HIE group had the highest sLOX-1 levels on day 0, which appeared to decline to the same levels as seen in controls by days 5-9; however, only 1 infant provided data for days 5-9, and thus statistical analysis was not possible (**Figure 3**, D). The sLOX-1 level gradually decreased in the single patient with mild HIE and serial levels (**Figure 3**, B).

The neonatal outcomes at discharge are summarized in **Table IV**. One infant with mild HIE, 15 of 16 infants with moderate HIE, and all infants with severe HIE underwent therapeutic hypothermia. An infant with mild HIE was treated with therapeutic hypothermia due to marked acidemia (base deficit, 25.1 mmol/L) at birth, and this infant's clinical data were excluded in the subsequent outcome analyses. One infant with moderate HIE could not undergo therapeutic hypothermia because of disseminated intravascular coagulation and severe pulmonary hypertension. Rates of death and neurosensory impairment in survivors are presented in **Table IV**. Hospital length of stay ($P = .02$), use of oxygen inhalation ($P = .004$), use of mechanical ventilation ($P < .001$), and days to full feeding ($P < .001$) were positively correlated with individual sLOX-1 level in all infants.

We assessed sLOX-1 level in the first 6 hours after birth as a biomarker of outcome at discharge. To exclude bias due to treatment, we included the 19 infants (14 with moderate HIE and 5 with severe HIE) who underwent therapeutic hypothermia and sampling within 6 hours after birth. Five infants died or had a neurosensory impairment, and the other 14 had intact survival; sLOX-1 levels in the former group (median, 1947 pg/ml; IQR, 1325-2506 pg/ml) were significantly higher than those in the intact group (median, 761 pg/ml; IQR, 533-1610 pg/ml) (**Figure 4**, A). Furthermore, in the former group, sLOX-1 levels increased with the increasing severity of outcome. The deceased infant had the highest level, 6077 pg/mL at 3 hours after birth. sLOX-1 levels were higher in the 2 infants with 2 neurosensory impairments each (1947 and 2506 pg/ml) compared with the 2 infants with 1 neurosensory impairment each (1075 and 1325 pg/mL) ($P = .014$).

ROC analysis for discriminating death or neurosensory impairments revealed an AUC value of 0.857 (95% CI, 0.684-1.000) (**Figure 4**, B; available at www.jpeds.com). The sensitivity and specificity were 60.0% and 92.9%, respectively, and PPV and NPV were 73.8% and 87.0%, respectively, when the cutoff value was set to ≥1900 pg/mL. The sensitivity and specificity for intact survival were 71.4% and 100% respectively, when the value was <1000 pg/ml. The PPV and NPV were 100% and 53.8% respectively.

Discussion

In the present study, we measured sLOX-1 level as a possible biomarker of HIE in infants and found that sLOX-1 levels parallel the severity of HIE and are useful for discriminating

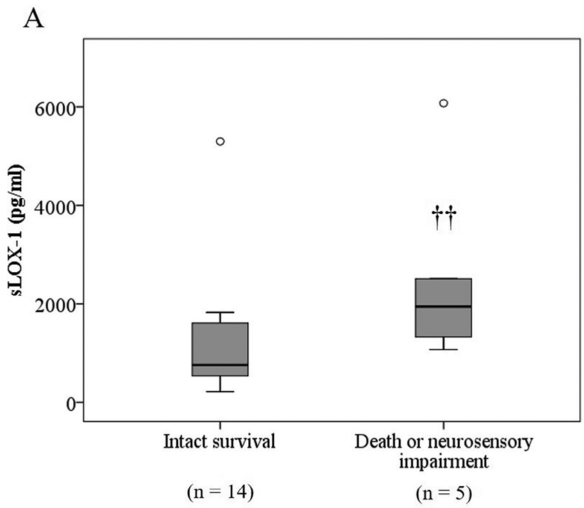

Figure 4. The clinical utility of sLOX-1 as a prognostic biomarker. **A,** Circles denote samples with extremely high levels. ††*P* < .01 vs intact survival.

moderate and severe HIE from mild or no HIE. Our findings also show that sLOX-1 levels decline rapidly after birth in infants with HIE and those without HIE, and that the sLOX-1 level in the first 6 hours is a useful prognostic biomarker of HIE.

The correlation between sLOX-1 level and the clinical severity of HIE and neurologic symptoms at discharge indicates that sLOX-1 may be useful as a severity staging and prognostic biomarker of HIE. We speculated that sLOX-1 blood levels would be high in infants with HIE, given the high sLOX-1 levels previously observed in a rat model of HIE (unpublished data). In fact, sLOX-1 levels in the first 6 hours were elevated in a stepwise manner according to the severity of HIE, a finding in accordance with a previous report linking sLOX-1 level to the number of lesions in acute coronary syndrome.[30] Although the clinical data (Apgar score, resuscitation, and blood gas values) revealed no significant differences among the 3 HIE groups at birth, sLOX-1 level differentiated moderate and severe HIE from mild HIE; thus, sLOX-1 level might be useful for identifying candidates for therapeutic hypothermia. Moreover, sLOX-1 levels differed between infants with intact survival and those who died or exhibited neurosensory impairment. The NPV of death or neurosensory impairment with a cutoff value of ≥1000 pg/mL was 100%, meaning that all infants in this study with an sLOX-1 level <1000 pg/mL and therapeutic hypothermia had intact survival at discharge, thus implying that sLOX-1 may be useful as a marker of intact survival. Although the PPV of death or neurosensory impairment with a cutoff value of ≥1900 pg/mL was as low as 73.8%, the fact that therapeutic hypothermia prevented death or neurosensory impairment in therapeutic hypothermia trials[2-5] might be a reason for the low PPV in this study. When applied to the patients without therapeutic hypothermia, sLOX-1 measurement may better predict death or neurosensory impairment.

We found a rapid decline in sLOX-1 levels after birth in our cohort. This finding corresponds to reports showing a rapid decline in sLOX-1 level after acute coronary syndrome.[25] The placenta strongly expresses LOX-1,[31] which may explain the physiologically increased sLOX-1 levels seen in control group infants at birth. Although a rise in maternal sLOX-1 level in preeclampsia has been reported,[32] none of mothers in this study had preeclampsia, and thus the sLOX-1 levels on day 0 in our cohort of infants were not related to maternal sLOX-1 induced by preeclampsia.

In the present study, individual sLOX-1 levels were positively correlated with nonneurologic indicators of illness severity, such as hospital length of stay. These findings suggest that the sLOX-1 level may reflect the severity of systemic illness as well as HIE severity.

Early severity staging of HIE is important for the initiation of therapeutic hypothermia within the first 6 hours. MRI and amplitude-integrated electroencephalography (aEEG) may be useful as biomarkers of HIE.[33-35] The NICHD MRI brain injury pattern of HIE is simple and closely correlated with the developmental outcome of HIE.[18,29] In our cohort, all infants with class 2B-3 brain injury (the most severe) had a poor outcome. MRI is not appropriate for severity staging in the acute phase of HIE, however. In a meta-analysis, Chandrasekaran et al concluded that a persistently abnormal aEEG at postnatal 48 hours or later was related to poor outcome, but that aEEG at postnatal 6 hours was not related to outcome.[36] Our data suggest that sLOX-1 may be superior to these physiological biomarkers, given that levels in the first 6 hours were associated with both early severity of HIE and neurosensory impairments.

LOX-1 can be induced by ischemia reperfusion and cytokines, and its activation induces functional deficit and apoptosis of endothelial cells in atherosclerosis.[37] Microglia are related to neural inflammation in HIE, and their activation is induced by hypoxic-ischemic insult.[38] We have confirmed LOX-1 expression in human HIE brain in endothelial cells and microglia (unpublished data); thus, we speculate that hypoxic-ischemic insult induces LOX-1 up-regulation and activation in endothelial cells and microglia through cytokines or other stress materials derived from neural cells, and that microglial LOX-1 may act as an apoptosis inducer in the brain. Some brain-specific proteins and cytokines have been described as biomarkers of HIE in clinical experiments[8-17]; however, measuring these molecules is difficult because they readily fluctuate with any change in condition. The expression of LOX-1 has been found to be closely related to the pathology of brain injuries in HIE rats and humans.[19] The combination of these factors with sLOX-1 may be a powerful biomarker of HIE. As a biomarker, sLOX-1 level may be useful for selecting or immediately inducing hypothermia and/or other treatments. In addition, sLOX-1 level may be useful for evaluating the undertreatment and overtreatment of patients, predicting prognosis, and planning medical care.

Limitations of this study include the small number of subjects, especially those with mild and severe HIE, and the lack of assessment of longer-term developmental outcomes. We did not find clearly stratified relationships between mild and moderate HIE and between moderate and severe HIE. Confirmation of sLOX-1 as a useful staging biomarker of HIE requires further studies with larger samples. Further assessment of long-term outcomes in a large-scale study with multiple hospitals will support the usefulness of sLOX-1 as a diagnostic and prognostic biomarker of HIE. In the meantime, although further investigation with a larger samples and long-term follow-up is necessary before clinical use, measurement of sLOX-1 can be useful for severity staging of HIE, determining the application of therapeutic hypothermia, and predicting outcomes. ■

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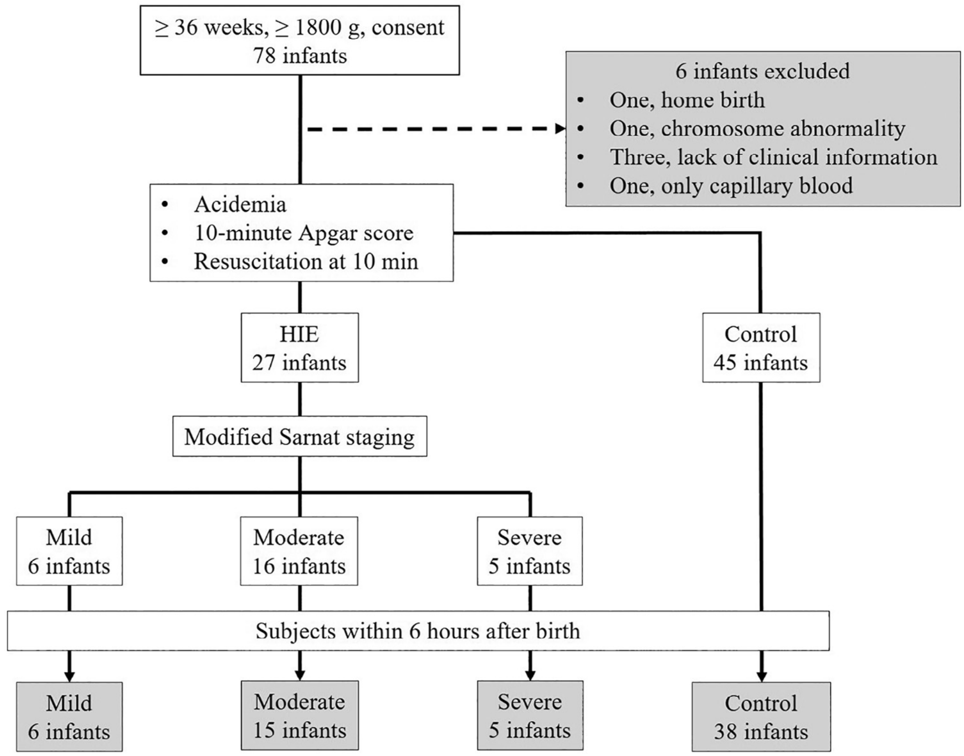

Figure 1. Recruitment and classification of infants.

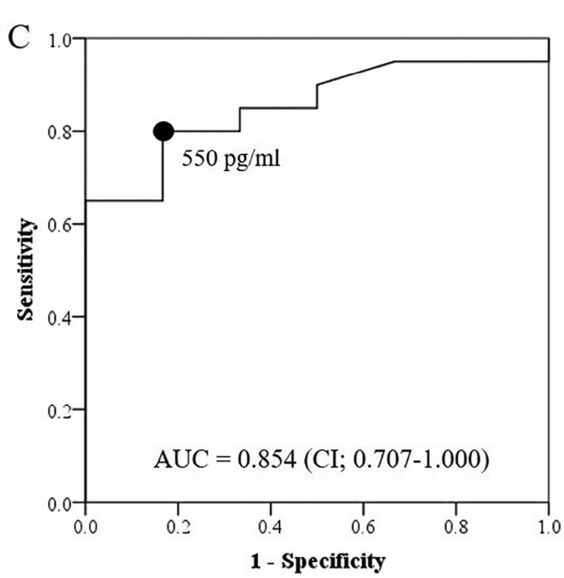

Figure 2. **C,** The black circle in the ROC curve shows the cutoff point of 550 pg/mL.

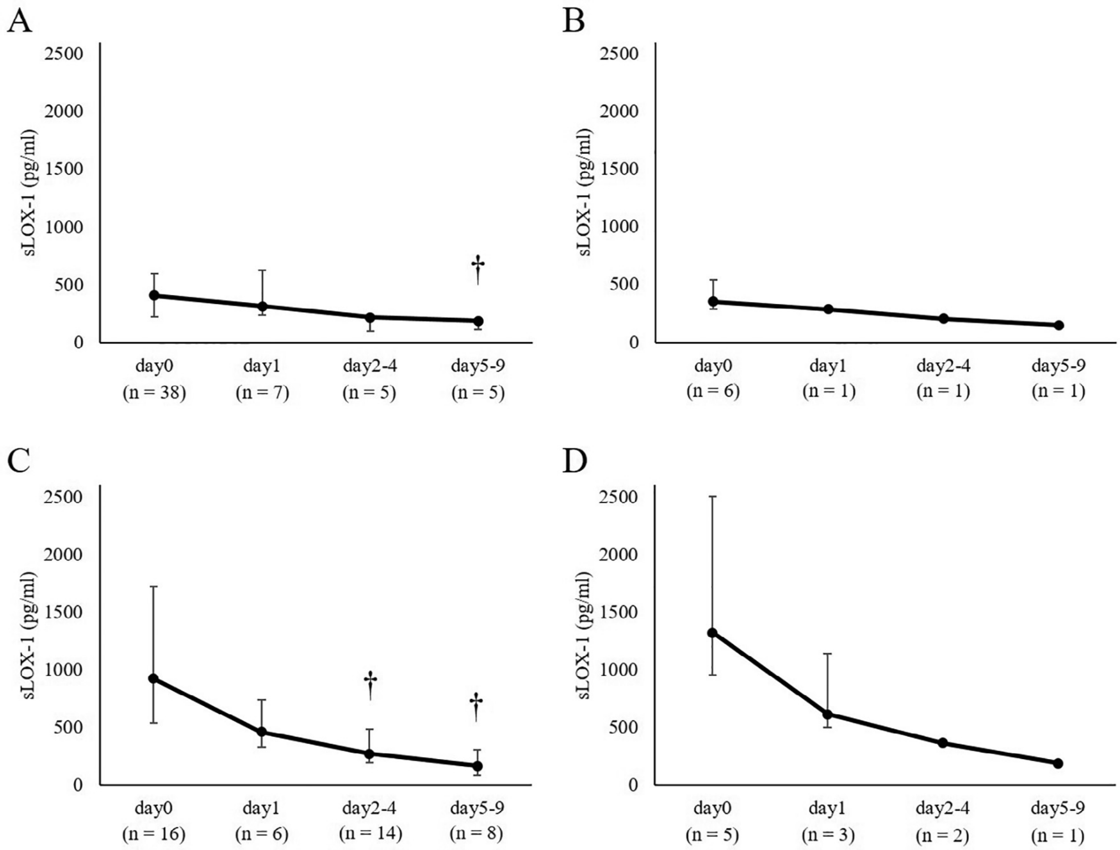

Figure 3. Temporal changes in sLOX-1 levels in **A,** control infants; **B,** infants with mild HIE; **C,** infants with moderate HIE; and **D,** infants with severe HIE. †$P < .05$ vs day0.

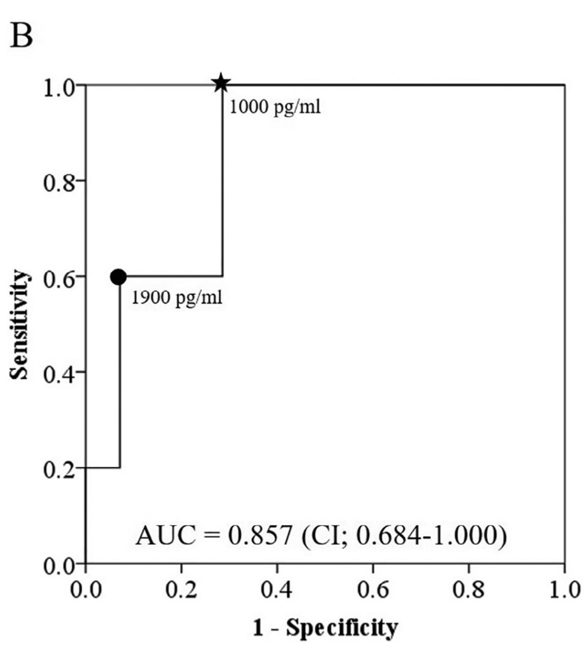

Figure 4. **B,** The black star and circle on the ROC curve denote the cutoff points of 1000 and 1900 pg/mL, respectively.

Table II. Complications during pregnancy and delivery

Complications	Control (n = 45)	Mild HIE (n = 6)	Moderate HIE (n = 16)	Severe HIE (n = 5)	*P* value All	*P* value Control vs HIE	*P* value Mild vs M/S HIE
Complications of pregnancy, n (%)	12 (27)	2 (33)	4 (25)	2 (40)	.85	.79	.55
Hypertension	5 (11)	0 (0)	0 (0)	0 (0)	.61	.09	NA
Diabetes	6 (13)	0 (0)	0 (0)	0 (0)	.47	.05	NA
Placental abruption	0 (0)	2 (33)	4 (25)	2 (40)	<.001	<.001	.59
Pre-eclampsia	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	NA
Intrapartum complications, n (%)	12 (27)	5 (83)	15 (94)	4 (80)	<.001	<.001	.54
Nonreassuring fetal status	9 (20)	4 (67)	14 (88)	4 (80)	<.001	<.001	.30
Cord prolapse	0 (0)	0 (0)	1 (6)	1 (20)	.05	.14	.60
Coiling of cord	2 (4)	0 (0)	3 (19)	0 (0)	.23	.27	.46
Maternal fever	0 (0)	0 (0)	1 (6)	1 (20)	.05	.14	.60

Comparisons were made using the χ^2 test, Fisher exact test, 1-way ANOVA, and Kruskal-Wallis test among 4 groups and the Student *t* test and Mann-Whitney *U* test between 2 groups.

Table III. sLOX-1 levels in the assessments in each severity

	Postnatal day (s) (pg/ml) Day 0	Day 1	Days 2-4	Days 5-9	*P* value
Control	411 (229-595) n = 38	316 (238-631) n = 7	220 (96-238) n = 5	191 (112-204) n = 5	.03*
Mild	354 (288-546) n = 6	288 n = 1	206 n = 1	151 n = 1	NA
Moderate	924 (543-1725) n = 16	465 (330-743) n = 6	272 (197-488) n = 14	170 (87-308) n = 8	.02† .01‡
Severe	1325 (957-2506) n = 5	618 (501-1140) n = 3	366 (358-374) n = 2	192 n = 1	NA

NA, not available.
Data are expressed as median (IQR). The Kruskal-Wallis test followed by Mann-Whitney *U* test with Bonferroni correction was used.
*Day 0 vs days 5-9.
†Day 0 vs days 2-4.
‡Day 0 vs days 5-9.