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HEART RATE VARIABILITY IN ENCEPHALOPATHIC NEWBORNS DURING AND AFTER THERAPEUTIC HYPOTHERMIA

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Abstract

Objective—To evaluate whether HRV measures are predictive of neurological outcome in babies with hypoxic ischemic encephalopathy (HIE).

Study Design—This case-control investigation included 20 term encephalopathic newborns treated with systemic hypothermia in a regional NICU. Electrocardiographic data were collected continuously during hypothermia. Spectral analysis of beat-to-beat heart rate interval was used to quantify HRV. HRV measures were compared between infants with adverse outcome (death or neurodevelopmental impairment at 15 months, n=10) and those with favorable outcome (survivors without impairment, n=10).

Results—HRV differentiated infants by outcome during hypothermia through post-rewarming, with the best distinction between groups at 24 hours and after 80 hours of life.

Conclusions—HRV during hypothermia treatment distinguished HIE babies who subsequently died or had neurodevelopmental impairment from intact survivors. This physiological biomarker may identify infants in need of adjuvant neuroprotective interventions. These findings warrant further investigation in a larger population of infants with HIE.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

Perinatal hypoxic ischemic encephalopathy (HIE) is a major cause of death and long-term disability in children.^{1, 2} Although therapeutic hypothermia improves outcomes in babies with HIE, approximately half of treated infants continue to suffer death or disability despite treatment with cooling.³⁻⁶ Additional work is needed, and ongoing, to identify adjuvant therapies aimed to further improve outcome in this high-risk population. Key to advancing neuroprotective interventions in babies with HIE, is the ability to tailor therapies to an individual's biological profile and ongoing response (or non-response) to treatment. Physiological biomarkers of brain injury can help to identify appropriate candidates for treatment, gauge treatment failure with need for escalation of therapy, and offer means for prognostication during and after intervention in babies with HIE.

A major impediment to the design of more effective neuroprotection is the lack of continuous real-time monitoring techniques capable of identifying critical thresholds in the early evolution of neonatal brain injury, and its response to intervention. This problem is compounded by the narrow therapeutic window for most neuroprotective agents. Current methods to assess brain injury risk in the critically-ill newborn are inadequate. Clinical exam is subjective, often confounded by neuroactive medications or medical devices, and evolves over time^{7, 8} requiring serial assessments that can be time and resource consuming. While magnetic resonance imaging (MRI) is helpful to discern structural brain injury, its utility is limited by the need for transport, requisite neuroradiology personnel with neonatal expertise for accurate interpretation, and limited sensitivity in the first 24 hours of life when key therapeutic decisions are often made.^{9, 10} Although advances in digital electroencephalography (EEG) monitors have improved continuous neuromonitoring capabilities, these tools likewise require specialized equipment and interpretive expertise. Thus, a bedside tool that provides an easily interpretable measure of neurological risk is needed in the intensive care unit.

Continuous cardiorespiratory monitoring has long aided clinicians in caring for the most critically ill infants. However, these physiological data are currently used only at the most basic level for clinical decision-making. This results in identifying critical events well after their occurrences, such as recognition of bradycardia or systemic oxygen desaturation. Advanced physiological signals processing of data acquired during critical care may augment the current monitoring scheme by detecting patterned changes that can serve as earlier indicators of autonomic failure, allowing for preventative interventions before systemic or neurological compromise to the patient. Specifically, interrogation of heart rate variability (HRV) via quantitative means may provide such actionable information. Few studies have evaluated HRV in babies with HIE.¹¹⁻¹³ While preliminary studies have demonstrated reduced HRV in more severely affected infants, no studies have evaluated changes in HRV over time in HIE newborns undergoing hypothermia. This study aims to evaluate the evolution of HRV during the first 4 days of life and whether HRV measures are predictive of neurological outcome in babies with HIE.

METHODS

Study Population

This study included newborns who were part of an ongoing prospective longitudinal study evaluating physiological and biochemical biomarkers of brain injury in babies with HIE. Infants were treated with whole-body hypothermia according to the NICHD Neonatal Research Network protocol, with inclusion criteria according to established NICHD criteria (i.e. infants were greater than 36 weeks gestational age, greater than 1800 grams at birth, demonstrated metabolic acidosis and/or low Apgar scores, and exhibited signs of moderate to severe clinical encephalopathy).³ Infants were cooled using the Blanketrol® II cooling unit (Cincinnati Sub-Zero, Cincinnati OH) for 72 hours followed by rewarming over 6 hours. Twenty infants with available outcome data were randomly selected from the overall cohort of 64 patients for this nested case-control secondary study. Infants were stratified by outcome into two groups: 1) cases with adverse outcome defined as death in the neonatal period or Bayley Developmental Index scores $>2SD$ below the mean at 15 month follow-up and 2) controls with favorable outcome defined as survivors with Bayley scores within $2SD$ at 15 months. The study was approved by the Children's National Medical Center Institutional Review Board. Informed consent and Health Insurance Portability and Authorization Act Authorization was obtained from the parent of the participant for the parent study, which included provisions for secondary analyses of clinical data collected during the participants' NICU course.

Clinical and Outcome Data Collection

Clinical and outcome data were extracted from the parent study database that includes data collected from birth hospital and study site medical records. Presenting characteristics were noted, including umbilical cord or arterial blood gas obtained in the first hour of life, Apgar scores and initial grade of encephalopathy classified according to modified Sarnat criteria.^{3, 14} Data from the infants' NICU hospitalization was also collected including hour of cooling initiation, need for vasoactive therapy, and mortality. Enrolled participants were followed longitudinally in our developmental follow-up program and assessed with the Bayley Scales of Infant Development – Second Edition (BSID-II).¹⁵ The BSID-II is a standardized assessment that evaluates a child's level of cognitive/language skills (reflected by the Mental Developmental Index- MDI) and fine and gross motor development (reflected by the Psychomotor Developmental Index – PDI). MDI or PDI scores of 100 ± 15 represent the mean $\pm 1sd$. Although the Third Edition of the Bayley Scales became available in 2006,¹⁶ our clinical program continued to use the BSID-II for developmental assessment during the study period in order to have comparable data to previously described infants treated for HIE.^{3, 6} Evaluations were performed by an experienced developmental psychologist who was blinded to the clinical history of the child and scores were entered into the database following each subject's visit to the clinic.

EKG Signal Processing

Electrocardiogram (EKG) data were retrieved from archived continuous EEG recordings obtained from each participant. Infants treated with hypothermia during the study period underwent routine continuous EEG monitoring (NicoletOne™ system, Viasys Healthcare,

San Diego CA) initiated as soon as possible after admission and continued through at least 12 hours after completion of rewarming. Signal processing was done using MATLAB (Mathworks, Inc., MA, USA). The data was sampled at 256 Hz. EKG data was isolated and bandpass filtered between 0.5–70Hz using Butterworth filter with zero-phase distortion. Artifacts caused by missing data were excluded. The R-wave was identified using adaptive Hilbert transform approach^{17, 18} and beat-to-beat interval (RR interval) was computed as successive difference of R-wave occurrence expressed in seconds. The RR interval was converted into evenly sampled data using cubic-spline interpolation at a sample rate of 4 Hz. The RR interval was divided into 10-minute windows. In each 10-minute window, the power spectrum was estimated via previously described methods.^{19, 20} The relative low-frequency (LF) and high frequency (HF) power were analyzed as measures of autonomic nervous system (ANS) regulation of HRV.^{21–23} The power in the LF band quantifies predominantly the sympathetic components, while the HF band quantifies the parasympathetic (vagal) components of the ANS. The LF power was calculated as the sum of power in the frequency bands covering 0.05–0.25 Hz¹¹, while HF power was calculated as the sum of power in the frequency bands covering 0.3–0.8 Hz.^{11, 24} Both LF and HF power were divided by the total power (sum of power in the frequency bands covering 0.05–2Hz) to calculate the relative LF and HF power. Continuous HRV data were partitioned into 3-hour windows for analyses over time. If patients were off EEG at any point during the 3-hour window (e.g. for imaging studies, for EEG electrode replacement or other clinical reasons), those particular 10-minute epochs were excluded. Group comparisons were made between all power spectrum calculations within a given 3-hour time window.

Statistical Analysis

Descriptive statistics included standard measures of central tendency and variability for continuous data and frequencies for categorical variables. Non-parametric testing (Wilcoxin Rank-Sum Test) was used to evaluate the differences in HRV measures in each 3-hour window between outcome groups. Intra-subject correlation was not considered in these analyses. Correction for multiple comparisons across the total number of 3-hour time windows (n=34) was performed with the Bonferroni method. The predictive ability of HRV to distinguish outcome groups was further evaluated by receiver operating curve (ROC) analyses where the area under the curve (AUC) of 1 denotes 100% model discrimination, whereas an AUC of 0.5 signifies no significant ability of the test (HRV) to distinguish between outcome groups. Values of 0.7–0.8 are considered acceptable, and values of greater than 0.8 are considered excellent. Thus, the magnitude of the AUC is a reflection of the predictive ability of HRV at a given time point to predict adverse outcome.

RESULTS

Ten infants were included in each outcome group. Infants with adverse outcome were more likely to present with severe clinical encephalopathy and had lower presenting pH and 10-minute Apgar score. Otherwise, demographic and clinical characteristics were similar between groups (Table 1). The adverse outcome group was comprised of 5 infants who died in the neonatal period and 5 infants who survived with neurodevelopmental impairment (4 infants with Bayley MDI and PDI <50 and 1 infant with PDI <70). All deaths were due to

withdrawal of care due to poor neurological prognosis (based on severely abnormal EEG background pattern, clinical exam and neuroimaging findings). Three infants were withdrawn prior to the completion of rewarming but after 48 hours of life. The favorable outcome group included 10 infants who survived with developmental scores within the normal range: MDI median (range)= 92 (81–103), PDI= 89 (77–101) at 15-month follow-up assessment.

EEG/EKG monitoring began at a median 11.6 (Range 4.7–40.7) hours of life. Total study duration varied between 20–110 hours (median 90.05 hours). Shorter duration studies were, as expected, present in the infants who died during cooling. Evolution of HRV over time for each outcome group is depicted in Figure 1. LF relative power was lower at nearly all timepoints in infants in the adverse outcome group compared to those with favorable outcome. These differences remained statistically significant ($p<0.01$) between 18–54 hours (except at 39 hours) and after 80 hours when controlling for multiple comparisons. In contrast, HF relative power was significantly higher in the adverse outcome group at nearly all time points.

Area under the receiver operating curve for discrimination of outcome group is plotted over time in Figure 2. The highest AUC values for LF relative power were demonstrated around 24 hours of life and after 80 hours of life. This signifies two key periods of vulnerability, during which time the separation between outcome groups became most evident. For HF relative power, a similar peak AUC was observed around 24 hours of life, while the second increase in AUC was observed after 96 hours of life. Selected cutpoints at key time periods of peak AUC and their respective sensitivities and specificities are presented in Table 2.

DISCUSSION

This is the first study to evaluate the evolution of HRV longitudinally over the course of hypothermia and rewarming in newborns with HIE. Consistent with prior work evaluating short periods of EKG data in babies with HIE,^{12, 13} we demonstrated that HIE infants with adverse outcome have decreased HRV compared to infants with favorable outcome. However, this study suggests that HRV is most affected in brain-injured patients during two key periods of vulnerability: 1) at 24 hours of life coincident with the peak of secondary energy failure, and 2) after 80 hours of life upon the completion of rewarming. That the discriminatory power of HRV reflects these known pathophysiological and clinical events supports its validity as a biomarker of ongoing brain injury in this population.

The clinical significance of HRV was first described as a harbinger of fetal distress²⁶. HRV has since been described as a predictor of outcome in other high-risk populations including neurosurgical patients,²⁷ patients traumatic brain injury²⁸, preterm infants^{29, 30}, newborns with sepsis³¹ and necrotizing enterocolitis,³² and other critically ill populations.³³ Few studies have evaluated HRV in newborns with HIE. Aliefendioglu and colleagues¹² evaluated HRV on EKG data from 22 HIE infants (of whom 10 had severe HIE) at one week of life. While they found HRV to differentiate HIE infants with moderate versus severe encephalopathy, distinction between these groups after the acute phase of injury has limited therapeutic implications. More recently, Matic and colleagues evaluated EKG data from 19

HIE infants during the first 48 hours of life and found several heart rate characteristics to be predictive of outcome by MRI and developmental assessment.¹³ The investigators evaluated 2-hour EKG segments recorded any time during the first 18–48 hours of life. This intermittent sampling at variable ages did not allow the investigators to establish the role of HRV in the context of the evolving process of brain injury. The advantages of our study include the longitudinal recordings and comparative analyses between groups based on time from birth. This approach enabled inferences that could account for recovery from initial insult and any maturational effects of postnatal age. One prior case report described HRV changes during the rewarming phase,¹¹ supporting the importance of group comparisons at similar stages of physiological response to injury and critical care interventions.

While other methods have been described to characterize HRV,³⁴ power spectral analysis of the beat to beat (RR) interval was first described by Akselrod and colleagues³⁵ as an advanced signals processing approach for quantitative analysis of HRV in continuous EKG data. Both animal and human studies have supported the notion that reduced spectral power in the low-frequency component of the EKG signal is indicative of impaired autonomic nervous system (ANS) function.^{21–23} In the aforementioned study by Matic and colleagues, LF power was amongst the best discriminators of outcome selected from 24 different quantitative HRV parameters evaluated.¹³ Relative HF power also discriminated between groups. Increased HF power within the adverse outcome group likely reflects increased ventilator dependency in these critically-ill patients, as the respiratory sinus arrhythmia is represented in the HF band.²⁴

A key finding of the current study is the temporal evolution of HRV in the two outcome groups. It is well established that hypoxia-ischemia triggers a delayed series of events (i.e. excitotoxic, pro-inflammatory, oxidative stress, and pro-apoptotic cell signaling pathways) that lead to cell death in the brain. The timing of this secondary injury cascade has been demonstrated via both laboratory and clinical observations to peak after 24 hours post-insult. This secondary injury is mitigated by hypothermia treatment. However, it is known that hypothermia does not benefit all patients, and that death and disability are frequent outcomes despite treatment with cooling. The prominent reduction in HRV seen around 24 hours of life in the adverse outcome group may identify infants in whom hypothermia is failing to prevent secondary energy failure and subsequent brain injury. Thus, HRV may be helpful in selecting patients for adjuvant neuroprotective therapies. The second significant distinguishing HRV time period occurred after 80 hours of life following the completion of rewarming. It is possible that HRV changes in the adverse outcome group signified progression of injury after the cessation of cooling. Thus, HRV may help determine the adequate duration of cooling on an individualized basis, which is currently unknown.

There are limitations to the current study. The association between hypoxic-ischemic insults and reduced HRV may be related to several factors. Whether decreased HRV is related to direct effects of asphyxia on the myocardium, the impact of medications or other critical care interventions, brainstem injury leading to autonomic dysfunction, presence of seizures or a combination of these factors cannot be elucidated by this study. That patients in the adverse outcome group required more vasopressor support is perhaps indicative of myocardial dysfunction that could be contributory to reduced HRV. It is important to note

that we did not control for the effect of seizures in these analyses. Seizure has been reported to have complex interaction with HRV depending on the location, propagation and duration of the ictal activity.^{37–39} It is possible that some of the alterations seen in HRV in the adverse outcome group may therefore be attributable to seizure activity, as the predominant seizure burden was observed in this group. However, given the aim of this pilot study was to evaluate the temporal evolution of HRV and its relation to outcome, elucidating the effect of seizures and other possible etiological factors that could explain why HRV relates to poor outcomes was beyond the scope of this study. The interaction of these factors with the association between HRV and outcome warrant further study in a larger population. The assessment of developmental outcome at 15-months in surviving infants was performed according to our clinical protocol during the study period. It is recognized that neurodevelopmental assessment at this relatively early age may be considered less reliable and further work is needed (on ongoing) to correlate HRV measures with longer-term outcome. Missing data was another important consideration and potential source of bias. Initiation (and cessation) of EEG/EKG recordings was influenced by many clinical and logistic factors that could not be controlled for the purposes of this study. Artifact in the EKG recordings could also impact results, as this is not an uncommon occurrence in the intensive care unit setting. However, our analytical approach incorporated an automated artifact rejection method and a modification that mitigated effects of non-stationarity in the data that could result from signal artifact.²⁰ It should be noted that although we used a well established method to interrogate HRV, other methods to quantify heart rate characteristics have been proposed. For example, while evaluation of LF/HF ratio as a measure of sympho-vagal balance has also been reported, we did not evaluate this metric as recent reports have questioned whether this measure accurately reflects the pathophysiological condition.³⁶ Another limitation of this preliminary study is the small sample size, although this was mitigated by the large number of observations provided by a continuous dataset. However, as we did not consider intra-subject correlation in these analyses, and the few patients included may not be representative of a larger HIE population, we consider these analyses hypothesis generating that warrant confirmation in a larger prospective study.

CONCLUSIONS

Heart rate variability during hypothermia treatment and after rewarming distinguished HIE infants who died or had neurodevelopmental impairment from intact survivors. Quantitative measures of HRV may provide a robust bedside physiological biomarker of ongoing brain injury that can help direct therapeutic interventions in babies with HIE.

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Time	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	
# subjects	4	4	6	7	8	8	9	9	8	8	9	10	10	10	9	10	10	10	10	9	9	9	10	10	9	10	10	7	7	6	5	5	3	□ Normal outcome
# epochs	41	72	93	119	139	143	149	152	141	140	134	162	178	160	157	158	172	178	157	162	160	158	171	158	152	157	145	126	118	98	74	53	40	
# subjects	7	6	6	8	9	9	8	7	7	7	8	8	8	8	7	5	6	6	7	7	6	6	6	6	6	5	7	7	6	6	6	4	3	▲ Adverse outcome
# epochs	100	90	96	115	142	132	121	123	116	105	138	141	134	131	107	78	91	103	105	114	105	104	103	101	96	88	98	123	98	103	63	52	53	

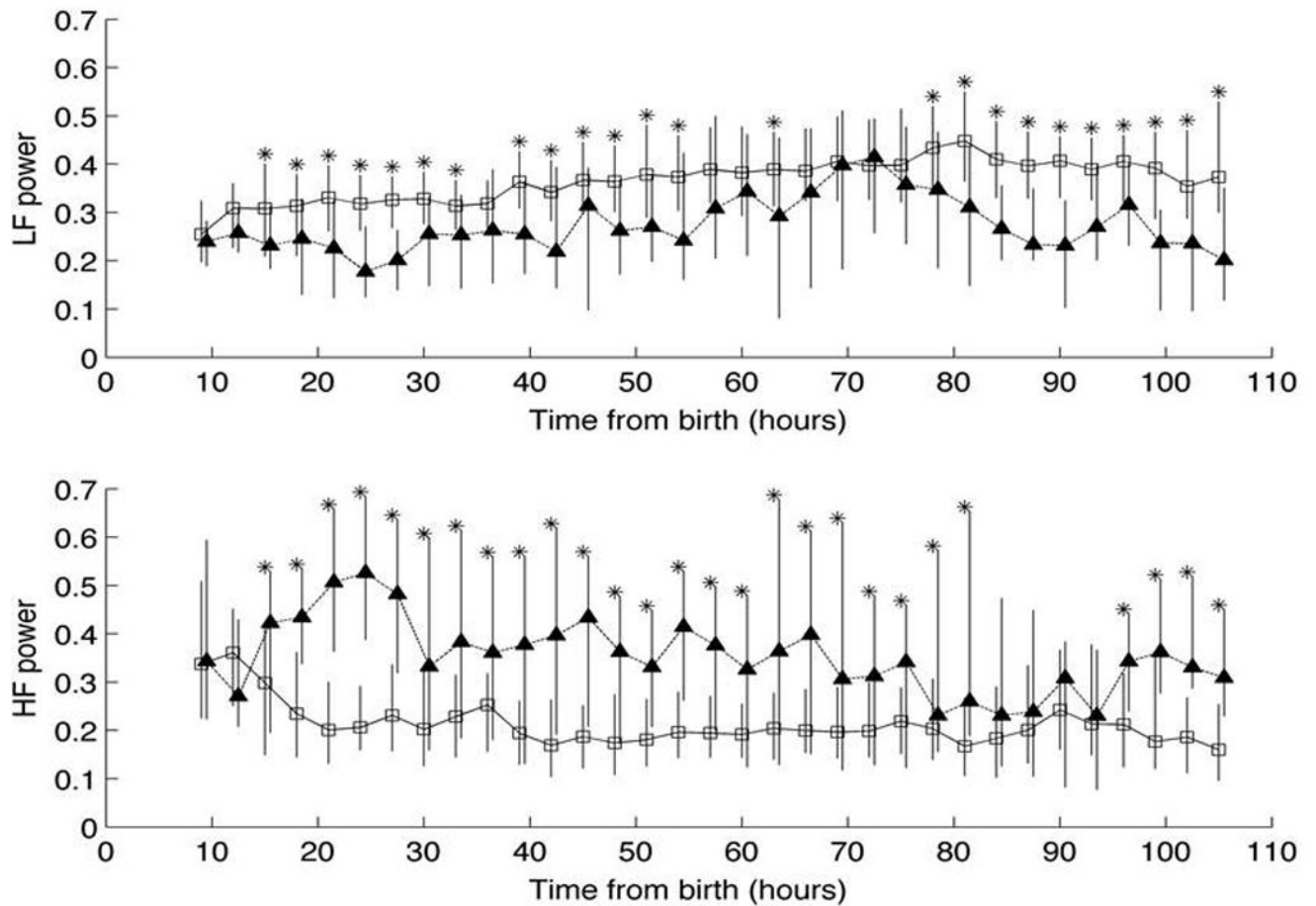


Figure 1. Heart rate variability (HRV), expressed as relative [A] low-frequency (LF) and [B] high-frequency (HF) power, over time from birth in favorable outcome (open squares) versus adverse outcome (filled triangles) groups. Data is depicted as median (IQR). Number of epochs per comparison and number of patients from whom data were available are shown in the embedded table. Asterisks mark significant differences ($p < 0.01$) between groups after Bonferroni correction.

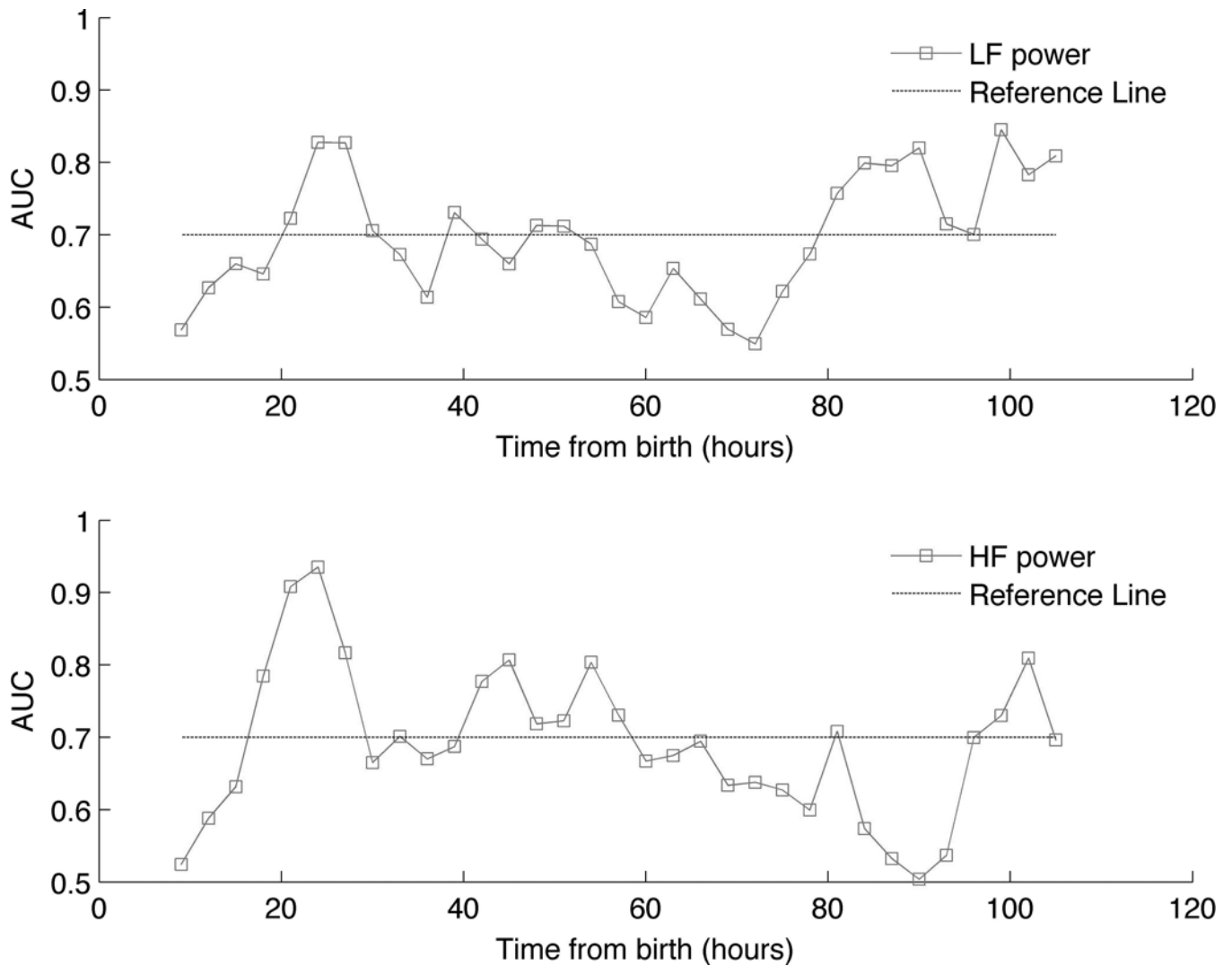


Figure 2. Area under the receiver operating curve (AUC) values plotted over time for ability of relative [A] low-frequency (LF) and [B] high-frequency (HF) power to discriminate between outcome groups. Dashed reference line is provided at the AUC value of 0.7. Note the relative peaks at 24 hours and after 80 hours of life.

Table 1

	Adverse Outcome (n=10)	Favorable Outcome (n=10)	P Value
Gestational Age (wks)	38.9±0.4	38.5±0.6	0.628
Birth weight (kg)	3.63±0.15	3.33±0.29	0.375
Head Circumference (cm)	35.0±0.4	34.1±0.7	0.294
Gender (n male)	7	4	0.178
Initial pH*^a	6.80 (6.50–7.15)	7.00 (6.79–7.30)	0.026
Initial Base Deficit^b	19.2±2.9	20.1±2.7	0.829
Apgars*			
1 minute	0 (0–4)	2 (0–6)	0.168
5 minute	1 (0–5)	5 (0–7)	0.065
10 minute^c	3 (0–6)	6.5 (2–7)	0.040
Cesarean Delivery (n)	8	5	0.160
Encephalopathy Grade			0.002
Moderate (n)	2	9	
Severe (n)	8	1	
Clinical Seizures at Presentation (n)	4	2	0.329
Patients with EEG Seizures during monitoring (n)	7	1	0.006
Hour of Life Cooling Started	4.0±0.4	4.6±0.3	0.297
Hour of Life HRV Started	11.1±1.6	13.3±1.8	0.397
Patients on Vasoactives (n)	6	3	0.178
Ventilator Days*	8 (4–22) ^d	2 (1–5)	0.003
Age at Follow-up	15.6±0.5	15.2±0.3	0.235

Data presented as mean ± SEM except where noted, *median (range)

^a Documented for 9/10 patients

^b Documented for 6/10 patients and 8/10 patients

^c Documented for 8/10 patients and 6/10 patients

^d Duration of mechanical ventilation presented for surviving infants

HRV = Heart rate variability recording

Table 2

Selected Cutpoints and Predictive Values from the ROC

	Time Window (hour of life)	Cutpoint	Sensitivity	Specificity	AUC
LF Relative Power	24–27	0.21	0.92	0.7	0.83
	93–96	0.32	0.8	0.65	0.82
HF Relative Power	24–27	0.34	0.84	0.87	0.94
	102–105	0.27	0.81	0.76	0.81