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Spectral Analysis of Nonstationary Heart Rate of Neonates Receiving Therapeutic Hypothermia Treatment

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Spectral Analysis of Nonstationary Heart Rate of Neonates
Receiving Therapeutic Hypothermia Treatment

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University.

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List of Abbreviations

HIE: Hypoxic Ischemic Encephalopathy.

H-I: Hypoxic Ischemic

PA: Perinatal Asphyxia.

MRI: Magnetic Resonance Imaging.

EEG: Electroencephalography.

SDNN: Standard Deviation of Normal to Normal Interval.

RMSSD: Root Mean Square of Successive Differences.

BSID-II: Bayley Scales of Infant Development – Second Edition.

MDI: Mental Developmental Index.

PDI: Psychomotor Developmental Index.

LF: Low Frequency.

HF: High Frequency.

HRV: Heart Rate Variability.

FFT: Fast Fourier Transform.

SA: Spectral Analysis.

RR: Time interval from one R peak to another R peak.

ATP: Adinosine triphosphate.

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Abstract

SPECTRAL ANALYSIS OF NONSTATIONARY HEART RATE OF NEONATES
RECEIVING THERAPEUTIC HYPOTHERMIA TREATMENT

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University.

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We studied Heart Rate Variability (HRV) evolution during therapeutic hypothermia in
newborns with hypoxic ischemic encephalopathy (HIE) using spectral analysis. We
hypothesized that HRV measures are predictive of neurological outcome in babies with
HIE.

Non-stationarity in the data causes inaccurate quantification of the spectral power. A
modification was proposed to power spectral analysis approach to mitigate the effect of
non-stationarity. The modified and the standard approaches were applied to cardiac
beat-to-beat intervals of newborns receiving hypothermia treatment. The performance of

the approaches in distinguishing the RRi dynamics of two groups of newborns was assessed using area under the receiver operating characteristic (ROC) curve. Our results showed that the modified spectral analysis distinguished the two groups of neonates better than the standard approach.

These results may be useful in identifying the deteriorating physiology of the infants receiving hypothermia treatment early in time and strategize alternate interventions for them.

Background and Introduction

Hypoxic Ischemic Encephalopathy (HIE) is a condition in which the brain does not receive enough Oxygen. HIE is a major cause of death and long-term disability in children (Shankaran S, 1991) (Dilenge ME, 2001). Abnormal findings on the neurologic examination in first few days after birth is the single most useful predictor in childhood that a brain insult has occurred in the perinatal period.

Brain malformations due to hypoxic ischemic encephalopathy are caused by the following mechanism: The low Adenosine Triphosphate (ATP) levels cause failure of many of the mechanisms that maintain cell integrity, particularly the sodium/potassium (Na/K) pumps and mechanisms to maintain low intracellular calcium. When the Na/K pumps fail, an excessive influx of the positively charged sodium ions precipitate massive depolarization of neurons. This leads to the release of glutamate, a prominent excitatory neurotransmitter. The glutamate binds to glutamate receptors allowing additional influx of intracellular calcium and sodium. Increased intracellular calcium has significant detrimental effects leading to cerebral edema, ischemia, microvascular damage with resultant necrosis and/or apoptosis (Allen K. A., 2011). The severity and extension of brain damage are strictly related to intensity, timing and duration of hypoxic-ischemic (H-I) insult. The more intense and long-lasting it is, the greater is the number of neuronal and glial cells which die (Distefano G., 2010).

Thus, HIE damage varies. The Sarnat stages (I-III) used to classify post-hypoxic encephalopathy represent a convenient description to characterize the extent of neurologic involvement (Acarregui M. "Neurological Disorders: Asphyxia". Web. 02 Dec. 2013).

Autonomic symptoms reported of a child that was diagnosed with HIE are listed below. These symptoms show how the autonomic nervous system is affected in those patients:

1. Does not regulate temperature appropriately; is typically hypothermic with body temperatures as low as 90F; can become hyperthermic (feverish) when exposed to hot temperatures.

2. Does not sweat when hot

3. Does not shiver when cold

4. Does not get goose bumps when she is cold (gets them when she is hot instead)

5. Eyes are always dilated and do not respond appropriately to light

6. Typically has high blood pressure

7. Typically is tachycardic (fast heart rate)

8. Has circulation problems, including cold, mottled, purple extremities and no peripheral pulses

9. Develops red blotches, patches on skin, and flushing, especially when stressed

10. Does not sense temperature on skin well

11. Does not sense pain on skin well

12. Neuropathic and visceral pain throughout body

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13. Sphincters in bladder are too tight, while muscles are too floppy, requiring cathing

14. Sphincters and muscles in GI tract do not work appropriately, causing motility problems

15. Nerves in GI tract are hypersensitive, causing pain, vomiting, and impaired motility, and requiring parenteral nutrition

16. Bone Marrow production is impaired during stress (drops platelets and Hemoglobin).

Hypothermia is a cooling procedure. In this study, the infants were cooled for three days, and then rewarmed no faster than half a degree Celsius per hour. Hypothermia is thought to be effective because it reduces free radicals and glutamate levels, decreases oxygen demand, and decreases apoptosis (Allen K. A., 2011)

Mechanism of action of hypothermia includes the following:

1. Reduces cerebral metabolism, prevents edema.

2. Decreases energy utilization.

3. Reduces/suppresses cytotoxic amino acid accumulation and nitric oxide.

4. Inhibits platelet-activating factor, inflammatory cascade.

5. Suppresses free radical activity.

6. Attenuates secondary energy failure.

7. Inhibits apoptosis (cell death).

8. Reduces extent of brain injury (S, 2009).

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Although therapeutic hypothermia improves outcomes in babies with HIE, approximately half of eligible infants continue to suffer death or disability despite treatment with cooling (Shankaran S, 2005) (Azzopardi DV, 2009) (Jacobs SE, 2011). 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, gage treatment failure with need for escalation of therapy, and offer means for prognostication during and after intervention in babies with HIE.

Advances in neonatal neurocritical care have paralleled the incorporation of hypothermia into standard of care. However, acute bedside assessment and ongoing monitoring of brain-injury risk in the critically ill newborn remains problematic. Clinical exam is subjective. It is confounded by neuroactive medications or medical devices. It evolves over time (Gunn AJ, 2008) (Shankaran S, 2012) 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 (McKinstry RC, 2002) (Barkovich AJ, 2006). Although advances in digital electroencephalography (EEG) monitors have improved continuous neuromonitoring

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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. 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. Specifically, interrogation of heart rate variability (HRV) via quantitative means can provide actionable information that can enable intervention before systemic or neurological compromise. Few studies have evaluated HRV in babies with HIE (Lasky RE, 2009) (Aliefendioglu D, 2012) (Matic V, 2013). 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.

Cardiac beat-to-beat interval (RR interval or RRi) is the time interval between two consecutive R peaks in the cardiogram. Figure 1 shows EKG tracing from one of the subjects. It shows how the RRi is computed.
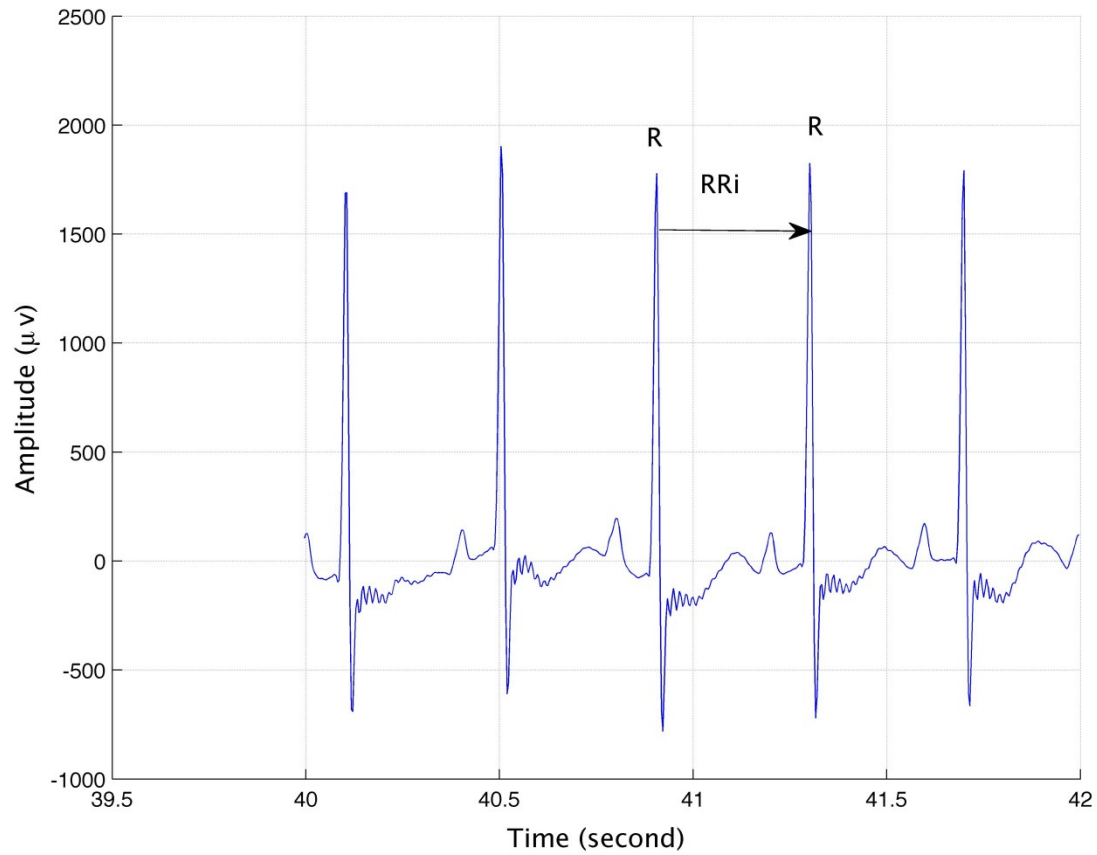
Fig. 1. EKG tracing from a patient from favorable outcome group. The tracing shows the R peaks and how RRi is computed. x-axis shows time in seconds referenced from starting time of recording.

RRi provides a potentially important window on autonomic function. Spectral analysis of RRi has been used to quantify the sympathetic and parasympathetic components of the autonomic nervous system (ANS) (Anon., 1996) (Cerutti S, 1986) (Akselrod S, 1981) (Appel ML, 1989) (Berger RD, 1986). Using the modulations caused by respirations on the RRi, the respiratory sinus arrhythmia (RSA) can also be quantified using spectral analysis of the RRi (Hamann C., 2009) (Andriessen P, 2005). Time domain quantification of the sympathetic and parasympathetic components can be done using standard deviation of normal-to-normal interval (SDNN) and root mean square of the successive differences (RMSSD) of RRis, respectively (Anon., 1996). Further, pNNx, probability that the current interval is greater than x-milliseconds from the previous interval has been used to quantify the parasympathetic component of RRi (Anon., 1996). Recently, several novel time domain approaches based on the concepts derived from statistical physics (Peng CK, 1995) (Arneodo A, 1995), nonlinear dynamics (Kantelhardt JW, 2007) (Govindan RB, 2011) and information theory (Schneider U, 2008) have been developed to characterize the RRi. Spectral analysis provides a direct measure of the cyclic changes in the RRi. LF power was amongst the best discriminators of outcome selected from 24 different quantitative HRV parameters evaluated by Matic and colleagues. Thus, LF power was chosen to be the primary variable of interest in this study.

Recently, a modification was proposed to the spectral analysis to mitigate the effect of non-stationarity in RRi (Govindan RB, 2013). Non-stationarity can be caused by

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extrinsic factors (such as movement of the subject) or by intrinsic factors such as; tachycardia, bradycardia, missing beats/data, or a gradual acceleration in the heart rate. Spectral analysis is sensitive to the presence of spikes in the RRi (Govindan RB, 2013) (Chen Z, 2002). In scenarios, such as continuous real-time processing of physiological signals acquired from patients receiving treatment in critical care unit, it is hard to check whether the stationarity condition required for the analysis is satisfied or failed. Hence, robust techniques have to be employed to reliably characterize the underlying physiology. In this study, a modified spectral analysis is applied to the RRi of neonates receiving hypothermia treatment. We hypothesize that the modified approach would differentiate better the two groups of newborns, favorable and adverse outcome compared to the standard approach.

Materials

Study Population

This study included term 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

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scores, and exhibited signs of moderate to severe clinical encephalopathy) (Shankaran S, 2005). Infants were cooled using the Blanketrol® II cooling unit (Cincinnati Sub- Zero, Cincinnati OH) for 72 hours followed by rewarming no faster than half a degree Celsius per hour. Twenty infants were selected from the overall cohort for this nested case-control pilot 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, and an informed consent and Health Insurance Portability and Authorization Act Authorization was obtained from the parent of the participant.

Clinical and Outcome Data Collection

Clinical and outcome data were extracted from the clinical server that includes data collected from birth hospital and study site medical records like time of birth and time of cooling initiation. Enrolled participants were followed longitudinally in our developmental follow-up program and assessed with the Bayley Scales of Infant Development – Second Edition (BSID-II) (Anon., 1969). 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

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Psychomotor Developmental Index – PDI). MDI or PDI scores of 100 $\pm$ 15 represent the mean $\pm$ 1sd. 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.

Ten infants were included in each outcome group. Infants with adverse outcome were more likely to present with severe clinical encephalopathy. Otherwise, demographic and clinical characteristics were similar between groups. The adverse outcome group was comprised of 5 infants who died in the neonatal period and 5 infants who survived with neurodevelopmental impairment. The favorable outcome group included 10 infants who survived with developmental scores within the normal range at 15-month follow-up assessment.

Methods

ECG Signal Processing

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. Electrocardiogram (ECG) was acquired using a chest electrode as a part of clinical practice and was retrieved from the clinical server from archived continuous EEG recordings obtained from each participant. Signal processing

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was done using MATLAB 2011 (Mathworks, Inc., MA, USA) installed on MAC computer having RAM of 8 GB and memory of 2 TB. The data were saved in the clinical server in a format that was not ready to be processed by MATLAB. A proprietary software (Persyst) was used to read the data and save them into text format files; a process that took an average of half an hour for each file. It went up to an hour or more depending on the size of the file. Each patient of the 20 patients had the data saved in a number of files that was 10 in average. After saving the files in text format, they were saved in mat format using MATLAB and thus they were ready to be processed. If the files were big in size, they had to be broken down into smaller files using Shell programming and then the smaller text files were saved into mat format. The EKG 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 manually. The R-wave was identified using adaptive Hilbert transform approach (Govindan RB, 2011) (Ulusar UD, 2009) 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, with a sliding overlap of two minutes, via previously described methods (Halliday DM, 1995) (Govindan RB, 2013). RRi provides a window of opportunity to characterize the ANS. The sympathetic and parasympathetic arms of the ANS can be quantified from the low- and high-frequency

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components of the RRi (Anon., 1996) (Cerutti S, 1986) (Akselrod S, 1981) (Berger RD, 1986) (HON EH, 1960). The relative low-frequency (LF) power was analyzed as the primary variable of interest as it quantifies the sympathetic and parasympathetic components of the autonomic nervous system, the former being dominant and reflective of HRV (Piccirillo G, 2009) (Piccirillo G, 2009) (Shah AJ, 2013). Large variability in RRi has been associated with healthy status of subjects. Based on this fact, the high power in the low-frequency band observed for the infants with favorable outcome indicates large variability in the RRi, and possibly a positive response of these infants to hypothermic treatment. In contrast, lower power values observed for the infants with adverse outcome (see table 1) show small variability in RRi and may indicate compromised health. Also, power in the high-frequency band was analyzed. Power in this band quantifies the parasympathetic component. RRi of infants with adverse outcome show higher power in this band when compared to infants with favorable outcome (see table 1). This may be due to the predominance of the parasympathetic component or due to an extrinsic factor; such as an increased dependency on the external ventilator support. The LF power (sum of power in the frequency bands covering 0.05-0.25 Hz (Lasky RE, 2009)) was divided by the total power (sum of power in the frequency bands covering 0.05-2Hz) to calculate the relative LF-power used as a measure of HRV in these analyses.

$$P_{LF} = \frac{\sum_k P(w_k)}{\sum_{k2} P(w_{k2})} \tag{1}$$

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Where, P_{LF} is power in LF band.

k is the index of the frequency bin in the low frequency band.

$P(w_k)$ is power at the frequency bin specified by w_k.

$w_k \in [0.05\ 0.25]$

$k2$ is the index of the frequency bin in the low and high frequency bands.

$P(w_{k2})$ is power at the frequency bin specified by w_{k2}.

$w_{k2} \in [0.05\ 2]$.

Similarly, the HF power (sum of the power in the frequency bands covering 0.3-1 Hz) was divided by the total power to calculate the relative HF-power.

$$P_{HF} = \left.\sum_{k1} P(w_{k1})\middle/\sum_{k2} P(w_{k2})\right. \tag{2}$$

Where, P_{HF} is power in HF band.

$k1$ is the index of the frequency bin in the high frequency band.

$P(w_{k1})$ is power at the frequency bin specified by w_{k1}.

$w_{k1} \in [0.3\ 1]$.

Total power was defined as the sum of the powers in the frequency band of 0.05-2 Hz.

$$P_{Tot} = \sum_{k2} P(w_{k2}) \tag{3}$$

Where P_{Tot} is total power.

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Table 1. Heart rate variability (HRV) low frequency (LF) and high frequency (HF) powers in each group as compared to the other group.

	HRV LF Power (as compared to the other category)	HRV HF Power (as compared to the other category)	Pathophysiology Characteristics
Infants with favorable outcome	Higher	Lower	Had higher power in low frequency band which indicated more heart rate variability as reflected by higher sympathetic and parasympathetic components activities.
Infants with adverse outcome	Lower	Higher	Had higher power in high frequency band which indicated predominance of parasympathetic component or high activity of respiratory sinus arrhythmia.

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Continuous LF power of HRV data were partitioned into 3-hour segments and the median of HRV LF power over the next 3-hour window was plotted as a function of time with respect to birth. LF and HF powers of HRV were compared between infants with adverse outcome (death or neurodevelopmental impairment at 15 months) and those with favorable outcome (survivors without impairment).

Power spectrum estimation

Spectral estimation (standard approach) involves the following steps (Halliday DM, 1995):

- Partition the data into smaller time windows of suitable duration decided based on the desired frequency resolution (also called Fast Fourier Transform, FFT, length). In this work, we used 60 seconds as FFT length. The choice of 60 seconds was to capture low frequency changes down to 0.05 Hz.

- Compute periodogram which is square of the magnitude of the Fourier transform of the data in each window.

- Average the periodograms over all windows (in this work 10 windows).

If the data have nonstationary regions, the periodograms from those regions will have different amplitude and frequency characteristics compared to the periodograms from stationary regions of the data. Thus, the spectral estimate, which is average of all the

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periodograms, will not represent the correct characterization of the power spectrum. Thus, RRis that have non-stationarity are compromised in the spectral analysis.

In spectral analysis, the total variance of a signal is decomposed into different frequency components. In other words, the power spectrum is a frequency-domain description of the variance of the RRi. Based on this fact, in a stationary dataset the sum of the powers over all frequency bands is equal to the total variance of the RRis. Thus, for the periodogram from $k-\text{th}$ 60 second window, we have the following relation:

$$\int_{-\infty}^{\infty} Per_k(w)dw = var(x^{(k)}) \tag{4}$$

Where $Per_k(w)$ is the periodogram in the frequency bin specified by w.

dw is the frequency resolution of the spectral estimate.

$x^{(k)}$ is a vector that has the RRis values in the kth 60-second window.

$var(.)$ indicates the variance function.

In practice, this integration is performed in the frequency bands from negative half of the sample frequency to positive half of the sample frequency, where the power is calculated.

The proposed modification involves dividing the data after Step 1 by its standard deviation. Thus, in the modified approach the periodogram is divided by the variance (recall that the calculation of the spectrum involves squaring of the standard deviation

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which converts the latter into variance) of the data and the above integral will yield a value of one. Because when we divide the data in each time window by its standard deviation, the variance of the data in that window will be one.

$$\int_{-\infty}^{\infty} Per_{(k)}(w)dw = 1 \tag{5}$$

Since power is the frequency domain description of the variance, the integral of power of the data which was previously normalized by standard deviation will be one.

For a stationary dataset, the total variance is approximately same as the average of the variance in all the windows.

$$\langle var(x^{(k)})\rangle_k \approx var(x) \tag{6}$$

Where $\langle . \rangle$ denotes the average.

x denotes 10-minutes of RRis.

k in the subscript is the index of the 60 second window.

Based on this relation, the integration of the spectral estimate $P(w)$ obtained using the modified approach, will also be one. Further, the power spectrum obtained through this modified scheme will be dimensionless because of the normalization by standard deviation. To compare the spectrum obtained using modified approach with standard approach, we rescaled the former by multiplying it with $\langle var(x^{(k)})\rangle_k$. This rescaling

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allows expressing power in the unit of $second^2$. Thus, for stationary data, both the standard and modified approaches should yield the same result within the numerical precision of the computation.

However, for non-stationary data, the normalization by standard deviation will ensure that the periodogram corresponding to the non-stationary region (with sudden changes in the signal amplitude) will assume the same or comparable amplitude as rest of the data from stationary regions. Therefore, the periodogram from the non-stationary region will not dominate the average in the calculation of the spectrum. However, the periodogram from the non-stationary region will have a different frequency distribution (i.e. the power may be concentrated in different frequency bands). This effect will be minimized in the subsequent averaging part, as the periodograms from stationary parts of the signal will have power concentration in a different region of the spectrum.

The above working principle of the modified spectral estimation approach can be explained by the example illustrated in figure 2. Figure 2 (a) shows 10 minutes of RRi data that has non-stationarity. Figure 2 (b) shows the periodograms obtained using the standard approach. The figure shows that some of the periodograms have higher value at different frequencies due to the segments that have non-stationarity. Those periodograms dominate the average and lead to the power spectrum shown in figure 2 (d). However, figure 2 (c) that have the periodograms from modified approach shows that all the periodograms are in comparable range due to normalization by standard

deviation. The periodograms still have different frequency distribution of the power. That is minimized when taking the average and obtaining the power spectrum shown in figure 2 (e).
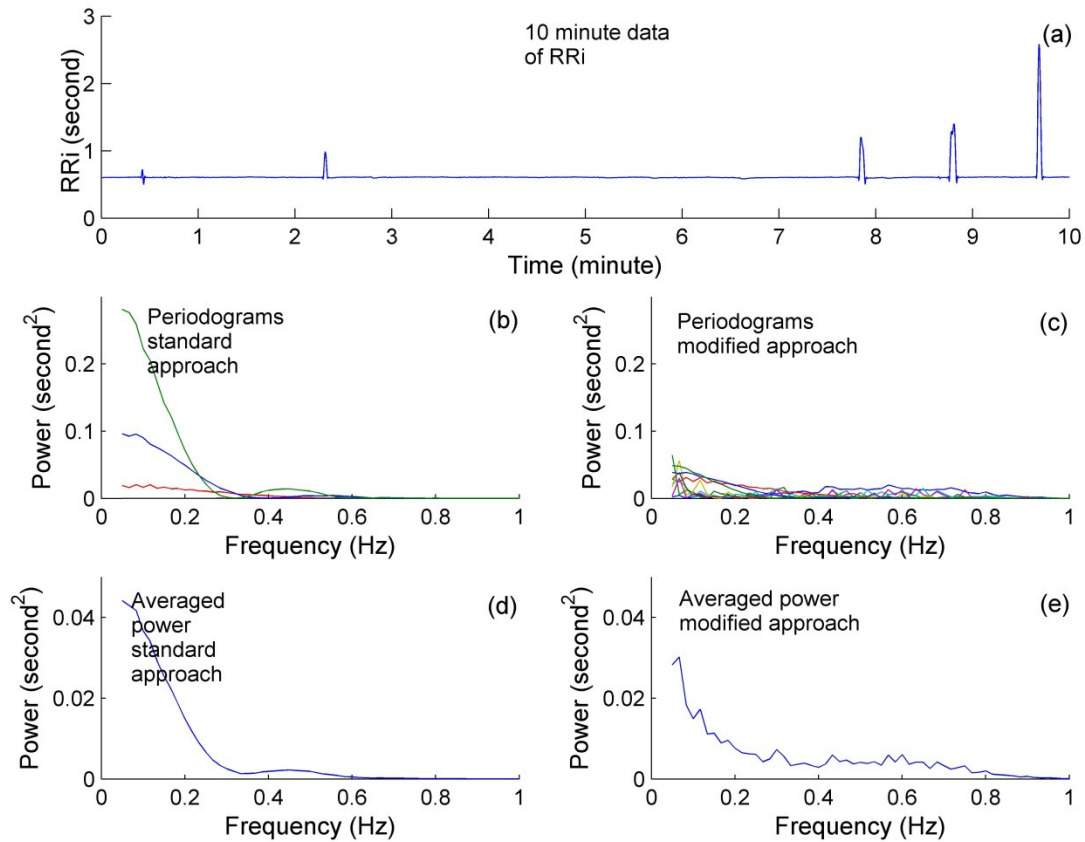
Fig. 2. Non-stationary RRi data in (a). (b) shows the periodograms obtained for the RRi using the standard approach. (c) shows the periodograms obtained using the modified approach. (d) shows the power spectrum using the standard approach. (e) shows the power spectrum using the modified approach.

The type and frequency of occurrence of the non-stationary region will determine the performance of the modified approach in mitigating the non-stationarity in the data.

Analysis of neonatal RRi

We partitioned the RRi of the newborns into 10 minute windows, each 10 minute window has 10 sixty second windows, and performed spectral analysis using standard and modified approaches with no overlapping. The instances containing missing data were visually identified and discarded from further analysis. From the power spectra of each 10 minute window, we quantified the power in the low-frequency band as sum of the power in the frequency band of 0.05-0.25Hz divided by the total power (see equation (1))

Similarly, we quantified the power in the high-frequency band as the sum of the power in the frequency band of 0.3-1 Hz divided by the total power (see equation (2)).

Total power was defined as the sum of the powers in the frequency band of 0.05-2 Hz (see equation (3)).

In a clinical setting, we would like to know how specifically the heart rate characteristic is distinguishing the two groups of infants, hence we used receiver operating characteristics (ROC) curve to study the differences between the two groups by comparing the spectral powers of the favorable and adverse outcome newborns. We performed ROC analysis for every three hours starting from the time of birth of the newborns. We compared the area under the ROC curve (AUC) obtained for the

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standard and modified approaches using paired t -test. We considered the AUC values greater than 0.7 as significant separation between the two groups. A p < 0.05 was considered statistically significant.

RESULTS

A 10 minute segment of RRi from an adverse outcome infant containing missed beats is shown in Figure 3(a). The inset shows the magnitude of the spikes in this data. In Figure 3(b), we replaced the spikes with the same number of data taken equally from either side of the spike to take away the non-stationarity in the data. For the RRi shown in Figure 3a, the power spectrum obtained using the standard approach showed higher power in the low-frequency band and lower power in the high-frequency band compared to the spectrum obtained from the modified approach (Figure 3(c)). However, as shown in figure 3d, spectra obtained from both the modified and standard approaches showed similar magnitude for the stationary RRi shown in Figure 3(b). The spectral powers both in low- and high-frequency bands obtained from the modified approach were almost same in both Figure 3(c) and Figure 3(d). However, the spectral power obtained by the standard approach differed significantly between Figure 3(c) and Figure 3(d).

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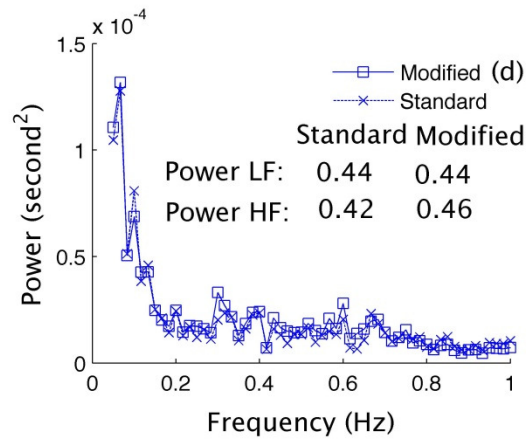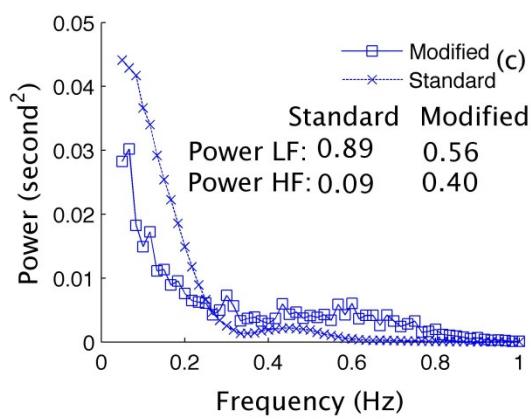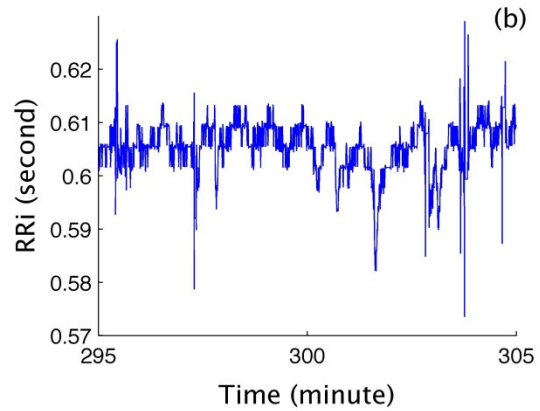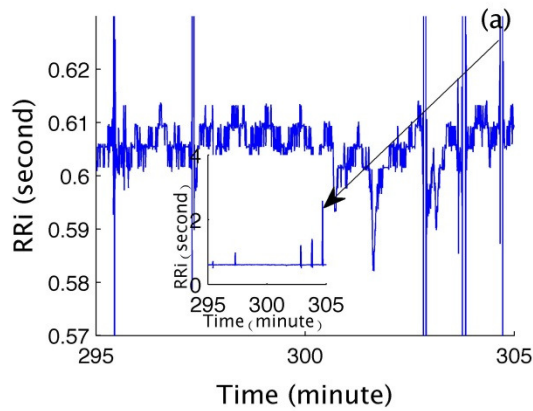
FIG. 3. Comparison between the modified approach and the standard approach in characterizing the non-stationary RRi. For an infant in adverse outcome group (a) non-stationary RRi for a period of 10 minutes (b) RRi after replacing the spikes by the points on either side of the spike. Power spectra obtained using standard and modified approaches (c) for non-stationary RRi shown in (a) and (d) for the stationary RRi shown in (b). The normalized powers in low- and high-frequency bands measured from the spectra are given in inset.

Spectra obtained from an infant in the favorable outcome group at different stages of treatment are shown in Figure 4(a) from the standard approach and in Figure 4(b) from the modified approach. The spectra obtained from standard approach displayed higher power than the spectra from modified approach. Further, the modified approach showed a clear increase in the spectral power during the course of the treatment. Also standard approach showed an increase in the power but displayed spuriously higher power around 75 hours when the RRi was non-stationary.

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FIG. 4. Comparison of power spectra obtained from the standard and the modified approaches at different states of hypothermia treatment. For an infant from favorable outcome group (a) spectra obtained from the standard approach (b) spectra obtained from the modified approach.

Figure 5 and figure 6 show the power values obtained using the standard and the modified approaches for subjects from favorable outcome group. Looking at these examples, we can see how the non-stationarities lead to a different frequency distribution of the power.

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FIG. 5. Comparison of power spectra obtained from the standard and the modified approaches at different states of hypothermia treatment for an infant from favorable outcome group (a) spectra obtained from the standard approach (b) spectra obtained from the modified approach.

FIG. 6. Comparison of power spectra obtained from the standard and the modified approaches at different states of hypothermia treatment for an infant from favorable outcome group (a) spectra obtained from the standard approach (b) spectra obtained from the modified approach.

Similarly, the spectra obtained from an infant in the adverse outcome group at different stages of treatment are shown in figure 7(a) from the standard approach and in figure 7(b) from the modified approach. Standard approach showed lower power compared to the power obtained from the modified approach. Further, the spectra obtained from the modified approach showed peaks around 0.75Hz corresponding to the respiratory sinus arrhythmia (RSA) while the standard approach showed no discernible peak around this frequency band. It is worth mentioning here that dividing the data by the standard deviation in the modified approach might lead to the assumption that if the standard deviation was small, then a huge peak would show and that is what we see in figure 7(b) at RSA frequency, however, since we divide all data points by the same standard deviation, then the effect of enlarging should be the same at all frequency bins and no peak would have been discerned around RSA frequency particularly. The standard approach failed to characterize RSA because the presence of non-stationarities redistributed the power in different frequency bins as we see in figure 7(a).
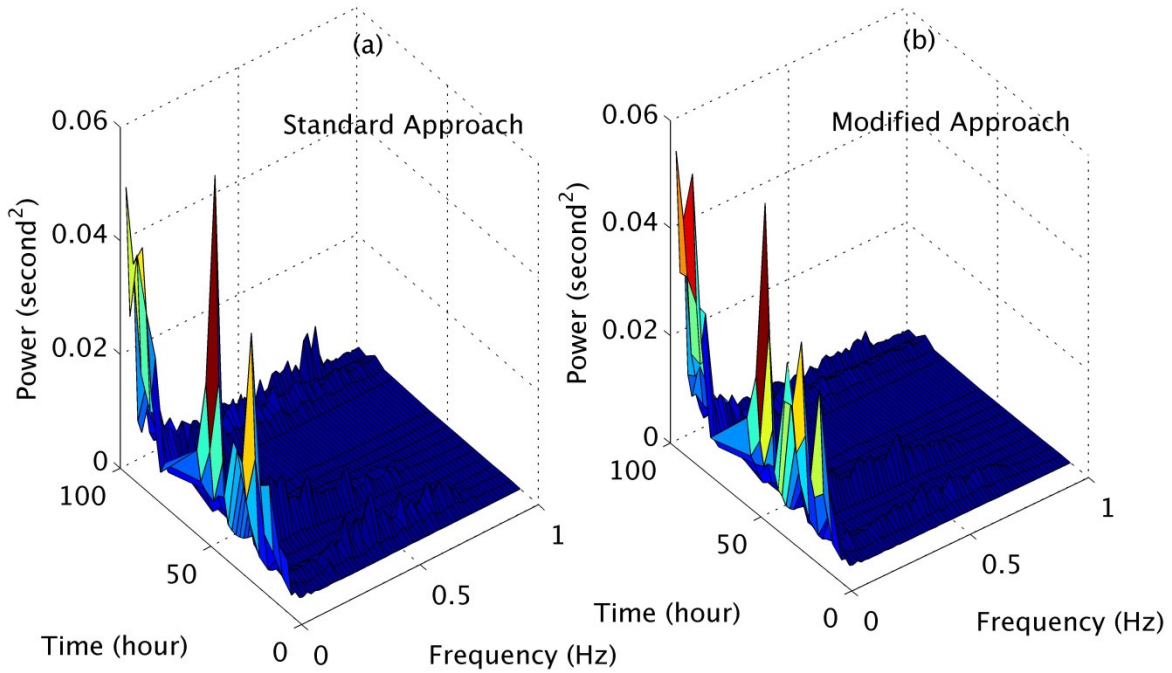
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FIG. 7. Comparison of power spectra obtained from the standard and the modified approaches at different states of hypothermia treatment for an infant from adverse outcome group (a) spectra obtained from the standard approach (b) spectra obtained from the modified approach.

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Figure 8 and figure 9 show the power values obtained using the standard and the modified approaches for subjects from adverse outcome group. Looking at these examples, we can see how the non-stationarities lead to a different frequency distribution of the power.
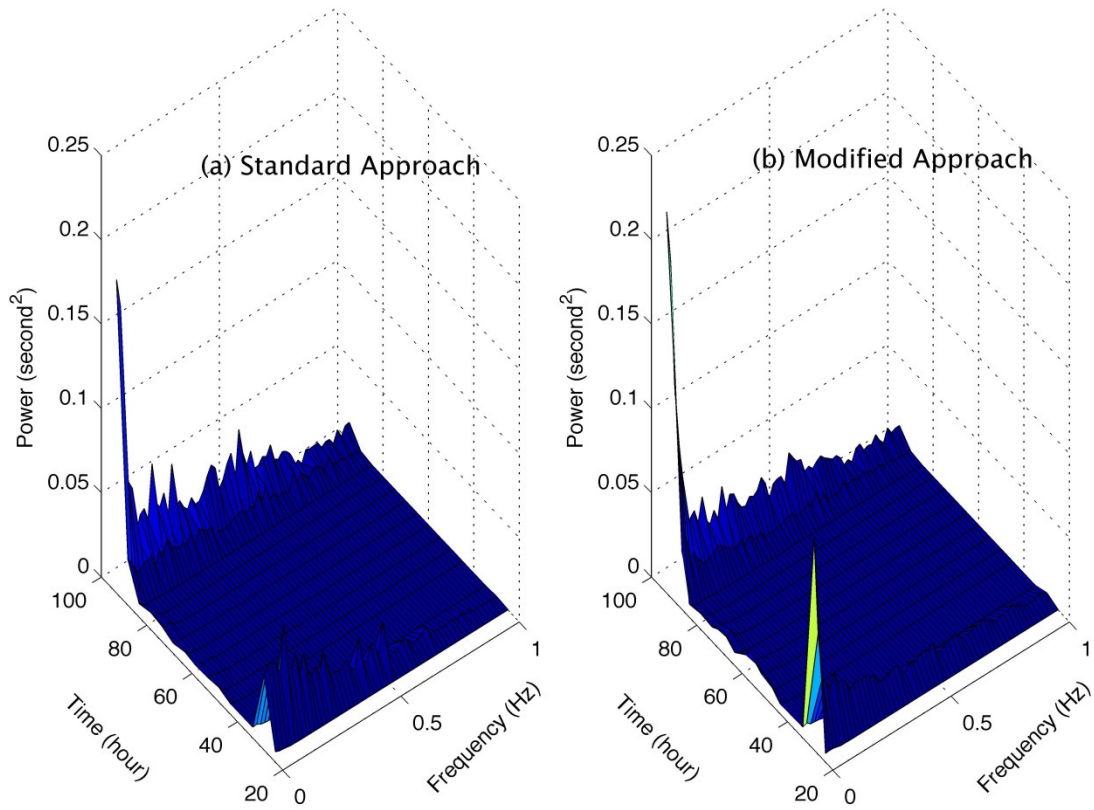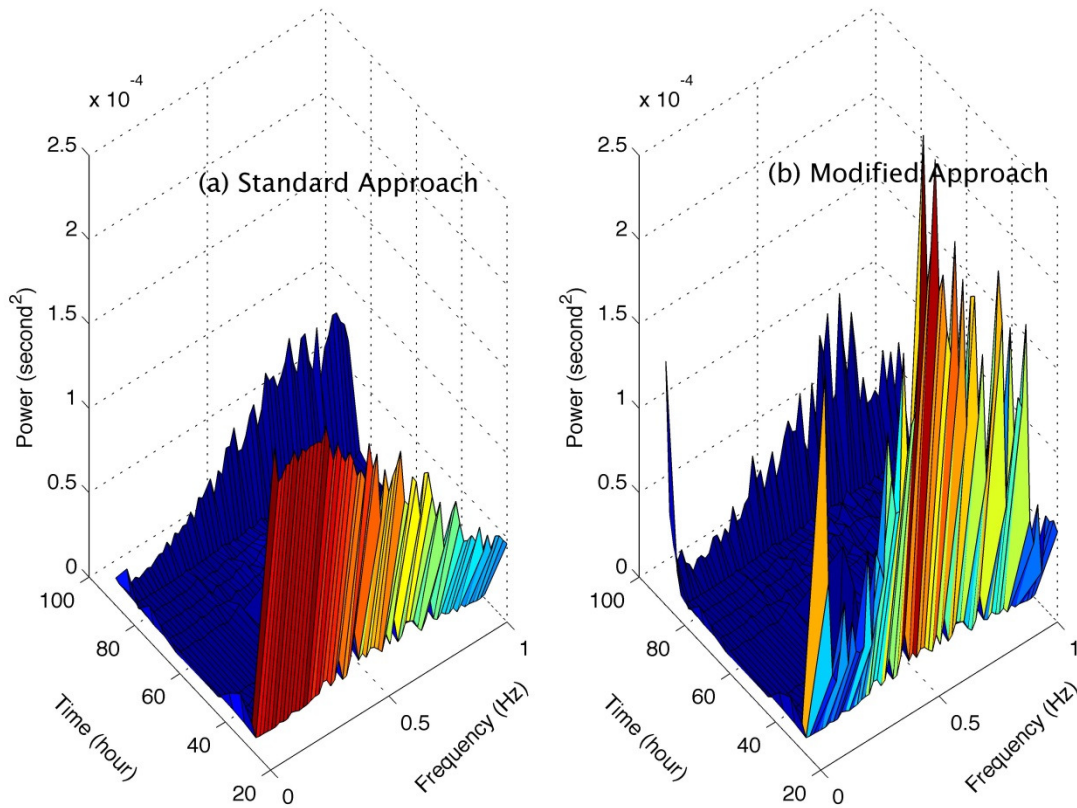
FIG. 8. Comparison of power spectra obtained from the standard and the modified approaches at different states of hypothermia treatment for an infant from adverse outcome group (a) spectra obtained from the standard approach (b) spectra obtained from the modified approach.
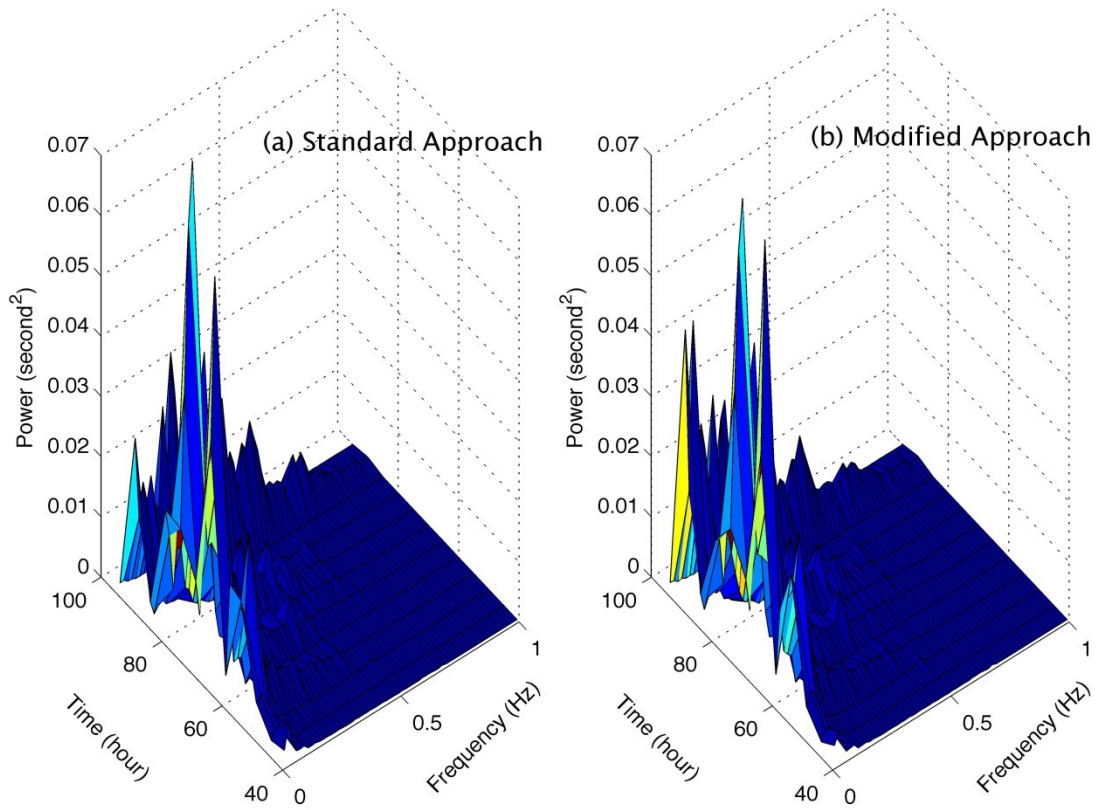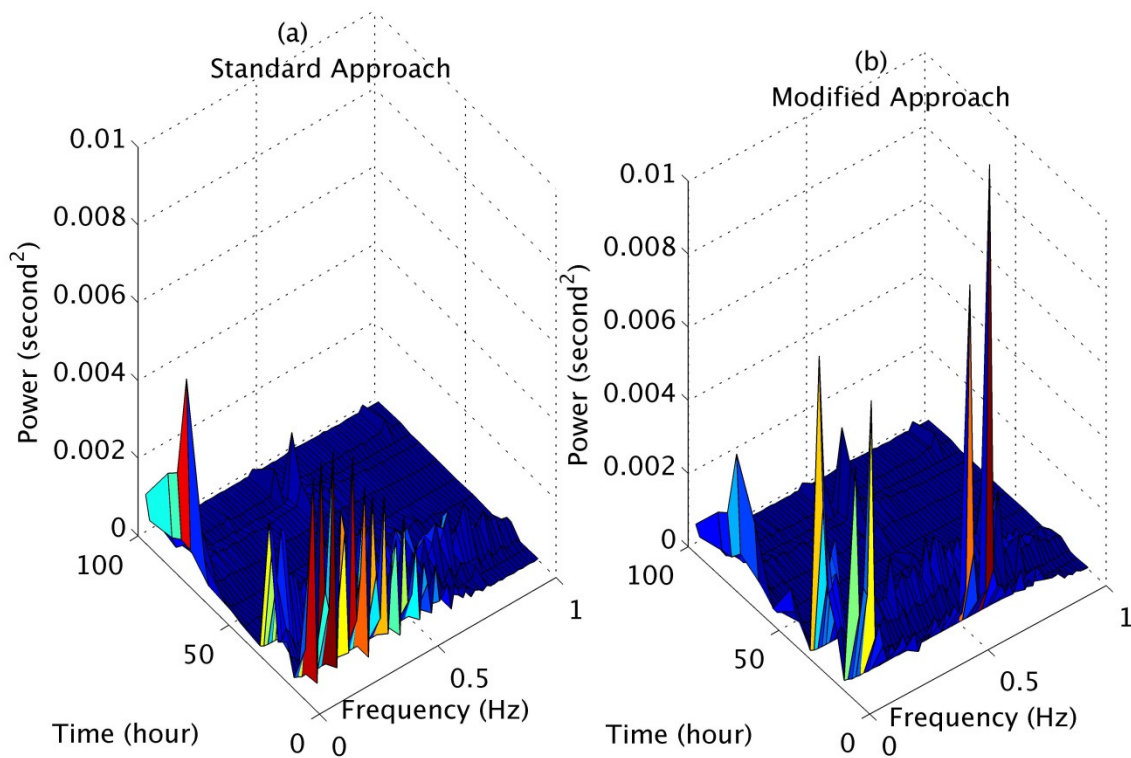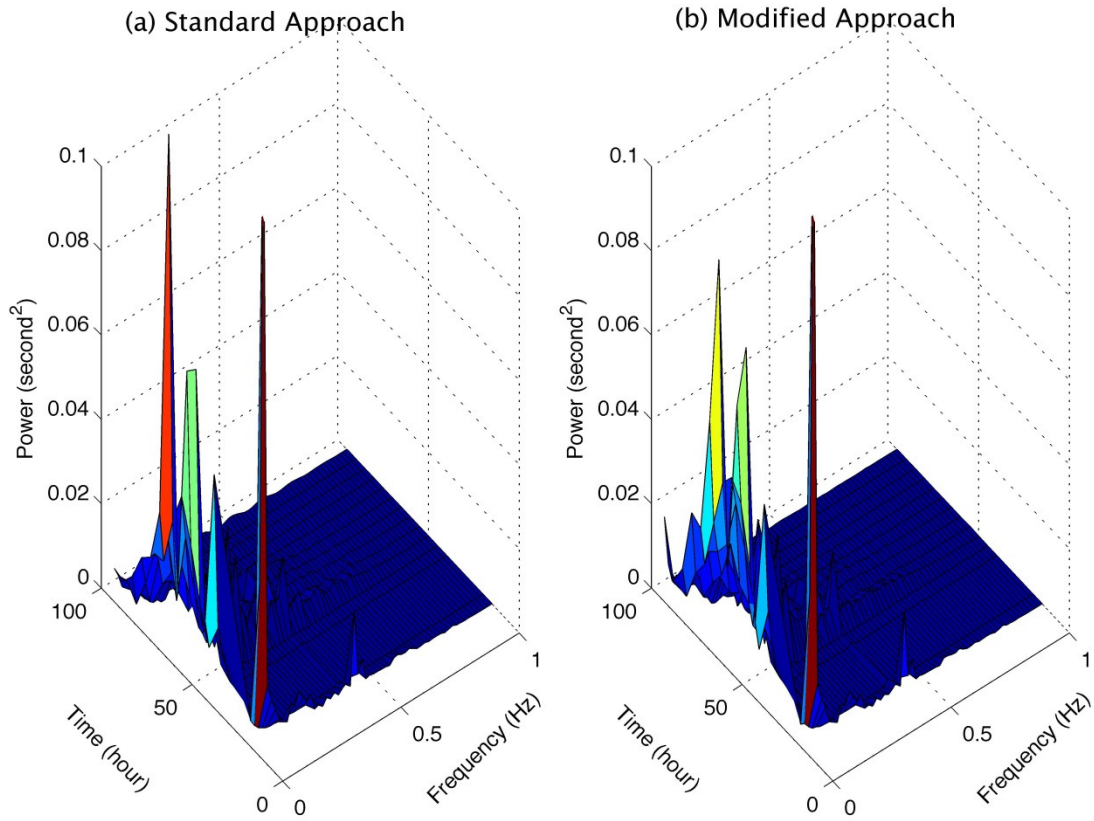
FIG. 9. Comparison of power spectra obtained from the standard and the modified approaches at different states of hypothermia treatment for an infant from adverse outcome group (a) spectra obtained from the standard approach (b) spectra obtained from the modified approach.

Statistical Analysis

Descriptive statistics included standard measures of central tendency using the median, variability for continuous data using the interquartile range and frequencies for categorical variables by showing how many subjects were available from each outcome group at the different time points where the comparisons were made. Non-parametric testing (Wilcoxin Rank-Sum Test) was used to evaluate the differences in HRV between outcome groups at each time point. Correction for multiple comparisons 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.

EEG/EKG monitoring began at a median 11.6 (mean: 14.6 hours) (Range 4.7-40.7) hours of life. Total study duration varied between 20-110 hours (mean: 77.2 hours and median 90.05 hours). Shorter duration studies were, as expected, present in the infants who died during cooling. Additionally, 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), that particular epoch was excluded. Thus, individual comparisons were made

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between subjects with available data for each given 3-hour time epoch. Evolution of HRV over time for each outcome group is depicted in Figure 10. HRV was lower at nearly all time points 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.
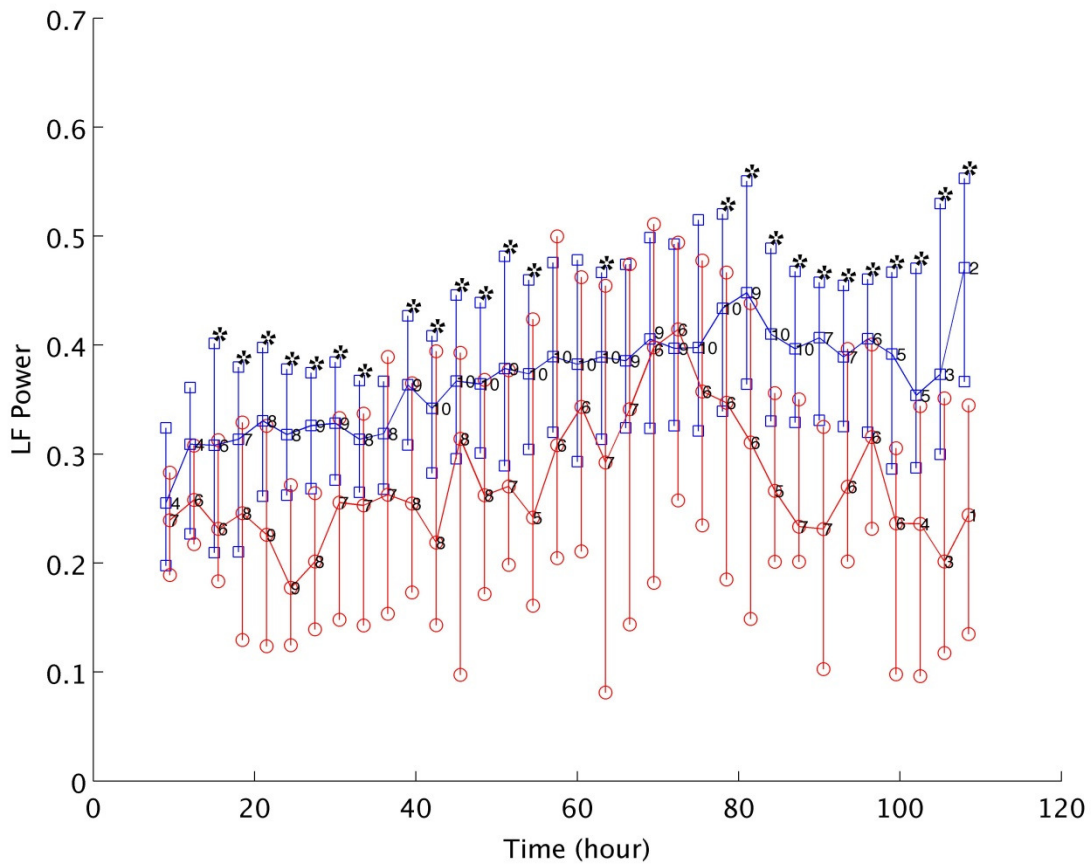
Fig. 10. A graph that shows the HRV LF power median and interquartile range for each group (favorable and adverse outcome) for every three hours of data. Number of subjects involved to make the calculations is shown. Asterisks denote where the difference was significant.

Area under the receiver operating curve for discrimination of outcome group is plotted over time in Figure 11(a). The highest AUC values 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. This was attributable to a decrease in HRV in the adverse outcome group, signaling potential response to ongoing brain injury in these infants.

For low-frequency power, the modified approach showed higher values of AUC compared to the standard approach. In both standard and modified approaches, the adverse group showed lower power compared to the favorable group (Figure 11a). Also, for high-frequency power, the modified approach showed higher values of AUC compared to the standard approach (Figure 11b). In this band, newborns in adverse group displayed higher power compared to the newborns in the favorable group. In both low- and high-frequency bands, there is a statistically significant difference between the AUCs obtained from modified and standard approaches ($P < 0.05$) using paired t-test and using all AUC values. The earliest AUC value that reached 0.7 was at 21 hours.
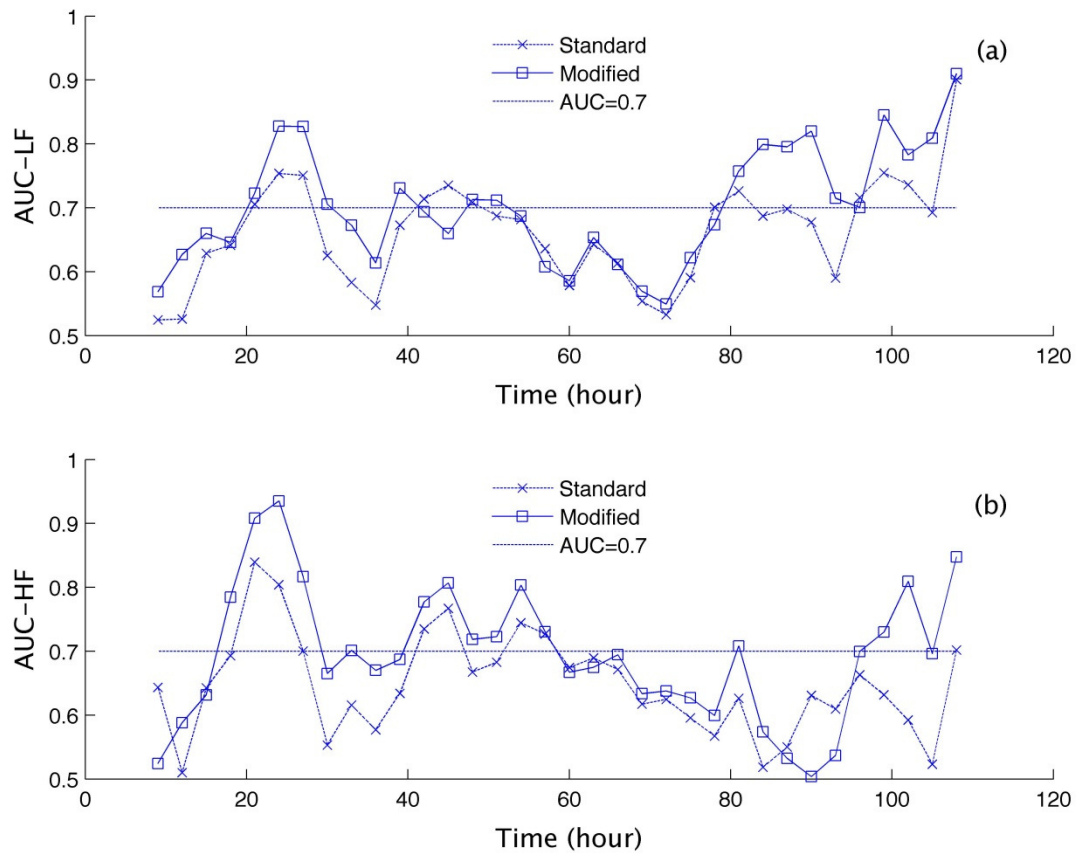
FIG. 11. Comparison of AUC obtained from the modified and standard approaches in distinguishing the spectral power of the two groups of newborns in (a) low-frequency and (b) high-frequency. The dashed line represents the AUC value of 0.7 and is shown for reference.

Further, since most of the differences in AUC values between the modified approach and the standard approach are between time of birth and 30 hours from birth. Also, a difference in the AUC values is shown to be between 78 hours from birth to 108 hours from birth, AUC values showed in figure 11a are divided into three figures (figure 12, figure 13 and figure 14) to show these differences. Each of these figures shows the median and interquartile range of AUC values in the time periods from birth to 30 hours of birth, from 30 hours from birth to 78 hours from birth, and from 78 hours from birth to 108 hours from birth, respectively. The first time period include the 24 hour from birth time point that corresponds to the secondary injury cascade, while the third time period include the after 80 hour time points that correspond to the rewarming period. We see the greatest difference between heart rate variability values of the infants with favorable outcome and infants with adverse outcome at these time points. The modified approach shows this difference more clearly than the standard approach.

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Fig. 12. Median, 75th percentile and 25th percentile of AUC values obtained by comparing the low frequency power of heart rate variability of infants with favorable outcome and infants with adverse outcome. AUC values were taken from the time periods from birth until 30 hours of birth. 1 refers to AUC values obtained from the standard approach. 2 refers to AUC values obtained from the modified approach.
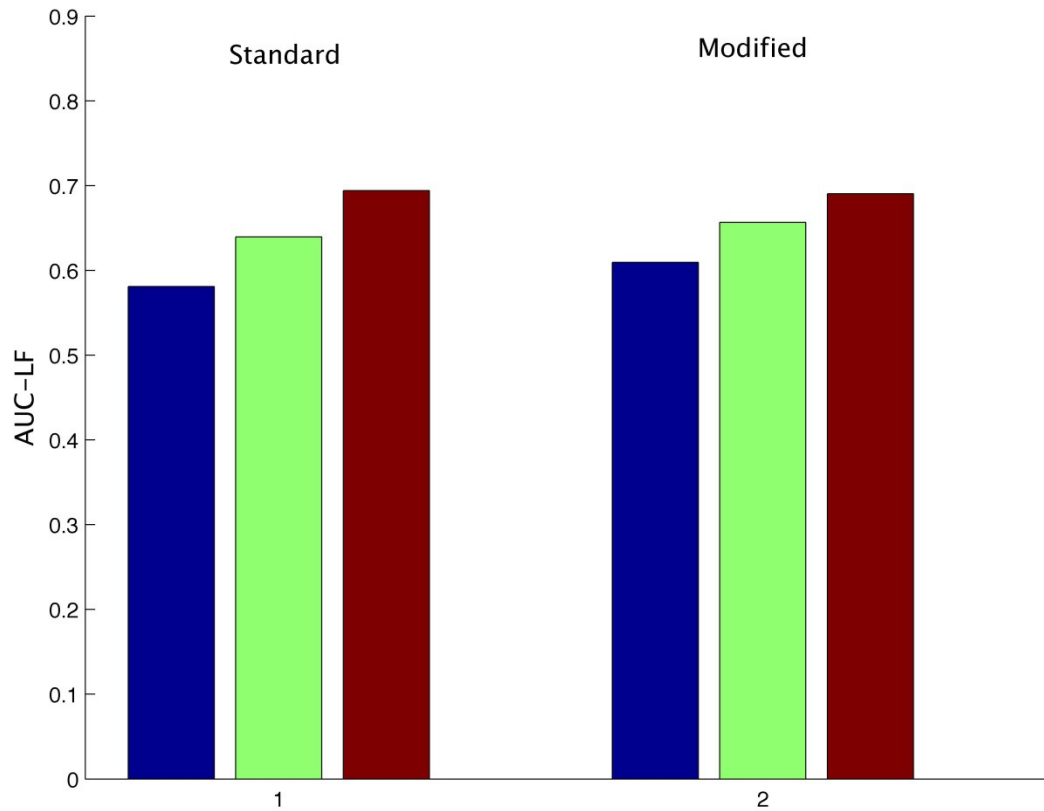
Fig. 13. Median, 75th percentile and 25th percentile of AUC values obtained by comparing the low frequency power of heart rate variability of infants with favorable outcome and infants with adverse outcome. AUC values were taken from the time periods from 30 hours of birth until 78 hours of birth. 1 refers to AUC values obtained from the standard approach. 2 refers to AUC values obtained from the modified approach.
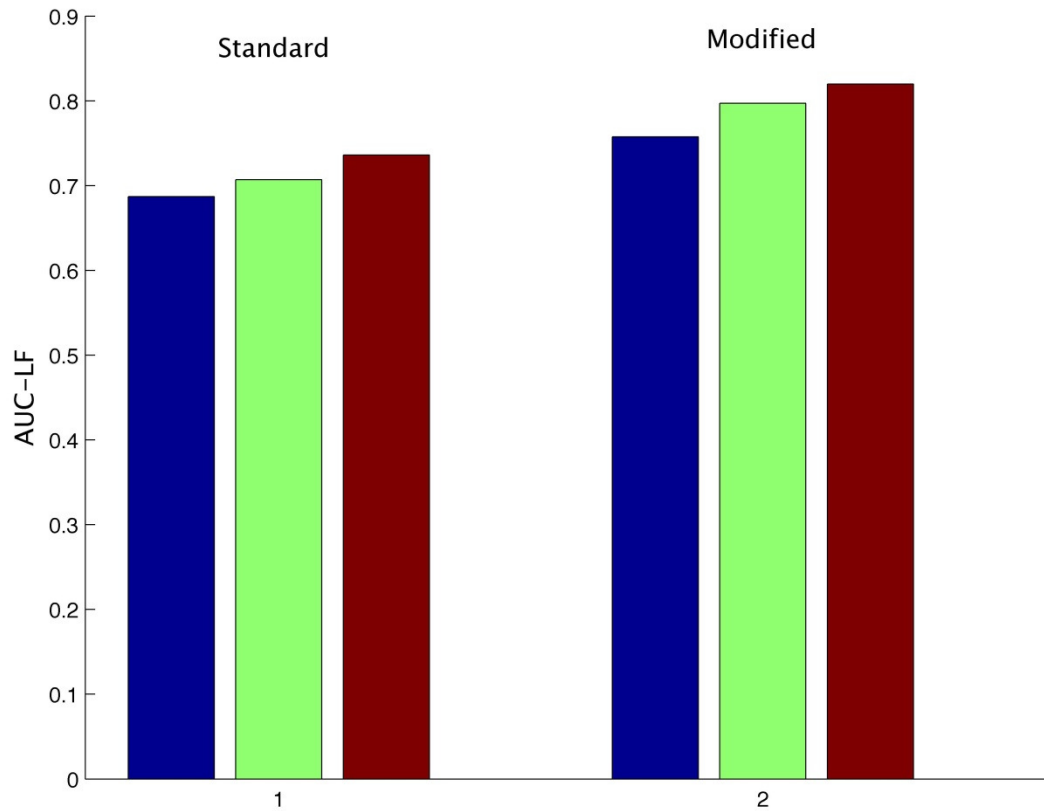
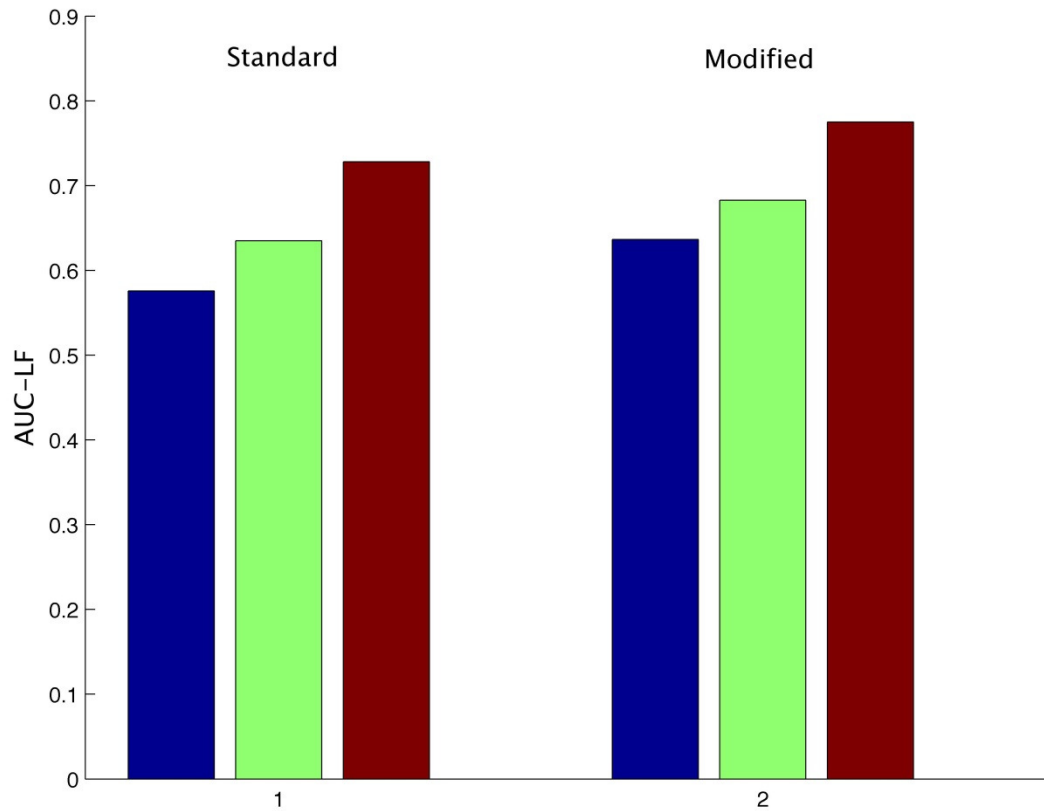Fig. 14. Median, 75th percentile and 25th percentile of AUC values obtained by comparing the low frequency power of heart rate variability of infants with favorable outcome and infants with adverse outcome. AUC values were taken from the time periods from 78 hours of birth until 108 hours of birth. 1 refers to AUC values obtained from the standard approach. 2 refers to AUC values obtained from the modified approach.

DISCUSSION

This study evaluates the evolution of HRV over the course of hypothermia and rewarming in newborns with HIE. Consistent with prior work (Aliefendioglu D, 2012) (Matic V, 2013), we demonstrated that HIE infants with adverse outcome have decreased HRV compared to infants with favorable outcome as has been shown using ROC analysis for low frequency power of heart rate variability. 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 previously reported peak of secondary energy failure after hypoxic ischemic injury (Johnston MV, 2011), and 2) after 80 hours of life upon the completion of rewarming. The discriminatory power of HRV reflecting 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 distress (EH., 1996). HRV has since been described as a predictor of outcome in other high-risk populations including neurosurgical patients (Haji-Michael PG, 2000), patients traumatic brain injury (Biswas AK, 2000), preterm infants (Yiallourou SR, 2013) (Golder V, 2013), newborns with sepsis (Fairchild KD, 2010) and necrotizing enterocolitis (Stone ML, 2013), and other critically ill populations (Gang Y, 2002). Few studies have evaluated HRV in newborns with HIE. Aliefendioglu and colleagues (Aliefendioglu D, 2012) 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

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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 (Matic V, 2013). The investigators evaluated 2-hour EKG segments recorded any time during the first 18-48 hours of life. The advantages of the current 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 (Lasky RE, 2009), 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 (Anon., 1996), power spectral analysis of the beat to beat (RR) interval was first described by Akselrod and colleagues (Akselrod S, 1981) 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 (Piccirillo G, 2009) (Piccirillo G, 2009) (Shah AJ, 2013). In the aforementioned study by Matic and colleagues, LF power was amongst the best discriminators of outcome

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selected from 24 different quantitative HRV parameters evaluated (Matic V, 2013). Thus, LF spectral power was evaluated as the primary measure of HRV in this study.

A key finding of the current study is the temporal evolution of HRV (as shown by different AUC values and different low frequency power of heart rate variability with time) in the two outcome groups as shown in figure 10 and figure 11. 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 the ongoing secondary injury cascade in the adverse outcome infants was partially mitigated by hypothermia and that this process was left unhindered by the cessation of cooling. Thus, HRV may help determine the adequate duration of cooling 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, or a combination of these factors cannot be elucidated by this study. The fact that patients in the adverse outcome group required more vasopressor support is perhaps indicative of myocardial dysfunction that could be contributory to reduced HRV. The interaction of these factors with the association between HRV and outcome warrant further study in a larger population. 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, the analytical approach utilized incorporated an automated artifact rejection method and a modification that mitigated effects of non-stationarity in the data that could occur as a result of signal artifact (Govindan RB, 2013).

We did the analysis using and comparing the performance of a recently introduced modified spectral estimation against the standard spectral power estimation in characterizing RRi of term birth asphyxiated newborns receiving therapeutic hypothermia. The modified power spectrum distinguishes the RRi characteristics of infants with favorable versus adverse outcome better than the standard approach. The

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modified spectral approach captures the difference between infants with favorable versus adverse outcome at 21 hours from birth. Furthermore, the difference peaks around 24 hours and after 80 hours.

Several factors related to cardiac dynamics can cause non-stationarity to RRi and they include the occurrence of tachycardic (Govindan RB, 2013) and bradycardic (Lasky RE, 2009) beats. Further, ongoing interventions and critical care events can contribute to the non-stationarity. We have shown that the power spectra obtained by the modified and standard approaches yield the same results when applied to the stationary RRi.

As mentioned earlier; RRi of infants with adverse outcome showed predominance of the parasympathetic component that may be due to an extrinsic factor, such as an increased dependency on the external ventilator support. The modified approach quantifies this feature correctly. The standard spectral approach is highly sensitive to the sudden changes in the RRi and its characterization is severely affected by the presence of the spurious and arrhythmic beats. AUC results show that the modified approach is able to better distinguish the two groups of infants compared to the standard approach. Based on animal studies (Piccirillo G, 2009) (Piccirillo G, 2009) and studies of critically ill humans (Shah AJ, 2013), the low power in the low-frequency component of the infants with adverse outcome group may indicate impaired autonomic function. The high spectral power in high-frequency band of the infants with adverse outcome may also indicate increased dependency on ventilator support. It may also

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indicate predominance of the parasympathetic component over the sympathetic components in adverse outcome infants.

RRi obtained in a clinical setting displays non-stationary segments that include missing beats, extra beats, slow changes lasting for a long period of time and arrhythmic beats. Each one of these affects the characterization of RRi in a different way (Govindan RB, 2013) (Chen Z, 2002). Sleep cycles are also known to modulate the RRi (Bunde A, 2000). Further, fetuses and neonates display acceleration patterns during the sleep states (Nijhuis JG, 1982) which may further confound the characterization of RRi. Our results show that the modified spectral estimation approach ,even though does a better job in addressing forms of non-stationarities when compared to the standard spectral estimation approach, is not able to adequately address other forms.

CONCLUSION

Using the modified and standard spectral approaches to study the RRi of birth asphyxiated newborns, the standard approach (owing to nonstationarity in the RRi) was not able to adequately characterize the dynamics and distinguish between newborns with favorable versus adverse outcome. In contrast, the modified spectral approach better distinguished the heart rate variability of the two groups of newborns during hypothermia treatment and after rewarming.

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 by

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identifying infants with poor response to hypothermia treatment and direct adjuvant neuroprotective therapies.

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