



Identifying an optimal epoch length for spectral analysis of heart rate of critically-ill infants



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ABSTRACT

Background and objective: To identify the optimal epoch length for power spectral analysis of cardiac beat-to-beat intervals (BBI) in critically ill newborns.

Materials and method: BBI of 49 term newborns undergoing therapeutic hypothermia for hypoxic-ischemic encephalopathy with well-defined outcomes (good outcome (n = 28): no or mild brain injury and adverse outcome (n = 21): moderate or severe brain injury or death) served as test population. A power spectrum of BBI was calculated with an autoregressive model in three different epoch lengths: 2 min, 5 min, and 10 min. Spectral power was quantified in three different frequency bands: very low-frequency (0.016–0.04 Hz), low-frequency (0.05–0.25 Hz), and high-frequency (0.3–1 Hz). In each frequency band, the absolute power and the normalized power were calculated. Furthermore, standard deviation (SDNN) of BBI was calculated. These metrics were compared between the outcome groups with a receiver operator characteristic (ROC) analysis in 3-h windows. The ROC curve area > 0.7 was regarded as a significant separation.

Results: The absolute spectral powers in all three epoch lengths in all three frequency bands and SDNN distinguished the two outcome groups consistently for most time points. The spectral metrics calculated with a 2-min epoch length performed as well as the five- and 10-min epoch lengths (paired *t* – test *P* < 0.05).

Conclusion: Spectral analysis of BBI in 2-min epoch shows a similar discriminatory power as longer epoch lengths. A shorter epoch also has clinical advantages for translation into a continuous real-time bedside monitor of heart rate variability in the intensive care unit.

1. Introduction

The clinical significance of fetal electrocardiogram (ECG) was recognized when Hon and Hess demonstrated the relation between intrapartum fetal heart rate variability (HRV) and fetal distress [1]. Newborn HRV has been shown to be inversely related to respiratory distress [2]. Impaired parasympathetic tone during preterm infants' first week of life has been shown to be related to the development of infection [3]. Furthermore, an altered parasympathetic tone on the first day of life has been shown to be associated with the development of intraventricular hemorrhage [4].

The central autonomic network refers to connected brain regions that modulate autonomic tone. These brain regions have been established using animal model, humans undergoing brain surgery [5,6] and functional image analysis [7]. In our earlier studies, we have used brain

injury as a model and identified the relationship between the region of brain injury and heart rate variability. In term hypoxic-ischemic encephalopathic (HIE) infants, injury in the right cerebral hemisphere has been associated with a decrease in the low-frequency power whereas injury in the left cerebral hemisphere has been associated with a decrease in the high-frequency power [8,9]. Furthermore, injuries in the deep brain regions were associated with higher depression of heart rate variability compared to absence of injury [10]. These studies highlight the importance of monitoring HRV of newborns, which can be performed easily at the bedside.

Several methods have been used to characterize the normal beat-to-beat interval (BBI) [11–14]. Among all methods, power spectral analysis is commonly used since it allows for characterization of slow and fast changes in the BBI in selected frequency bands. Initial studies focused on calculating the spectrum using the square of the magnitude of

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the Fourier coefficients; whereas later studies used estimation techniques [11,15]. In general, estimation techniques such as Welch periodogram or autoregressive model (AR), attenuate stochastic (statistical) fluctuations in the BBi and allow reliable characterization of the changes in the autonomic tone. For short datasets, the AR approach yields reliable results compared to the Welch periodogram approach [16].

The Task Force recommends using 5-min epoch length of heart rate for HRV analysis. Yet different periods are currently used to examine critically ill infants' HRV. In our previous studies, we have used 10-min epoch length of BBi [17]. Others have used 5-min epoch lengths [18–20] or 20-min epoch length of BBi [21]. This difference in choice of epoch length is dependent on the technique used to characterize heart rate. The heart rate during early development is high and it decreases with maturation [12]. This reflects the increase in parasympathetic tone, and the consequent decrease in the sympathovagal tone ratio, with maturation [12]. The number of beats sampled in a given period is greater when the heart rate is high compared to when the heart rate is low. When the dynamics of the system is changing quickly it can be more effectively characterized using short measurements. In addition to these inherent dynamics, disease dynamics (e.g., evolving encephalopathy) may change the course of the physiology from its natural path. A system that exhibits fast change in dynamics can be more effectively characterized using a short epoch length of data as the essential features of the dynamics may be diluted when a long period of data is used. This higher heart rate in newborns may allow characterization of the HRV in shorter epoch lengths compared to older children and adults. Thus, the goal of this manuscript is to test the feasibility of characterizing the autonomic tone using 2-min epoch length of heart rate in term newborns. To test this hypothesis, we consider term newborns undergoing therapeutic hypothermia for hypoxic-ischemic encephalopathy (HIE) with well-defined clinical outcome groups (good versus adverse outcomes [10]) based on MRI findings. We hypothesize that the HRV characterized using a 2-min epoch will distinguish the two outcome groups of cooled HIE newborns to the same degree or better than the HRV of longer (5-min and 10-min) epoch lengths.

2. Materials and Methods

In this prospective study, acutely ill HIE newborns referred to our level 4 neonatal intensive care unit for therapeutic hypothermia were examined. The newborns were undergoing whole-body therapeutic hypothermia based on a well-established National Institute of Child Health and Human Development protocol [22]. Briefly, the protocol includes maintaining the newborn's core temperature at 33.5 °C for 72 h and gradually rewarming to normothermia at a rate ≤ 0.5 °C/hour over 6 h. The Children's National Institutional Review Board approved the study and informed consent was obtained.

We have abstracted the clinical variables from the clinical server. The clinical variables are shown in Table 1. The mean gestational age at

Table 1
Clinical variables (n = 49).

Clinical variable	Mean \pm standard deviation or median (minimum, maximum)
GA	38.65 \pm 1.54
Weight	3.2 \pm 0.78
APGAR1*	1 (0, 9)
APGAR5**	3 (0, 9)
APGAR10***	5 (0, 9)
BD****	19.79 \pm 5.41
Male (n %) ^a	25 (51%)

*, **, ***, **** indicate that their statistics were calculated only from 48, 47, 42 and 38 subjects, respectively.

^a Expressed as number (percentage).

birth is 38.65 weeks and 51% of the infants were male. The average weight of the infants was 3.2 kg. The medians of APGAR scores at 1, 5, and 10 min were 1, 3, and 5, respectively. The mean base deficit was 19.79.

Their electrocardiograph (ECG) and vital signs were monitored with a cardiorespiratory monitor (Intellivue MP70, Philips, MA, USA). The ECG waveform was retrieved and digitized at a rate of 1000 Hz using custom software developed in Labview (National Instruments, TX, USA). Brain magnetic resonance (MR) imaging (Discovery MR750, GE Healthcare, WI, USA) was performed in surviving infants at a median age of 10 days (range 3–18 days). The MR images were read and scored [23] by an experienced neuroradiologist (G.V.), who was blinded to the clinical course of the infant. Outcomes were classified as good (basal ganglia score < 3 , or watershed score < 4) or adverse (for details refer [10]).

ECG was bandpass filtered between 0.5 and 70 Hz using the 4th order Butterworth filter with zero phase distortion. The QRS complexes were identified using a combination of Hilbert transform and an adaptive threshold detection approach [24], and the BBi was calculated. The spikes in the BBi were corrected using a computer-based approach [25]. For spectral analysis, the BBi was converted into evenly sampled data with a cubic spline interpolation scheme with a sample period of 0.25 s.

The BBi was partitioned into 2-min, non-overlapping epochs. A spectral analysis was performed for BBi in each epoch using an autoregressive (AR) model. The AR coefficients were solved with the modified Yule-Walker approach [26,27]. The model order was systematically increased from 1 to 20. The spectrum was calculated as the linear combination of the product of AR coefficients and Fourier coefficients. The spectrum was estimated for every model order. The order of the AR model was determined with an intraclass correlation coefficient. If the intraclass correlation coefficient between the spectra calculated from two successive model orders was greater than 0.95, the spectrum was assumed to have converged and the calculation was terminated. Usually, the spectrum converged around the model order 6 or 7. Spectral power was calculated from the power spectrum for three frequency bands of very low-frequency (VLF) (0.016–0.04 Hz), low-frequency (LF) (0.05–0.25 Hz) and high-frequency (HF) (0.3–1 Hz). In each frequency band, the absolute and normalized power were calculated. The normalized spectral power was obtained by calculating the sum of spectral powers in that band divided by the sum of powers in the frequencies below 2 Hz for each frequency band and denoted nVLF, nLF, nHF, for very low-frequency, low-frequency, and high-frequency, respectively. The original power values were not normally distributed (Jarque-Bera test $P < 10^{-3}$) and hence the absolute power was calculated as the median of the logarithm of power in that frequency band and denoted VLF, LF, HF, respectively. Of note, the direct component which reflects the square of the mean BBi at zero frequency is not included in the calculation. In addition to spectral metrics, an asymmetric index (R) was calculated for each 2-min epoch as follows: Let the median BBi be μ . Let the sequence of BBi less or equal to μ be S_2 and the sequence of BBi greater than μ be S_1 . R_1 was calculated as the mean of the square of the deviations of S_1 from μ . Similarly, R_2 was calculated as the mean of the square of the deviations of S_2 from μ . To this end, R was calculated as the ratio of R_1 to R_2 . The metrics calculated for the BBi in a 2-min epoch was considered for comparison if R was within 0.8 and 2.

The very low-frequency (0.016–0.04 Hz) and low-frequency power (0.05–0.25 Hz) quantify the sympathetic arm of the autonomic nervous system (ANS) whereas the high-frequency power quantifies the parasympathetic arm of the ANS [10,11]. However, low-frequency power has been regarded as the interaction between sympathetic and parasympathetic nervous systems [28]. In addition to spectral metrics, we also calculated the standard deviation of the beat-to-beat intervals (SDNN) in every epoch. SDNN is also regarded as a measure of total power of a signal.

The asymmetric index is not a commonly used approach. It is being used in the Heart Rate Observation (HeRO) monitor [29]. The premise of this metric is that a stationary beat-to-beat interval will yield a distribution that will be symmetric around the median value. The presence of any outliers (bradycardia, tachyarrhythmia, missed beats or extra beats) will make the distribution of BBI asymmetric around the median value. There are several approaches to quantify any asymmetry in the BBI distribution (e.g., kurtosis); however, Kovatchev et al. have shown that asymmetric index calculated with the approach explained in this manuscript characterizes the asymmetry in the BBI distribution better than the traditional approaches [29,30]. We used this approach to identify spurious beats that escaped our spike-correction approach. It merely serves as a beat-to-beat interval quality gauging metric.

2.1. Statistical analysis

We checked the spectral metrics for Normality condition using the Jarque-Bera test before the receiver operating characteristic (ROC) analysis. The normalized and absolute spectral powers for each frequency band were compared between the good and adverse outcome groups using a ROC analysis. For each newborn, the median of the normalized or absolute spectral power was calculated for every 3-h period of time until 93 h of life. The median spectral power findings for the outcome groups were compared using a ROC analysis. Since the infants in the good outcome group were expected to show increased variability, this group was treated as a positive group. An area under the ROC curve (AUC) greater than 0.7 was regarded as a clinically meaningful separation [31–33].

The above analysis and comparison were repeated for the BBI partitioned into 5-min and 10-min epoch lengths. For every 3-h period, for every metric, there were three AUCs calculated for two-, five-, and 10-min epoch lengths. To identify the epoch length that best distinguished the outcome groups, the three AUCs from every 3-h epoch were compared using a paired *t*-test. In this exploratory study, the *P*-values were not adjusted for multiple comparisons. All analyses were performed offline in MATLAB 2018a (Mathworks Inc, MA, USA).

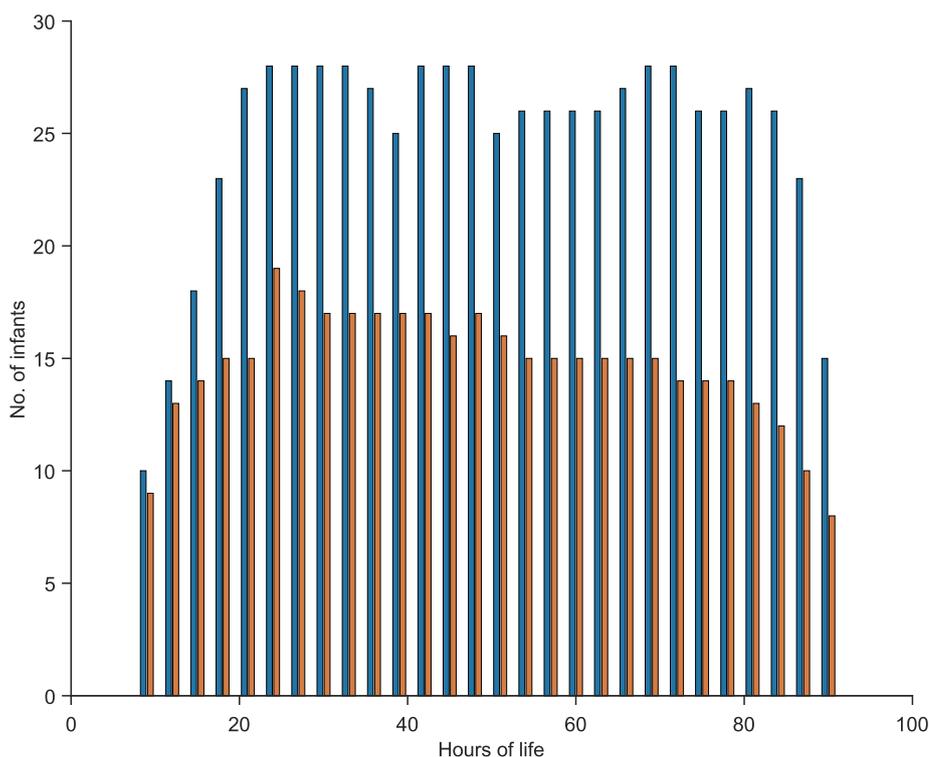


Fig. 1. (Color online) Histogram of the number of infants compared in every 3-h epoch. The good outcome group is shown in blue bar and the adverse outcome group in red bar. This distribution is the same for all three epoch lengths. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Results

A total of 49 newborns were included in this study. The median (minimum, maximum) duration of ECG monitoring was 74.03 (4.633, 91.8) hours. The median hours of life at study onset was 13.97 (5.81, 25.24) hours and the median hours of life at study completion was 89.15 (14.05, 111.61) hours. Twenty-eight newborns had a good outcome, and 21 newborns had an adverse outcome (11 had moderate to severe brain injury and 10 died).

The number of infants analyzed in every 3-h period for the good and adverse outcome groups is shown in Fig. 1. Except for the first (9–12 h), and the last 3-h period of life (90–93 h), all epoch lengths had at least ten infants in each group.

The mean and standard deviation of the spectral power in each band are shown in Fig. 2. For clarity, we have shown mean plus standard deviation of the spectral metrics of the good outcome infants and mean minus standard deviation of the spectral metrics of the adverse outcome infants. Except for nHF, almost all metrics are different between the two outcome groups. The differences in the metrics are quantitated using a receiver operating characteristic (ROC) analysis and the ROC AUCs are shown in Fig. 3.

The Jarque-Bera test showed that all of the metrics (nVLF, nLF, nHF, VLF, LF, HF, and SDNN) were Normally distributed ($P < 0.001$). The comparison AUC values for normalized and absolute spectral powers for each frequency band calculated for 2, 5, and 10-min epoch lengths are shown in Fig. 3a–f. The normalized spectral power distinguished the good versus adverse outcome groups only at narrow time points. In the nVLF, all three epoch lengths distinguished the outcome groups at approximately 21–42 h of life although there is a drop in the AUCs around 36 h for the 2-min results and drops at 36 and 38 h. In the nLF, the two groups were distinguished at 20–30 h of life. In the nHF, the two groups were distinguishable at 30–33 h of life and again after 80 h of life.

The absolute spectral power for all three frequency bands was superior to the normalized spectral power in distinguishing the outcome groups in each frequency band between 20 and 80 h of life (Fig. 3 d–f). This was true whether the BBI was partitioned at two, five, or 10-min epoch lengths.

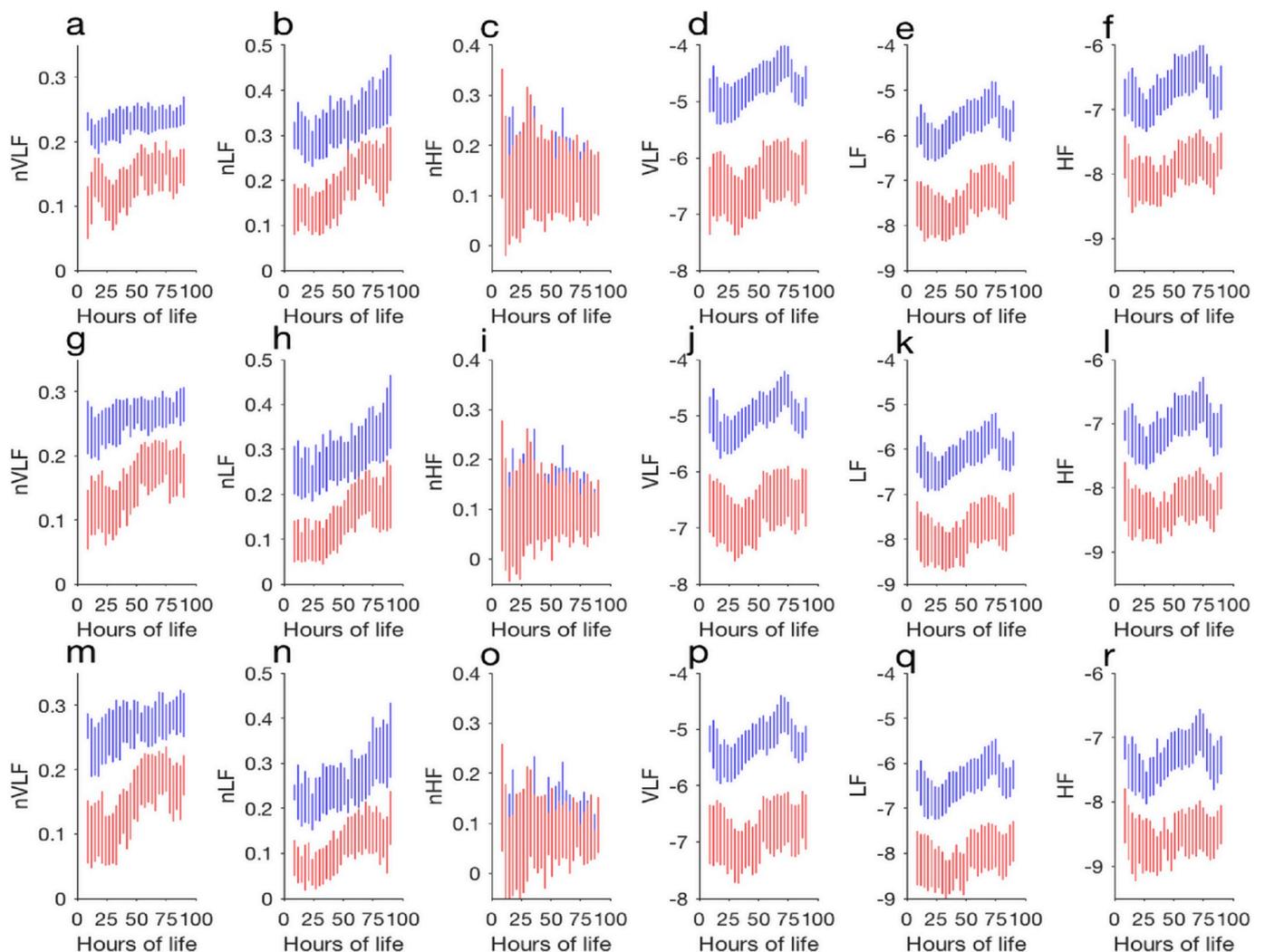


Fig. 2. (color online). Summary of HRV metrics in every 3 h. In each plot, mean plus standard deviation of the spectral power of the good outcome group and mean minus standard deviation of the spectral power of the adverse outcome group are shown. The spectral metrics from 2-min analysis are shown in a) nVLF, b) nLF, c) nHF, d) VLF, e) LF, and f) HF. The results shown in g-i, and m-r are the same as shown in a-f but obtained for 5-min and 10-min analyses, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The p-values comparing the AUCs of the spectral power analysis at two, five and 10-min partition epoch lengths are shown in Table 2. Only the normalized spectral powers for BBI in the very low-frequency band showed a distinction between two, five, and 10-min partition epochs lengths. Although nLF and nHF exhibited $P < 0.00001$, the AUC values obtained for these metrics were less than 0.7 for majority of the time points and hence they are not discussed further. The magnitude and direction of separation can be inferred from the t-scores shown in Table 2. For absolute spectral powers, the 2-min epoch partition yielded higher AUC values each in all three frequency bands (see the t-score values reported for 2-min vs 5-min and 2-min vs 10-min) compared to five and 10-min epoch lengths.

The AUCs obtained from the comparison of the SDNN of BBI of the two groups in each epoch length are shown in Fig. 4. Similar to the absolute power in the VLF, the SDNN also distinguished the two groups better in all three epoch lengths. The paired *t*-test comparison of AUCs obtained for SDNN and those obtained for very low-frequency (VLF) showed the spectral powers yielded higher AUCs in all three epoch lengths (2-min: $P = 0.0025$; 5-min: $P = 0.0455$; 10-min = 0.0088). Furthermore, the paired *t*-test analysis of AUCs obtained using SDNN comparison showed they were not different from each other; that is *t*-test showed AUC-two-minute versus AUC-five-minute, AUC-five-minute versus AUC-ten-minute as well as AUC-five-minute versus AUC-ten-

minute were not different from each other ($P > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

The AR model allows characterizing spectral power of short BBI data. The HRV metrics obtained from 2-min analysis performed as well as those obtained from 5 to 10 min. The absolute spectral power in the different frequency bands distinguished the two outcome groups of HIE infants better than their normalized spectral power counter parts. Both SDNN and spectral power showed the variability in the BBI was higher in good outcome group infants compared to adverse outcome group infants.

The sympathetic and parasympathetic components of the autonomic nervous system mature at different rates during early human development [34]. Several fetal electro/magnetocardiogram studies have shown an increase in the high-frequency spectral power after 31 weeks of gestational age [35–37]. The rapid maturation of the parasympathetic component in the third trimester is regarded as an indication of increased respiratory activity and increased presence of the active behavioral state [35,38]. Also, newborn studies have shown an increase in the high-frequency spectral power and a decrease in the

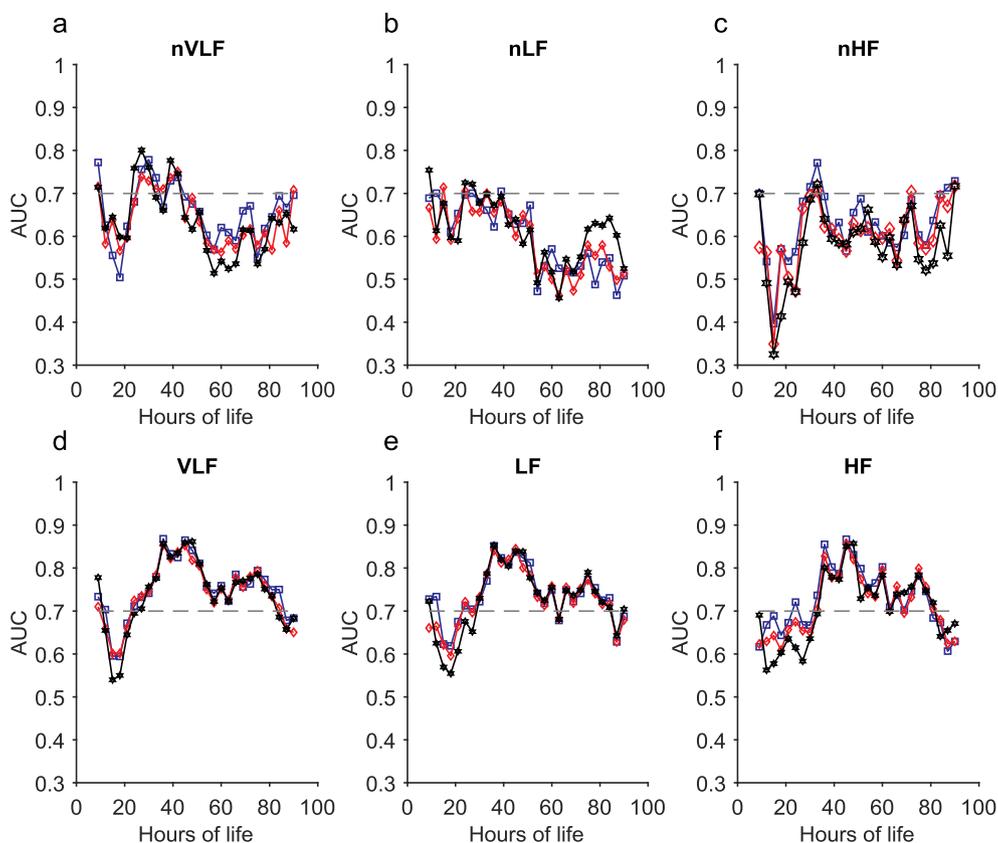


Fig. 3. (Color online) The area under the ROC curve (AUC) obtained from the six spectral metrics are shown as a function of hours of life. a) normalized very low-frequency (nVLF), b) normalized low-frequency (nLF), c) normalized high-frequency (nHF), d) very low-frequency (VLF), e) low-frequency (LF), and f) high-frequency (HF). The results from two-, five-, and 10-min analyses are shown in blue, red, and black, respectively. The horizontal dashed line at AUC = 0.7 is a reference line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Comparison of the area under the ROC obtained for every spectral metric for each length. P-values obtained from a paired t-test are given. The significant P values are shown in bold face. The t-values calculated for every pair are shown also in this table.

	2-min vs 5-min		2-min vs 10-min		5-min vs 10-min	
	t-score	P-value	t-score	P-value	t-score	P-value
nVLF	3.034	0.005	4.942	0.000	4.140	0.000
nLF	0.891	0.381	-1.376	0.180	-2.675	0.013
nHF	-3.526	0.002	-5.130	0.000	-2.304	0.029
VLF	2.480	0.020	2.758	0.010	1.478	0.151
LF	2.185	0.038	2.034	0.052	0.719	0.478
HF	3.147	0.004	2.167	0.039	1.184	0.247

ratio of the low-frequency to high-frequency spectral powers [12,39] with maturation. These studies indicate the maturation of the parasympathetic component and a relative decrease in the ratio of sympathovagal tone during development.

The Task Force recommendations for BBI analysis are made for adult studies [11]. The current epoch length for BBI analysis of newborns and frequency bands used to define low-frequency and high-frequency spectral power of BBI are taken from adult studies. These definitions may not apply to newborns. For example, the probability that a normal-to-normal interval to be greater than x milliseconds from its previous interval denoted as (pNNx, x = 50) metric that distinguished BBI of normal volunteers from individuals with congestive heart failure did not work for fetal data. For fetal BBI, owing to the shorter interbeat intervals, pNN10 has been shown to distinguish low-risk and high-risk fetuses [40]. Since the heart rate of newborns is faster than that of adults, BBI from short time epochs should be sufficient to distinguish the heart rate variability of two groups of HIE infants. In particular, in HF band the AUC was higher in the 2-min epoch than that were

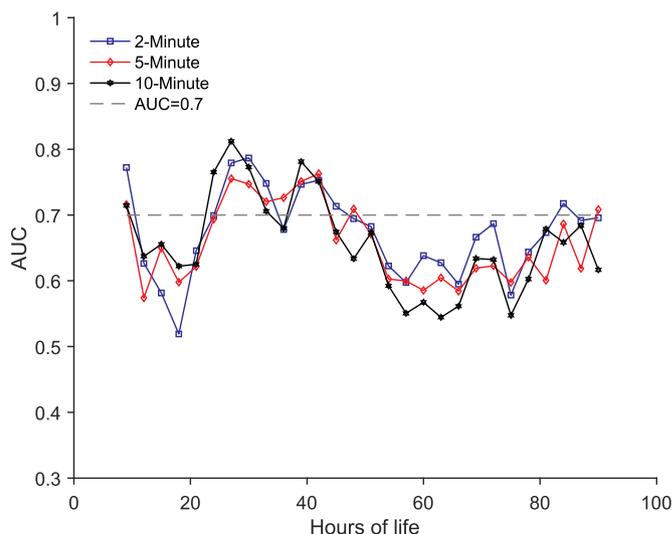


Fig. 4. AUC obtained for the comparison of standard deviation of BBI in 2-min, 5-min, and 10-min between good outcome and adverse outcome groups.

obtained with five- and 10-min epoch lengths.

We and others have shown that infants with HIE who had adverse neurological outcomes had depressed autonomic tone [41–44]. Earlier studies have shown a correlation between HRV and qualitative and quantitative electroencephalographic characteristics of encephalopathy [43,44]. In our earlier study [42], we demonstrated a high predictive ability of nLF and nHF to discriminate neurodevelopmental outcomes in infants with HIE. Our results indicate that the higher heart rate during early human development enables 2 min of heart rate to be sufficient to quantify HRV, exhibiting the same discriminatory power as those of 5-min and 10-min epochs.

Currently, there are no gold standard indices for characterizing

autonomic tone. While the AUC obtained from 2-min analysis is, on an average, higher than the AUC values obtained from the five- and ten-minute analyses, we can only safely conclude that 2 min of BBi are sufficient to distinguish the two groups of HIE infants. Further studies are needed to validate if 2 min of BBi are sufficient, or most optimal, to characterize autonomic tones in other populations of high-risk newborns.

In the current study, we observed poor predictive abilities for nLF and nHF, whereas absolute LF and HF power demonstrated better discrimination of outcome groups. The differences between the two studies may be attributable to several factors. First, in our earlier study, the spectral powers from individual 10-min epochs were used in the comparison whereas in the current study the spectral powers of individual epochs were averaged over 3-h period. Additionally, we confined our analyses to the comparison of epochs with a specified asymmetry index as an additional quality assurance metric, which was not used in the prior study. We did not consider the impact of clinical variables on the predictive value of the various HRV for discriminating outcome groups. As the goal of this work was to evaluate the comparability of varying time windows on the estimation of HRV, evaluating whether clinical variables mediated relationships observed between HRV and outcome was beyond the scope of this project. We have previously demonstrated that clinical factors, including encephalopathy grade, gestational age, and temperature, can impact the relationships between HRV and outcomes [10]. Finally, we evaluated neurological outcomes defined by developmental assessments in the earlier study whereas the current study defined outcomes based on severity of brain injury by MRI. Side by side comparison of these methods will be considered in detail in our future studies.

Early studies of HIE infants have shown an evolution of sleep-wake cycles in infants that had good outcome whereas this evolution was not observed in infants with adverse outcomes [45,46]. The higher spectral power in good outcome infants might represent an evolution of sleep-wake cycling and the depressed spectral power in the adverse outcome infants might represent a lack of the sleep-wake cycling in these newborns. Furthermore, the altered spectral power in adverse outcome group infants might be due to impairment of central autonomic network, brain centers that orchestrate autonomic modulation – caused by brain injury, however further studies are needed to confirm these inferences.

We also compared the spectral powers of the two groups of infants in LF calculated between 0.05 and 0.3 Hz and high-frequency calculated between 0.3 and 1.5 Hz. We found the LF frequency comparisons were not affected when extending the frequency band from 0.05 – 0.25 Hz to 0.05–0.3 Hz. However, in the HF power defined between 0.3 and 1.5 Hz the discriminatory power was compromised. These results clearly justify the choice of our frequency bands.

The absolute power in the very low-frequency can be regarded as a quantification of slow changes in the beat-to-beat intervals, which can also be quantified with SDNN. This relationship between very low-frequency power and SDNN shows the similarity between these measures in distinguishing the two groups of infants. However, the failure of SDNN to distinguish among three epoch lengths might indicate that total power (as it includes power below 0.04 Hz as well above 1 Hz) is not as robust as the power in the very low-frequency.

The strengths of this study include the evaluation of long recordings of BBi from a well-characterized cohort and the use of advanced signal processing techniques with robust approaches for artifact rejection and quality assurance. This study also has certain limitations. As a free-standing children's hospital without an attached labor and delivery unit, we do not have access to low-risk newborns. Hence, we have included only acutely ill HIE newborns in this study. While examining a healthy term cohort would be of interest, we feel that the application of this work would be most clinically relevant to distinguish acutely ill newborns (i.e., normal and pathological tracings). Because the AR modeling has been shown to yield reliable spectral estimation from

short dataset, we chose this approach in this study [16]. The model order of the AR process was fixed based on the convergence of the spectrum which is different from the traditional way wherein the model order is determined by the Akaike Information Criterion (AIC). Although the spectral convergence and AIC are closely related, this needs further investigation. We would also like to mention that the commonly used Akaike Information Criterion (AIC) is not reliable to identify the model order for short datasets. In this study, only the epochs with asymmetric index in a heuristically selected range were used. This might have confined the analysis to a certain behavioral (quiet) state of the infant. Further studies are needed to relate the AI to behavioral states. The characterization of sleep-wake cycles can help to interpret the spectral power differences between the two groups however, this has not been done in our cohort. We have also not corrected for any influence of behavioral states on variability analysis. Because low-frequency to high-frequency ratio has been shown to be an unreliable measure of sympathovagal balance [47], we did not use this metric in this study. Finally, although this method is demonstrated using HIE population with a well-characterized outcome, this method could be generalized to other types of critically-ill infants.

5. Conclusion

Analysis of BBi of newborns in 2-min epoch length is capable of distinguishing clinical groups. Extension of this approach to a larger cohort of HIE infant is needed and is currently underway with plans to translate this approach to real-time analysis for bedside application. Acknowledgment

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Conflicts of interest

The authors declare no conflict of interest.

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