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Inflammatory cytokine response and reduced heart rate variability in newborns with hypoxic ischemic encephalopathy

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Abstract

Objective—To determine whether systemic inflammation-modulating cytokine expression is related to heart rate variability (HRV) in newborns with hypoxic ischemic encephalopathy (HIE).

STUDY DESIGN—Data from 30 newborns with HIE were analyzed. Cytokine levels (IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-1β, TNF-α, IFN-λ) were measured either at 24 hours of cooling (n=5), 72 hours of cooling (n=4), or at both timepoints (n=21). The following HRV metrics were quantified in the time domain: alpha_S, alpha_L, root mean square (RMS) at short time scales (RMS_S), RMS at long time scales (RMS_L), while low frequency power (LF) and high frequency power (HF) were quantified in the frequency domain. The relationships between HRV metrics and cytokines were evaluated using mixed-models.

Results—IL-6, IL-8, IL-10, and IL-13 levels were inversely related to selected HRV metrics.

Conclusion—Inflammation-modulating cytokines may be important mediators in the autonomic dysfunction observed in newborns with HIE.

Introduction

Perinatal asphyxia can lead to multisystem organ dysfunction in the newborn, including possible effects on the pulmonary, renal, hematologic and cardiovascular systems. While these injuries are often reversible, damage to the central nervous system can lead to hypoxic-ischemic encephalopathy (HIE) and cause devastating long-term disability - and in severe

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Conflict of interest

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cases - even death[1, 2]. Therapeutic hypothermia (TH) has been shown to improve outcomes in newborns with HIE. However, about half of all treated infants continue to suffer death or disability despite treatment with TH[3, 4, 5, 6]. In order to improve outcomes, additional treatment options need to be implemented for those infants failing to respond to TH alone. Therefore, it is crucial to be able to assess an individual infant's response to treatment over time in order to gauge the need for escalation of care. Hence, research is directed towards understanding the pathophysiological mechanisms leading to irreversible brain injury after a perinatal hypoxic ischemic insult, and identifying real-time methods to assess these ongoing processes at the bedside.

In an earlier report, we described the use of heart rate variability (HRV) metrics as physiological biomarkers signifying the evolution of neonatal brain injury in patients with HIE[7]. The mechanism by which HRV is depressed after hypoxia-ischemia, however, remains unclear. Possible etiologies include autonomic dysfunction resulting from direct brainstem injury, hypoxia induced cardiac dysfunction affecting regulation of heart rate at the nodal level, or systemic inflammatory response triggering adrenergic and cholinergic desensitization[8]. While the association between decreased HRV and systemic inflammation has been described in animal models of sepsis[9] and adult human patients with heart failure[10], diabetes[11], and sepsis[12], only one study has evaluated the relationship between HRV and cytokines in newborns with sepsis[13]. No studies have evaluated the link between inflammatory cytokine response and reduced HRV in critically-ill newborns with HIE.

We recently demonstrated the presence of elevated plasma inflammation-modulating cytokine levels in newborns with HIE who died or had severe brain injury on MRI compared to survivors with minimal or no brain injury[14]. This study aims to examine the association between inflammation-modulating cytokine response and HRV metrics in HIE newborns. We hypothesized that increased inflammation-modulating cytokine levels would be associated with reduced HRV in newborns with HIE.

Subjects and Methods

Study Population and Data Collection

Newborns with HIE meeting the criteria for TH using the National Institute of Child Health and Development Protocol[3] were enrolled in this prospective observational study evaluating biomarkers of brain injury. Cytokine and MRI data have been previously reported from this cohort[14] and the current study includes patients with available HRV data. Demographic and clinical data were collected from the birth hospital and the NICU medical records. Serum samples were collected 24 hours after cooling initiation and at 72 hours of cooling (i.e. initiation of rewarming) and frozen at −80 °C for later bulk assay. Cytokine (IL-10, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, IFN-λ) concentrations were measured using a commercially available multiplex electrochemiluminesce-based assay (Meso Scale Discovery; Rockville, MD, USA). Continuous recordings of electrocardiogram (EKG) from the NICU bedside cardiorespiratory monitor (Philips IntelliVue MP70, MA, USA) were collected prospectively at a rate of 500 Hz and up-sampled to 1 kHz using custom software developed in LabView (National Instruments, TX, USA). For newborns not enrolled within 24 hours of life, EKG data were collected if available from an institutional Research Data

Export (RDE) archive (IntelliVue Information Center, Philips Healthcare, MA, USA), which allowed for adequate data resolution for calculation of all HRV metrics. The study was approved by the Children's National Institutional Review Board and informed consent was obtained from the parent of each participant.

EKG Signal Processing

To attenuate the baseline wandering and high-frequency noise, the EKG data were bandpass-filtered between 1 – 60 Hz using a Butterworth filter with zero-phase distortion. The R-waves were identified using a combination of Hilbert transform and an adaptive threshold detection approach,[15] and heart rate was defined. The artifacts in the heart rate data were cleaned using an automated approach[16]. Heart rate was converted into RR intervals and partitioned into 10-minute epochs for further processing. For frequency-domain spectral analysis, the RR intervals in each 10-minute epoch were converted into uniformly sampled data using a cubic spline interpolation at a rate of 4 Hz. We estimated power spectra of the RR intervals in each 10-minute epoch using the Welch-periodogram approach with a frequency resolution of 0.0167 Hz. Using the estimated spectrum, we calculated LF and HF powers as the sum of the spectral powers in 0.05–0.25 Hz and 0.3–1 Hz, respectively. For further analysis, we divided LF and HF powers by the total power (sum of powers in 0.05–2 Hz) and used the normalized representation of the spectral powers[17, 18]. The normalized LF and HF powers characterize the sympathetic and parasympathetic tones of the autonomic nervous system, respectively[17, 19]. For time domain, we used the detrended fluctuation analysis (DFA) to calculate scaling exponents α_S(15–30 beats) and α_L(35–150 beats) for short and long time scales, respectively. We also calculated root means square (RMS_S, 15–50 beats) and (RMS_L, 100–150 beats) at short and long time scales, respectively. The α exponent characterizes the auto-correlations within the specified number of beats whereas the RMS characterizes the variability within the specified number of beats[20]. All analyses were performed off-line using MATLAB (Mathworks Inc, MA, USA). Code availability can be accessed by contacting the authors.

Statistical Analysis

The median value of the HRV metric in the two hours preceding the cytokine measurement was used for analysis. Unadjusted analyses were performed by generating a local regression line using LOWESS (LOcally WEighted Scatter-plot Smoother) to fit the corresponding variables. LOESS performs nonparametric local regression smoothing for estimating regression surfaces and does not require any assumptions about the parametric relationship between variables. To account for multiple measurements per subject, the relationships between HRV metrics and cytokines were evaluated using mixed-models adjusting for gestational age, birth weight, gender and time of measurement. Both the HRV metrics and the cytokine levels were log-transformed to meet the required normality assumption. The t-statistic was calculated as the ratio of the regression model estimate divided by the standard error for a given variable, providing a measure of the strength and direction of the relationship between the cytokine and HRV metric. The significance level was set at $p<0.01$ given multiple comparisons. We used a convenience sample of 30 patients with available cytokine and HRV data for these analyses. This sample provided 80% statistical power with

α=0.05 to detect a strong (r=0.5) correlation between cytokine and HRV metric, not accounting for repeated measures in subjects.

Results

A total of 30 newborns with moderate-to-severe HIE were included in this study. HRV was prospectively monitored in the majority of patients (n=23), while 7 patients were enrolled after 24 hours of life with data retrieved from the RDE archive. The median gestational age was 39 weeks, median birth weight was 2.96 kg and 33% were male. None had culture-positive sepsis during their NICU stay. Of the 22 patients for whom placental pathology information was available, 8 (36%) had chorioamnionitis. Other baseline and clinical characteristics are shown in Table 1.

Cytokine levels were measured at both timepoints of interest in 21 infants. Nine infants only had single measurements: this was due either to a blood sample that was insufficient for analysis (n=4) or because death occurred before 72 hours of cooling (n=5). Consequently, we entered into analyses with 51 observations.

IL-6, IL-8, IL-10, and IL-13 demonstrated statistically significant ($p<0.01$) negative associations with certain HRV metrics, indicating reduced HRV with an increasing cytokine level (Figure 1). These relationships remained significant after adjustment for baseline characteristics including gestational age, birth weight, gender, and time of measurement. The results of the regression models are presented in Table 2. Sensitivity analyses were also performed evaluating the subset of infants with available information regarding chorioamnionitis status and the results were similar. Of note, the infants who died had significantly lower RMS_S and RMS_L and higher IL-8 and IL-10 levels compared to those who survived.

Discussion

To our knowledge, this is the first study to demonstrate the association of elevated inflammation-modulating cytokine levels with depressed heart rate variability in newborns with HIE undergoing TH. In earlier studies, we demonstrated that inflammation-modulating cytokines are able to differentiate newborns with adverse neurological outcomes[14]. We expand upon this work to elucidate a possible pathophysiological link between systemic inflammatory response and disease evolution in HIE. The relationship between inflammation-modulating cytokine release and reduced HRV supports the notion that ANS dysfunction in newborns with HIE is mediated by inflammatory processes triggered by hypoxia-ischemia. As one of the proposed mechanisms of TH includes reduction of inflammation[21, 22], results of this study suggest that one of the downstream neuroprotective properties of TH may be the preservation of ANS function during the 72 hours following an asphyxial insult.

Several studies have evaluated the relationship between inflammatory cytokines and brain injury in HIE. Elevated levels of IL-6[23, 24, 25, 26, 27], IL-8[25, 26, 27] IL-10[14, 27] and IL-13[14], specifically, have been related to brain injury based on severity of clinical encephalopathy grade, MRI abnormalities and/or later neurodevelopmental impairments. The fact that we

did not find a relationship between HRV and IL-1β, TNF-α and IFN-γ is of interest, as early measurements of these cytokines have also been linked to brain injury in HIE[24, 25, 27]. Whether this lack of relationship relates to timing of measurement or represents a specificity of certain cytokines in the pathogenesis of ANS dysfunction warrants further study in a larger population of infants with HIE.

The timing of cytokine responses and HRV evolution also supports a mechanistic link between the peak of inflammation and reduced HRV. Based on animal[28, 29] and human studies[25, 27], cytokines have been shown to peak within 12–24 hours after a hypoxic-ischemic insult, with some having a biphasic response.[27] Mirroring this time course, we demonstrated that HRV most differentiated HIE infants by outcome at 24 hours of life[30] - which is the time when the peak of secondary injury is thought to happen[31] - and again later after 72 hours post-rewarming. Based on this rationale, we evaluated the relationship between the HRV metrics and cytokines measured at these two timepoints with key clinical implications.

The biological plausibility of the association between cytokine levels and reduced HRV is based on observations from several animal studies and limited human data. Both parasympathetic and sympathetic inputs have been demonstrated to have immunomodulatory effects. Vagal activation has been shown to attenuate proinflammatory cytokine release[32]. Bernik et al showed that vagus nerve stimulation inhibited TNF synthesis[33], while vagotomy was associated with worsening inflammation[34]. The sympathetic nervous system also plays a role in the regulation of the immune system, possessing both pro- and anti-inflammatory properties.[35] It is possible that primary ANS dysfunction leads to loss of these immunomodulatory functions and an unregulated pro-inflammatory state. Other data, however, support the notion that elevated cytokine levels, whether triggered by sepsis or hypoxia-ischemia, lead to reduced HRV. Fairchild et al showed that HRV was depressed in mice in the setting of a pro-inflammatory cytokine response after injecting them with endotoxins[9]. These investigators proposed that while cytokines are expected to activate the efferent vagal nerve and thus increase HRV, repeated vagal nerve activations cause desensitization of the cholinergic receptors in the heart, which results in decreased HRV[8]. In fact, cytokine-mediated desensitization of cholinergic receptors has been shown in several organ systems[36, 37, 38]. The negative association between inflammatory mediators and HRV was recently demonstrated in a large nationally representative sample of adults, where HRV assessed by HF and LF was inversely associated with fibrinogen, CRP, and IL-6[39]. Our findings are consistent with this inverse relationship between inflammation-modulating cytokines and HRV. Only one prior study has evaluated the relationship between cytokines and HRV in neonatal patients. Raynor et al showed an association of cytokines with HRV in newborns diagnosed with sepsis[13]. Our study confirms the association reported between IL-8 and HRV and revealed additional associations of IL-6, IL-10, and IL-13 with HRV.

Our study has limitations. We made assumptions regarding the temporal scale of the cytokine-HRV response. We evaluated the median of HRV metrics in the two hours preceding the cytokine measurement as we felt our measurement reflected circulating levels in the time period immediately preceding the blood sampling time. Given their dynamic

nature and variable time-dependency, this 2-hour window may not be ideal for evaluating the relationship with all cytokines. The fact that not all subjects had cytokines measured at both timepoints of interest influenced our already relatively small sample size. Future studies with more frequent collection of samples during cooling and rewarming will be useful to further define the relationship between inflammation-modulating cytokine profiles, HRV, and brain injury in newborns with HIE. Despite these limitations, this study highlights the importance of inflammation in the evolution of brain injury and provides insights into the pathogenesis of autonomic dysfunction that is observed in HIE.

Conclusions

Elevated cytokines IL-6, IL-8, IL-10, and IL-13 showed an association with quantitative measures of reduced HRV. These data suggest a possible role of inflammation-modulating cytokines in mediating ANS dysfunction in infants with HIE.

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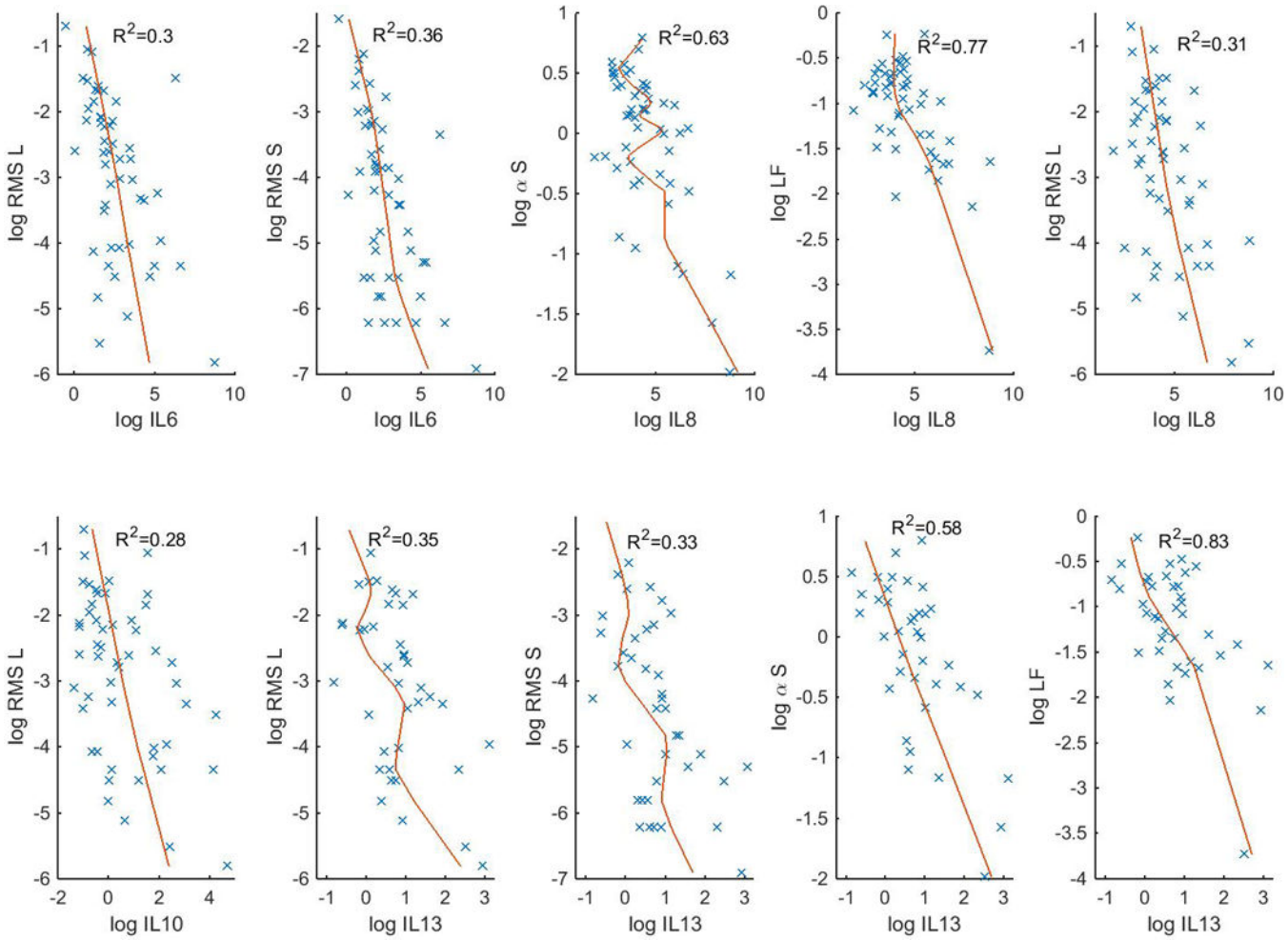

Figure 1.

Relationship between HRV metrics and cytokine concentration shown by scatterplot and local regression line.

Table 1

Clinical characteristics of study population

Gestational Age (weeks)	39, [35, 40]
Birth Weight (Kilograms)	2.96, [1.98,6.3]
Presenting pH	6.9, [6.6,7.11]
Gender (n, % male)	16 (53)
APGARS	
1 Minute	1, [0,4]
5 Minutes	3, [0,7]
10 Minutes	5, [0,8]
Encephalopathy Grade	
% Moderate	92.8%
% Severe	7.2%
Hour of life on cooling	2:24 (4:04, 6:00)
Electrographic Seizures (n, %)	10 (33)
Chorioamnionitis (n, % of patients with placental pathology available)	8 (36) *
Received hydrocortisone (n, %)	8 (27)
Brain Injury by MRI (n, %)	
None	12 (40)
Mild	6 (20)
Moderate/Severe	7 (23)
Died before MRI	5 (17)

Data presented as median [range] unless otherwise indicated

* Placental information available for 22/30 patients

Table 2

Association between HRV metrics and cytokine levels

Cytokine	HRV Metric	*t*-value	P value*	Significant Covariates
Log IL-6	Log RMS_S	−4.12	0.0006	
Log IL-6	Log RMS_L	−3.34	0.0034	
Log IL-8	Log alpha_S	−4.81	0.0001	
Log IL-8	Log LF	−4.53	0.0002	
Log IL-8	Log RMS_L	−3.42	0.0029	
Log IL-10	Log RMS_L	−3.12	0.0056	
Log IL-13	Log alpha_S	−6.73	<.0001	GA (t=2.6, p=0.016)
Log IL-13	Log LF	−5.14	<.0001	GA (t=2.4, p=0.028)
Log IL-13	Log RMS_L	−3.94	0.0009	
Log IL-13	Log RMS_S	−3.75	0.0014	

*P-values indicate significance of association between HRV metric and cytokine after adjusting for gestational age (GA), birth weight, gender and time of measurement