Impaired cerebral autoregulation and brain injury in newborns with hypoxic-ischemic encephalopathy treated with hypothermia

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Massaro AN, Govindan RB, Vezina G, Chang T, Andescavage NN, Wang Y, Al-Shargabi T, Metzler M, Harris K, du Plessis AJ. Impaired cerebral autoregulation and brain injury in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. J Neurophysiol 114: 818–824, 2015. First published 10 June 2015; doi:10.1152/jn.00353.2015.—Impaired cerebral autoregulation may contribute to secondary injury in newborns with hypoxic-ischemic encephalopathy (HIE). Continuous, noninvasive assessment of cerebral pressure autoregulation can be achieved with bedside near-infrared spectroscopy (NIRS) and systemic mean arterial blood pressure (MAP) monitoring. This study aimed to evaluate whether impaired cerebral autoregulation measured by NIRS-MAP monitoring during therapeutic hypothermia and rewarming relates to outcome in 36 newborns with HIE. Spectral coherence analysis between NIRS and MAP was used to quantify changes in the duration [pressure passivity index (PPI)] and magnitude (gain) of cerebral autoregulatory impairment. Higher PPI in both cerebral hemispheres and gain in the right hemisphere were associated with neonatal adverse outcomes [death or detectable brain injury by magnetic resonance imaging (MRI), $P < 0.001$]. NIRS-MAP monitoring of cerebral autoregulation can provide an ongoing physiological biomarker that may help direct care in perinatal brain injury.

hypoxic-ischemic encephalopathy; cerebral blood flow; magnetic resonance imaging; newborn

PERINATAL HYPOXIC-ISCHEMIC encephalopathy (HIE) is an important cause of neonatal mortality and long-term neurodisability (Dilenge et al. 2001; Liu et al. 2012). While therapeutic hypothermia improves outcome in HIE and is the current standard of care, a significant proportion of treated infants continue to suffer adverse outcomes after therapy (Gluckman et al. 2005; Shankaran et al. 2005). Adjuvant neuroprotective interventions are needed to further improve outcomes for this devastating condition. Unfortunately, methods to acutely identify infants with inadequate response to hypothermia and evolving brain injury are limited in the neonatal intensive care unit (NICU). Clinicians often rely on magnetic resonance imaging (MRI) performed after hypothermia to determine the presence and extent of brain injury that has occurred. This approach does not enable therapeutic interventions aimed to prevent injury progression. Thus bedside methods to monitor risk for evolving brain injury are needed to direct clinical care and rational therapeutic interventions.

Ongoing assessment of cerebral pressure autoregulation integrity can provide an assessment of the physiological response to hypoxia and/or ischemia, offering a window into evolving brain injury. Impaired cerebral pressure autoregulation has been implicated in the pathogenesis of secondary injury in HIE, as cerebral vasoparalysis following a hypoxic and/or ischemic insult has been well documented in both animal and human studies (Meek et al. 1999; Pryds et al. 1990; Short et al. 1994). Cerebral pressure passivity, or the inability to regulate cerebral blood flow (CBF) in the setting of changes in systemic blood pressure, can lead to uncoupling of CBF and cerebral energy metabolism that can contribute to secondary energy failure and structural brain injury in HIE.

We previously described methods to monitor cerebral pressure autoregulation by quantifying frequency domain spectral coherence between near-infrared spectroscopy (NIRS)-derived cerebral oxyhemoglobin-deoxyhemoglobin (HbD), a surrogate measure of CBF (Tsuji et al. 1998), and systemic mean arterial blood pressure (MAP) (Govindan et al. 2014; Soul et al. 2007). Spectral coherence is a technique that allows quantification of the relationship between changes in MAP and changes in CBF (or HbD), with pressure-passive CBF indicated by increased coherence between the two physiological signals. This approach allows for quantification of both the duration [pressure passivity index (PPI)] and magnitude (gain) of cerebral pressure passivity. While cerebral pressure passivity assessed by this approach has been proposed as a risk factor for brain injury in the preterm infant (O’Leary et al. 2009), its significance in term newborns with HIE is unknown. This study aimed to investigate whether quantified changes of the duration and magnitude of cerebral pressure passivity during therapeutic hypothermia are associated with adverse neonatal outcomes.
were also collected, including use of vasoactive therapy, presence of encephalopathy as classified by modified Sarnat criteria (Sarnat and Sarnat 1976; Shankaran et al. 2005). Data from NICU hospitalizations of encephalopathy from birth hospital and study site medical records. Presenting characteristics of the models were evaluated with receiver operating characteristic (ROC) analysis to determine their ability to distinguish infants with adverse outcomes. Performance characteristics of the models were evaluated with receiver operating characteristic curves, where an area under the curve (AUC) of 1 represents high diagnostic accuracy, in contrast to a value of 0.5 that signifies poor discrimination of outcome groups. Because of sample size limitations, these secondary analyses were not adjusted for covariates. Statistical analysis was performed with SAS 9.3 (SAS Institute, Cary, NC).

RESULTS

Description of study population. Of the 59 eligible patients with moderate to severe HIE admitted during the study period (November 2012–July 2014), 9 infants were not enrolled because of extremes presentation and death prior to consent (n = 1), parental refusal (n = 3), or inability to obtain consent estimation, which was 20 in our case, and a set to 0.999 (Govindan et al. 2014; Halliday et al. 1995). Based on the knowledge that impulse response time of cerebral autoregulation is expected to operate at 5–20 s, pressure passivity was defined as coherence greater than the threshold in the frequency band of 0.05–0.25 Hz (O’Leary et al. 2009). The PPI was then calculated as the total percentage of pressure-passive 10-min epochs per 6 h of recording. We also calculated transfer gain (Halliday et al. 1995) within the pressure-passive epochs and averaged over 6 h of recording to match the PPI calculation. Gain function provides a measure of the magnitude of pressure passivity. All processing was done off-line with MATLAB software (MathWorks, Natick, MA).

Magnetic resonance imaging. MRI was performed according to our institutional clinical protocol based on established guidelines (2014). Surviving infants underwent routine MRI at target age 3–5 days (to evaluate early diffusion changes) and 10–12 days (to evaluate extent of lesions) of life on a 3-T scanner (Discovery MR750, GE Healthcare, Milwaukee, WI) using a 32-channel receive-only head coil (MR Instruments, Minneapolis, MN). Standard anatomical sequences included 3D T1-weighted spoiled gradient recalled, double-acquisition axial FSE T2 proton density, axial T2 propeller (in cases of patient motion), axial T2-star-weighted susceptibility imaging, coronal T1 FLAIR propeller, and axial 30-direction DTI with generation of ADC maps off-line. Images were scored according to a validated scoring system that assigns a score ranging from 0 to 4 for the degree of basal ganglia/thalamic (BG) injury and from 0 to 5 for the degree of watershed cortical (WS) injury (Barkovich et al. 1998) by an experienced neuroradiologist (G. Vezina) masked to clinical and physiological data. If an infant had more than one MRI, the higher injury score was used for analysis. Adverse outcome was defined as death during hospitalization or moderate to severe brain injury by MRI (BG score ≥ 3 or WS score ≥ 4).

Statistical analysis. Descriptive statistics included means (standard deviations) and medians (ranges) for continuous parametric and non-parametric variables, respectively, as well as counts (percentages) for categorical data. Generalized estimating equations (GEEs) were used to evaluate the relationship between repeated measures of PPI/gain over time and outcome classification adjusting for baseline and treatment effects. Statistical analysis was performed with SAS 9.3 (SAS Institute, Cary, NC).
because of parental (n = 2) or investigator (n = 3) unavailability. Of the 50 patients enrolled for physiological monitoring, 14 infants did not have indwelling arterial catheters for continuous blood pressure monitoring. Thus data from 36 patients with complete MAP and NIRS data were included in the study. The adverse outcome group comprised seven infants who died (because of withdrawal of medical care based on poor neurological prognosis) and nine infants with moderate/severe MRI brain injury (severe BG injury n = 5, severe WS injury n = 4). Infants in the adverse outcome group had lower birth weight, lower Apgar score at 5 min, and higher frequency of electrographic seizures compared with the favorable outcome group (P < 0.05). Otherwise, baseline characteristics were similar between groups (Table 1).

The majority of infants received vasoactive medications (dopamine n = 23, 67%; epinephrine n = 7, 19%) and sedatives (morphine n = 31, 86%; midazolam n = 12, 33%). MAP over time did not differentiate the two outcome groups (Fig. 1). Phenobarbital was given for seizures in 12 (33%) patients. Frequency of these medical therapies did not differ between outcome groups (P > 0.05). Temperature and laboratory data monitored through hypothermia and rewarming are presented in Table 2. Median temperatures, PaCO2, and hemoglobin levels did not differ between groups (P > 0.05). Continuous NIRS/MAP recordings were initiated at a median age of 13 (range 6–25) h of life. The median duration of recording was 73 (range 5–91) h.

Early MRI was performed in 24 infants at a median age of 4 (range 3–6) days of life, while a later MRI (median 10, range 7–16 days) was performed in 25 infants. Seventeen infants had two scans performed. Of the nine infants with moderate/severe MRI brain injury in the adverse outcome group, the majority (n = 8, 89%) had severe BG injury while only one infant had severe WS injury. The favorable outcome group comprised 14

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### Table 1. Characteristics of study population

<table>
<thead>
<tr>
<th></th>
<th>Favorable Outcome (n = 20)</th>
<th>Adverse Outcome (n = 16)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk (mean ± SD)</td>
<td>38.6 ± 1.7</td>
<td>39.2 ± 1.3</td>
<td>0.294</td>
</tr>
<tr>
<td>Birth weight, kg (mean ± SD)</td>
<td>3.5 ± 1.0</td>
<td>2.9 ± 0.3</td>
<td>0.016</td>
</tr>
<tr>
<td>Sex, n males (%)</td>
<td>12 (60)</td>
<td>46 (37.5)</td>
<td>0.180</td>
</tr>
<tr>
<td>Initial pH</td>
<td>6.91 (6.50–7.25)</td>
<td>6.9 (6.65–7.14)</td>
<td>0.814</td>
</tr>
<tr>
<td>Apgars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>1 (0–9)</td>
<td>1 (0–8)*</td>
<td>0.479</td>
</tr>
<tr>
<td>5 min</td>
<td>4 (1–8)</td>
<td>2 (0–9)*</td>
<td>0.023</td>
</tr>
<tr>
<td>10 min</td>
<td>6 (2–8)†</td>
<td>3 (0–7)‡</td>
<td>0.059</td>
</tr>
<tr>
<td>Encephalopathy grade, n (%)</td>
<td></td>
<td></td>
<td>0.180</td>
</tr>
<tr>
<td>Moderate</td>
<td>16 (80)</td>
<td>10 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>4 (20)</td>
<td>6 (37.5)</td>
<td></td>
</tr>
<tr>
<td>EEG seizures, n (%)</td>
<td>2 (10)</td>
<td>8 (50)</td>
<td>0.008</td>
</tr>
<tr>
<td>Received vasoactives, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>13 (65)</td>
<td>11 (69)</td>
<td>0.983</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>2 (10)</td>
<td>5 (31)</td>
<td>0.109</td>
</tr>
<tr>
<td>Age monitoring initiated, h</td>
<td>13.4 (5.9–23.2)</td>
<td>13.3 (7.4–25.1)</td>
<td>0.987</td>
</tr>
</tbody>
</table>

Data presented as median (range) except where indicated. *Documented for 15/16 patients; †documented for 17/20 patients; ‡documented for 13/16 patients.
infants with normal MRI and 6 infants with mild BG injury (score ≤ 2). There was no laterality to injury pattern in any of the infants.

Pressure passivity index. A total of 442 observations (i.e., PPI calculated in a 6-h window) were included in the PPI analyses for each cerebral hemisphere. Unadjusted median PPI values over time are shown in Fig. 2. After adjusting for baseline and clinical covariates, higher PPIs measured from both the left (LH) and right (RH) hemispheres were significantly associated with adverse outcome (P < 0.01). There was no significant time effect on these relationships, and, similarly, no interaction was detected between time and outcome group. Regression model results are summarized in Table 3.

HbD-MAP spectral gain. Given that gain function can only be calculated within pressure-passive epochs, fewer observations (LH, n = 332; RH, n = 334) were included in the gain analyses. While gain in the RH was significantly higher in the adverse outcome group (P < 0.001), gain from the LH was not significantly associated with outcome (P > 0.05) after adjusting for covariates. Similar to PPI, there was no significant time effect on the relationship between gain and outcome. Regression model results are included in Table 3.

Secondary PPI/gain predictive models. Only average PPI (over the recording period) measured from the LH was significantly associated with adverse outcome (AUC = 0.7193, 95% CI: 0.5430–0.896, P = 0.034). There was a trend toward association between PPI from the RH and adverse outcome (AUC = 0.646, 95% CI: 0.480–0.849, P = 0.082), while gains from both the LH and RH were not significantly predictive of adverse outcome (P > 0.05). A probability cutoff point of 0.378 for average PPI value measured from the LH had 87% sensitivity and 58% specificity for prediction of adverse outcome.

DISCUSSION

We demonstrate that measurements of spectral coherence between MAP and NIRS HbD, reflecting impaired cerebral autoregulation, differ between newborns with HIE who die or have moderate to severe MRI brain injury and survivors with normal to mild injury. Specifically, infants with adverse outcomes exhibited higher PPI and gain, indicating longer duration and higher magnitude of cerebral pressure passivity following hypoxia-ischemia. Monitoring of blood pressure alone did not differentiate outcome groups. These data support the notion that quantifying cerebral autoregulation integrity can provide a viable physiological biomarker of brain injury. A bedside tool that can assess evolving cerebral injury is needed to individualize therapy in newborns with HIE. Such a tool would enable the timely identification of patients for whom hypothermia alone is providing inadequate neuroprotection and could be used to direct the rational introduction of adjuvant therapies. Continuous real-time assessment of spectral coherence...
ence between MAP and NIRS HbD offers a promising candidate for a bedside brain injury monitor.

Cerebral pressure autoregulation is a physiological compensatory mechanism that maintains CBF relatively constant during changes in systemic blood pressure. Its importance in maintaining cerebral integrity has been recognized for over 50 years (Lassen 1964). Several animal and human studies have demonstrated loss of cerebral autoregulation (i.e., pressure passivity) following hypoxia and/or ischemia (Marks et al. 1996; Meek et al. 1999; Pryds et al. 1990; Short et al. 1994). Proposed mechanisms include release of vasoactive agents such as extracellular hydrogen ions, adenosine, endothelins, prostaglandins, reactive oxygen species, and nitric oxide. These mediators lead to dysregulation of cerebral vascular tone (Lassen 1964; Meek et al. 1999). The association between cerebrovascular dysfunction and cerebral injury has been supported by studies linking CBF abnormalities with proton magnetic resonance spectroscopy markers of cerebral energy failure (Tsuji et al. 1995; Winter et al. 2009) and adverse neurological outcomes (Meek et al. 1999; Pryds et al. 1990; Toet et al. 2006; Zaramella et al. 2007).

Prior human studies evaluating CBF in newborns have utilized technically challenging methods such as contrast-enhanced imaging modalities [e.g., positron emission tomography (Duncan and Ment 1982; Volpe et al. 1985), single-photon emission computed tomography (Haddad et al. 1994; Konishi et al. 1994), perfusion-weighted MRI (Laswad et al. 2009; Wintermark et al. 2008)] or other point-in-time measures [e.g., Doppler ultrasound cerebral blood velocity (Archer et al. 1986; Bennhagen et al. 1998; Fukuda et al. 2008; Ilves et al. 2004), xenon-133 clearance method (Pryds et al. 1990; Younkin et al. 1982)]. More recently, NIRS has emerged as an alternative method to interrogate CBF continuously and noninvasively at the bedside (Edwards et al. 1988; Skov et al. 1991). While prior studies have linked NIRS measures with outcomes in HIE (Ancora et al. 2013; Lemmers et al. 2013; Meek et al. 1999; Toet et al. 2006; van Bel et al. 1993; Zaramella et al. 2007), few studies have focused on evaluating the relationship between MAP and NIRS as a method to interrogate autoregulation (Howlett et al. 2013; Tekes et al. 2015). Signs of impaired cerebral pressure autoregulation may precede the presence of unregulated CBF, giving this approach the potential advantage of directing intervention before irreversible injury has occurred.

To our knowledge, only one other investigative group has evaluated the relationship between impaired cerebral autoregulation and brain injury in newborns undergoing therapeutic hypothermia. Howlett and colleagues recently described the use of the hemoglobin volume index (HVx), calculated as a moving correlation coefficient between MAP and relative tissue hemoglobin (a surrogate measure of cerebral blood volume obtained by NIRS) (Howlett et al. 2013). HVx was used to identify the optimal MAP where vasoreactivity was preserved. These authors demonstrated a significant relationship between increased time spent below optimal MAP and brain injury assessed by both qualitative (Howlett et al. 2013) and quantitative (Tekes et al. 2015) MRI. Of note, this approach was not able to identify optimal MAP in all patients (79%, 77%, and 86% of patients during hypothermia, rewarming, and normothermia, respectively) and evaluated changes occurring in a narrower frequency range (<0.1 Hz). While we similarly demonstrated a relationship between indexes of cerebral autoregulation and brain injury, we used a frequency-based spectral analysis approach. This approach provides a measure of the duration and magnitude of autoregulation impairment for each patient across a wider range of frequencies (0.05–0.25 Hz). Both approaches require time for baseline data collection but have the potential for real-time ongoing quantification of cerebral autoregulation.

Our study has limitations. Although the repeated measurements over time enabled the inclusion of a relatively large number of observations in our primary GEE analyses, the small patient sample size precluded the development of robust predictive models. While our data support the association between impaired cerebral autoregulation and adverse outcome, further study is needed to establish the predictive value of PPI and gain measures, to compare these values with other clinical indicators of severity (e.g., Apgar score, seizure), and to determine thresholds that signify brain injury with high sensitivity and specificity at various time points during treatment. While we did not detect a significant time effect in our statistical analyses, graphical representation of our unadjusted data (Fig. 1) suggests that PPI may increase in the adverse outcome group after 72 h. That this time period coincides with rewarming may reflect that PPI monitoring can demonstrate withdrawal of the therapeutic effect of hypothermia. We speculate that PPI monitoring could be useful in identifying infants who may benefit from continued hypothermia or adjuvant therapies. This potential clinical application will need validation in future studies. Additionally, future investigations will be needed to confirm whether there is vulnerability in a particular cerebral hemisphere with regard to autoregulation control (i.e., gain in the RH), as we acknowledge that technical complications leading to the observation of laterality cannot be excluded. This study did not include a control group of healthy, nonencephalopathic infants, as the feasibility of continuous and prolonged invasive blood pressure monitoring is problematic in such a population. However, since the primary goal of our study was to evaluate whether cerebral hemodynamic biomarkers could identify infants with significant brain injury among at-risk infants, inclu-

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**Table 3. GEE model results**

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>95% CI</th>
<th>P Value</th>
<th>Other Significant Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI-left</td>
<td>0.92</td>
<td>0.28</td>
<td>0.37–1.48</td>
<td>0.001</td>
<td>BW, GA, severe HIE, initial pH, 5-min Apgar, EEG Sz, Hb, PaCO2, sex, vasopressor use</td>
</tr>
<tr>
<td>PPI-right</td>
<td>1.24</td>
<td>0.25</td>
<td>0.75–1.74</td>
<td>&lt;0.001</td>
<td>BW, GA, severe HIE, initial pH, 5-min Apgar, EEG Sz, Hb, PaCO2, sex, vasopressor use</td>
</tr>
<tr>
<td>Gain-left</td>
<td>0.11</td>
<td>0.12</td>
<td>−0.11–0.34</td>
<td>0.322</td>
<td>PaCO2, vasopressor use</td>
</tr>
<tr>
<td>Gain-right</td>
<td>1.64</td>
<td>0.41</td>
<td>0.84–4.44</td>
<td>&lt;0.001</td>
<td>Severe HIE, 5-min Apgar, EEG Sz, PaCO2, sex, vasopressor use</td>
</tr>
</tbody>
</table>

B, outcome group regression coefficient; SE, standard error; CI, confidence interval; PPI, pressure passivity index; BW, birth weight; GA, gestational age; HIE, hypoxic-ischemic encephalopathy; EEG Sz, electrographic seizure; Hb, hemoglobin level.
sion of a healthy control group was not needed to achieve study aims. Our study cannot address how these methods would characterize cerebral autoregulation in healthy newborns. While we included in the analyses many factors that may impact CBF and/or systemic blood pressure including presence of seizure, temperature, hemoglobin level, Pao2/CO2, and use of sedative and vasoactive medications in our primary analyses, sample size limitations precluded our ability to adjust for all factors in the predictive models. These and possibly other variables will need to be considered in future studies. Finally, we used a short-term outcome of death or MRI brain injury. Qualitative MRI injury has been established as a reliable predictor of developmental outcome, particularly moderate-severe MRI injury is sensitive and specific for later motor and cognitive impairment (Azzopardi and Edwards 2010; Barkovich et al. 1998; Rutherford et al. 2010). Validation of PPI/gain as brain injury biomarkers, however, will require correlation with long-term functional outcomes.

Conclusions. In this small observational study, quantifiable measures of the duration (PPI) and magnitude (gain) of cerebral pressure passivity differentiate newborns with HIE who die or have moderate to severe MRI brain injury from survivors with mild to no detectable injury. Ongoing monitoring of spectral coherence between systemic MAP and cerebral NIRS HbD may provide a bedside tool to assess evolving brain injury in the critically ill newborn. Further study is warranted to establish the role of cerebral hemodynamic monitoring in the care of infants at risk for brain injury in the intensive care unit.

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