Published in final edited form as:

J Ultrasound Med. 2018 April ; 37(4): 913–920. doi:10.1002/jum.14426.

Evaluating a targeted bedside measure of cerebral perfusion in a non-human primate model of neonatal hypoxic-ischemic encephalopathy

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Abstract

Objective—To compare ultrasound-derived resistive indices (RI) obtained at the level of the thalamus via fast Doppler with traditional anterior cerebral artery (ACA) measures in a model of neonatal hypoxic-ischemic encephalopathy and correlate each with clinical outcomes.

Methods—Nine non-human primate infants underwent no umbilical cord occlusion (UCO) (n=3), UCO without hypothermia (n=3), or UCO + hypothermia (n=3). RI was measured in the ACA and thalamus at days 0, 1, and 4 of life. Magnetic resonance imaging with spectroscopy was performed at day 4.

Results—Mean thalamus and ACA RI values in the first 36 hours of life were statistically different in infants who died (+0.13, p=0.019) or developed cerebral palsy (−0.08, p=0.003). Thalamic RI values showed stronger association with serum and spectroscopy lactate values than those in the ACA. The UCO + hypothermia group demonstrated a significant increase in RI in the thalamus, but not the ACA.

Conclusion—RI measurements in the thalamus may eventually supplement other bedside measures for predicting outcomes in the HIE population, but further studies need to differentiate the effect of hypothermia from illness severity on thalamic perfusion.

Keywords

fast Doppler; resistive index; newborn; ultrasound

Introduction

Hypoxic-ischemic encephalopathy (HIE) is estimated to occur in 1.3–1.7 per 1000 live births in the United States.¹ The standard use of therapeutic hypothermia for moderate to severe HIE has significantly reduced morbidity and mortality, but despite its widespread use,

48% of these infants die or develop severe neurodevelopmental disability at 18 months of life.² Alterations to the standard hypothermia protocol have been investigated, as have numerous supplemental neuroprotective therapies such as erythropoietin, melatonin, xenon, and autologous stem cells. To maximize the therapeutic efficacy of these additional treatments, though, we must first be able to identify infants who are unlikely to respond to hypothermia alone.

Perinatal clinical indicators of distress, such as the Apgar score, have poor predictive value for outcomes in infants with HIE. Even without treatment with hypothermia, a 5-minute Apgar score of 5 has a sensitivity of 13% and specificity of 67% for poor neurodevelopmental outcomes.³ As such, response to treatment and outcome prediction are currently based on a combination of clinical examination, electroencephalography (EEG), and magnetic resonance imaging (MRI). Full EEG is not always easily available at all sites, and both clinical examination findings and amplitude-integrated EEG may be affected by hypothermia.⁴ Although MRI and magnetic resonance spectroscopy (MRS) have shown promise in guiding prognosis,^{5,6} up to 26% of infants treated for HIE had normal MRI findings and abnormal neurodevelopmental outcomes.⁷ Additionally, the utility of MRI is restricted by the need to transport and frequently sedate these critically ill infants to obtain adequate images.

Ultrasound and near-infrared spectroscopy (NIRS) assessments of cerebral perfusion have been promoted as beneficial additions to bedside monitoring that may allow clinicians to better monitor response to therapy at the bedside. Ultrasound measures have historically focused on the middle cerebral or anterior cerebral artery (ACA). Like NIRS, these regions of interest provide global or regional white matter perfusion measures. As such, NIRS measures have been found to correlate with MRI perfusion values in severe HIE – where the entire circulation is likely affected – but do not appear to correlate in moderate HIE.⁸ ACA resistive indices have been utilized to differentiate between asphyxiated and healthy infants,⁹ and their positive and negative predictive values are 75% and 85%, respectively, for the development of death or neurodevelopmental disability.¹⁰ These moderate predictive values may reflect the difficulty in assuming the vascular tone of the ACA accurately reflects the tone in the arteries perfusing the thalamus and basal ganglia, especially when imaging a disease process such as HIE in which the basal ganglia and thalamus are primarily affected.¹¹

Fast Doppler (also referred to as UltraFast™ Doppler) utilizes plane-wave imaging to capture Doppler maps up to 100 times faster than conventional ultrasound systems. This speed allows for high sensitivity, especially in deeper tissues, and the ability to quantify flow information over entire regions of interest. Our group previously demonstrated that fast Doppler could reliably measure blood flow velocities in the deep gray matter of newborn human infants.¹² We hypothesized that measuring resistive index at the level of the thalamus using fast Doppler in a non-human primate model of HIE would demonstrate a stronger association with adverse neurological outcomes, clinical illness severity, and death than measuring resistive index in the anterior cerebral artery.

Materials and Methods

The University of Washington animal care and use committee approved all procedures. These data represent a pilot imaging protocol performed on a series of animals that were undergoing previously reported study procedures.¹³ Pertinent methods are summarized below.

Delivery

Nine *Macaca nemestrina* dams underwent general anesthesia with sevoflurane and hysterotomy at 168 ± 2 days gestation (term = 173 days). In the umbilical cord occlusion (UCO) groups, the umbilical cord was clamped *in utero* for 18–20 minutes, and a 2.5 French umbilical arterial catheter was placed before delivery for emergent medication administration and parenteral nutrition. Fetuses were delivered and resuscitated as needed by a team of neonatologists following standard neonatal resuscitation procedures. Resuscitation included positive pressure ventilation, endotracheal intubation, chest compressions, and bolus epinephrine as indicated. A covered heating pad, radiant warmer, and polyethylene sheet provided thermal support during stabilization. Upon delivery, Apgar scores, vital signs, and serial laboratory results were recorded. The animals were monitored using pulse oximetry and a rectal thermometer.

Treatment groups

UCO animals were managed under hypothermia (HT, n=3) or normothermia (n=3). Three additional animals did not undergo UCO and acted as controls. As part of the primary study, animals undergoing hypothermia also received erythropoietin intravenously at a dose of 1,000–3,000 Units/kg/day $\times$ 4, and the other groups received normal saline at equivalent time points. Active HT was initiated in the HT group within 3 hours after resuscitation of the infants and continued for 72 hours, after which the infants were rewarmed slowly at about 0.5°C per hour.

Imaging Protocol

Transcranial color Doppler ultrasound images were assessed at the bedside using the Aixplorer ultrasound system (Supersonic Imagine, Aix-en-Provence, France) equipped with an SL 10-2 linear array probe. As the *Macaca nemestrina* infants do not possess fontanels, fast Doppler image sequences of the ACA and thalamus were obtained through the cranium at the superior aspect of the skull. The thalamus was chosen as the primary region of interest because of previous reports demonstrating preferential injury to the thalamus in HIE. Additionally, the thalamic blood flow lies at the midline inferior to the ACA, so we were able to use the ACA as an anatomical landmark in a population where 2D grayscale imaging for landmarks is limited by signal propagation through the skull (Figure 1).

All images were obtained at three time points on all subjects (Figure 2). The first was within 12 hours of birth, the second between 24 and 36 hours, and the last at four days. To assess test-retest reliability, we obtained up to six image sequences at each time point, performed consecutively to minimize physiologic variability between measures. Ideal Doppler images with good color flow and minimal aliasing were found within a range of -27 to 27 cm/s for

the ACA and -20 to 20 cm/s for the thalamus. The ACA measures were obtained at a depth of 1.5–2 cm, while the thalamus images were at a depth of 2–2.5 cm, allowing for a 60 Hz compounded frame rate in the ACA and 52 Hz in the thalamus. Each fast Doppler video clip lasted for two seconds.

The detailed MRI and spectroscopy protocol has been previously reported.¹³ All infants underwent MRI at day 4 of life. Animals were anesthetized for MRI with intramuscular ketamine (5–10 mg/kg) and atropine (0.04 mg/kg), then maintained on sevoflurane (0.8–2.5%) and 100% oxygen. Pulse oximetry and single channel ECG were monitored with an MRI-compatible device (Invivo PrecessTM) and the temperature maintained with warm packs. Sequences included magnetization-prepared rapid gradient echo (MP-RAGE) high-resolution T₁-weighted imaging and single voxel proton spectroscopy (MRS) acquired on a Phillips Achieva 3.0-tesla magnet with an X-series Quasar Dual gradient system. Two 8-channel array head coils were custom made to fit neonatal and juvenile macaques.

Post-Imaging Processing and Analysis

Doppler video clips were extracted from the Aixplorer device and post-processed using code created in Matlab (Mathworks, Natick, MA) to calculate systolic (V_s) and diastolic (V_d) velocity. These values were used to calculate resistive indices by the equation $V_s - V_d / V_s$ for each cardiac cycle, which were averaged over the 2-second video clip. We chose to use Matlab-derived values instead of hand-extraction due to concerns of poor sampling repeatability in hand extraction of velocities, which has been recently confirmed in a large study.¹⁴ It is possible that this limitation could be overcome by obtaining several values from each image, as the Aixplorer system allows for post-acquisition flow velocity measures within any voxel in the region of interest (Figure 1). Due to the small sample size in this study, we were unable to adequately compare the Matlab- and hand-derived values, but would consider this for future studies.

Each of the frames was converted into an image file for processing in Matlab. The Doppler data was extracted from each pixel within the specific region of interest by translating RGB values from the images into blood-flow velocity using the velocity versus color scale within each image. The ACA velocity was obtained from the anterior ascending portion of the artery, and the thalamus velocity was obtained from the entire visible Doppler area within the region of interest around the thalamus. Application of a Matlab-based thresholding function then eliminated all gray background noise, leaving only the blood flow velocity data within the region of interest.

Systolic and diastolic values were obtained across the duration of the clip using a peak and trough detection algorithm based on the mean values of velocity across all pixels for one image. To avoid aliasing, the peak and trough detection compared every data point along the cardiac cycle to two adjacent points on either side. As neonates frequently have upwards of three cardiac cycles per second, attempting to compare more than two points on either side would have exceeded the temporal resolution of the compounding rate. Mean systolic, diastolic, and mean velocity were calculated from data collected across the entire time span of the 44 frames.

Statistics

Data were analyzed using STATA (version 12.1, StataCorp, College Station, TX). Measured descriptive covariates are listed in Table 1, stratified by treatment group. Continuous variables are described as mean $\pm$ one standard deviation, and categorical variables as the number and percent in each study population.

The relative test-retest reliability was quantified by an intraclass correlation coefficient (ICC) of single measures using a mixed-effects model,¹⁵ which takes into account both systematic and random errors in the data. All study images were obtained by the same investigator (EP) to avoid inter-observer variability. It has been suggested that an ICC < 0.4 indicates poor, $0.4 \leq \text{ICC} < 0.75$ indicates fair to good, and ≥ 0.75 indicates excellent reliability.¹⁶ Alternatively, a single cut-off of ≥ 0.6 has been used to indicate clinically useful measures.¹⁷ To provide additional comparison with recent reports of neonatal ultrasonographic repeatability, we also calculated the repeatability index as described by Froeling, et al.¹⁸

Correlations between RI and serum or MRS lactate levels were assessed using classic linear regression with robust standard errors. The R-squared values provided an estimate of goodness-of-fit. Differences between groups over time were evaluated using repeated measures analysis of variance (ANOVA). P-values and 95% confidence intervals (CI) were evaluated when appropriate, using an alpha level of 0.05 to test for statistical significance.

Results

Nine infant macaques were enrolled into this pilot phase of the study. Three were control infants with no umbilical cord occlusion (UCO), three underwent UCO with no therapy, and three underwent UCO with 72 hours of hypothermia. Subject characteristics are described in Table 1.

Reproducibility and repeatability were estimated using an intraclass correlation coefficient (ICC) and repeatability index. The ICC for ACA RI measures was 0.73 (95% CI: 0.58 – 0.85) and for thalamus measures was 0.60 (95% CI: 0.38 – 0.78). The repeatability index for ACA RI was 24.6% (95% CI: 18.8 – 30.4%) and for thalamus measures was 37.8% (95% CI: 31.5 – 44.2%). The thalamus repeatability improved with time, from 40.0% (95% CI: 33.6 – 46.5%) within the first 36 hours of life to 33.5% (95% CI: 19.2 – 47.8%) at four days of life.

Figure 3 demonstrates the RI stratified by study group and outcome, as defined by the development of cerebral palsy (CP) or death. Measuring RI in the ACA demonstrated no difference between groups at any of the time points and no significant change within the groups over time. Measurements in the thalamus within the first 36 hours of life demonstrated a significant increase in the mean RI in the UCO + hypothermia group (0.58 in the UCO + hypothermia group versus 0.31 in the control group [-0.27 , $p=0.002$] and 0.39 in the UCO group [-0.19 , $p<0.001$]). The thalamus RI continued to be elevated in the UCO + hypothermia group versus the control and UCO groups at day 4, but the difference was smaller (0.41 in the UCO + hypothermia group versus 0.35 in the control group [-0.06 ,

$p=0.033$] and 0.32 in the UCO group [-0.09 , $p=0.074$]). When grouped by control versus asphyxia (i.e. combining the UCO group with UCO + hypothermia), there was no difference in thalamus RI values between groups regardless of time point or region of interest.

Mean ACA RI in the first 36 hours of life was on average 0.08 lower in infants who died or developed cerebral palsy (CP) compared with controls ($p=0.003$). Thalamus measures in the first 36 hours were 0.13 higher in the death or CP group relative to controls ($p=0.019$). There was no difference in ACA or thalamus RI measures at day 4 for infants who died or developed CP relative to controls. All of the MR images demonstrated normal myelination pattern for term gestation and no diffusion restriction on day 4 or day 7. MR spectroscopy lactate values were higher in the UCO + hypothermia group (Table 1), corresponding to higher serum lactate levels in that group.

Discussion

We demonstrated good intraobserver repeatability with ultrasonographic imaging of the thalamus in this primate model of HIE, which is consistent with our previous report of imaging the human basal ganglia¹² as well as reports of other cerebral hemodynamic measures, such as SVC flow.¹⁹ The thalamus measures of RI did not demonstrate improved ability to predict outcomes over ACA measures (Figure 3), but they had a consistently stronger association with clinical measures of disease severity such as serum lactate and MRS lactate outside the first 24 hours of life (Table 2). Additionally, there was a significant increase in the thalamus RI in the first 36 hours in the group who received hypothermia. The difference decreased but maintained statistical significance at four days of life.

Both the ACA and thalamus RI measures in the first 36 hours of life were statistically different in the group who died or developed CP. It is also worth noting the difference in trajectory between the ACA and thalamus RI measures. Where the ACA RI decreased in the CP or death group, demonstrating increased global perfusion, the thalamus RI increased in the same group, demonstrating a relative regional hypoperfusion. This is consistent with a study by Wintermark et al. that demonstrated a significant early hypoperfusion in the basal ganglia and cortical gray matter followed by a hyperperfusion on days 2–6 in the infants who developed brain injury despite hypothermia.²⁰ They did not see any difference in the perfusion values of the white matter. There was overlap in the RI range between the outcome groups in our study, so it is unlikely that a single measurement would adequately identify infants who would benefit from additional therapy. It still needs to be determined whether these values in combination with NIRS and/or EEG measures would be able to better differentiate prognosis. Additionally, using serial ultrasound measures, it may be possible to monitor response to therapy.

Previous reports found that ACA RI can differentiate between asphyxiated and healthy infants.⁹ We did not find significant differences between the control group and the combined asphyxiated group in either the ACA or thalamus RI measures. This finding may be related to differences in RI between humans and non-human primates, as we were unable to find studies in the literature that documented normal RI values in non-human primates for comparison. A recent study of 28 infants demonstrated that an RI value of <0.6 before and

following cooling was associated with death or severe neurodevelopmental disability,²¹ and essentially all of our measures, even in the control infants, had RI <0.6. This study would need to be repeated in human neonates to evaluate if the difference in RI was a difference in species or imaging technique.

Lastly, we demonstrated a significant increase in the RI of the thalamus during hypothermia, which was not seen in the other two study groups, nor in the ACA RI measure during hypothermia. We pose two potential hypotheses to support this finding. The first is that because the hypothermia group was sicker – as demonstrated by lower 5-minute Apgar and initial pH, as well as higher serum lactate, base deficit, and MRS lactate peak – the RI was elevated due to the worsened pathology. This hypothesis supports the view that hypothermia produces a clinically positive effect since the RI difference had considerably decreased by day 4 of life. The second hypothesis is that the hypothermia itself has a larger effect on the perfusion of the thalamus than the ACA and that the change in RI difference at day 4 was due to return to normothermia at that time. Previous studies demonstrated decreased positive predictive value of the anterior cerebral artery RI to predict death or severe disability when infants are undergoing hypothermia versus measurements obtained during normothermia.^{10,22} Because all three infants in the hypothermia group developed our primary outcome of death or CP, we are unable to differentiate between these two hypotheses with the current study.

This small pilot study had a number of limitations. First, as demonstrated in Table 1, the UCO + hypothermia group ended up being a sicker group of infants at birth than either of the other groups as defined by 5-minute Apgar and initial blood gas. These values, especially at the levels demonstrated in our study, have been shown to have poor predictive value in the HIE population,^{3,23} so we do not feel that this limitation alters the validity of our current findings. Additionally, all three of those infants had death or CP, so there was no ability to make within-group comparisons using outcome as a measure; we were therefore unable to separate the effect of hypothermia on perfusion from that of illness severity. Lastly, the described non-human primate model was developed to create a severe hypoxic-ischemic injury. Severe HIE is the group where ACA RI has the best prognostic ability, so if the thalamus is better at predicting outcomes, the inclusion of moderate injury may be necessary to demonstrate a difference between the two measures.

Conclusion

The fast Doppler measure of thalamic perfusion demonstrated good intraobserver reliability and good correlation with measures of clinical severity. The measures in the thalamus demonstrated a large difference in perfusion in the hypothermic group that was not seen by imaging the ACA. Future studies should further evaluate the effects of hypothermia on perfusion measures within the deep gray matter, and attempt to correlate those measures with outcomes. If the changes seen here are due to injury as opposed to hypothermia alone, these ultrasound measures of the thalamic RI could be a valuable addition to the standard MRI and EEG (or amplitude-integrated EEG) in monitoring for therapeutic response in infants with HIE.

Acknowledgments

We would like to thank the staff of the University of Washington infant primate lab for all of their assistance in caring for the infants in this study. We would also like to thank Dr. Nina Natarajan for providing MRI interpretation. This study was supported by a National Institutes of Health grant R01-HD-52820-01 and an American Recovery and Reinvestment Act supplement to R01-HD-52820-01 and P30 HD02274 through the Washington Regional Primate Research Center and the National Institute of Child Health and Human Development.

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Figure 1.
 Ultrasound imaging. A. Sagittal image of anterior cerebral artery (ACA) and thalamus; B. Sagittal image with region of interest focused on thalamic blood flow; C. Demonstration of post-acquisition flow velocity measurements

Figure 2.
Study imaging protocol
UCO, umbilical cord occlusion; MRI, magnetic resonance imaging

Figure 3.

Comparisons of resistive indices over time, by study group (A,B: open box = control; light shading = umbilical cord occlusion (UCO); diagonal marks = UCO + hypothermia) and by outcomes (C,D: open box = no death or cerebral palsy (CP); dark shading = died or developed CP).

* $p < 0.001$, ** $p = 0.002$

Table 1

Subject Demographics

	Control (n=3)	UCO (n=3)	UCO + hypothermia (n=3)
Male	2 (66.7)	2 (66.7)	1 (33.3)
Birth Weight	630 ± 156	638 ± 83	646 ± 127
5-minute Apgar	8 ± 2	4 ± 4	1 ± 1
Initial pH	7.26 ± 0.04	7.27 ± 0.09	7.11 ± 0.05
Initial Base Deficit	6.7 ± 1.7	3.7 ± 5.6	16.8 ± 3.1
Initial Lactate	3.8 ± 3.6	3.0 ± 1.7	14.2 ± 5.0
Day 4 MRS Lactate	0.2 ± 0.2	0.2 ± 0.1	1.2 ± 1.4
Death or CP	0 (0.0)	1 (33.3)	3 (100.0)

UCO, umbilical cord occlusion; MRS, magnetic resonance spectroscopy; CP, cerebral palsy

Association between mean resistive indices in the anterior cerebral artery (ACA) or thalamus and serum or magnetic resonance spectroscopy (MRS) lactate

Table 2

	Day of Life 0		Day of Life 1		Day of Life 4	
	R ²	P Value	R ²	P Value	R ²	P Value
ACA : serum lactate	0.138	0.412	0.004	0.889	0.123	0.440
Thalamus : serum lactate	0.023	0.776	0.937	0.002	0.351	0.161
ACA : MRS lactate	0.518	0.068	0.170	0.358	0.103	0.525
Thalamus : MRS lactate	0.574	0.081	0.236	0.329	0.596	0.072