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Ultrasound predicts white matter integrity after hypothermia therapy in neonatal hypoxic-ischemic injury

Jacqueline Salas^{1,2}, Nihaal Reddy^{2,3}, Kathryn A. Carson⁴, Frances J. Northington^{2,5}, Thierry A.G.M. Huisman^{2,3}

¹Division of Neonatology, Department of Pediatrics, New-York Presbyterian Brooklyn Methodist Hospital, Brooklyn, NY, USA

²Neurosciences Intensive Care Nursery Group and Division of Neonatology, Johns Hopkins University School of Medicine

³Division of Pediatric Neuroradiology, Division of Pediatric Radiology, Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁴Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

⁵Division of Neonatology, Department of Pediatrics, Johns Hopkins School of Medicine

Abstract

Background: Hypoxic-ischemic injury (HII) is a major cause of neonatal death and neurodevelopmental disability. Head ultrasounds (HUS) in neonates with HII often show enhanced gray/white matter differentiation. We assessed the significance of this finding in predicting white matter structural integrity measured by diffusion tensor imaging (DTI) in neonates with HII.

Methods: We performed a quantitative region of interest-based analysis of white and gray matter echogenicity within the cingulate gyrus on pre- and post-hypothermia HUS. We also completed a quantitative analysis of fractional anisotropy (FA) and mean (MD), axial (AD), and radial (RD) diffusivity within the bilateral anterior and posterior centrum semiovale (CSO) on post-hypothermia brain MRI. For HUS studies, we calculated a white-to-gray matter echogenicity ratio (WGR) and subsequently correlated it to DTI measurements.

Results: Forty-two term neonates with HII who underwent hypothermia therapy were included. Significant correlation was found between pre-hypothermia WGR and MD, AD and RD values in the left anterior CSO ($r=0.38-0.40$, $p=0.02$). Pre-hypothermia WGR also correlated with the following: MD and RD in the right anterior CSO ($r=0.35-0.36$, $p=0.04$), MD and AD in the right posterior CSO ($r=0.32-0.45$, $p=0.008-0.03$), and AD in the left posterior CSO ($r=0.47$, $p=0.005$). No significant correlation was found either between pre-hypothermia WGR and FA values in the bilateral anterior and posterior CSO or between post-hypothermia WGR and DTI scalars in the bilateral anterior and posterior CSO.

Conclusions: Pre-hypothermia HUS WGR may predict post-hypothermia white matter structural integrity and is potentially an early and easily obtainable biomarker of severity in neonatal HII.

Keywords

Diffusion tensor imaging; hypoxic-ischemic encephalopathy; head ultrasound; brain injury

INTRODUCTION

Hypoxic ischemic injury (HII) in full and near-term neonates remains one of the major causes for neonatal brain injury and death despite new approaches in diagnosis and treatment.¹ Early diagnosis is essential in determining the appropriate clinical management. Therapeutic hypothermia (TH) after perinatal hypoxia-ischemia is currently the only therapy known to improve outcomes in randomized controlled trials such including the Cool Cap, TOBY and NICHD Body Cooling trials.^{2–4} Despite this, there is prognostic uncertainty for any particular neonate with HII. Imaging biomarkers may aid in elucidating the severity of the injury, guide treatment decisions and predict prognosis for an infant with this diagnosis.

MRI is the gold standard imaging modality for assessing the pattern and severity of injury and for prediction of prognosis.⁵ However, developmental delays are possible despite a normal appearing conventional MRI.⁶ Diffusion tensor imaging (DTI) evaluates the functional integrity of the cerebral gray and white matter. DTI has shown improved sensitivity and specificity in the acute and subacute phase for brain injury compared to conventional MRI.⁷ Diffusion changes on DTI in neonates with HII likely result from a combination of hypoxia related acute global edema and cerebral tissue injury.⁸ Powerful post-processing algorithms generate maps of isotropic and anisotropic diffusion. Fractional anisotropy (FA), mean diffusivity (MD), radial and axial diffusivity (RD, AD) maps can be utilized to quantify tissue integrity using a region of interest (ROI) approach. DTI scalars measure distinct diffusion characteristics of the sampled tissue and consequently quantify various components of tissue injury and may predict prognosis. For example, low FA values in the corpus callosum, centrum semiovale (CSO) and limbs of the internal capsule have been shown to correlate to an unfavorable neurodevelopmental outcome.^{9–11} Elevated MD values are associated with tissue edema and may also be of prognostic value.^{12,13}

Despite the wealth of information provided by MRI, there are often multiple obstacles to obtaining an MRI in the acute period. MRI/DTI requires transportation of the neonate to the MRI suite, the neonate may be unstable, and may require sedation to prevent motion artifacts. Head ultrasound (HUS) provides a fast and safe alternative which can be performed bedside with an option for serial examination. HUS can be done without the need for sedation. Recent research suggests that data collected by HUS is of prognostic significance in HII.^{14–16} In addition to subjective image interpretation, objective, quantitative data collection in HUS can be correlated with follow-up DTI scalars.

Cerebral edema is a common early finding after HII, demonstrated by an increase in echogenicity of the cerebral white matter (WM) compared to the overlying cortical gray matter (GM) on HUS. Until recently, the evaluation of edema has relied on a subjective

review by a radiologist, limiting objective comparability of study results. Even for experienced pediatric neuroradiologists, multiple factors including the size of the fontanelle, presence of any extracalvarial obscuring fluid collections, and differences in imaging techniques impact reproducible image interpretation.⁹ HUS quantitative scalars such as the WM-to-GM ratio (WGR) have emerged as a more user-independent tool with the potential for objectively diagnosing common hypoxic-ischemic changes in the brain such as WM edema. A previous study from our institution demonstrated that a quantitative WGR as measured by HUS was significantly increased in neonates who suffered from HII compared with the same measure on the HUS of healthy controls.¹⁷ Therefore, the WGR has the potential to provide an early quantitative marker of injury in HII. However, this marker has never been compared to objective DTI scalars or long-term outcomes after TH.

We assessed and analyzed 1) the HUS scalar of WGR for pre- and post- hypothermia neonates, 2) the correlation between the pre- hypothermia WGR and post- hypothermia DTI FA, MD, RD, and AD values in the CSO, 3) the correlation between post- hypothermia WGR and post- hypothermia DTI scalar values and 4) the relationship between the difference of pre- to post- hypothermia WGR to post-hypothermia DTI scalars. We hypothesized that markers of diffusion would correlate to the pre- and post-hypothermia WGR obtained on HUS.

METHODS

This study was approved by the Johns Hopkins Institutional Review Board. A consent waiver was obtained due to the retrospective nature of the study.

Patients and controls

The inclusion criteria for patients were the following: 1) a diagnosis of HII and completion of therapeutic hypothermia, 2) availability of HUS and DTI data without artifacts and 3) neuroimaging at day of life 5-14. Exclusion criteria included 1) early discontinuation of therapeutic hypothermia, 2) alternative or additional diagnoses that may explain neonatal encephalopathy, and 3) presence of severe hypoglycemia defined as a blood glucose level < 30 mg/dL prior to imaging studies being completed.

Patients were enrolled in an ongoing research cohort that included all neonates with HII who underwent therapeutic hypothermia from January 1, 2010 to March 1, 2016, in the Johns Hopkins Neuro-Intensive Care Nursery. The research cohort data was reviewed retrospectively for neonates who met criteria for inclusion in our study. Neonatal controls were selected from our pediatric MRI database using the following criteria: 1) age at MRI between 1 and 28 days, 2) age at birth between 36 and 41 weeks of gestation, 3) normal brain anatomy, 4) absence of known or suspected neurological disorders, and 5) availability of DTI data without artifacts. All MRI studies included in the control population were performed for clinical indications, primarily for the evaluation of soft tissue pathologies in the head and neck region or facial lesions not involving the brain.

Demographic data and clinical information, such as maternal and birth history, Apgar score, and cord blood gas measurements, were available for all studied neonates.

Acquisition of HUS data

All neonates were examined in the neonatal intensive care unit. No sedation was performed for the purpose of the HUS studies. HUS was performed by experienced pediatric sonographers, using state-of-the-art ultrasound (US) equipment (Zonare, Medical Systems, Mountain View, CA, USA). A standardized set of images were obtained through the anterior fontanel in coronal and sagittal planes using curved and linear array transducers (8 to 17 MHz). (Figures 1a and 1b) The angle of the transducer was varied when needed to evaluate the periphery of the brain. Penetration depth, number of US foci, US gain and tissue brightness were optimized for each individual examination.

US Image Analysis

Of the 42 patients, six neonates did not have a HUS after hypothermia was completed, therefore were not included in the analysis of post-hypothermia DTI and HUS. All 42 patients were included in the analysis that compared pre-hypothermia HUS scalars with post-hypothermia DTI scalars. All images were evaluated for diagnostic quality, technique and completeness of data. The WM echogenicity was measured by manually positioning a ROI within the WM of the cingulate gyrus on a paramedian sagittal image using the Patient Archiving and Communication system (PACS). Similarly, the GM echogenicity was measured on the same paramedian sagittal image using a separate ROI analysis (Figure 1). Care was given to prevent partial volume effects. Cross-sectional areas for WM and GM were approximately equal in size. All measurements were repeated twice and the measurement with the smallest standard deviation was selected for further statistical analysis. The pediatric neuroradiologist outlining the ROI was blinded to the clinical findings on both occasions.

The measured values of echogenicity (mean pixel brightness) of WM and GM including the standard deviation and size of the cross-sectional areas were transferred into our database for further analysis. WGR was consequently calculated for each HUS study and recorded.

Acquisition of MRI data

Using the standard protocol for neonatal brain imaging previously described by our institution,¹⁸ a 1.5 T clinical scanner (Siemens, Erlangen, Germany) was used to perform all brain MRI studies. Sequences included isotropic three dimensional (3D)-T1-weighted and axial T2-weighted images, a single-shot spin echo, echo planar axial DTI sequence with diffusion gradients along 20 non-collinear directions (effective high b-value of 800 s/mm²) and susceptibility weighted imaging. An additional measurement without diffusion weighting (b=0 s/mm²) was performed. The following parameters were used for DTI acquisition: repetition time (TR) 8500 ms, echo time (TE) 86 ms, slice thickness 2.0 mm, field-of-view (FOV) 240 × 240 mm, and matrix size 192×192. Parallel imaging iPAT=2 with auto-calibrating partial parallel acquisition reconstruction was used. The acquisition was repeated twice to optimize signal-to-noise ratio. In rare circumstances, rectal midazolam was needed due to agitation for image acquisition. Otherwise all studies were performed in natural sleep.

Quantitative DTI analysis

DTIStudio, DiffeoMap, and ROEditor software were used for analysis.^{19–21} The raw DTI data were collected and co-registered to a template image. A 12-mode affine transformation was used to correct for eddy current and subject motion. Maps of FA, color-coded FA, and trace of diffusion were generated. A rigid transformation was performed to our atlas to standardize the orientation of all images. Using axial trace of diffusion and axial color-coded FA maps, ROIs were manually drawn individually for each of the following: bilateral anterior and posterior CSO (Figure 2), creating four separate areas of measurement. For each analyzed structure, ROIs were placed on three contiguous slices and the mean FA and trace of diffusion values were generated. MD values were then calculated ($MD = \text{trace of diffusion} / 3$).

Statistical analysis

First, we assessed the distribution of the DTI scalar values of the anterior and posterior centrum semiovale. The AD measures were not normally distributed. We then performed a non-parametric Wilcoxon signed rank tests to test for the differences between left and right-sided DTI scalars. There were significant differences between left and right AD both anteriorly and posteriorly, thus the left and right measures were not averaged. Patient characteristics were summarized using descriptive statistics. Partial Spearman rank correlations, controlling for age at MRI, were used to test the association of DTI scalars with HUS measures. Analyses were performed using SAS software, version 9.3 (SAS Institute, Inc., Cary, NC, USA). All tests were two-sided and observed differences were considered statistically significant if $p\text{-value} < 0.05$. No correction was made for multiple comparisons, but false discovery rates were calculated for each ROI region using the Benjamini and Hochberg method.²² All statistical analyses were performed by a trained statistician (KAC).

RESULTS

Patients

One hundred and fifty-six neonates with HII underwent TH at our institution during the study period. One hundred and fourteen neonates were excluded due to lack of high quality DTI data or significant artifacts ($n=81$), gestational age ($n=11$), premature discontinuation of therapeutic hypothermia ($n=5$), suspected infection ($n=4$), MRI obtained after 14 days of life ($n=3$), concurrent severe congenital heart disease ($n=1$) or severe hypoglycemia defined as a serum glucose of < 30 mg/dL ($n=9$). Forty-two term neonates with HII met the inclusion criteria for the study. 37 subjects (65%) were male. Median gestational age at birth was 39 weeks (range: 36–41 weeks). MRI was performed at a median of 8 days after birth (range 4–11 days). Additional cohort characteristics are presented in Table 1.

Imaging Analysis

Post-hypothermia DTI data were acquired between 4 and 11 days of age. CSO DTI scalars and their correlation to pre- and post-hypothermia WGR are summarized in Tables 2 and 3. A positive correlation was found between pre-hypothermia WGR and MD ($r=0.40$, $p=0.02$), AD ($r=0.40$, $p=0.02$), and RD values (0.38 , $p=0.02$) in the left anterior CSO. The following

DTI scalars correlated with pre-hypothermia WGRs: right anterior CSO MD ($r=0.35$, $p=0.04$) and RD ($r=0.36$, $p=0.04$), right posterior CSO MD ($r=0.38$, $p=0.03$) and AD ($r=0.45$, $p=0.008$), left posterior CSO AD ($r=0.47$, $p=0.005$). Graphical representation of the correlation of MD values in the right anterior CSO with pre- and post-hypothermia WM/GM ratio is shown in Figure 3. No correlation was found between FA values in any region and the pre-hypothermia WGR. None of the DTI scalars in the bilateral anterior and posterior CSO were associated with the post-hypothermia WGR. The following DTI measures were inversely correlated with the difference between pre- and post-hypothermia WGRs: left anterior CSO MD ($r=-0.37$, $p=0.03$) and AD ($r=-0.36$, $p=0.04$), and left posterior CSO AD ($r=-0.39$, $p=0.02$).

DISCUSSION

Our study compared a quantifiable, acute HUS measure of echogenicity of the brain with follow-up DTI markers of white matter injury in neonates with HII. We found that the WGR determined by pre-hypothermia HUS studies of neonates with HII correlated with multiple diffusion scalars in the CSO on DTI following hypothermia therapy. Fractional anisotropy values on DTI were not associated with the WGR, whether obtained from HUS studies prior to or post-hypothermia therapy. Post-hypothermia WGR did not correlate with any diffusion measures on DTI in the same cohort. Correlation was only noted in three of the twelve scalars analyzed when the difference between pre- and post-hypothermia WGRs was compared to MD, AD and RD values from all four regions on DTI.

Early, easily obtainable and objective biomarkers of HII are yet to be defined. Given its bedside availability, low cost and lack of radiation, HUS is an appealing modality for examining acutely injured neonates. Subjective evaluation of the HUS of affected neonates often does not recognize acute changes in the first 24-48 hours following injury, however repeat ultrasound studies in the same neonates after 48 hours may reveal significant brain injury.^{16,23,24} Thus, subjective assessment is limited in its use to determine diagnosis and predict prognosis in this early time frame. Past studies have relied on the subjective evaluation of the HUS images, and enhanced WM and GM differentiation was not evaluated. Significant abnormalities on high-end US correlate (to up to 95% accuracy) to concurrently performed MRI.²⁵ Enhanced WM and GM differentiation may be the only finding in the acute period and may remain unrecognized by subjective assessment in the first 24-48 hours following injury. An objective HUS image analysis that can identify subtle injuries early in HII course may be an essential and helpful tool in the acute phase of injury to determine the severity and likely prognosis of the neonate when combined with the clinical evaluation and EEG findings.

TH is effective when instituted within the first six hours of life in neonates with HII. Clinically, it can be challenging to identify neonates who present with mild encephalopathy but will progress to moderate encephalopathy. Given that TH is most effective in moderate to severe encephalopathy when initiated within the first six hours of life, these infants are often not treated with TH from which they would potentially benefit. Reliable, early markers of injury could aid in identifying neonates who benefit from intervention. The identification of effective adjunctive therapies is also the target of emerging research. Imaging and other

biological markers of injury could play an essential role in determining eligibility and effectiveness of such therapies. Reliable DTI data without motion artifact can also be difficult to obtain in neonates as evidenced by the number of subjects necessitating exclusion from the study due to this. HUS may be a more reliable alternative to provide biomarkers of injury.

DTI is a sensitive, non-invasive imaging sequence for detecting abnormalities, such as damage and rarefaction of the white matter fiber tracts in HII, through quantification of the diffusion characteristics of water molecules.^{26–28} DTI also provides indirect evidence about the tissue microstructure of the brain, through different DTI scalars such as FA, MD, RD, and AD. The correlation to MD, AD and RD values on DTI with pre-hypothermia WGR was inconsistent in all areas. This could be due to regional differences in restricted diffusion; however this seems unlikely given the global nature of HII. The lack of significance in these areas may also be due to a lack of adequate power. Accurate values also depend on the consistency of ROI placement. This was optimized by using the same observer, placing standards on the size of area measured and obtaining multiple measurements for each region.

FA values did not correlate to WGR performed pre-hypothermia therapy. This may be a reflection of the tissue level diffusion characteristics that each gradient is measuring. AD represents the coherence of axonal membranes and fiber structure.²⁹ RD represents the degree and integrity of white matter myelination, while MD is a parameter gauging the overall microstructural integrity of white/gray matter structures.³⁰ FA is determined by a combination of the former and other factors including fiber density and diameter, along with extracellular and interaxonal spacing.²⁹ Typically, there is a reduction in MD values in the acute phase of ischemia, and in FA values in the subacute/chronic phase.²⁶ Given the multifactorial nature of FA, there may be factors that negate each other and thus the combined marker fails to correlate to the WGR. WGR in the acute phase may simply be an inadequate measure to predict FA value changes at the time point in which MRIs were being performed in our study.

Despite the global nature of HII, there are multiple typical injury patterns depending on the degree and type of white and gray matter involvement.^{31,32} Given that the CSO encompasses the bulk of hemispheric WM of the brain, it is unclear if this measurement of diffusion would be a consistently accurate evaluation of injury in those neonates that have a predominant deep GM injury. Edema is a common finding found clinically in the initial HUS of neonates with HII, regardless of the resulting injury pattern. Inclusion in our study was not influenced by resulting injury pattern. It is encouraging that most MD, RD and AD values correlated to HUS WGR measurements despite the fact that all injury patterns (white versus gray matter) were included. Additional studies could be performed to understand the role of this marker in specific injury patterns given that our sample size limited our ability to do so.

WGR on studies performed following hypothermia failed to correlate to DTI scalars in the CSO. Enhanced white to gray matter differentiation has been correlated to acute edema in the acute period of injury. The fact that post-hypothermia WGR does not correlate to DTI markers may imply that the WGR is not sensitive enough to detect the resultant

microstructural changes that DTI can. It is also unclear what effect hypothermia has on the WGR, which may also account for lack of correlation of post-hypothermia WGR with DTI values. Hypothermia is recognized to reduce injury in infants with moderate hypoxic-ischemic encephalopathy, but its influence on brain edema is still lacking in the literature. Presumably there is a reduction in edema from a reduction in microstructural injury but the exact mechanisms continue to be evaluated.³³ Although the same cohort of neonates was utilized for pre-hypothermia and post-hypothermia analysis when possible, not all neonates had both pre- and post-hypothermia ultrasound studies performed. It is not known if the severity of injury had any influence on which neonates did not receive both ultrasounds. Future studies should be performed to confirm these results.

We appreciate limitations in our study. It is retrospective in design and was limited in sample size primarily by the presence of adequate DTI data for analysis in neonates for possible inclusion. Another limitation is the lack of post-hypothermia HUS for 6 of 42 of the neonates. WGR of the cingulate gyrus and ROI analyses on DTI are dependent on semi-subjective placement of ROI. We feel confident that this has not affected our outcomes. Variability in obtained measurements was limited by using experienced neuroradiologists as observers, taking multiple measurements and using ROI areas with the lowest standard deviation. Automated software could ensure the uniformity of measurements and provide rapid analysis of both HUS and DTI for academic and non-academic centers in the future. Nevertheless, we remain confident in our findings that the WGR on pre-hypothermia HUS correlates with DTI abnormalities in the white matter.

The significance of the WGR as a biomarker of HII is only fully defined once its potential correlation to long-term neurodevelopmental outcomes is known. We showed that the pre-hypothermia WGR correlates to multiple diffusion scalars on DTI. DTI changes in several known ROIs correlate to long term neurodevelopmental outcomes in multiple studies.^{9,10} Commonly, studies that examine the association between DTI and long-term neonatal outcomes primarily assess FA as the main diffusion scalar although it is recognized that other diffusion markers have important implications as well. We saw no correlation of the WGR to FA values. The predictive value of the WGR for long term outcomes will need to be defined independently. Future studies are needed to determine the value of this biomarker as a predictor of neurologic outcomes. With standardized ROI placement, prognosis could be predicted for an individual neonate once normal values of WGR are defined. Comparative studies may then be required to assess whether the WGR or DTI scalars are the best predictors of outcome in different types of injury. The WGR may also have utility as an adjunctive measure to DTI scalars in order to enhance their predictive capability. In addition, the role of the WGR and its association to different patterns of injury needs further clarification. We agree that HUS does not replace an MRI study for its diagnostic and prognostic value. However, when MRI is not possible, particularly in the acute phase of illness, the WGR could be an invaluable tool for practitioners. It has the potential to aid discussions regarding prognosis in the acute phase of injury to guide families regarding the plan of care. A biomarker such as this could also be useful in determining who would benefit from novel adjunct therapies.

In summary, our results suggest that the WGR on HUS performed prior to hypothermia therapy may predict post-hypothermia white matter structural integrity. Thus, it is potentially a very early and easily obtainable biomarker of severity in neonatal HIE even in resource limited settings. Future studies should confirm the clinical significance of the WGR as a quantitative measure in outcomes research.

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Figure 1.**Region of Interest Delineation on Head Ultrasound**

Sample 'regions of interest' (ROI) on a sagittal head ultrasound images in a neonate with hypoxic-ischemic injury. In panel (A), the dark cortical gray matter in the cingulate gyrus is outlined for ROI analysis. Panel (B) demonstrates positioning of the ROI for the underlying relatively echogenic white matter of the cingulate gyrus.

Figure 2.
Region of Interest Delineation on Diffusion Tensor Imaging
Sample 'regions of interest' positioned within the bilateral anterior and posterior centrum semiovale in axial diffusion tensor imaging on fractional anisotropy map(A) and apparent diffusion coefficient map (B) in a neonate affected by hypoxic-ischemic injury.

Figure 3.

Mean Diffusivity Correlation to White Matter/Gray Matter Ratio in Right Anterior Centrum Semiovale

Scatterplots and linear regression of mean diffusivity (MD) of right anterior (RA) centrum semiovale in relation to white-matter/gray-matter ratio on head ultrasound performed pre-hypothermia (A) and post-hypothermia (B). There is a positive correlation between the mean diffusivity values and the white-matter/gray-matter ratios prior to hypothermia; however, this correlation is not present when MD is compared to post-hypothermia ratios. Correlation

coefficients (r) and p-values are shown in upper right corner of graphs with the significant p-value starred. All patients with available data for each cohort were included in the graphs. Post-hypothermia ultrasounds were not available for all those with pre-hypothermia ultrasounds.

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Table 1.

Demographic and Clinical Characteristics of Neonates with Hypoxic-Ischemic Injury

Characteristic	Value
Male, n (%)	27 (64)
Gestational age, weeks	39 (36-41)
APGAR at 5 min	5 (0-9)
APGAR at 10 min	6 (2-9)
Cord pH	6.97 (6.63-7.44)
Base deficit, mmol/L	15.5 (4.5-30)

All values are medians (range) unless otherwise noted.

Table 2.

Results of Spearman rank correlation between DTI scalars in the anterior CSO and quantitative ultrasonography measurements.

ROI	DTI scalars	WGR pre-hypothermia		WGR post-hypothermia		Pre-/post-hypothermia differences in WGR	
		Spearman CC	p value (FDR)	Spearman CC	p value (FDR)	Spearman CC	p value (FDR)
Right anterior CSO	FA	−0.14	0.43 (0.64)	−0.07	0.69 (0.87)	0.04	0.82 (0.92)
	MD (x10 ^{−3} mm ² /s)	0.35	0.04 * (0.18)	−0.01	0.96 (0.98)	−0.31	0.07 (0.22)
	AD (x10 ^{−3} mm ² /s)	0.27	0.13 (0.31)	−0.05	0.76 (0.89)	−0.28	0.11 (0.28)
	RD (x10 ^{−3} mm ² /s)	0.36	0.04 * (0.18)	0.02	0.93 (0.98)	−0.29	0.09 (0.25)
Left anterior CSO	FA	−0.17	0.33 (0.56)	0.09	0.63 (0.87)	0.14	0.42 (0.65)
	MD (x10 ^{−3} mm ² /s)	0.40	0.02 * (0.18)	−0.05	0.76 (0.89)	−0.37	0.03 * (0.18)
	AD (x10 ^{−3} mm ² /s)	0.40	0.02 * (0.18)	−0.04	0.84 (0.92)	−0.36	0.04 * (0.18)
	RD (x10 ^{−3} mm ² /s)	0.38	0.02 * (0.18)	−0.04	0.82 (0.92)	−0.34	0.05 (0.20)

AD, axial diffusivity; CC, correlation coefficient; CSO, centrum semiovale; DTI, diffusion tensor imaging; FA, fractional anisotropy; MD, mean diffusivity; ROI, region of interest; RD, radial diffusivity; WGM, white matter-gray matter ratio;

* p value is from the Spearman rank partial correlation; statistically significant correlations are starred. Correlations adjusted for age at time of MRI. FDR represents the false discovery rate.

Table 3.

Results of Spearman rank correlation between DTI scalars in the posterior CSO and quantitative ultrasonography measurements.

ROI	DTI scalars	WGR pre-hypothermia		WGR post-hypothermia		Pre-/post-hypothermia differences in WGR	
		Spearman CC	p value (FDR)	Spearman CC	p value (FDR)	Spearman CC	p value (FDR)
Right Posterior CSO	FA	-0.16	0.37 (0.62)	-0.11	0.53 (0.77)	0.05	0.76 (0.89)
	MD (x10 ⁻³ mm ² /s)	0.38	0.03 * (0.18)	0.14	0.43 (0.65)	-0.24	0.17 (0.40)
	AD (x10 ⁻³ mm ² /s)	0.45	0.008 * (0.18)	0.07	0.69 (0.88)	-0.33	0.06 (0.20)
	RD (x10 ⁻³ mm ² /s)	0.32	0.06 (0.20)	0.19	0.29 (0.56)	-0.18	0.32 (0.56)
Left Posterior CSO	FA	-0.07	0.69 (0.88)	-0.11	0.54 (0.77)	-0.001	0.99 (1.00)
	MD (x10 ⁻³ mm ² /s)	0.29	0.10 (0.25)	0.17	0.33(0.56)	-0.19	0.28 (0.56)
	AD (x10 ⁻³ mm ² /s)	0.47	0.005 * (0.18)	0.01	0.94 (0.98)	-0.39	0.02 * (0.18)
	RD (x10 ⁻³ mm ² /s)	0.32	0.06 (0.20)	0.21	0.22 (0.49)	-0.18	0.30 (0.56)

AD, axial diffusivity; CC, correlation coefficient; CSO, centrum semiovale; DTI, diffusion tensor imaging; FA, fractional anisotropy; MD, mean diffusivity; ROI, region of interest; RD, radial diffusivity; WGM, white matter-gray matter ratio;

* p value is from the Spearman rank partial correlation; statistically significant correlations are starred. Correlations adjusted for age at time of MRI. FDR represents the false discovery rate.