Journal of Perinatology (2009) 29, 143–149

www.nature.com/jp

ORIGINAL ARTICLE

Volumetric and anatomical MRI for hypoxic–ischemic encephalopathy: relationship to hypothermia therapy and neurosensory impairments

NA Parikh[1], RE Lasky[1,2], CN Garza[1], E Bonfante-Mejia[3,4], S Shankaran[5] and JE Tyson[1,2]

[1]Division of Neonatal-Perinatal Medicine, Department of Pediatrics, The University of Texas Medical School at Houston, Houston, TX, USA; [2]Center for Clinical Research and Evidence-Based Medicine, The University of Texas Medical School at Houston, Houston, TX, USA; [3]Department of Neuroradiology, The University of Texas Medical School at Houston, Houston, TX, USA; [4]Department of Neuroradiology, Memorial Hermann Hospital, Houston, TX, USA and [5]Division of Neonatal-Perinatal Medicine, Department of Pediatrics, Wayne State University, Detroit, MI, USA

Objective: To relate volumetric magnetic resonance imaging (MRI) findings to hypothermia therapy and neurosensory impairments.

Study Design: Newborns ⩾36 weeks' gestation with hypoxic–ischemic encephalopathy who participated in the National Institute of Child Health and Human Development hypothermia randomized trial at our center were eligible. We determined the relationship between hypothermia treatment and usual care (control) to absolute and relative cerebral tissue volumes. Furthermore, we correlated brain volumes with death or neurosensory impairments at 18 to 22 months.

Result: Both treatment groups were comparable before randomization. Total brain tissue volumes did not differ in relation to treatment assignment. However, relative volumes of subcortical white matter were significantly larger in hypothermia-treated than control infants. Furthermore, relative total brain volumes correlated significantly with death or neurosensory impairments. Relative volumes of the cortical gray and subcortical white matter also correlated significantly with Bayley Scales psychomotor development index.

Conclusion: Selected volumetric MRI findings correlated with hypothermia therapy and neurosensory impairments. Larger studies using MRI brain volumes as a secondary outcome measure are needed.
Journal of Perinatology (2009) **29,** 143–149; doi:10.1038/jp.2008.184; published online 20 November 2008

Keywords: hypoxia–ischemia; brain; hypothermia; induced; magnetic resonance imaging; cerebral palsy; outcome assessment

Correspondence: Dr NA Parikh, Division of Neonatal-Perinatal Medicine, Department of Pediatrics, The University of Texas Medical School at Houston, 6431 Fannin, MSB 3.236B, Houston, TX 77030, USA.
E-mail: Nehal.A.Parikh@uth.tmc.edu
Received 7 April 2008; revised 17 September 2008; accepted 29 September 2008; published online 20 November 2008

Introduction

Neonatal encephalopathy resulting from suspected acute hypoxic–ischemic perinatal events affects 1 to 2 near-term and term newborns per 1000 births in the developed world.[1–3] Up to 40% of infants with moderate hypoxic–ischemic encephalopathy (HIE) and 100% of those with severe HIE either die or develop neurosensory impairments, including cerebral palsy, mental retardation and deafness.[4] Until recently, treatment for this disorder was primarily supportive. Randomized therapy with selective head or systemic induced hypothermia therapy has been administered to more than 700 newborns with HIE.[5–10] The National Institute of Child Health and Human Development (NICHD) whole-body hypothermia trial[9] showed a 28% relative reduction in death or moderate or severe disability at 18 to 22 months in hypothermia-treated infants compared with control infants. Although how hypothermia confers neuroprotection is not fully understood, hypothermia reduces cerebral metabolism, inhibits glutamate release, preserves high-energy phosphates, reduces neuronal nitric oxide production, preserves endogenous antioxidants and ameliorates apoptotic neuronal death in experimental models.[11,12]

Qualitative anatomical magnetic resonance imaging (MRI) studies have helped elucidate the potential short-term effects of hypothermia on the extent and pattern of cerebral injury following HIE[13,14] and in predicting neurosensory disability.[15,16] However, quantitative volumetric MRI—an emerging tool to assess subtle neuroanatomic treatment effects[17]—may provide complementary information to anatomical MRI scans by objectively assessing regional or global cerebral atrophy, a common sequelae of HIE,[15,18] and improving prediction of neurosensory impairments.[19,20] In this pilot investigation, we determined the feasibility and reliability of using volumetric MRI to investigate the effects of systemic hypothermia therapy on regional brain volumes for neonates with HIE and to establish whether MRI brain volumes

correlate with neurosensory outcomes. We hypothesized that encephalopathic newborns randomized to whole-body hypothermia therapy[8,9] will have larger total brain tissue volumes than those randomized to the usual care. We further hypothesized that total brain volumes will correlate with death or neurosensory impairments at 18 to 22 months of age.

Methods

Subjects

All infants at our institution who were enrolled in the NICHD Neonatal Research Network whole body hypothermia trial[8,9] were eligible for this substudy. All of the following criteria were required for enrollment in the trial: gestational age $\geqslant 36$ weeks; NICU admission with a diagnosis of neonatal depression, asphyxia or encephalopathy; signs of acute perinatal asphyxia (cord or neonatal gas within the first hour with pH of $\leqslant 7.0$ and/or base deficit of $\geqslant 16\,\mathrm{mmol\,l^{-1}}$; if pH was between 7.01 and 7.15 or a base deficit was between 10 and $15.9\,\mathrm{mmol\,l^{-1}}$, or a blood gas was not available, additional history of an acute perinatal event and an Apgar score at 10 min of $\leqslant 5$ or need for assisted ventilation at birth was required); and seizures or moderate or severe encephalopathy by standardized neurological examination. Of the 24 eligible infants, 10 subjects, 5 each in the hypothermia treatment and control (usual care) groups, were excluded: 4 because of early death (3 receiving hypothermia treatment and 1 control); 3 (all controls) because they were discharged home without receiving a brain MRI; 2 because of incomplete MRI scans (1 hypothermia-treated and 1 control); and 1 due to poor image quality resulting from significant motion artifacts (hypothermia-treated). Of the 14 infants assessed, 8 were randomized to hypothermia treatment and 6 to the control usual care arm within 6 h after birth. Seven of the 8 infants in the hypothermia arm were systemically cooled to a core temperature of 33.5 °C and one from the pilot trial[8] was cooled to 34.5 °C with a cooling blanket (Blanketrol II Hyper-Hypothermia System, Cincinnati Sub-Zero). Hypothermia was maintained for 72 h at this temperature, followed by gradual rewarming over a 6-h period (0.5 °C per hour) until the core temperature reached 36.5 °C. Core temperature was monitored with an esophageal probe and skin temperature with a probe placed on the abdominal wall. Infants in the control group were cared for on overhead radiant warmers and their abdominal-wall skin and esophageal temperatures were recorded every 4 h. Although informed consent was obtained for each infant in the hypothermia trial, a separate institutional review board approval was obtained for this substudy.

Anatomical MRI acquisition and analysis

Standard anatomical MRI and diffusion-weighted images were obtained with a GE-LX or GE-Horizon 1.5 Tesla scanner (General Electric, Milwaukee, WI, USA). Axial T2 scans (TE 85; TR 4500; slice thickness 4 mm; gap 2 mm). One of the authors (EBM), blinded to clinical history and treatment assignment, interpreted all anatomical MRI scans using a modified HIE MRI scoring system on the basis of studies by Barkovich *et al*.[21] and Mercuri *et al*.[22] (0 = normal; 1 = abnormal signal in either basal ganglia (putamen, caudate and globus pallidus), thalamus or cortex; 2 = abnormal signal in cortex and either basal ganglia or thalamus; or abnormal signal in entire cortex; 3 = abnormal signal in cortex, basal ganglia and thalami; 4 = abnormal signal in entire cortex, basal ganglia and thalamus). Degree of myelination/signal intensity of the posterior limb of the internal capsule[23] and presence of other abnormalities, such as brain atrophy and vascular territory infarct, were also assessed.

Volumetric MRI analysis

The primary anatomical references used were the Haines atlas of neuroanatomy,[24] Bayer and Altman atlas of human central nervous system development[25] and two online human atlases.[26,27] Axial T2-weighted images were imported into Analyze 7.0 software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN, USA) for manual and semi-automated whole brain segmentation and volume rendering. We estimated brain volumes for the 2-mm gap using Analyze. After standardization of image intensity, readily identifiable structures were manually segmented on the basis of predefined algorithms, including pixel intensity and known spatial neuroanatomical boundaries, in the following order: brain stem, fourth ventricle, cerebellum, amygdala, corpus callosum, thalamus, lenticular nucleus, caudate nucleus and third ventricle (Figure 1b). The lateral ventricles, subcortical/periventricular white matter, cortical gray matter and extra-axial cerebrospinal fluid (CSF) were segmented last using semiautomated algorithms (Figure 1c).

The ventricles, cerebellum, corpus callosum, cortical gray matter, subcortical white matter and extra-axial CSF were identified and segmented as described previously.[17] The brain stem was defined by its central location and anterior placement to the cerebellum and fourth ventricle. The amygdalae were bound by the hippocampi inferiorly, the lateral ventricles inferiolaterally and the mamillary bodies superiorly.[28,29] The thalamic nuclei were defined as anterior to the posterior commissure, floor of the lateral ventricles posteriorly, posterior limb of the internal capsule medially and third ventricle laterally. The lenticular nuclei were bounded by the internal capsule laterally and external capsule medially. The caudate nuclei were located lateral to the anterior horn of the lateral ventricle, lateral and ventral to the anterior limb of internal capsule and anterior to the lenticular nucleus. The inferior boundaries of the caudate head began at the level of the anterior commissure and the most inferior aspect of the third ventricle.[30,31]

Subcortical gray matter was defined as comprising thalamic, lenticular (putamen and globus pallidum), amygdalae and

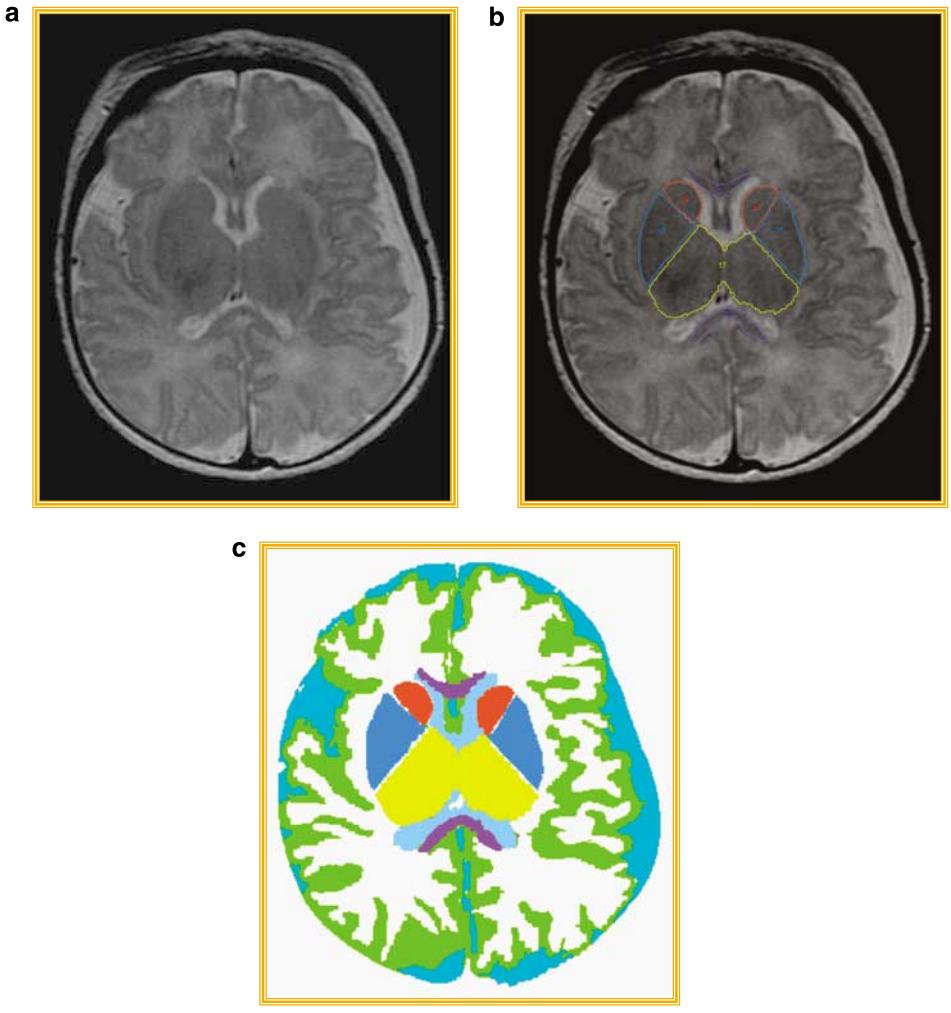

Figure 1 Representative example of brain volume segmentation and labeling methodology for an axial T2-weighted brain MRI. (**a**) Unsegmented T2-weighted midbrain MRI slice. (**b**) Manual segmentation and labeling of smaller subcortical structures: (purple), corpus callosum; (yellow), thalamus; (blue), lenticular nucleus; (red), caudate nucleus. (**c**) Fully segmented mid-axial slice. Representative pixels were individually sampled from the lateral ventricles (light blue), subcortical white matter (white) and cortical gray matter (green) and segmented using a semiautomatic segmentation tool. Extra-axial CSF (cyan) spaces were sampled and labeled last, on the basis of location and clear regional intensity differences.

caudate nuclei. Total intracranial volume was defined as all brain tissue and CSF spaces combined. Total brain tissue volume was defined as the total intracranial volume minus all CSF spaces. One of the authors (CNG), blinded to clinical history and group treatment assignment, segmented all the MR images.

Volumetric MRI reliability

Regional and global brain segmentation for all 14 MRI scans was repeated by the same author (CNG) a fourth time and results were compared with the third iteration to determine the intra-observer correlation coefficients for all segmented brain regions. Interobserver correlation coefficients were determined by comparing segmentation performed by CNG to segmentation performed by another experienced evaluator in a separate group of 10 preterm subjects scanned at term-equivalent age using the same methodology and landmark definitions.

Data collection and neurosensory assessment

Research nurses using prespecified definitions and data forms collected clinical data prospectively.[9] Anatomical brain MRI was recommended at 44 weeks postmenstrual age or at discharge home, whichever occurred first. Data on vision and audiometric characteristics were obtained from parental report and standardized neurological and developmental testing were performed by trained and certified examiners blinded to intervention status at 18 to 22 months of age.[9] An assessment of neuromotor disability was based on the presence of cerebral palsy, and functional disability was graded according to the Gross Motor Function Classification System (range 1 to 5)[32] (level 1 includes children who walk independently with some gait abnormalities; level 5 includes those who require adult assistance to move). Cognitive outcomes were assessed by using Bayley Scales of Infant Development II.[33] Moderate disability was defined as a Bayley Mental Development Index (MDI) scores 1

to 2 s.d. below the mean score (that is, 70 to 84) in addition to one or more of the following: a Gross Motor Function Classification System grade of level 2, hearing impairment with no amplification or a persistent seizure disorder. Severe disability was defined as any of the following: MDI score more than 2 s.d. below the mean score (that is, below 70), a Gross Motor Function Classification System grade of level 3 to 5, hearing impairment requiring hearing aids or blindness.[9]

Statistical analysis

Baseline variables known or suspected to affect the primary outcome were compared in the hypothermia-treated and control groups using the Mann–Whitney *U* test for continuous variables and Fisher's exact test for categorical variables. To control for the effects of small and statistically nonsignificant group differences in head circumference and/or postmenstrual age at MRI, we also tested relative brain volumes (absolute volume divided by total intracranial volume). Therefore, all analyses examined the effect of absolute and relative regional brain volumes. Outcomes were analyzed by Fisher's exact test or the Mann–Whitney *U* test as appropriate. Correlations of relative brain volumes with the Bayley subscales were assessed using Spearman rank correlation (ρ). The analyses were conducted using NCSS (version NCSS 2004; NCSS, Kaysville, UT, USA) statistical software. Intra-observer and inter-observer correlation coefficients were calculated using SPSS software (version 10.0.7; June 2000; SPSS Inc., Chicago, IL, USA). Multiple secondary analyses were conducted to identify significant relationships and to generate new hypotheses. A *P*-value of <0.05 was considered significant. All reported *P*-values are two-sided and uncorrected for multiple comparisons.[34]

Results

Subjects

Before randomization, the eight hypothermia-treated infants and six usual care control infants were comparable with respect to important baseline variables ($P =$ NS; Table 1). Whereas only large differences would be statistically significant with the modest number of infants studied, with the exception of cord pH, there was no overall tendency for the hypothermia group to be lower risk than the usual care group. Median postmenstrual age at MRI scan was also comparable in hypothermia-treated infants (41.0 weeks; 95% CI: 38.6 to 43.3) and control infants (40.4 weeks; 95% CI: 38.7 to 45.9) ($P = 0.60$). An MRI scan was performed within 10 days of life in four infants in the usual care and three in the hypothermia group.

Perinatal variables and total and regional brain volumes

As expected, head circumference, gestational age and postmenstrual age at MRI scan all correlated with total brain intracranial volume (correlation coefficients = 0.55 to 0.67; $P = 0.008$ to 0.05). These covariates were comparable in our two treatment groups. Potential residual scaling effects were controlled by including relative regional brain volumes in the analysis (absolute volume divided by total intracranial volume).

Table 1 Baseline characteristics of hypothermia-treated and usual care control infants

Variables	*Hypothermia-treated (N = 8)*	*Controls (N = 6)*
Gestational age, week[a]	38.5 (36–40)	39.5 (37–41)
Birth weight, g[a]	3288 (2775–3850)	3415 (2385–4885)
Head circumference, cm[a]	34.3 (32.5–35.5)	35.0 (33.0–37.0)
Male, *n* (%)	5 (63%)	3 (50%)
Non-Caucasian, *n* (%)	7 (88%)	6 (100%)
Chest compressions at birth	4 (50%)	1 (17%)
Resuscitation at 10 min	6 (75%)	4 (67%)
Apgar score (5 min)[a]	3 (0–6)	3.5 (1–5)
Apgar score (10 min)[a]	4 (0–5)	5 (3–7)
Cord pH[a]	6.96 (6.57–7.25)	6.77 (6.63–7.18)
Outborn	3 (38%)	1 (17%)
Moderate encephalopathy, *n* (%)	6 (75%)	5 (83%)
Severe encephalopathy, *n* (%)	2 (25%)	1 (17%)

[a]Median and 95% CI.

Hypothermia and anatomical MRI outcomes

Three of the eight hypothermia-treated infants and three of the six controls had abnormal anatomical brain MRIs before discharge home ($P =$ NS). Two of the three hypothermia-treated infants with abnormal MRI scans had cortical lesions sparing the basal ganglia/thalamus and the third had cortex-sparing lesions in the basal ganglia/thalamus. Among the control infants, one infant had isolated basal ganglia/thalamic lesions, one had cortical lesions only and the third had severe lesions to the basal nuclei and cerebral cortex.

Volumetric MRI reliability

The intra-observer correlation coefficients ranged from 0.80 to 0.83 for the amygdalae, thalamic and caudate nuclei and 0.90 to 0.99 for all the other segmented structures and tissue classes. The inter-observer correlation coefficient was 0.88 for the lenticular nucleus, 0.89 for the corpus callosum and 0.93 to 1.00 for all the other segmented structures and tissue classes.

Hypothermia and regional brain volumes

Table 2 presents the absolute and relative brain tissue volumes. Although hypothermia-treated infants had larger absolute total brain tissue volumes, our primary outcome, this difference was not statistically significant. There was no significant difference in relative total brain tissue volumes either. We did, however, observe significantly larger relative subcortical white matter volumes in

Table 2 Absolute and relative brain tissue volumes (median and 95% CI) in hypothermia-treated and control infants

Tissue class	*Hypothermia-treated (N = 8)*	*Control (N = 6)*	P-*value*
Total brain tissue, total			
Absolute volume, ml	373.1 (314.0–396.5)	348.1 (216.2–448.6)	0.34
Relative volume, %	84.3 (78.1–87.4)	84.2 (46.8–89.8)	0.85
Subcortical white matter			
Absolute volume, ml	152.7 (119.3–173.0)	134.8 (119.4–188.8)	0.18
Relative volume, %	35.2 (33.2–36.6)	33.5 (29.6–34.9)	0.02
Cortical gray matter			
Absolute volume, ml	157.7 (146.1–170.3)	163.4 (27.4–190.2)	0.45
Relative volume, %	35.9 (32.5–40.4)	39.2 (5.9–43.7)	0.57
Subcortical gray matter[a]			
Absolute volume, ml	18.7 (16.4–22.3)	18.0 (12.4–25.5)	0.75
Relative volume, %	4.2 (4.0–5.0)	4.5 (2.7–5.3)	0.66
Corpus callosum			
Absolute volume, ml	1.0 (0.6–1.6)	1.0 (0.2–1.9)	0.75
Relative volume, %	0.2 (0.1–0.4)	0.2 (0.1–0.5)	0.95
Brain stem			
Absolute volume, ml	8.3 (6.3–8.6)	7.8 (7.2–9.3)	0.85
Relative volume, %	1.8 (1.5–2.0)	1.9 (1.6–2.2)	0.75
Cerebellum			
Absolute volume, ml	25.2 (18.4–27.5)	25.5 (21.5–34.1)	0.85
Relative volume, %	5.8 (4.9–6.1)	6.1 (5.4–6.3)	0.14
Total CSF			
Absolute volume, ml	67.4 (45.2–89.5)	66.8 (39.4–245.7)	0.95
Relative volume, %	15.7 (12.2–19.1)	15.8 (10.2–53.2)	0.85
Total intracranial volume			
Absolute volume, ml	437.3 (359.2–486.0)	419.5 (357.4–562.2)	0.66

Abbreviation: CSF, cerebrospinal fluid.
[a]Comprising caudate, amygdalae, thalamic and lenticular nuclei.

hypothermia-treated infants as compared with control infants ($P = 0.02$).

Hypothermia, MRI and neurosensory outcomes

Mortality and neurosensory outcome data at 18 to 22 months were available for all 14 infants. Bayley Scales testing, however, was not performed for two infants (evaluator unavailable). These two infants (one from each treatment arm) had normal hearing, vision and motor function on standardized neurological exam. One hypothermia-treated infant died after nursery discharge but before follow-up. Of the 11 infants with a complete standardized neurosensory examination (median age 19 months; 95% CI: 18 to 28), 6 were diagnosed as having severe neurosensory impairments and the other 5 had mild to no impairments. In our small sample, treatment assignment did not correlate with death or moderate or severe neurosensory impairments (four of seven hypothermia-treated infants and three of five controls; $P =$ NS).

Anatomical MRI findings before nursery discharge were strongly associated with death or neurosensory impairments: all five infants with abnormal anatomical MRI scans either died or developed severe neurosensory impairments (sixth infant with an abnormal MRI did not receive a Bayley exam); of the seven infants with normal MRI scans (eighth infant with a normal MRI did not receive a Bayley exam), five were developing normally at $\geqslant 18$ months and two developed severe impairments ($P = 0.03$). These two infants with a normal MRI and severe impairments were both treated with hypothermia, did not have a history of seizures, showed a normal standardized neurological exam at discharge and normal clinical anatomical MRI readings as well. One of these MRI scans was performed at 21 days of life and the second at 5 days of life (122 h post-birth). In the latter of these two cases, diffusion-weighted images did show restricted diffusion in the left occipital periventricular white matter; however, no signal changes were evident on T1- or T2-weighted scans. At 18 to 22 months, both these infants had a normal motor exam and psychomotor development index (PDI) score at 2 years of age, but Bayley MDI scores two s.d. below the mean (<70). Overall, anatomical MRI findings did not significantly correlate with Bayley MDI ($P = 0.18$) or PDI scores ($P = 0.21$).

Relative total brain tissue volumes were significantly associated with death or neurosensory impairments ($P = 0.048$). Similarly, relative total CSF volumes also correlated with such adverse outcomes ($P = 0.048$). However, there was no relationship between absolute total brain tissue volumes or total CSF volumes and adverse outcomes. Neither absolute or relative subcortical white matter nor cortical gray matter significantly correlated with death or disability ($P =$ NS). Relative cortical gray matter and subcortical white matter combined volume showed a nonsignificant trend toward correlation with death or neurological disability ($P = 0.11$). No other cerebral regions correlated with death or neurosensory disability. The two hypothermia infants with a normal anatomical MRI that developed severe cognitive delays had absolute and relative total brain tissue volumes close to the median for the hypothermia group.

Last, we observed a significant correlation between relative cortical gray matter and subcortical white matter combined volume and Bayley PDI ($\rho = 0.69$; $P = 0.02$). No significant relationship existed between relative cortical gray matter and subcortical white matter combined volume and Bayley MDI ($\rho = 0.36$; $P = 0.28$). Similarly, relative volumes of total brain tissue did not significantly correlate with PDI ($\rho = 0.51$; $P = 0.11$) or MDI scores ($\rho = 0.43$;

$P = 0.19$). None of the other regional volumes correlated with Bayley II MDI or PDI scores.

Discussion

In a single-center substudy of the NICHD randomized controlled trial of systemic hypothermia, we showed the feasibility and reliability of measuring regional brain volumes in infants with HIE. We did not observe significantly larger total brain tissue volumes in infants randomized to hypothermia as compared with usual care. Except for subcortical white matter, hypothermia was not associated with significantly larger absolute or relative volumes in the multiple regions assessed. This absence of differences may reflect the small sample size or multiple small baseline differences between the groups favoring the usual care group. Alternatively, systemic hypothermia may exert beneficial effects primarily through preservation of subcortical/periventicular white matter, a vulnerable region that is often injured in infants with HIE. We also observed a significant correlation between qualitative and quantitative measure of brain injury on MRI and death or neurosensory impairments at $\geqslant 18$ months of age. While volumetric MRI did not appear to provide an incremental benefit over anatomical MRI in the prediction of neurosensory impairments, our study lacked the power to detect smaller differences in diagnosis. Additionally, because half of the subjects had their MRI scans within 10 days post-birth, we may not have captured the full extent of the cerebral atrophy that follows perinatal hypoxia–ischemia.[15,18]

To our knowledge, this is the first study to use volumetric brain MRI to assess the effect of hypothermia therapy on regional and global cerebral volumes in term infants with HIE and to correlate such volumes with their neurosensory impairments. Volumetric MRI may yield information complementary to that of qualitative MRI, and volumetric sequences can be readily incorporated into routinely performed anatomical MRI scans. Moreover, because brain volumes are a continuous outcome measure, significant group differences can be readily detected, even with small sample sizes. For these reasons, investigators should consider including volumetric analyses along with qualitative MRI assessments. We were able to reliably segment not only the three major tissue classes, but also smaller subcortical structures commonly involved in hypoxic–ischemic injury. Atrophy and cellular loss, an important pathological feature of cerebral injury following hypoxia–ischemia,[15,35] can be more objectively assessed using three-dimensional quantitative analyses than with qualitative assessments using conventional MRI.

We were able to measure brain volumes using the existing NICHD hypothermia study anatomical brain MRI data and commercially available segmentation software. Although this type of analysis can be performed on regular T_2-weighted MRI scans, ideally spoiled gradient thin isotropic contiguous slices are required to accurately assess brain volumes for smaller structures. As the anatomical MRI scans we analyzed were performed with a 2 mm gap, brain volumes for this gap were estimated using the Analyze software. Although this should have adequately compensated for the slice gap for larger structures and tissue classes, the reliability of volume measurements for smaller structures may not be as robust. This limitation may have masked true differences in volumes for vulnerable structures, such as the lenticular nucleus and thalamus. Furthermore, our small sample size lacked the power to detect smaller, yet meaningful, differences in volumes. However, because the primary intervention was randomized, our findings are not subject to some of the limitations inherent in observational studies.

Unlike Inder *et al.*,[13] we observed selective neuroprotection of the subcortical white matter rather than cortical gray matter after systemic hypothermia therapy. Their study[13] also observed more frequent isolated basal ganglia injury in hypothermia-treated infants than in controls. In contrast, Rutherford and colleagues,[14] in an observational study, reported significantly fewer lesions in the basal ganglia and thalamus after whole-body or selective head cooling than in unmatched controls with HIE. In our small, randomized study, we observed no qualitative or quantitative differences in basal nuclei injury. Similar but larger quantitative studies, including volumetric MRI and diffusion tensor imaging, performed at discharge and at neurodevelopmental follow-up are required to delineate the regional effects of hypothermia therapy.

As in several previous HIE studies,[15,16] we observed a strong relationship between structural lesions detected by anatomical MRI and neurosensory impairments at $\geqslant 18$ months of age. Similarly, total brain tissue volume correlated with this important adverse outcome. Relative cortical gray matter and subcortical white matter combined volumes also correlated significantly with Bayley PDI scores. To our knowledge, only one other study has correlated *in vivo* brain volumes, as measured by volumetric MRI at term equivalent age, with neurosensory outcomes at $\geqslant 18$ months of age.[20] That pilot investigation of preterm infants and term controls showed a significant correlation between several hemispheric white and cortical gray matter regional volumes with Bayley MDI and PDI scores. One larger cohort study related brain volumes in preterm infants to object working memory at 2 years but not motor or sensory impairments as in our study.[36] Our study provides preliminary evidence that volumetric MRI is also a significant predictor of neurosensory outcomes in term infants with HIE. Advanced quantitative MRI is emerging as a valid tool to assess the short-term neurological effects of neonatal interventions[17] and facilitate prediction of neurosensory outcomes.

Conclusion

In a single-center substudy of the NICHD randomized whole-body hypothermia trial, we showed that volumetric brain MRI is a feasible and reliable surrogate measure predictive of neurosensory

impairments. Larger multicenter studies are needed to fully reveal the potential benefits and limitations of volumetric MRI for assessing the effects of neuroprotective interventions for infants with HIE. In centers where it is feasible to reliably assess quantitative MRI, we recommend that future randomized trials of neuroprotection assess *in vivo* brain volumes as a secondary outcome measure in all or a representative sample of treated infants.

Acknowledgments

This work was supported in part by National Institutes of Health Grant 5K23NS048152 to Nehal Parikh. We thank the NICHD Neonatal Research Network for access to hypothermia study data.

Conflict of interest

The authors have no conflicts of interest to disclose.

References

1 Hull J, Dodd KL. Falling incidence of hypoxic–ischaemic encephalopathy in term infants. *Br J Obstet Gynaecol* 1992; **99**(5): 386–391.
2 Thornberg E, Thiringer K, Odeback A, Milsom I. Birth asphyxia: incidence, clinical course and outcome in a Swedish population. *Acta Paediatr* 1995; **84**(8): 927–932.
3 Smith J, Wells L, Dodd K. The continuing fall in incidence of hypoxic–ischaemic encephalopathy in term infants. *BJOG* 2000; **107**(4): 461–466.
4 Robertson CMT, Finer NN, Grace MGA. School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. *J Pediatr* 1989; **114**: 753–760.
5 Gunn AJ, Gluckman PD, Gunn TR. Selective head cooling in newborn infants after perinatal asphyxia: a safety study. *Pediatrics* 1998; **102**: 885–892.
6 Eicher DJ, Wagner CL, Katikaneni LP, Hulsey TC, Bass WT, Kaufman DA *et al.* Moderate hypothermia in neonatal encephalopathy: efficacy outcomes. *Pediatr Neurol* 2005; **32**(1): 11–17.
7 Gluckman PD, Wyatt JS, Azopardi D, Ballard R, Edwards AD, Ferriero DM *et al.* Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomized trial. *Lancet* 2005; **365**: 663–670.
8 Shankaran S, Laptook A, Wright LL, Ehrenkranz RA, Donovan EF, Fanaroff AA *et al.* Whole-body hypothermia for neonatal encephalopathy: animal observations as a basis for a randomized, controlled pilot study in term infants. *Pediatrics* 2002; **110**(2 Part 1): 377–385.
9 Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, *et al.*, National Institute of Child Health and Human Development Neonatal Research Network. Whole-body hypothermia for neonates with hypoxic–ischemic encephalopathy. *N Engl J Med* 2005; **353**: 1574–1584.
10 Shao X, Zhou W, Cheng G. Head cooling in neonatal hypoxic–ischemic encephalopathy-multicenter randomized trial from China. Presented at *Hot Topics in Neonatology* 2005,. Washington, DC.
11 Laptook AR, Corbett RJ. The effects of temperature on hypoxic–ischemic brain injury. *Clin Perinatol* 2002; **29**: 623–649.
12 Safar PJ, Kochanek PM. Therapeutic hypothermia after cardiac arrest. *N Engl J Med* 2002; **346**(8): 612–613.
13 Inder TE, Hunt RW, Morley CJ, Coleman L, Stewart M, Doyle LW *et al.* Randomized trial of systemic hypothermia selectively protects the cortex on MRI in term hypoxic–ischemic encephalopathy. *J Pediatr* 2004; **145**(6): 835–837.
14 Rutherford MA, Azzopardi D, Whitelaw A, Cowan F, Renowden S, Edwards AD *et al.* Mild hypothermia and the distribution of cerebral lesions in neonates with hypoxic–ischemic encephalopathy. *Pediatrics* 2005; **116**(4): 1001–1006.
15 Belet N, Belet U, Incesu L, Uysal S, Ozinal S, Keskin T *et al.* Hypoxic–ischemic encephalopathy: correlation of serial MRI and outcome. *Pediatr Neurol* 2004; **31**: 267–274.
16 Ment LR, Bada HS, Barnes P, Grant PE, Hirtz D, Papile LA *et al.* Practice parameter: neuroimaging of the neonate: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology* 2002; **58**: 1726–1738.
17 Parikh NA, Lasky RE, Kennedy KA, Moya FR, Hochhauser L, Romo S *et al.* Postnatal dexamethasone therapy and cerebral tissue volumes in extremely low birth weight infants. *Pediatrics* 2007; **119**(2): 265–272.
18 Sie LT, van der Knaap MS, Oosting J, de Vries LS, Lafeber HN, Valk J. MR patterns of hypoxic–ischemic brain damage after prenatal, perinatal or postnatal asphyxia. *Neuropediatrics* 2000; **31**: 128–136.
19 Inder TE, Warfield SK, Wang H, Huppi PS, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. *Pediatrics* 2005; **115**(2): 286–294.
20 Peterson BS, Anderson AW, Ehrenkranz R, Staib LH, Tageldin M, Colson E *et al.* Regional brain volumes and their later neurodevelopmental correlates in term and preterm infants. *Pediatrics* 2003; **111**: 939–948.
21 Barkovich AJ, Hajnal BL, Vigneron D, Sola A, Partridge JC, Allen F *et al.* Prediction of neuromotor outcome in perinatal asphyxia: evaluation of MR scoring systems. *AJNR Am J Neuroradiol* 1998; **19**(1): 143–149.
22 Mercuri E, Rutherford M, Barnett A, Foglia C, Haataja L, Counsell S *et al.* MRI lesions and infants with neonatal encephalopathy. Is the Apgar score predictive? *Neuropediatrics* 2002; **33**(3): 150–156.
23 Rutherford MA, Pennock JM, Counsell SJ, Mercuri E, Cowan FM, Dubowitz LM *et al.* Abnormal magnetic resonance signal in the internal capsule predicts poor neurodevelopmental outcome in infants with hypoxic–ischemic encephalopathy. *Pediatrics* 1998; **102**: 323–328.
24 Haines DE. *Neuroanatomy: An Atlas of Structures, Sections and Systems*, 3rd edn. Williams & Wilkins: Baltimore, MD, 1991.
25 Bayer SA, Altman J. *The Human Brain During the Third Trimester (Atlas of Human Central Nervous System Development)*. CRC Press: Boca Raton, FL., 2003.
26 University of Michigan. The Navigable Atlas of the Human Brain. Available at: http://www.msu.edu/∼brains/brains/human/index.htmlAccessed August 14, 2007.
27 Interactive Atlases. Digital Anatomist: Interactive Brain Atlas. University of Washington. Available at: http://www9.biostr.washington.edu/da.htmlAccessed August 14, 2007.
28 Honeycutt NA, Smith PD, Aylward E, Li Q, Chan M, Barta PE. Mesial temporal lobe measurements on magnetic resonance imaging scans. *Psychiatry Res* 1998; **83**(2): 85–94.
29 Hastings RS, Parsey RV, Oquendo MA, Arango V, Mann JJ. Volumetric analysis of the prefrontal cortex, amygdala, and hippocampus in major depression. *Neuropsychopharmacology* 2004; **29**: 952–959.
30 Gur RE, Maany V, Mozley PD, Swanson C, Bilker W, Gur RC. Subcortical MRI volumes in neuroleptic-naive and treated patients with schizophrenia. *Am J Psychiatry* 1998; **155**(12): 1711–1717.
31 Postle BR, D′Esposito M. Spatial working memory activity of the caudate nucleus is sensitive to frame of reference. *Cogn Affect Behav Neurosci* 2003; **3**: 133–144.
32 Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Dev Med Child Neurol* 1997; **39**(4): 214–223.
33 Bayley N. *Bayley Scales of Infant Development-II*. Psychological Corporation: San Antonio, TX, 1993.
34 Rothman KJ, Greenland S. *Modern Epidemiology*, 2nd edn. Lippincott-Raven: Philadelphia, PA, 1998.
35 Gluckman PD, Pinal CS, Gunn AJ. Hypoxic–ischemic brain injury in the newborn: pathophysiology and potential strategies for intervention. *Semin Neonatol* 2001; **6**: 109–120.
36 Woodward LJ, Edgin JO, Thompson D, Inder TE. Object working memory deficits predicted by early brain injury and development in the preterm infant. *Brain* 2005; **128**: 2578–2587.