Published in final edited form as:

J Magn Reson Imaging. 2015 December ; 42(6): 1689–1697. doi:10.1002/jmri.24930.

Comparing tract-based spatial statistics and manual region-of-interest labeling as diffusion analysis methods to detect white matter abnormalities in infants with hypoxic-ischemic encephalopathy

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Abstract

Purpose—To compare manual region of interest (ROI) labeling and tract-based spatial statistics (TBSS) by their ability to detect group-wise differences in fractional anisotropy (FA) in the neonatal brain.

Materials and Methods—Diffusion weighted data were obtained for nine infants with hypoxic-ischemic encephalopathy (HIE) (6 males, 3 females; gestational age (GA) range, 36–40 weeks; mean GA, 37.8 weeks) and eleven healthy-control infants (10 males, 1 female; GA range, 36–40 weeks; mean GA, 38.4 weeks) at 3T. For manual ROI labeling, ROIs were drawn freehand for each subject in eight, clinically relevant brain regions. For TBSS, all FA data underwent an optimized, automated protocol for neonates. Each method was evaluated for detection of decreased FA in HIE infants, sensitivity, specificity, and variability.

Results—FA values from manual ROI and TBSS were strongly correlated ($r=0.94$, $p<0.0001$). Both methods found decreased FA in most ROIs for HIE infants. There was no significant interaction between method and group, indicating a similar ability to detect FA differences ($F(1,19)=0.599$, $p=0.449$). Sensitivity (manual: 0.709, TBSS: 0.694, 95% CI $[-0.136, .163]$, $p=0.856$), specificity (manual and TBSS: 0.716, 95% CI $[-0.133, 0.133]$, $p=1$), and standard error (manual: 0.009, TBSS: 0.007) were comparable.

Conclusion—Manual ROI labeling and TBSS are comparable methods of diffusion analysis to detect group differences in FA in the neonatal brain.

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Disclosures: The authors have declared that there are no competing financial interests in relation to the work described. The submitted material is that of original research and has not been previously published, nor has it been submitted for publication elsewhere while under consideration.

Keywords

fractional anisotropy; tract-based spatial statistics; hypoxic-ischemic encephalopathy; diffusion tensor imaging; neonates

Introduction

Hypoxic-ischemic encephalopathy (HIE) remains one of the largest causes of neonatal death and long-term disability in young children (1, 2). Though more severe cases of HIE can be diagnosed through conventional MRI after approximately two weeks postnatally, a more objective and immediate measure is necessary to improve detection of mild-to-moderate injury and for quantification of severity (3). A primary advantage to using diffusion tensor imaging is its ability to quantify microstructural changes during the acute phase of injury.

Fractional anisotropy measures directional diffusivity in the brain and reflects axon maturation and myelination (4). Decreased FA in patients with HIE has been shown to indicate microstructural changes undetectable by conventional MRI (5). Several studies have shown reduced FA in surviving infants with HIE, reflecting a possible breakdown or delay in white matter organization that can lead to adverse neurodevelopmental outcome (3, 6, 7). Manual labeling of ROIs has been the traditional method of analysis for functional and diffusion MRI (8). FA is extracted from the ROIs drawn for each subject. Despite the potentially large inter-rater variability due to a bias in ROI selection, this method has been the default first step for many developmental studies. Given the high variability of infant brain shape and structure especially when damaged by ischemic injury, manual labeling is advantageous in that it allows placement of regions specific to each infant. In addition, labeling is performed on native space so it requires minimal processing of original data. TBSS is an automated approach that does not require pre-specified ROIs and therefore can perform whole-brain group comparisons (9). However, this method is based on the realignment of all subjects' FA maps onto a mean FA image, where the several alignment steps involved may distort individual FA data. Due to the utilization of adult normalization templates, concerns existed for using TBSS in neonates; however, a modification of the method has recently been introduced to improve its reliability in neonates (10).

One recent study provides a direct comparison of fractional anisotropy and other diffusivity measures derived from each method, but only for a wide age range of healthy neonates (11). The purpose of the present study was to compare manual labeling and TBSS for their consistency as well as their ability to detect group differences in a patients vs. controls analysis, specifically during the acute phase of ischemic injury.

Materials and Methods

Study Subjects

This study was approved by the West Virginia University Institutional Review Board, and informed consent was obtained from subjects' parents or legal guardians. Twelve infants with HIE (8 males, 4 females; gestational age range, 36–40 weeks; mean gestational age, 37.3 weeks) and eleven healthy controls (10 males, 1 female; gestational age range, 36–40

weeks; mean gestational age, 38.4 weeks) underwent MRI including diffusion weighted imaging between July 2011 and June 2014. All scans for infants with HIE occurred within 4–10 days since birth. All scans for control infants occurred within 2–5 days since birth.

MR Acquisition

All subjects were imaged with a 3.0T Siemens scanner (Verio; Siemens Medical System, Malvern, PA, USA). For temperature control, neonates were placed in an incubator equipped with an 8-channel head coil. 15 minutes prior to the scan, neonates were fed, swaddled, and positioned in the isolette. Infants wore earmuffs for hearing protection. Vitals were continuously monitored throughout the procedure and no sedation was used. A pediatric neurologist or neonatology fellow was present during all MRI acquisitions.

Diffusion weighted images were acquired within a conventional MRI sequence according to the clinical protocol used at the institution. Conventional MRI included high resolution T1-weighted images (MP-RAGE) (repetition time (TR), 2300 msec; inversion recovery time (TI), 1100 msec; echo time (TE), 3.2 msec; voxel size, $1.3 \times 1.3 \times 1.3$); axial T2-weighted images (TR, 4370 msec; TE, 84 msec; voxel size, $0.7 \times 0.7 \times 3.5$); T2 FLAIR images (TR, 9000 msec; TI, 2500 msec; TE, 96 msec; voxel size, $0.7 \times 0.7 \times 3.5$). Diffusion weighted data were acquired with single shot echo planar sequence: TR, 7600 msec; TE, 91 msec; FOV, 200 mm; voxel size: $2.3 \times 2.3 \times 3$; b-value, 1000; 30 directions, acquisition time, 5 minutes.

Preprocessing Steps

Diffusion weighted data were preprocessed and FA maps were generated using DTI-Studio, version 3.0.3 (12). Preprocessing steps included registration to the b=0 image to minimize image artifacts due to eddy current distortion.

Method 1 – Manual ROI

Manually labeled ROIs were drawn by two blinded investigators (PP, 3 years experience; TN, 1 year experience) on anatomically defined areas on the mean diffusion map for each subject using MRICro, version 1.40 (13). The genu and splenium of the corpus callosum, left and right corticospinal tracts, and left and right temporal areas were drawn with the freehand paint tool. Corticospinal tracts were defined at the level of the mid-thalamus and temporal areas were defined in the white matter just laterally to the lateral ventricle. The left and right periventricular regions were drawn using the oval and fill tools in the fronto-parietal region, one slice above the ventricles on the axial plane. ROIs were overlaid on each subject's FA map and a mean FA value for each region was calculated (Figure 1). FA values were averaged across 4 voxels for the genu, 7 voxels for the splenium, 20–24 voxels for the corticospinal tracts, 14–16 voxels for the temporal tracts, and 40–50 voxels for the periventricular regions. The genu and splenium regions were small for consistency and to avoid including surrounding cerebrospinal fluid because the voxels were not isovoxels in all 3 directions. Placement of each ROI was repeated until the standard deviation of the consecutive measurements was less than 10%. Then, the three most recent consistent FA measurements were averaged. Inter-rater reliability for ROI placement was determined by intra-class correlations for a randomly selected group of six subjects.

Method 2 – TBSS

For TBSS, FA images were analyzed with the FMRIB software library, version 5.0.4 (14). Each subject's FA image was brain-extracted using the Brain Extraction Tool (BET) of FMRIB (15) at very low threshold to skull-strip and reduce halo noise effects. All subjects' FA data underwent an optimized TBSS protocol for neonates to reduce global misalignments (9, 10). Each FA map was registered to every other using a 6 df linear registration, a 12 df linear registration, and nonlinear warping. A target was selected by calculating mean warp displacement scores for each subject (min: 1.466, average: 2.221, max: 3.220). Median scores were also calculated for secondary consideration (min: 0.074, average: 0.240, max: 0.515). The subject with the lowest mean score was not chosen as target due to concerns regarding poor resolution in two of the imaging planes. The chosen target thus had the second lowest mean score of 1.493 and third lowest median score of 0.154. It was a healthy male infant of 36 weeks gestational age. Each subject's FA map was aligned to the target space and a mean FA map was created. A second iteration of registrations was performed from the original FA maps to the mean FA map. Those aligned images were then used to create another mean FA image and a mean FA skeleton (Figure 2). The FA skeleton, which represents the center of all common tracts, was thresholded at FA 0.18 because using the standard threshold for adults of 0.20 did not yield contiguous tracts. The FA for most white matter pathways was, as expected due to lack of development, lower in the infants than they would be in adults so a lower threshold ensured that all regions of interest were detected. All FA images were visually inspected after linear and non-linear registration to ensure that no major misalignments or distortions had occurred.

Statistical Analysis

For TBSS, voxelwise cross-subjects statistics were performed with Randomise (16) using a general linear model to find the FA skeleton voxels that showed a significant difference between the control and HIE groups. The results were fully corrected for multiple comparisons across space following threshold-free cluster enhancement. In order to directly compare the TBSS results to the manually labeled ROIs, a single set of the eight ROIs was created on the mean FA skeleton using the same guidelines as in the manual ROI method. FA values from each subjects' mean-aligned image were extracted for each skeletal region.

Statistical analysis was performed using SPSS, version 21.0 (IBM Corp., Chicago, IL, USA). A linear correlation was performed on the FA values from each region of each subject to assess the similarity between the two methods. Bland-Altman plots were used to further assess agreement between the methods. To determine whether the differences in FA showed proportional bias, linear regression was performed with and without outliers. A repeated-measures analysis of variance was used to investigate the effects of method, group (control or HIE) and region on FA. For each method separately, one-tailed t-tests were performed for each region to detect significant differences in FA between groups. Reported p-values for the t-tests were not corrected for multiple comparisons to avoid low power due to the large number of regions in consideration and the small number of subjects in each group. $P = 0.05$ was considered significant for all statistical tests.

Sensitivity and specificity were calculated for each method. The test criteria for detecting HIE was set as the average FA value between HIE and control infants for each region. Both measures were compared using a difference of two proportions test and the Yates continuity correction.

Results

Subjects

Two of the infants with HIE – who were identified retrospectively – were excluded due to insufficient volumes acquired from the diffusion imaging. One infant with HIE was excluded due to extensive motion artifacts. The remaining nine infants with HIE (6 males, 3 females; gestational age range, 36–40 weeks; mean gestational age, 37.8 weeks) and eleven healthy controls were included in this study.

Manual ROI

Inter-rater reliability as determined by intra-class correlations between the two blinded investigators was high ($r_s > 0.90$). Infants with HIE showed significantly lower FA than controls in 6 of the 8 ROIs, with the exception of the genu and splenium (Table 1).

TBSS

The optimized TBSS protocol resulted in a mean FA skeleton that successfully captured the major white matter tracts, including all of the regions of interest (Figure 2). Upon preliminary examination of the whole-brain significance map, many of the voxels on the mean FA skeleton showed statistically significant differences for all of the ROIs except for the splenium, with lower FA in the infants with HIE compared to the controls (Figure 3). No major clusters or tracts showed a statistically significant difference where FA was higher in infants with HIE compared to controls.

After masking for only the ROIs on the mean FA skeleton and extracting mean FA values for each subject, infants with HIE showed significantly lower FA than controls in 7 of the 8 ROIs, with the exception of the splenium (Table 1).

Comparison

FA values extracted from manually drawn and TBSS-aligned ROIs for each subject showed a very strong correlation, reflecting a high similarity between the proportional outputs of the two methods ($r=0.94$, $p<0.0001$, Figure 4). Agreement between the two methods was confirmed in the Bland-Altman plots for each region (Figure 5). There were few outliers beyond the 95% limits of agreement for any of the regions. Excluding outliers, the left temporal region ($R^2=0.223$, Slope=0.61, $t(18)=2.271$, $p=0.036$), genu ($R^2=0.553$, Slope=0.798, $t(18)=4.587$, $p<0.001$), and splenium ($R^2=0.211$, Slope=0.43, $t(18)=2.132$, $p=0.048$) showed proportional bias where higher differences in FA were associated with higher average FAs between the two methods.

Both methods showed the expected pattern of decreased FA in infants with HIE in all ROIs (Figure 6). Across both methods and all regions, FA was significantly lower in HIE infants

compared to controls ($F(1,19)=14.179, p=0.001$). The FA values extracted from TBSS were significantly lower than those extracted from the manually drawn ROIs, with a mean difference of 0.015 ($F(1,19)=6.55, p=0.02$).

The two methods were only inconsistent in detecting a statistically significant FA difference for the genu of the corpus callosum (Table 1). TBSS found a significant difference in FA between HIE and control infants for the genu while manual labeling did not. P-values per region tended to be lower for TBSS, which may reflect an improved ability to detect significance, but omnibus analysis found no significant interaction between method and group ($F(1,19)=0.599, p=0.449$). Neither method found the splenium to have significantly lower FA in HIE infants. Sensitivity, specificity, and standard error were very similar for both methods (Table 2). Neither the sensitivities (95% CI [-0.136, 0.163], $p=0.856$) nor the specificities (95% CI [-0.133, 0.133], $p=1$) differed significantly.

Discussion

In this study, we compared manual ROI labeling and TBSS as diffusion analysis methods and found that they are highly similar in sensitivity, specificity, and the ability to detect significant differences. Manual ROI labeling is commonly used in functional MRI analysis in order to limit testing and control for Type I error (8). ROIs drawn using structural features for reference are most useful when defined for each individual subject. Since neonatal brains tend to vary in shape, size, and structure much more than adult brains, uniquely labeled ROIs are advantageous. However, the process of manually drawing ROIs and confirming inter-rater reliability is time-intensive and may be biased. Tract-based spatial statistics sacrifices subject uniqueness and utilizes alignment steps and skeletonization of white matter tracts in order to conduct a whole-brain group comparison. It is more efficient, but by registering all subjects' FA maps to a target, individual differences of interest may be lost. Alignment is especially difficult when individual data were collected using different parameters. Though alignment of the highly variable neonatal brains may raise issues regarding distortion, the output significance map provides exploratory data that can be used to generate a single set of ROIs for further analysis. When working with large numbers of subjects, this automated approach may be preferable for its ease of operation. TBSS has been supported by several studies to be a qualified biomarker for efficient evaluation of neural insult and intervention (17–19).

A comparison of the two methods' abilities to detect significant group differences in FA was necessary to ensure the viability of TBSS for use in neonatal studies, and particularly in injured brains. As manual labeling has served as the traditional method of FA analysis, the strong correlation between FA values extracted from the two different methods demonstrated that TBSS would provide proportionally very similar results to manual labeling. The narrow limits of agreement and low number of outliers in the Bland-Altman plots further supported the concordance of the two methods. The association between higher differences in FA and average FA was not a major concern in the left temporal region given the low variation in agreement. In the genu and splenium, however, the bias indicated that manual labeling sometimes yielded much higher FA values than TBSS. This could be explained by our use of very few voxels to label the regions in order to avoid the

surrounding cerebrospinal fluid. Manual labeling may have been more precise in yielding maximum FA since the TBSS skeleton regions mapped to individual registered FA images may not have corresponded exactly to the few voxels of white matter. Notably, the genu and splenium also had the widest limits of agreement and high inter-subject variability, which implies that the variation in measurements affected the differences between the two methods. This reflects a need to refine the TBSS registration protocol for neonates to ensure accurate and reliable alignment of small regions surrounded by cerebrospinal fluid.

The major concern that individual data would be significantly distorted through TBSS realignment appears to be a non-issue. All registered FA maps underwent visual inspection to check for distortions, and a past comparison of manual labeling and TBSS showed that the degree of nonlinear warping does not affect the differences in FA between the two methods (11). The slight but significant difference in extracted FA values between the two methods found in this study can be explained by the smaller size of the TBSS-generated ROIs, which comprised only of voxels on the mean FA skeleton. The mean FA skeleton represents the center of all white matter tracts as they appear when averaged across all subjects. When extracting the few skeleton voxels from each individual's aligned images, the ROIs may not have been located right at the center of the individual's tract where FA would be highest, hence causing extracted values to be lower than expected. This discrepancy could be considered a weakness in using TBSS for neonates since with manual labeling, ROIs would almost always be placed at the location of maximum FA for each individual.

Despite the potential added variability from alignment to a mean FA image, TBSS yielded results that matched those of manual labeling. Both methods found that infants with HIE have significantly lower FA than controls in the bilateral corticospinal tract, bilateral periventricular regions, and bilateral temporal tracts. Decreased diffusion measures in the corticospinal tract, especially the posterior limb of the internal capsule, have been associated with hypoxic injury and its consequent effect on neuromotor outcome (3, 20, 21). Lower FA measures in the periventricular regions are clinically significant in premature infants due to the presence of premyelinating oligodendrocytes that are sensitive to hypoxia and therefore prone to ischemic injuries (22). These lower FA measures could be predictors of clinical outcome in term neonates with HIE (23). The genu of the corpus callosum was the only area of discrepancy between the two methods, with TBSS finding a significant difference while manual labeling did not. In contrast to several past findings of restricted diffusion within the corpus callosum (20, 24, 25), neither method found a significant difference in FA at the splenium. These results can be explained by the high inter-subject variability within groups that was only present for the corpus callosum. Considering the easy visibility and high FA intensities of the corpus callosum, it was surprising that the genu and splenium showed such variability, but there was a trend towards lower FA in the infants with HIE that would become more significant with a larger sample size. In addition, our population mostly included subjects with mild to moderate HIE (only two subjects had severe disease). The severity of HIE injury is known to influence which brain regions are impacted: mild insults tend to affect watershed distributions while severe, prolonged insults affect deep nuclear structures and tend to be more global (26). Hence, detecting large differences in the corpus callosum with a group of mostly mild HIE infants would have been difficult. Our results

demonstrated that though manual ROI and TBSS may have discrepancies in extracted FA values, they are both able to detect differences in FA between patient and control groups of infants.

A recent study by Seo and colleagues conducted a direct comparison of manual ROI labeling and TBSS as diffusion analysis methods in healthy neonates, but concluded that the metrics derived were not consistently comparable (11). Though the analysis included axial and radial diffusivity as well, the study actually found high correlations between FA values for the genu, splenium, and left posterior internal capsule (PLIC). Our results may have shown higher correlations due to larger defined areas for most ROIs: each corticospinal tract consisted of an average of 20–24 voxels while their study labeled only 12–15 voxels for each PLIC. Conversely, FA values for the genu and splenium in our study show much more variability because they were defined with fewer voxels than in Seo's work. Their work included older infants (up to 9 weeks of age) within a larger age range while our subjects were all examined at less than two weeks after birth, and mostly within five days since birth. A wide subject age range raises concerns regarding increased FA variability due to the greater variation in brain development. However, the decreased variability in brain shape as well as the better defined white matter tracts in older infants likely aided in TBSS alignment and skeletonization, which would contribute to their finding that TBSS-derived FA is systemically higher than manually-derived FA (11). This is the opposite pattern to our results because with very young neonates, our TBSS skeletal regions were smaller and likely did not exactly correspond to the small areas of maximum FA for every individual. Manual ROI labeling in Seo's study was done on color maps as opposed to diffusion images, which may have also resulted in lower FA readings due to inaccurate placement. The ability to demonstrate significant correlations for FA and other diffusion metrics may have been constrained by Seo's subject population, as only healthy infants of an older and larger age range were used (11). When considering the consistency between manual ROI and TBSS for FA, their correlation results in fact provide some support for the conclusion that the two methods are comparable. This study builds upon the previous finding by not only assessing the consistency of the two methods, but also testing for their ability to detect group differences in a patients vs. controls analysis.

The limitations of this study should be considered in interpreting the results. The study sample was relatively small, which may have prevented several of the regions of interest from reaching statistical significance even though they showed a trend towards a group difference. Since the primary output of TBSS is only relevant to the mean FA skeleton, it was difficult to directly compare the two methods. We chose to generate a new set of ROIs from the mean FA skeleton because of the greater difficulty of aligning and merging all individual ROIs, which would have been reduced to the skeleton for analysis in any case. The TBSS ROIs located at the calculated center of the white matter tracts still provided adequate estimates of relative FA between groups. A third limitation was that scanning parameters differed for many of the subjects. As the diffusion scans were included within a clinical sequence, we were unable to consistently control the exact parameters for the infants with HIE. Some of the data were also collected retrospectively from prior clinical scans.

These results demonstrate that TBSS is a viable option for diffusion analysis as compared to manual ROI labeling, but this study can be extended to a larger study population as well as to other neonatal patient groups. Examining correlations between FA and severity of HIE is another route of exploration beyond the scope of this study. Recent studies have begun to correlate TBSS findings with neurodevelopmental outcome in HIE and preterm infants, suggesting the eventual use of TBSS as a predictor for cognitive, behavioral, or motor impairment (17, 19, 23).

In conclusion, tract-based spatial statistics and manual region of interest labeling are comparable methods of diffusion analysis in terms of their sensitivity, specificity, and ability to detect significant differences in FA in the neonatal brain. By confirming that the methods are comparable, this study supports the increasing use of TBSS for faster and more objective analyses. Given adequate resources to run the computations, TBSS can provide results much faster than manual labeling when dealing with large sample sizes. TBSS may eventually be of interest to clinicians, but at the moment it can only be used for group analyses. Since the clinical significance of FA is still unclear, use of both methods and neuroimaging technology as a whole requires much more refinement before diffusion tensor imaging can be used as an individual diagnostic tool.

Acknowledgments

This project was completed through the Center for Neuroscience's Summer Undergraduate Research Internships Program, funded by the Center for Neuroscience and the NIH/NIGMS CoBRE Grant 8P30GM103503. Support was also given by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number U54GM104942. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Lai MC, Yang SN. Perinatal hypoxic-ischemic encephalopathy. *J Biomed Biotechnol.* 2010; 2011:609813. [PubMed: 21197402]
2. Cowan F, Azzopardi D. Hypoxic-ischaemic encephalopathy. *Paediatr Child Health (Oxford).* 2007; 17:47–57.
3. Ward P, Counsell S, Allsop J, Cowan F, Shen Y, Edwards D, Rutherford M. Reduced fractional anisotropy on diffusion tensor magnetic resonance imaging after hypoxic-ischemic encephalopathy. *Pediatrics.* 2006; 117:e619–30. [PubMed: 16510613]
4. Lodygensky GA, Vasung L, Sizonenko SV, Hüppi PS. Neuroimaging of cortical development and brain connectivity in human newborns and animal models. *J Anat.* 2010; 217:418–28. [PubMed: 20979587]
5. De Vries LS, Groenendaal F. Patterns of neonatal hypoxic-ischaemic brain injury. *Neuroradiology.* 2010; 52:555–66. [PubMed: 20390260]
6. Brissaud O, Amirault M, Villega F, Periot O, Chateil JF, Allard M. Efficiency of fractional anisotropy and apparent diffusion coefficient on diffusion tensor imaging in prognosis of neonates with hypoxic-ischemic encephalopathy: a methodologic prospective pilot study. *AJNR Am J Neuroradiol.* 2010; 31:282–7. [PubMed: 19959775]
7. Malik GK, Trivedi R, Gupta RK, Hasan KM, Hasan M, Gupta A, Pandey CM, Narayana P a. Serial quantitative diffusion tensor MRI of the term neonates with hypoxic-ischemic encephalopathy (HIE). *Neuropediatrics.* 2007; 37:337–43. [PubMed: 17357035]
8. Poldrack RA. Region of interest analysis for fMRI. *Soc Cogn Affect Neurosci.* 2007; 2:67–70. [PubMed: 18985121]

9. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, Behrens TEJ. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage*. 2006; 31:1487–505. [PubMed: 16624579]
10. Ball G, Counsell SJ, Anjari M, Merchant N, Arichi T, Doria V, Rutherford MA, Edwards AD, Rueckert D, Boardman JP. An optimised tract-based spatial statistics protocol for neonates: applications to prematurity and chronic lung disease. *Neuroimage*. 2010; 53:94–102. [PubMed: 20510375]
11. Seo Y, Wang ZJ, Ball G, Rollins NK. Diffusion tensor imaging metrics in neonates—a comparison of manual region-of-interest analysis vs. tract-based spatial statistics. *Pediatr Radiol*. 2013; 43:69–79. [PubMed: 23179484]
12. Jiang H, van Zijl PCM, Kim J, Pearlson GD, Mori S. DtiStudio: resource program for diffusion tensor computation and fiber bundle tracking. *Comput Methods Programs Biomed*. 2006; 81:106–16. [PubMed: 16413083]
13. Rorden C, Brett M. Stereotaxic display of brain lesions. *Behav Neurol*. 2000; 12:191–200. [PubMed: 11568431]
14. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM. Fsl. *Neuroimage*. 2012; 62:782–90. [PubMed: 21979382]
15. Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp*. 2002; 17:143–55. [PubMed: 12391568]
16. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. *Neuroimage*. 2014; 92:381–97. [PubMed: 24530839]
17. Porter EJ, Counsell SJ, Edwards AD, Allsop J, Azzopardi D. Tract-Based Spatial Statistics of Magnetic Resonance Images to Assess Disease and Treatment Effects in Perinatal Asphyxial Encephalopathy. *Pediatr Res*. 2010; 68:205–9. [PubMed: 20520585]
18. Ball G, Boardman JP, Arichi T, Merchant N, Rueckert D, Edwards AD, Counsell SJ. Testing the sensitivity of Tract-Based Spatial Statistics to simulated treatment effects in preterm neonates. *PLoS One*. 2013; 8:e67706. [PubMed: 23844070]
19. Van Kooij BJM, de Vries LS, Ball G, van Haastert IC, Benders MJNL, Groenendaal F, Counsell SJ. Neonatal tract-based spatial statistics findings and outcome in preterm infants. *AJNR Am J Neuroradiol*. 2012; 33:188–94. [PubMed: 21998101]
20. Ancora G, Testa C, Grandi S, et al. Prognostic value of brain proton MR spectroscopy and diffusion tensor imaging in newborns with hypoxic-ischemic encephalopathy treated by brain cooling. *Neuroradiology*. 2013; 55:1017–25. [PubMed: 23703033]
21. Hunt RW, Neil JJ, Coleman LT, Kean MJ, Inder TE. Apparent diffusion coefficient in the posterior limb of the internal capsule predicts outcome after perinatal asphyxia. *Pediatrics*. 2004; 114:999–1003. [PubMed: 15466097]
22. Volpe JJ, Kinney HC, Jensen FE, Rosenberg PA. The developing oligodendrocyte: key cellular target in brain injury in the premature infant. *Int J Dev Neurosci*. 2011; 29:423–40. [PubMed: 21382469]
23. Tusor N, Wusthoff C, Smee N, et al. Prediction of neurodevelopmental outcome after hypoxic-ischemic encephalopathy treated with hypothermia by diffusion tensor imaging analyzed using tract-based spatial statistics. *Pediatr Res*. 2012; 72:63–9. [PubMed: 22447318]
24. Righini A, Doneda C, Parazzini C, Arrigoni F, Matta U, Triulzi F. Diffusion tensor imaging of early changes in corpus callosum after acute cerebral hemisphere lesions in newborns. *Neuroradiology*. 2010; 52:1025–35. [PubMed: 20625707]
25. Takenouchi T, Heier LA, Engel M, Perlman JM. Restricted diffusion in the corpus callosum in hypoxic-ischemic encephalopathy. *Pediatr Neurol*. 2010; 43:190–6. [PubMed: 20691941]
26. Volpe JJ. Neonatal encephalopathy: an inadequate term for hypoxic-ischemic encephalopathy. *Ann Neurol*. 2012; 72:156–66. [PubMed: 22926849]

Figure 1. Manually drawn ROIs (red) for a control subject overlaid on FA image: left (a) and right (b) periventricular regions, genu (c) and splenium (d) of the corpus callosum, left (e) and right (f) corticospinal tracts, and left (g) and right (h) temporal tracts. Radiological view.

Figure 2. Mean FA skeleton (green, thresholded at FA 0.18) overlaid on mean FA of all subjects. Axial (a) and sagittal (b) slice. Radiological view.

Figure 3.

Significance map of HIE < Control (red-yellow, $p < 0.05$), overlaid on mean FA skeleton (Green, thresholded at $FA > 0.18$) overlaid on mean FA. ROIs created on the skeleton are loosely circled in white: left and right periventricular regions (a), splenium (b), genu (c), left and right corticospinal tracts (b,c), left temporal tract (d), and right temporal tract (e). Radiological view.

Figure 4.

Correlation of FA values from the two methods: Manual ROI labeling and TBSS. Each data point represents the FA of one region of one subject as calculated by each method. 160 points for 8 regions from 20 total subjects. $r = 0.94$, $p < 0.0001$.

Figure 5. Bland-Altman plots for the left (a) and right (b) corticospinal tracts, left (c) and right (d) periventricular regions, genu (e) and splenium (f) of the corpus callosum, and left (g) and right (h) temporal regions. Mean difference in FA between the two methods shown in the dotted line. 95% limits of agreement shown in dashed lines (Mean difference $\pm 1.96 \times SD$).

Figure 6.

Comparison of FA values from each method and each region across groups. HIE infants consistently show lower FA than controls. Error bars were constructed from standard error of the mean. L=left, R=right, CST=corticospinal tract, PV=periventricular region, Spl=splenium, temp=temporal white matter.

Table 1
Detection of differences in FA between control and HIE infants using manual or TBSS methods

Region	Manual			TBSS		
	Control	HIE	% Difference	Control	HIE	% Difference
Left Corticospinal	0.46	0.42	0.09*	0.43	0.38	0.12**
Right Corticospinal	0.46	0.41	0.11*	0.43	0.39	0.01**
Left Periventricular	0.23	0.19	0.16*	0.23	0.19	0.16**
Right Periventricular	0.24	0.19	0.22**	0.22	0.20	0.11*
Genu	0.61	0.53	0.14	0.52	0.46	0.10*
Splenium	0.65	0.60	0.08	0.53	0.50	0.05
Left Temporal	0.28	0.21	0.24**	0.26	0.22	0.17**
Right Temporal	0.27	0.21	0.23**	0.24	0.19	0.21**

HIE=hypoxic-ischemic encephalopathy, TBSS=tract-based spatial statistics,

* p 0.05,
** p 0.01

Table 2

Method Evaluation

Method	Sensitivity	Specificity	Standard Error
Manual	0.708	0.716	0.009
TBSS	0.694	0.716	0.007

TBSS=tract-based spatial statistics