A prospective diffusion tensor imaging study in mild traumatic brain injury

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ABSTRACT

Objectives: Only a handful of studies have investigated the nature, functional significance, and course of white matter abnormalities associated with mild traumatic brain injury (mTBI) during the semi-acute stage of injury. The present study used diffusion tensor imaging (DTI) to investigate white matter integrity and compared the accuracy of traditional anatomic scans, neuropsychological testing, and DTI for objectively classifying mTBI patients from controls.

Methods: Twenty-two patients with semi-acute mTBI (mean = 12 days postinjury), 21 matched healthy controls, and a larger sample (n = 32) of healthy controls were studied with an extensive imaging and clinical battery. A subset of participants was examined longitudinally 3–5 months after their initial visit.

Results: mTBI patients did not differ from controls on clinical imaging scans or neuropsychological performance, although effect sizes were consistent with literature values. In contrast, mTBI patients demonstrated significantly greater fractional anisotropy as a result of reduced radial diffusivity in the corpus callosum and several left hemisphere tracts. DTI measures were more accurate than traditional clinical measures in classifying patients from controls. Longitudinal data provided preliminary evidence of partial normalization of DTI values in several white matter tracts.

Conclusions: Current findings of white matter abnormalities suggest that cytotoxic edema may be present during the semi-acute phase of mild traumatic brain injury (mTBI). Initial mechanical damage to axons disrupts ionic homeostasis and the ratio of intracellular and extracellular water, primarily affecting diffusion perpendicular to axons. Diffusion tensor imaging measurement may have utility for objectively classifying mTBI, and may serve as a potential biomarker of recovery.

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GLOSSARY

ADC = apparent diffusion coefficient; CC = corpus callosum; CCI = cortical impact injury model; CR = corona radiata; DTI = diffusion tensor imaging; EC = external capsule; FA = fractional anisotropy; FPI = fluid percussion injury model; HC = healthy controls; IC = internal capsule; JHU = Johns Hopkins University; MANCOVA = multivariate analysis of covariance; mTBI = mild traumatic brain injury; RD = radial diffusivity; ROI = region of interest; SCR = superior corona radiata; SLF = superior longitudinal fasciculus; UF = uncinate fasciculus.

Complex cognitive processes such as attention, executive functions, and memory depend on intact white matter tracts among frontal, parietal, and medial temporal lobes, which are likely disrupted following mild traumatic brain injury (mTBI). Histologic evidence of white matter changes have been observed in both human autopsy and animal studies of mTBI. Although traditional neuroimaging sequences (i.e., T1- and T2-weighted imaging) are typically insensitive to these putative white matter changes, diffusion tensor imaging (DTI) is capable of measuring white matter pathology with histologic correlates in animal models of injury.

The majority of human mTBI studies have been cross-sectional in nature, examining selected patients (i.e., those with persistent complaints) during the chronic (e.g., after several
Additionally, few studies have examined potential differences in axial diffusivity or radial diffusivity (RD) following mTBI in either selected or unselected populations. The distinction between axial diffusivity and RD is critical given that FA is determined from these measurements, and each is putatively associated with different pathologies. Specifically, animal models of retinal ischemia suggest that axial diffusivity corresponds to axonal pathology whereas RD measures myelin pathology.

The present study examined FA, axial diffusivity, and RD prospectively in an unselected sample of mTBI patients. Based on previous clinical studies, we predicted that FA and axial diffusivity would be reduced in the CC, IC, superior longitudinal fasciculus (SLF), uncinate fasciculus (UF), and corona radiata (CR) in mTBI patients compared to controls in the semi-acute phase of injury (21 days postinjury) with increased findings in terms of myelin integrity (RD) during the more chronic injury stages.

### METHODS

**Participants.** Twenty-two patients (recruited from the University Emergency Department) with mTBI and 21 sex-, age-, and education-matched controls participated in an ongoing study. DTI data from an independent sample of healthy controls (HC) were also collected.

All patients experienced a closed head injury resulting in an alteration in mental status (see table 1) and were evaluated within 21 days of injury (clinical examination = 11.75 ± 4.97 days postinjury; imaging examination = 12.50 ± 5.40 days postinjury). The majority (85%) of patients completed the imaging and clinical protocols within 3 days of each other. Inclusion criteria for the mTBI group were based on the American Congress of Rehabilitation Medicine (Glasgow Coma Score of 13–15, loss of consciousness <30 minutes, posttraumatic amnesia <24 hours). mTBI participants and controls were excluded if there was a positive history of neurologic disease, psychiatric disturbance, additional closed head injuries with more than 5 minutes loss of consciousness or any head injury within the last year, learning disorder, attention deficit hyperactivity disorder, or a history of substance or alcohol abuse.

**Standard protocol approvals, registrations, and patient consents.** Informed consent was obtained from all participants according to institutional guidelines at the University of New Mexico.

**Clinical assessment.** Similar to previous studies, composite indices were calculated for attention, working memory, process-

### Table 1 Mild traumatic brain injury patient information

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Mechanism of injury</th>
<th>AAN rating</th>
<th>Days postinjury MRI</th>
<th>Days postinjury NP</th>
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<tr>
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<td>III</td>
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Abbreviations: AAN = American Academy of Neurology; MVA = motor vehicle accident; NP = neuropsychological testing.
RESULTS Neuropsychological and clinical measures. A compilation of all major neuropsychological and clinical indices is presented in table 2. Results indicated an increase in emotional ($t_{1,38} = -3.11; p < 0.05$; mTBI > HC), cognitive ($t_{1,38} = -4.20; p < 0.001$), and somatic ($t_{1,38} = -3.62; p < 0.005$) complaints for mTBI patients compared to controls. Estimates of premorbid intellectual functioning were lower in mTBI patients ($t_{1,37} = 2.09; p < 0.05$) despite educational matching.

A multivariate analysis of covariance (MANCOVA) examining differences in neuropsychological testing using premorbid intelligence as a covariate was not significant for group differences. However, effect sizes (table 2) in the domains of attention, executive functioning, and memory were of similar magnitude to those reported in recent meta-analyses on cognitive deficits in mTBI.

Structural imaging data. Anatomic images were limited to T1- and T2-weighted images. These were found to be free of pathology for both groups of subjects by a board-certified neuroradiologist (i.e., all mTBI patients were classified as being noncomplicated).

ROI analyses. Three MANCOVAs were conducted to examine group differences (mTBI patients vs matched controls) in FA values within the corpus callosum and left and right hemisphere ROI (figure 1A) with estimates of premorbid intellectual functioning as a covariate. Results indicated a multivariate effect of group for both the CC ($F_{1,38} = 3.81; p < 0.05$) and the left ($F_{1,34} = 2.70; p < 0.05$) but not right ($p > 0.10$) hemisphere. Follow-up univariate tests indicated that mTBI patients had higher FA within the genu ($F_{1,38} = 7.52; p < 0.01$, $d = -0.91$), left SCR ($F_{1,38} = 5.54; p < 0.05$, $d = -0.77$), left CR ($F_{1,38} = 5.47; p < 0.05$, $d = -0.74$), and left UF ($F_{1,38} = 6.67; p < 0.05$, $d = -0.84$). Trends were observed for the left IC ($F_{1,38} = 3.69; p = 0.062$, $d = -0.62$) and the splenium ($F_{1,38} = 2.95; p = 0.094$, $d = -0.53$) with mTBI patients again exhibiting higher FA values than HC (see figure e-1 for normalized FA histograms).

HC were then compared with a larger normative sample. However, there were no multivariate effects of group for all multivariate analyses of variance ($p > 0.10$), suggesting that our control group was statistically similar to the larger normative sample in terms of FA.

Next, we compared axial diffusivity and RD values for the 6 ROI that exhibited significant or trend differences in FA using one-way analyses of covariance (figure 1B). There were no significant differences between patients and controls in terms of axial diffusivity. In contrast, RD was lower in mTBI patients within the genu ($F_{1,38} = 5.09; p < 0.05$, $d = 0.74$), the left UF ($F_{1,38} = 5.67; p < 0.05$, $d = 0.77$), and the left CR ($F_{1,38} = 4.42; p < 0.05$, $d = 0.66$), with trends present in the left SCR ($F_{1,38} = 3.58; p = 0.06$, $d = 0.59$) and left IC ($F_{1,38} = 2.95; p = 0.094$, $d = -0.53$).

<table>
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<tr>
<th>Table 2</th>
<th>Demographic and clinical measures for visit 1</th>
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<tr>
<td>Characteristic</td>
<td>Mild traumatic brain injury</td>
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<tr>
<td>Age</td>
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<td>Education</td>
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<tr>
<td>HQ</td>
<td>78.56 $\pm$ 37.92</td>
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<td>Attention</td>
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<tr>
<td>Mean</td>
<td>50.91</td>
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<td>SD</td>
<td>5.20</td>
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<tr>
<td>Effect size</td>
<td>0.29</td>
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</table>

Abbreviations: EF = executive function; HQ = handedness quotient; NBSI-Som = Neurobehavioral Symptom Inventory Somatic complaints (Cog = cognitive complaints); PS = processing speed; TOMM = Test of Memory Malingering; WM = working memory; WTAR = Wechsler Test of Adult Reading.

Denotes significant result.
Finally, a MANCOVA (figure 2) comparing variability in FA measurements between right and left hemisphere homologue ROI (SFL, IC, UF, SCR, and CR) revealed a group effect ($F_{5,34} = 4.53; p < 0.005$), with univariate tests indicating increased variability in patients compared to controls for the SCR ($F_{1,38} = 15.06; p < 0.001$, $d = 1.21$), with a trend for the UF ($F_{1,38} = 3.82, p = 0.058; d = 0.63$).

**DTI and clinical measures.** Hierarchical multiple regressions were performed on the 6 clinical measures with the largest effect sizes (attention, memory, executive functions, cognitive complaints, somatic complaints, and emotional complaints) using FA from the CC and right and left hemisphere ROI as the independent variables and premorbid intelligence as a covariate. Although premorbid intelligence accounted for significant variance in terms of both attentional and executive functioning, only FA levels in the right hemisphere ROI (SFL, IC, UF, SCR, and CR) predicted variance in attentional deficits (positive relationship) for the mTBI group.

Next we determined which of our objective measures of deficits (FA or neuropsychological testing) would more accurately classify mTBI patients and HC using binary logistical regression. Estimates of premorbid intelligence were entered into both models as it discriminated (Wald = 4.16; $p < 0.05$) be-
tween HC (65% accuracy) and mTBI patients (66.7%) at slightly above chance levels. Traditional neuropsychological measures (attention, memory, and executive function) did not significantly improve classification accuracy in the first model (HC = 60%; mTBI = 71.4%). In contrast, results from the second model indicated that both the left (Wald = 7.73; \( p < 0.05 \)) and right (Wald = 5.66; \( p < 0.05 \)) hemisphere FA indices improved classification accuracy (HC = 70%; mTBI = 81%), with a trend being noted for the CC (Wald = 3.59; \( p = 0.059 \)). A support vector machine analysis with the leave-one-out methodology confirmed the generality (HC = 65%; mTBI = 81%) of the classification findings.

Visit 2 data. To date, 10 out of 17 (59%) eligible mTBI patients and 15 out of 16 (94%) eligible HC participants have returned for their 3- to 5-month follow-up visit (see appendix e-1). Intraclass correlation coefficient values for FA were highly reliable (all ROI = 0.65 < \( r > 0.93; p < 0.01 \)) in the HC sample; however, reliability of homologue measures was much more variable (SCR \( r_{14} = 0.64, p < 0.01 \); SLF \( r_{14} = 0.81, p < 0.001 \); UF \( r_{14} = 0.22, p > 0.10 \); CR \( r_{14} = 0.71, p < 0.01 \); IC \( r_{14} = -0.26, p > 0.10 \)). There were no significant differences for all clinical measures for mTBI patients who returned and those who did not. Additionally, there were no significant differences in FA values between the 2 groups across the 3 sets of ROI (CC, right and left hemisphere).

Change scores in clinical measures were calculated (visit 2 — visit 1 data) for those measurements that were most suggestive (i.e., based on significance or effect sizes) of group differences at visit 1 (attention, memory, executive functions, emotional distress, somatic and cognitive complaints) using premorbid intelligence as a covariate. Although there were no significant group effects, effect sizes suggested that memory scores improved (\( d = 0.52 \)) and cognitive complaints decreased (\( d = 0.79 \)) for the returning mTBI group compared to their matched controls at visit 2.

Differences in visit 1 and 2 FA and RD measurements were compared separately across the 2 groups with paired samples \( t \) tests to maximize power (see figure 3). Tests were again limited to those ROI that exhibited significant or trend differences in mean FA and RD (genu, splenium, left SCR, left IC, left UF, left UF, left UF, left SCR, left internal capsule, left corona radiata, left genu, and the splenium). For the y-axis, the units of FA are dimensionless, whereas RD is equivalent to \( \text{mm}^2/\text{s} \).
and left CR) at visit 1. In HC, there were no significant differences for either FA or RD across the 2 visits. In contrast, partial normalization (i.e., decrease) in FA values was evident in the splenium ($t_8 = 4.17, p < 0.005$) and CR ($t_8 = 1.89, p = 0.09$) at visit 2 for mTBI patients. Although none of the RD effects reached statistical levels of significance, visual examination of the data suggests that RD differences may have partially normalized at visit 2 as well.

**DISCUSSION** The types of abnormalities seen in human neuropathology studies of mTBI are poorly revealed by neuroimaging techniques, limiting detection of potential white matter pathologies, and prediction of cognitive impairment and functional outcome. Hence, conventional imaging modalities cannot provide an objective measure of injury for the difficult differential diagnoses that most clinicians face when confronted with mTBI patients. Contrary to our initial hypothesis, mTBI patients demonstrated increased FA and reduced RD within the genu and several left hemisphere white matter tracts compared to age- and education-matched controls during the semi-acute phase of injury.

Animal research indicates that there are several morphologic changes, metabolic processes, and inflammatory responses that follow mTBI. Therefore, a definitive mechanistic explanation for current results is challenging at best given the many constraints of an in vivo human clinical imaging study. With this caveat in mind, perhaps the 2 most plausible explanations for the current and previous observations of increased diffusion anisotropy following mTBI are cytotoxic edema or changes in water content within the myelin sheath. The mechanical forces of mTBI typically result in the stretching of axons and related supporting structures such as oligodendrocytes, altering the function of gated ion channels and resulting in an increase in intracellular water and a decrease in extracellular water. The decrease in extracellular water leads to a decrease in diffusivity perpendicular to the axon (second and third eigenvalues; RD), secondary to more tightly compacted axons and potential differences in the tortuosity of intracellular and extracellular water. Modeling studies suggest that even small departures from the normal distribution of intracellular and extracellular water can lead to dramatic changes in perpendicular diffusion coefficients.

A central role for cytotoxic edema is also partially supported by animal models of both ischemic stroke and TBI, in which perilesional white matter shows increased FA in the first 3 hours of stroke followed by a reduction in FA and RD from 4 to 120 hours postinjury. Of note, the timeline from these animal models suggests that reduced rather than increased FA should be observed at days to weeks postinjury. However, cytotoxic edema may follow a somewhat more prolonged course in human TBI than in the animal models of TBI, peaking between 24 and 48 hours postinjury and persisting for days postinjury. An alternative explanation for our findings is that mTBI decreases water content in the myelin sheaths rather than in extracellular space. Although myelin only accounts for approximately 13% of total water in white matter compartments, a reduction in this percentage would theoretically also decrease diffusivity perpendicular to the axon.

At a more basic level, there may be qualitative differences in neuropathologic processes among appropriately diagnosed mTBI patients as illustrated by a recent study comparing the fluid percussion (FPI) and cortical impact (CCI) injury models. Injured animals from both groups differed from shams in terms of T2 values and apparent diffusion coefficients (ADC), but in opposite directions. The FPI injury, which might be a better model for mTBI injuries caused by motor vehicle accidents, showed decreased T2 and ADC, while the CCI injury, perhaps a better model for falls or assaults, showed increased ADC and elevated T2. Both groups showed evidence of increased immunoreactivity.

Magnetic resonance spectroscopy also captures unique information about white matter pathology that may elucidate potential mechanisms of pathology. Increased creatine-phosphocreatine concentrations in supraventricular white matter and in the splenium have been observed in mTBI, perhaps related to an increased need for energy resources (ATP) for repair. Though such metabolic derangements may follow a different recovery course than DTI abnormalities, they likely represent an important component of the suite of pathologic processes. A tentative hypothesis linking the 2 imaging modalities suggests that disruption of ionic homeostasis causes increased intracellular water, simultaneously reducing RD and increasing ATP demand so as to upregulate membrane pumps and restore ionic homeostasis.

Current results also suggest that DTI results are more accurate in objectively classifying mTBI patients from carefully matched HC. Although limited in nature, our anatomic protocol was completely insensitive (e.g., all mTBI and HC scans were interpreted as trauma-free) to the putative underlying pathology following trauma. Second, although our mTBI patients exhibited cognitive deficits on several neuropsychological domains (attention, memory, and executive functioning) that were consistent in magnitude with previous meta-analyses, these deficits did not substantially improve classification accu-
accuracy even though neuropsychological testing has traditionally served as the gold standard for differential diagnosis. In contrast, classification accuracy improved to 75% with data derived from DTI images. Future studies should examine the classification accuracy of DTI and neuropsychological measures in orthopedically injured patients or similar populations to better control for nonspecific effects of trauma.

Similarly, longitudinal studies with larger samples spanning the acute to chronic time frame are also needed to chart the evolving nature of mTBI, which has been documented in studies employing animal models. FA measurements appear to be relatively stable over month-long intervals in HC, rendering it an ideal mechanism for monitoring potential changes associated with recovery of function. Our preliminary longitudinal data suggest a partial normalization of FA (i.e., a decrease toward levels observed in HC) within several ROI in our mTBI group. Although others have examined more severely injured populations, we examined longitudinal DTI changes in mTBI. Consistent with patients’ self-report of continued cognitive and somatic symptoms at visit 2, not all of our ROI demonstrated significant changes as a function of time, suggesting that a more extensive postimaging interval may be necessary to track recovery.

ACKNOWLEDGMENT
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DISCLOSURE
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REFERENCES


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**CDC, AAN to Health Care Professionals: Monitor Patients for GBS**

The Centers for Disease Control and Prevention (CDC) and the American Academy of Neurology (AAN) collaborated to reach out to neurologists across the US to monitor and report any possible new cases of Guillain-Barré syndrome (GBS) following 2009 H1N1 flu vaccination.

Neurologists and health care professionals nationwide who diagnose patients with vaccine-associated GBS should use the CDC and FDA Vaccine Adverse Event Reporting System (VAERS) to report their observations.

In addition, neurologists and all health practitioners in the 10 Emerging Infections Program (EIP) states—California, Connecticut, Maryland, Minnesota, New Mexico, New York, Colorado, Oregon, Georgia, and Tennessee—are asked to report all new cases of GBS, regardless of vaccination status, to their state’s surveillance officer.

The AAN hosted a series of webinars providing an in-depth look at H1N1 vaccination and how it may pose a risk for GBS and information about the vaccination monitoring campaign.

For additional information about the monitoring campaign, or to watch the webinars or download VAERS form and information on reporting to surveillance officers in your state, visit the AAN’s GBS toolkit page, [www.aan.com/view/gbstoolkit](http://www.aan.com/view/gbstoolkit).