Detection of hemorrhagic and axonal pathology in mild traumatic brain injury using advanced MRI: Implications for neurorehabilitation

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Abstract. Introduction: There is a need to more accurately diagnose milder traumatic brain injuries with increasing awareness of the high prevalence in both military and civilian populations. Magnetic resonance imaging methods may be capable of detecting a number of the pathoanatomical and pathophysiological consequences of focal and diffuse traumatic brain injury. Susceptibility-weighted imaging (SWI) detects heme iron and reveals even small venous microhemorrhages occurring in diffuse vascular injury. Diffusion tensor imaging (DTI) reveals axonal injury by detecting alterations in water flow in and around injured axons. The overarching hypothesis of this paper is that newer, advanced MR imaging generates sensitive biomarkers of regional brain injury which allows for correlation with clinical signs and symptoms.

Methods: Studies involving subjects with a history of traumatic brain injury as well as healthy, non-trauma controls were used. Analysis involved comparison of TBI patients’ imaging results with healthy controls as well as correlation of imaging findings with clinical measures of injury severity. An additional animal study of Sprague-Dawley albino rats compared imaging results with histopathological findings after the animals were sacrificed and stained for b-APP.

Results: SWI revealed small foci of hemosiderin for some patients while aggregate lesion volume on SWI correlated with clinical injury severity indices. Similarly, DTI showed striking group differences for fractional anisotropy over the white matter globally, while tract and voxel-based regional results colocalized with SWI and FLAIR lesions in some cases and correlated with clinical deficits. For the rats, correlations were seen between imaging findings and staining of axonal injury.

Discussion: Animal data gave important tissue correlations with imaging results. SWI and DTI are commercially available sequences that can improve the diagnostic and prognostic ability of the trauma clinician. These biomarkers of regional brain injury which are present in imaging shortly after acute injury and persist indefinitely can inform clinicians and researchers about not only injury severity but also which neurobehavioral systems were injured. Analogous to stroke rehabilitation, having an understanding of the distribution of brain injury should ultimately allow for development of more effective rehabilitation strategies and more efficient clinical interventional trials.

Keywords: Diffusion tensor imaging, susceptibility-weighted imaging, hemorrhagic pathology, axonal pathology and mild traumatic brain injury

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1. Introduction: The need and urgency for more sensitive imaging of TBI

While brain injury in military personnel and in football has brought mild traumatic brain injury (mTBI) to the fore, other significant events have paved the way as well. The 21st century’s reliance on technology, information processing and speed has put an unprecedented premium on healthy cognitive functioning. Increased competition for jobs in a changing economy has meant that minor differences in cognitive and behavioral functioning are more important than ever [1–3]. In addition, automotive safety features in the last thirty years have reduced mortality and severe injuries but have increased the incidence of milder injuries [4]. The same is true in the military where Kevlar has protected the body, rendering the brain the most vulnerable to injury from improvised explosive devices [5]. The brain is the singularly most sensitive organ in the body to injury. More than a “mild” injury in the liver, kidney or spleen, for instance, can be tolerated and have no clinical consequences but this is not so for the brain. A 5% loss in capacity due to diffuse axonal injury results in loss in attention and working memory which has downstream consequences on longer term memory, both storage and retrieval, and ability to process verbal and nonverbal information in real time [3,6–13]. Fatigue or neurasthenia is a real phenomenon which also further reduces cognitive capacity [14]. This is to say nothing about emotional processing which is largely the domain of right hemisphere [15,16], medial and ventral frontal and temporal lobe structures. The interplay between mild cognitive and neuropsychiatric symptoms is often overlooked or incorrectly attributed to premorbid or post traumatic reactive psychological issues. The difficulty in objectively distinguishing between organic and nonorganic causes of posttraumaticic symptoms leads to frequent misdiagnosis and contentious litigation. People who experience a mild TBI are most often discharged from the emergency room the same day and told that they will likely recover fully. Nevertheless, clinical symptoms often worsen over days or longer, a function of the complex pathological cascade of TBI [17,18]. In 2005 the World Health Organization Collaborating Center Task Force on mTBI concluded that 80–90% of mTBI cases will fully recover in 3–12 months [19], a finding that has led to minimization of the significance of mTBI. Patients and concussed athletes often return to activity before they have fully recovered, which likely worsens outcomes. However, there remains no consensus on return to play or activity guidelines after single and repeat injuries, partly because objective indicators of mTBI such as brain imaging have been lacking [20,21]. The effects of repetitive concussive and “subconcussive” injuries long-term is also not known but could be informed by sensitive functional or structural imaging of mTBI [22,23]. To date there are no FDA approved pharmacological treatments which have shown efficacy despite significant effects in preclinical animal studies. An international working group (IMPACT) [24] concluded that MRI has a role in minimizing pathological heterogeneity in clinical trials which will improve the efficiency of the trials [25,26]. All of the aforementioned leads to the conclusion that there is an urgency or time sensitivity with regard to more accurate diagnosis of TBI, and in particular mTBI.

2. Approaches to diagnosing TBI

A number of different approaches have attempted to address the problem of diagnosing mTBI indirectly. This has been necessary since pathology, the gold standard, is reserved for postmortem investigation. Neuropsychological assessment, EEG, functional and structural neuroimaging seem to offer the greatest potential diagnostically with SPECT [32,33], PET [34,35], and MRI heading the way [36] MRI, on the other hand, singularly offers the ability to image several of the pathological consequences of TBI even in mild TBI [37]. This is a critical advantage of MRI since clinically the symptoms are often subjective and subtle in mTBI. This paper will focus on multi-modality MRI which produces unprecedented information regarding tissue energetics and physiology, network functionality, microstructural integrity, tissue perfusion, hemorrhage and iron deposition, inflammation and edema, regional volume loss and comorbid conditions such as hyper-
tensive vascular disease and degenerative diseases (e.g., Alzheimer’s disease, Parkinson disease, etc.). Beginning with an overview of TBI pathology and available MRI methods which detect these pathological changes, we will then narrow the focus of the paper to two MRI techniques which have been well studied by our group and others in TBI and more recently mTBI. We will then present an overview of the imaging methods used by our laboratory for investigating mTBI.

3. Advanced MRI – Sensitivities and general approaches

3.1. Circumscribing TBI

Mechanical trauma results in a number of consequences to the brain. Generally speaking we can categorize these consequences as focal and diffuse pathology which reflects a particular concentration of force to one portion of the brain or vasculature (focal) or a more spatially distributed perturbation of the brain (diffuse). In reality, while diffuse vascular and axonal pathologies are found segmentally (interspersed with normal tissue) in multifocal locations, there are areas of predilection which reflect both structural properties of the brain and supportive elements as well as the particulars of biomechanics. By example, the corpus callosum, and particularly the splenium, commonly shows axonal injury as does frontal subcortical white matter. Frontal or executive function impairment is one of the hallmark features of TBI. The pathologies themselves have both structural and physiological changes which are evolving after injury and are not maximal immediately after injury [18]. This pathological evolution which plays out over hours to days in mild cases likely underlies the clinical evolution that is observed but frequently misattributed to nonorganic factors [38]. Optimistically, the plurality of the pathology and the evolution over time would afford clinicians the opportunity for therapeutic intervention, analogous to current strategies for the acute treatment of stroke where there is a time-limited ischemic penumbra. Some of the pathologic and pathophysiologic findings in brain trauma include:

1. Hemorrhage – these include epidural, subdural, contusion, microhemorrhaging, macro or parenchymal hemorrhaging
2. Acute ischemia – this is a short-lived uncoupling between the metabolic demands of the neurons due to depolarization caused by trauma and a short lived hypoperfusion likely due to transiently disturbed neurovascular functioning [39].

3. Hypoxia – observed in severe TBI with compromise of ventilation or circulation. May result in laminar necrosis or injury to selectively vulnerable brain regions including hippocampus, dentate gyrus and basal ganglia [40].
4. Vasogenic edema – results from hemorrhaging and ischemia
5. Biochemical alterations – N-acetyl-aspartate (NAA), choline, myoinositol, glutamate, glumamine, lactate, ATP, ADP and Magnesium are but a few of the biochemical compounds whose concentrations change reflecting the metabolic, energetic and microstructural properties of the tissue [41].
6. Axonal injury – stretch, shear, compressive forces, anoxia and edema can lead to localized demyelination or deeper injury to axons resulting in degeneration of the axon known as Wallerian degeneration. The latter is characterized by progression from an undulated profile (potentially reversible) to segmental swelling to localized spheroids known as retraction balls [42,43].
7. Functional disconnection – With injury to neurons from contusions, ischemia, excitotoxicity, energy depletion, mass effect from edema and hemorrhaging, along with axonal and even glial cell injury, the pre-injury neural circuitry is compromised as are the functional neural networks. Diffuse injury results in a predictable degradation in signal strength within neural networks resulting in less efficient, lower capacitance systems [44]. Clinically, the latter results in decreased immediate and working memory, attentional impairment and neural fatigability [45]. Electroencephalography shows increases in local coherence and decreases in more distant coherence reflecting degradation of a weaker signal between distant (e.g., transcortical) neural units [46].
8. Parenchymal volume loss – in moderate to severe TBI, volume loss can be detected globally and regionally as early as two weeks. A progressive volume loss may occur over a year or longer reflecting the loss of gray and white matter from the above-listed pathologies including transsynaptic degeneration resulting in a more generalized pattern of atrophy than might be expected [47].

3.2. Advanced MRI and detection of TBI – pluri-pathology

1. Hemorrhage – Susceptibility-weighted imaging (SWI) exploits the magnetic susceptibility differ-
2. Acute ischemia – a number of advanced MRI techniques are capable of providing information which directly or indirectly suggests ischemia. The clinical neuroimaging method diffusion weighted imaging (DWI) [49], together with the apparent diffusion coefficient (ADC) [50], detects voxels with reduced diffusion of water caused by cytotoxic edema, itself the consequence of cytolemma ion pump failure. In addition, perfusion-weighted imaging (PWI) [51] using a contrast agent and arterial spin labeling (ASL) [52] using magnetic “spin tagging” are able to identify regions of reduced relative blood flow or volume. Finally, proton and phosphorous MR spectroscopy (MRS) reveal information regarding oxygenation of the tissue (lactate) as well as the energetics of the tissue (high energy phosphorous and creatine compounds) [53].

3. Hypoxia – This secondary effect of trauma (usually multiple trauma) due to hypventilation and/or circulatory failure may overlap with ischemia but should not include clear perfusion abnormalities and would include a lactate peak on H-MRS [54] and a reduction in ATP and ADP in P-MRS in selectively vulnerable gray matter nuclei [55].

4. Vasogenic edema – DWI and conventional imaging such as T2-weighted and fluid-attenuated inversion recovery (FLAIR) are most useful. DWI is more sensitive than T2/FLAIR early (i.e. within 12 hours) with the ADC increased over baseline since there is relatively more extracellular water diffusion compared with pre-injury baseline [56].

5. Biochemical alterations – MRS detects biochemical alterations as a consequence of TBI. In addition to ischemia and hypoxia (see above) in TBI MRS detects the compounds NAA, choline, creatine, myoinositol, Glx (glutamate/glutamine) which are variably altered in TBI [32,57].

6. Axonal injury – Diffusion Tensor Imaging (DTI) and the higher resolution Diffusion Spectrum Imaging (DSI) are able to detect alterations in intravoxel anisotropic diffusion which matches well with the morphological changes that occur to axons after they have been deformed beyond their elastic limit (our abstract). In the early stages after injury the axons swell, fragment and degenerate. The transition from packed coherent fibers to segmentally injured, swollen and degenerated fibers [58] causes a reduction in diffusion anisotropy [59,60] except in the hyperacute stage where ischemia caused by uncoupling between metabolism due to excitotoxicity and perfusion may lead to increased diffusion anisotropy [61]. In addition, T2/FLAIR sometimes reveals small hyperintense punctate foci at or near the gray-white junction which reflect axonal injury [62]. These same foci are usually detected by DTI. It is likely that a subset of axonal lesions revealed by DTI is detected by T2/FLAIR and reflect a spectrum of severity, a secondary insult resulting in edema or gliosis (unpublished observation).

7. Functional disconnection – BOLD-fMRI is a method that detects blood oxygenation level in veins. In brain regions which are activated there is a temporary uncoupling between oxygenation and perfusion resulting in an abnormally high oxyhemoglobin concentration in the draining veins. Paramagnetic deoxyhemoglobin is therefore reduced which results in less signal loss or a net signal gain. Biswal in 1995 discovered that at rest spontaneous fluctuations of BOLD fMRI revealed coherence between regions that should be functionally connected [63]. This method known as functional connectivity MRI (fcMRI) can be done at rest (no task, eyes closed) or during a non-rest state and reveal the “reorganization” of functional networks reflecting deployment of different neural networks under different conditions [64]. This method is now being used to study TBI with the hypothesis that functional networks might be weakened or altered reflecting depletion and neuroplasticity [44].

8. Parenchymal volume loss – Conventional MRI with high gray-white contrast and little spatial distortion is optimal for volumetric measurements globally and regionally. These analyses can be performed by sensitive computerized automated methods and compared with prior images or against age and gender-matched normative data from healthy volunteers [65,66].
4. Imaging hemorrhage and traumatic axonal injury

Of the eight pathologic sequelae of TBI noted above, two are relatively specific for TBI, i.e., microhemorrhaging and traumatic axonal injury. In addition, these two pathological findings are apparent early after injury and persist in some form permanently as macrostructural (hemorrhage) and microstructural (axonal injury) changes, respectively. The persistence of these injury markers has enhanced test-retest reliability of the imaging and prediction of global clinical outcome [37,60]. For the neurorehabilitation clinician, an understanding of a patient’s unique injury profile revealed by imaging and the ability to relate these lesions and their locations to neurocognitive and neurobehavioral symptoms presents an unprecedented opportunity to explore more effective rehabilitation techniques, potentially using some of the same imaging methods in parallel with rehabilitation to monitor the effectiveness of the intervention. For these reasons the remainder of this paper will explore advanced MR imaging of hemorrhage and traumatic axonal injury.

4.1. Susceptibility-weighted imaging – imaging of hemorrhage

SWI is a high-resolution, fully velocity-compensated, three-dimensional gradient echo imaging sequence
that is extremely sensitive to blood products in hemorrhage and deoxyhemoglobin in venous blood. Haacke et al. have given a detailed technical description of SWI [67], and Kou et al. have given a systematic review on the role of SWI in brain trauma [68]. SWI combines magnitude and phase images to maximize sensitivity to susceptibility contrast. Studies by Tong et al. have shown that SWI is 3–6 times more sensitive than conventional gradient echo imaging (GRE) in detecting hemorrhagic lesions and [48,69] and reveals about twice the aggregate hemorrhage volume (see Fig. 1).

Conventional CT and MRI studies have reported that traumatic hemorrhages are found in cortical gray matter (GM), subcortical white matter (WM), major WM tracts, including corpus callosum and internal capsule, brainstem, and within the ventricles [70]. In addition to these “traditional” locations reported using conventional imaging techniques, SWI has been shown to visualize microhemorrhages at the gray-white junction (see Fig. 2, red circle) as well as at the junction of branching vessels, especially the veins [68] (Fig. 2, arrows). Putting localization aside, total hemorrhagic lesion number and volume identified by SWI has been demonstrated to correlate with clinical severity of TBI indexed by duration of posttraumatic amnesia [69] (See Fig. 3).

With regard to mild TBI, mechanism of injury certainly affects presence or absence of bleeding. A blow to the head or a fall with head strike on a hard surface is more likely to produce a contusion with some subarachnoid blood than a pure inertial mechanism. Head rotation is more likely to cause subdural hemorrhage than pure translation. Small venous hemorrhages at branch points, flame or linearly shaped are occasionally seen on SWI in mTBI and likely is a marker of relatively greater local strain on the axonal and vascular elements. This is supported by the frequent proximity of these small venous hemorrhages to axonal injury revealed by DTI.

4.2. Diffusion tensor imaging – imaging traumatic axonal injury

Diffusion tensor imaging sequences are sensitive to traumatic axonal injury secondary to stretch, shear and compaction forces. DTI measures the bulk motion of water molecular diffusion in tissue. It is clinically useful to detect abnormal alterations of diffusion in tissues with a repeating, uniform fiber architecture such as muscle fibers and the white matter tracts of the brain. Histological data also validated the usefulness of DTI characterization of brain injury pathology by using either a focal injury model [71] or a DAI model [72].
Fig. 4. Comparison of TBI patients (n = 20) and controls (n = 14) for distribution of fractional anisotropy for all white matter voxels in all subjects. X-axis gives FA ranges and Y-axis gives the normalized voxel count for a FA bin range. Note the leftward shift of the TBI mean plot compared with the controls. Also, note the much larger error bars for the TBI group indicating much greater variability within the patient group. Also note the higher peak for the TBI group. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)

When axons are injured, as in acceleration/deceleration injuries normally high diffusion anisotropy (i.e., greater diffusion in the long axis dimension compared with radial dimension) is reduced because of early decreases in long axis (axial) axoplasmic flow and slightly later occurring increases in radial flow in swollen axons and leaky axonal membranes.

 Scalars derived from DTI have been applied to TBI to describe spatially nonselective and spatially selective diffusion properties, i.e., apparent diffusion coefficient (ADC) and fractional anisotropy (FA) [73,74]. ADC is an estimate of the average magnitude of water movement in a voxel, whereas FA estimates the spatial inhomogeneity of water diffusion within a voxel. ADC increases with vasogenic edema, when water flows out of capillaries into the interstitial space, and decreases with cytotoxic edema, where a greater proportion of water molecules is contained by ischemic, swollen cells. FA, which indexes the fraction of total intravoxel diffusion which is inhomogenous, or unequal spatially, is sensitive to white matter tract damage.

 FA in WM is greatest when fibers are long (relative to voxel dimension) and uniformly oriented within a voxel and reduced when fibers are oriented orthogonal to each other, i.e., “crossing fibers” or have been damaged. In general, several pathologies of WM injury will result in FA decrease, including (vasogenic) edema, ischemia, neurodegenerative, metabolic, and traumatic while few result in FA increase. FA increases have been demonstrated hyperacutely after trauma and may occur in voxels containing crossing fibers where one orientation of fibers is selectively injured. ADC, FA, and DTI directional diffusivities can be used together to characterize the pathology. For instance, decreased FA in association with increased ADC suggests vasogenic edema, whereas increased FA in association with decreased ADC suggests cytotoxic edema. Decreased FA in association with decreased longitudinal water diffusivity indicates impaired axonal transport. Decreased FA, together with slight or no increases in ADC with increased radial diffusivity is typical of subacute or more chronic axonal injury from trauma. Biomechanical forces that distort and strain axons beyond their elastic limit will result in a change in the morphology of the axon over hours to months, characterized by swelling and shortening of fibers and eventual fiber loss (i.e., Wallerian degeneration). It should be noted that microscopically one typically observes damaged, distorted fibers interspersed among normal appearing fibers; this intermixing of normal and abnormal axons would also be represented in a single voxel. Thus, the greater the proportion of damaged, distorted axons in a voxel, the lower the FA for the voxel. In the more chronic stage, fiber loss may occur, resulting in lower fiber density (also reducing FA) and decreased volume of WM structures on anatomical imaging.
4.2.1. Global white matter analysis with DTI

This global approach leverages the diffuse or multifocal nature of TBI pathology. The strength of the global approach is its simplicity. There is no need to transform the DTI image into a standard space since coregistration with other subjects is not required. Image processing does require segmentation of tissue classes in order to isolate the white matter component.

An initial study by our laboratory to investigate the utility of such a global approach used 21 heterogeneous, non-penetrating TBI patients and 14 healthy controls [60]. Using a histogram analysis of FA we found that the TBI patients had distributions that were globally reduced and had shape changes with higher peaks and a change in skew and kurtosis (see Fig. 4). Using a Student’s t-test, the global FA mean was found to distinguish the TBIs from controls better than global ADC, radial and axial diffusivities, trace, kurtosis, skew, or peak. Even the 6 mTBI patients had lower FA means than all 14 of the healthy controls, suggesting the sensitivity of a global approach. In addition, correlation of the various DTI indices with early clinical indicators of TBI severity (GCS score and PTA) revealed that FA mean was the best predictor of severity of injury (GCS, Spearman \( r = 0.47 \) and PTA, \( r = 0.64 \)). The validity of the histogram approach has been confirmed elsewhere [75].

A few cautionary points need to be mentioned, however: FA, while it is a scalar ratio of anisotropic to bulk diffusion, does vary as a function of age [76], field strength [77] voxel size [78] receiver coil type and other image-related parameters. These observations then necessitate that comparison between DTI datasets either between individuals or between time points be done using exactly the same imaging sequences, magnets and acquisition matrices (i.e., protocol) to minimize false positives and negatives. In between-group analyses where two groups differ on imaging parameters or magnets, it may be feasible to empirically measure and remove system (magnet, imaging) differences. This is far from trivial and is not as reliable as using the same protocol for both groups. With regard to age, it has been shown by our group and others that FA decreases linearly with age [79]. Therefore, use of diffusion anisotropy in DTI must adequately account for age differences in order to minimize false positives and negatives.

4.2.2. Strategies to improve the detection of mTBI with DTI

Because milder closed head injury shows a more spatially restricted distribution of axonal injury, use of a global approach method may not always distinguish TBI from normal brains. Note that in Fig. 5 the patients with moderate and severe injuries have a mean FA which is clearly lower than the range of normals. In contrast, the two mild TBI (GCS of 14 and 15) cases fall within the low normal range for their ages. Two
Fig. 6. Fusion of colorized white matter parcellation units from DTI-81 atlas (http://www.loni.ucla.edu/Atlases–ICBM) and an anatomical template. This atlas is based on probabilistic tensor maps obtained from 81 normal subjects acquired under an initiative of the International Consortium of Brain Mapping (ICBM). The subjects were normal right-handed adults ranging from 18 to 59 years of age. A hand-segmented white matter parcellation map was created from this averaged map. This map can be used for automated white matter parcellation. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)

possibilities exist for this observation: 1) one or both cases did not suffer axonal injury; 2) one or both suffered relatively restricted axonal injury but insufficient to reduce the global FA mean below the normal range.

To minimize false negative error we employ three additional analytic methods, each of which effectively subdivides the white matter into smaller units. These include an atlas-based regional approach, a voxel-based method and a tract-based method. All three methods necessitate that brain images for all subjects/patients be transformed (i.e., warped) into a standard space prior statistical comparison between brains. These approaches vary and have advantages and disadvantages:

a. **Atlas-based regional method** – Conceptually, this method utilizes a standard atlas with a priori defined white matter tracts or parcellation units (see Fig. 6). Since all FA images are warped into the same “atlas space”, statistical analysis between subjects can proceed for each of the atlas defined regions, with results presented in a spreadsheet format. Advantages of this method are that it: 1) reduces dilutional effects of the global analysis; 2) is automated; 3) results are easily correlated with clinical measures and; 4) allows for easy visualization of affected regions using color coding. Possible disadvantages: 1) Large regions may still suffer from dilutional effects; 2) some regions may not be adequately imaged for one or more subjects; 3) multiple comparisons being performed; 4) spatial registration errors.

b. **Voxel-based analysis** – Similar to the atlas-based method, all subjects’ FA images are warped into a standard space so that between-subjects sta-
Fig. 7. Schematic of voxel-based statistical analysis: Patient’s spatially normalized FA map on the left. Z-score map is created by subtracting the mean FA map of 19 controls in standard space from the patient’s FA map and dividing the result by the standard deviation map for the 19 controls. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)

Voxel-Based Statistical Analysis

Fig. 8

Fractional Anisotropy (FA image)

Mean of 19 normals

Z-score FA map

x WM Mask

Fig. 7. Schematic of voxel-based statistical analysis: Patient’s spatially normalized FA map on the left. Z-score map is created by subtracting the mean FA map of 19 controls in standard space from the patient’s FA map and dividing the result by the standard deviation map for the 19 controls. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)

tistical analysis can proceed voxel by voxel (see Fig. 7). We use the ‘spatial normalization’ module in SPM8 to spatially transform individual subject FA images into standard (MNI) space. We use a custom template created by averaging 50 healthy volunteer FA images together. In addition, we employ a weighting mask which is the subject’s FA image itself (scaled from 0 to 1) to maximize the quality of the white matter warp to the template. Only voxels which are classified as white matter in both template and subject’s image are included in statistical analysis. This step minimizes false positives caused by misregistration between images. The voxel-based method is best suited for milder injuries where anatomy is not greatly distorted. Figure 8 shows an example of voxel-based analysis in a severe TBI after smoothing thresholded at a $Z \leq 1$.

c. Tract-based spatial statistics – This method attempts to coregister white matter tracts between brains, i.e., between groups of brains. The motivation for doing a tract based analysis is to reduce misregistration related error occurring with non tract-based anatomical normalization methods. An initial nonlinear spatial normalization is performed on all FA images as a first step (using TBSS in FSL [80]) in order to get corresponding white matter fiber tracts approximately in register between brains. The next step creates a mean “skeleton” of the white matter tracts that are common to all brains entered into the analysis [81]. This mean skeleton is comprised of the central core of the tract and is one voxel in width. The group mean skeleton is then used to extract the corresponding skeleton for each brain in the analysis. Statistical analysis is performed between subjects using only the voxels contained in the white matter skeleton—not the full thickness of the tracts. The inherent assumption is that as one gradually moves perpendicular to the central core of a tract (away from it) misregistration increases due to differences in tract width and partial volume effects. Figure 9 shows the result of a TBSS analysis in a patient who was riding a bike when he was hit by a car and hit the pavement on the right side of his head.

4.3. Gray-white junction hyperintensities on FLAIR/T2 have reduced FA on DTI

The gray-white junction (GWJ) is a site of predilection for injury in TBI [82]. In our experience involving
over 100 mTBI patients (alteration in consciousness following mechanical trauma) in the chronic stage, a minority (< 20%) of them have small hyperintensities on FLAIR/T2 which are located at or just deep to the gray-white junction (GWJ) of the cerebral hemispheres, most commonly the frontal lobes. These lesions appear small, round, discrete and scattered. They can be distinguished from small vessel ischemic changes by their predilection for the GWJ at the periphery of the cerebral white matter. They are distinct from MS lesions which have an affinity for the commissural fiber tracts, are typically coalescent and may enhance with contrast. Virtually always we find a corresponding reduction in FA in the same location on DTI. On the other hand, for the minority of patients with these GWJ hyperintensities these lesions represent a small subset of the DTI lesions and do not represent the largest or most statistically significant lesions on DTI. We have, however, observed a clinical association in patients who may suffer a second injury (concussion) within weeks of the first injury or in patients who experience prolonged postconcussive syndromes consisting of headache, sensory irritability, vestibular symptoms and fatigue. An illustrative case was a professional football placekicker who was concussed twice in 15 days with brief LOC both times followed by a third game involving exertion but no trauma. He had not fully recovered from his prior concussions but had exacerbation of symptoms and neurocognitive impairment which began during the third game and persisted. MRI one month later revealed hyperintensities
Fig. 9. Tract-based spatial statistics (TBSS) – result from a 37 year old man who was riding a bike when he was hit by a truck to the left side of his body and went airborne before striking the pavement with the right side of his head. No LOC but some post traumatic amnesia. A. Coronal image; B. Axial image; C. Sagittal image. Tract-based analysis considers for analysis only the central skeleton of white matter fibers common to all subjects. A permutation-based inference method is performed voxel-wise on the skeleton with significant voxels ($t > 3$) colored red to orange. Note the prominent involvement of the right corticospinal tract indicated by dark blue arrows. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)

which have remained on scans three years later (see Fig. 10). We speculate that the hyperintense lesions on FLAIR/T2 scans (arrows pointing) result from the additional metabolic stress imposed on already injured axons. The latter may result in swelling, cytotoxic or vasogenic edema and gliosis which would be revealed by the conventional images.

4.4. Validation of imaging in an animal model of diffuse axonal injury

We have undertaken a study involving a rodent model of diffuse axonal injury with the goal of comparing imaging findings over time with postmortem histology. A weight drop (Marmarou) model was used with 4 groups of rats sacrificed at 4 hours, 24 hours, 3 days and 7 days, respectively. Histology used APP staining to identify injured axons. Imaging was done in vivo and ex vivo when possible. DTI image analysis used a tract-based statistical method adapted from TBSS. The approach utilized SPM8 for spatial normalization and TBSS for statistical analysis. Pre-trauma images were used as the reference group ($N = 16$). Figure 11 shows histology and imaging from one of the rats imaged at 4 hours. Panel A shows a photograph of the APP stained slice (red) corresponding to the SWI image (Panel B) and the DTI TBSS statistical image (Panel C). Panel A clearly shows APP staining more on the left than right corpus callosum (CC). Panel B shows an area of abnormal susceptibility only on the left CC while the DTI image shows reduced FA compared to the pre-trauma control images which is remarkably similar to the pattern of APP staining. DTI analysis used TBSS so only the central skeleton is statistically analyzed which may explain some of the non-overlap between the two. The altered susceptibility on SWI (ex vivo) which is in the nidus of the APP stained region may indicate a local

Fig. 10. Single slice of a fluid-attenuated inversion-recovery (FLAIR) image from a former professional football placekicker who suffered two concussions in a two week period (see text). His FLAIR MRI one month after the second concussion revealed hyperintensities which have remained on FLAIR three years later. Hyperintensities are singlet, spherical foci, located in subcortical white matter often close to the gray-white junction and are likely foci of axonal injury. Foci are indicated by red arrows in this slice. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)
Fig. 11. Comparison between imaging and histology in corpus callosum (CC) for the rat: A. b-APP staining of tissue slice indicating axonal injury using the impact-acceleration weight drop model; B. SWI image obtained 4 hours after impact showing a focal area of abnormal susceptibility in the left CC possibly indicating increased deoxyhemoglobin; C. TBSS analysis of DTI also at 4 hours after impact with voxels with significantly reduced FA shown in color ($t > 2$). Note the colocalization of pathology across the three images in the left medial CC. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)

Fig. 12. Comparison between FLAIR, SWI and DTI voxel based analysis for a 22 year old male in a head on collision with a GCS of 3 in the field. Scans are in standard space: A. shows the FLAIR image with a ventriculostomy tract (red arrow); B. shows the SWI image which reveals a number of small, venous hemorrhages (i.e., black “holes” in the frontal lobes bilaterally in addition to the right ventriculostomy tract (red arrow); C. shows the areas of reduced diffusion anisotropy in blue including bilateral CC genu. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)

decrease in oxygen or hemorrhage, although bleeding is not evident. We have observed an evolution from increased FA at 4 hours to decreased FA at later time points often in regions of abnormal susceptibility suggesting that hyperacute ischemia and cytotoxic edema may underlie the increased FA [83].

4.5. Clinical correlations: Case examples

Case 1: K.P. was a 22 year old male in a head on collision 3/18/08 with a GCS of 3 in the field. CT showed frontal lobe hemorrhaging. Early on he showed slowed information processing, language and memory issues
which improved. He is currently in college maintaining a B average. MRI was done in Detroit four years after injury. Global FA was 0.392 which is much lower than normals corrected for age. Figure 12A shows the FLAIR image with a ventriculostomy tract outside the right frontal horn. B shows the SWI image which reveals a number of small, venous hemorrhages in the frontal lobes bilaterally in addition to larger hemorrhagic lesions corresponding to the right ventriculostomy tract and left frontal contusion. C shows the areas of reduced diffusion anisotropy in blue.

Discussion: Note the similar distribution of hemorrhagic and non-hemorrhagic (reduced FA on DTI) lesions. Note that the DTI image is only sensitive to white matter pathology, so that small hemorrhages in the cortex will not have a corresponding lesion in the

![Fig. 13. Comparison between FLAIR and DTI in standard space for a 55-year-old man who was rear-ended by a semi-truck causing a second collision with the car in front of him. A. Note the ventricular enlargement along with multiple white matter hyperintensities, many of which are close to the gray-white junction. B. Shows a DTI focus (blue color) of reduced FA in the same anatomical location as the hyperintensity on FLAIR indicated by the crosshairs. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)](image1)

![Fig. 14. Volume rendering of the DTI data from Fig. 13 with the front of the brain removed to reveal injury to the right anterior limb internal capsule. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)](image2)
Fig. 15. DTI result for a 36 year old former professional football fullback who reported more than 50 episodes of transient visual loss after head collisions. DTI voxel and tract based analysis each revealed reduced FA only in the splenium of the corpus callosum (see text for discussion). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/NRE-2012-0795)

gray matter on DTI. Also, note that there are foci of reduced FA (denoting axonal injury) without proximal hemorrhages.

**Case 2:** S.M., a 55 year old successful Wall Street investor with a history of well-controlled bipolar disorder, was rear-ended by a semi-truck causing a second collision with the car in front of him. He struck his head on the steering wheel and had a brief LOC and post traumatic confusion. He had soft tissue swelling on the right superior scalp and a whiplash injury of his neck and a disk herniation at C5-6. Neuropsychological and neurobehavioral evaluations revealed cognitive deficits in memory, processing speed and attention, in addition to personality and behavioral changes consisting of apathy, irritability, emotional disengagement, flattened affect, and anhedonia without depression. MRI was performed 2 years after injury in Detroit. No hemorrhages were detected by SWI. His global FA was 0.403 which was at the 6th percentile for age. Figure 13 compares FLAIR and DTI results after spatial normalization. Note the ventricular enlargement in the FLAIR along with multiple white matter hyperintensities (Fig. 13A), many of which are close to the cortical gray-white junction. Note the right frontal FLAIR lesion and corresponding DTI focus (blue color) denoted by the crosshairs (Fig. 13B). In our experience we find that more often than not the larger FLAIR hyperintensities also have reduced FA on DTI images suggesting that each is a marker for axonal injury, with FLAIR lesions likely indicating edema or gliosis. Figure 14 shows a volume rendering with the front of the brain removed to reveal injury to the right anterior limb internal capsule.

**Discussion:** The right anterior limb contains limbic thalamocortical fibers which are still targeted in psychosurgery (i.e., capsulotomy) for OCD and refractory anxiety [84]. S.M.’s prominent emotional flattening and disengagement likely at least in part related to his TBI-induced right internal capsule lesion. His loss of cognitive efficiency, nearly ubiquitous in TBI, is due to the bilateral diffuse axonal injury [85] and is not well localized.

**Case 3:** C.S. was a 36 year old former professional football fullback who played 11 years in the league and reported more than 50 episodes of transient visual loss after head collisions. He was imaged as part of a research study two years after retirement. Imaging revealed no evidence of hemorrhaging but he did have
cavum septum pellucidum with large ventricles and a thin corpus callosum. DTI voxel and tract based analysis revealed reduced FA only in the splenium of the corpus callosum (Fig. 15).

Discussion: His congenitally small splenium may have rendered him vulnerable to the effects of repeated trauma to this region which is often injured in closed head injury [86]. The posterior ventral splenium is comprised of fibers which reciprocally connect areas 17 and 18 of the occipital lobes [87] and lesions here have been reported to cause transient loss of central vision [88].

Case 4: B.J. was a 43 year old right handed woman who was sitting in her parked car with one foot outside the car when a semi-truck struck the rear of her car hard. She had no LOC but was dazed and speaking slowly to witnesses. Clinically she had whiplash, a herniated disk at C3-4, cognitive slowing, stuttering, emotional lability (pseudobulbar affect) and loss of fine motor coordination. Her MRI twenty-one months after injury revealed on SWI a 5 mm long venous microhemorrhage in the left temporoparietal white matter (TP-WM) while her DTI revealed reduced FA in a number of regions including the same deep white matter region and bilateral corticospinal tracts. Figure 16A reveals the microhemorrhage (encircled) while Fig. 16B shows that the FA is reduced in the same region in addition to reduction of FA in bilateral corticospinal tracts at the level of the internal capsule.

Discussion: Although not common in mTBI, expressive speech can be affected including stuttering. This woman who was hit unexpectedly had axonal and vascular injury in the left TP white matter in addition to axonal injury in both corticospinal tracts. We speculate that her motor speech problem was the consequence of injury to both her language system (left TPWM) and the corticospinal tract which controls fine motor movements. In addition, our experience is that the corticospinal tract often shows reduced FA in the context of rear and front end impacts with resulting flexion/extension injuries of the neck and whiplash syndrome. This mechanism is described in animal models of closed head trauma as well [89]. Furthermore, we have observed an association between whiplash, corticospinal tract injury and emotional dyscontrol which may be a forme fruste of pseudobulbar affect [90] which is an under-recognized symptom of motor system disorders.

4.6. Summary and conclusions

1. There is a need and urgency to more sensitively detect and diagnose milder brain injuries which have significant consequences in an increasingly technologically driven society.
2. Advanced magnetic resonance imaging methods are capable of detecting several of the pathoanatomical and pathophysiological consequences of brain trauma.
3. SWI is very sensitive to alterations in tissue susceptibility as occurs in hemorrhage and has the requisite resolution to detect small venous (micro) hemorrhages which occur in diffuse vascular injury.

4. DTI complements SWI in revealing axonal injury by detecting altered water flow in swollen and truncated axons.

5. FLAIR detects a smaller percentage of axonal injuries than DTI which likely reflects additional insult or stress to injured axons.

6. SWI, DTI and FLAIR as biomarkers of regional brain injury allow for correlation with clinical signs and symptoms.

7. These biomarkers of regional brain injury which are present on acute imaging can inform clinicians about severity as well as which neurobehavioral systems are involved in injury. Analogous to stroke rehabilitation, having an understanding of the spatial distribution of brain injury should ultimately allow for development of more effective rehabilitation strategies rooted in new clinical classification schemes based as much on neuroanatomical information as neurocognitive and neurobehavioral deficits.

8. In addition to improving diagnosis and outcome prediction, it is likely that advanced MRI imaging of TBI will be the basis of a more specific, pathologically based system of classification of TBI which should improve the efficiency of interventional trials, which will hopefully translate into the first clinically effective primary treatments for TBI.

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References


