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Back to the future: Estimating pre-injury brain volume in patients with traumatic brain injury

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ABSTRACT

Introduction: A recent meta-analysis by Hedman et al. allows for accurate estimation of brain volume changes throughout the life span. Additionally, Tate et al. showed that intracranial volume at a later point in life can be used to estimate reliably brain volume at an earlier point in life. These advancements were combined to create a model which allowed the estimation of brain volume just prior to injury in a group of patients with mild or moderate traumatic brain injury (TBI). This volume estimation model was used in combination with actual measurements of brain volume to test hypotheses about progressive brain volume changes in the patients.
Methods: Twenty six patients with mild or moderate TBI were compared to 20 normal control subjects. NeuroQuant® was used to measure brain MRI volume. Brain volume after the injury (from MRI scans performed at t1 and t2) was compared to brain volume just before the injury (volume estimation at t0) using longitudinal designs. Groups were compared with respect to volume changes in whole brain parenchyma (WBP) and its 3 major subdivisions: cortical gray matter (GM), cerebral white matter (CWM) and subcortical nuclei + infratentorial regions (SCN + IFT).
Results: Using the normal control data, the volume estimation model was tested by comparing measured brain volume to estimated brain volume; reliability ranged from good to excellent. During the initial phase after injury (t0–t1), the TBI patients had abnormally rapid atrophy of WBP and CWM, and abnormally rapid enlargement of SCN + IFT. Rates of volume change during t0–t1 correlated with cross-sectional measures of volume change at t1, supporting the internal reliability of the volume estimation model. A logistic regression analysis using the volume change data produced a function which perfectly predicted group membership (TBI patients vs. normal control subjects).
Conclusions: During the first few months after injury, patients with mild or moderate TBI have rapid atrophy of WBP and CWM, and rapid enlargement of SCN + IFT. The magnitude and pattern of the changes in volume may allow for the eventual development of diagnostic tools based on the volume estimation approach.

Introduction

Decades of research have shown that traumatic brain injury (TBI) causes brain atrophy (Bigler, 2005, 2011). Despite this impressive body of work, brain structural studies before and after injury are rare. To our knowledge, there have been only two studies published using quantitative structural brain imaging before and after injury. In total, the 2 studies examined 2 patients with severe TBI who showed progressive brain atrophy and 4 patients with mild TBI who did not (Bigler and Snyder, 1995; Gale et al., 1995). The small number of patients and limited (by today's standards) volumetric methods may have decreased the ability to detect abnormalities in the patient group. In contrast, more recent longitudinal studies of mild or moderate TBI patients, which examined brain structure at two points after injury, have consistently found abnormalities (Hofman et al., 2001; MacKenzie et al., 2002; Ross et al., 2012a, 2012b, 2012c; Zhou et al., 2013).

The lack of studies before and after injury is understandable for several reasons. Since it is not known when an accident will occur, usually an MRI cannot be obtained just before the accident. Also it would be impractical to get baseline MRI scans on large groups of normal subjects and then study the small percentage who would have a TBI afterwards. However, it would be possible to overcome these challenges, at least in part, if it were possible to reliably estimate brain volume just before injury.

Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; CWM, cerebral white matter; GM, cortical gray matter; ICV, intracranial volume; IFT, infratentorial; MRI, magnetic resonance imaging; SCN, subcortical; t0, time of injury; t1, time of first MRI scan after injury; t2, time of second MRI scan after injury; TBI, traumatic brain injury; WBP, whole brain parenchyma.

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For many years, it has been known that normally the brain and skull change volume with a characteristic pattern throughout the life span (Courchesne et al., 2000). The brain and skull reach maximal volume around age 13, with the skull growing to be just big enough to cover the brain. Whole brain volume then changes relatively little overall until about age 35, when it begins to decrease. During later adulthood, the rate of atrophy progressively increases. In contrast, intracranial volume does not change during adult life. Based on these observations, Tate et al. (2011)—following the lead of Blatter et al. (1995)—showed that brain volume at an earlier point in life can be estimated reliably from intracranial volume measured later in life.

Further progress toward building a volume estimation model was achieved by Hedman et al., who conducted a meta-analysis of 56 studies (which included 2211 normal control subjects) of longitudinal change in MRI brain volume throughout the life span. Using curve-fitting regression techniques, they produced growth/atrophy curves for whole brain parenchyma (WBP), cortical gray matter (GM) and cerebral white matter (CWM). Thus, they created models which allow for accurate estimation of brain volume changes throughout the life span.

By considering combining the work of these researchers, it seemed possible to create a volume estimation method which could be used to test hypotheses about patients with traumatic brain injury (TBI). Intracranial volume could be measured in TBI patients (after the accident) and used to estimate brain volume for each patient just before the accident. The reliability of this method could be tested in a group of normal control subjects.

Accordingly, the aims of the current study were as follows: (1) develop methods for estimating brain volume throughout the life span; (2) use total intracranial volume (ICV), in combination with the Hedman growth/atrophy curves, to predict brain volume just before injury (t0); (3) compare TBI patients to normal control subjects, using longitudinal changes in brain volume (from t0 to t1), to test the hypothesis that patients have more rapid volume changes than normal control subjects; and (4) explore the relationship between longitudinal changes (t0–t1) and traditional brain volume measures (t1 cross sectional measures, and t1–t2 longitudinal measures).

Methods

Subjects

Patients

Selection criteria. Included in this study were outpatients consecutively admitted to the Virginia Institute of Neuropsychiatry who had mild or moderate TBI and no medical or neuropsychiatric disorders which would affect brain volume or its measurement with MRI. For details, see Ross et al. (2012a, 2012b, 2012c), or Inline Supplementary Methods 1.

This study was approved by the New England Institutional Review Board and satisfied the requirements of the Code of Ethics of the World Medical Association (Declaration of Helsinki) for human research.

Description of patient sample. 26 patients met the selection criteria. Demographic characteristics were as follows: fifteen men and eleven women; mean number of years of education was 14.3 (SD 2.7; range 10–20); mean age in years at the time of the injury was 45.3 (SD 9.7; range 25.3–62.0); mean age in years at the time of the first MRI scan was 47.0 (SD 9.5; range 29.6–62.9).

A subset consisting of 21 patients had a second MRI scan. The mean age in years at the time of the second MRI scan was 48.7 (SD 9.1; range 30.1–63.7). The mean duration between the first and second MRIs was 0.70 years (SD 0.47; range 0.32–2.59).

A subset consisting of thirteen patients had a third MRI scan; mean age in years at the time of the third MRI scan was 50.7 (SD 8.0; range 33.8–64.2). The mean duration between the second and third MRIs was 0.65 years (SD 0.26; range 0.32–1.12).

Causes of injury included motor vehicle accident (N = 23), motor vehicle vs. pedestrian (N = 1), train accident (N = 1) and others (metal gate fell on head) (N = 1).

24 patients had mild TBI and 2 patients had moderate TBI. The mean GCS score was 14.7, median 15.0, range 11–15. The mean duration of loss of consciousness was 3.8 min, median 0, range 0–30 min. The mean duration of posttraumatic amnesia was 21.1 min, median 4.0, range 0–90.

Regarding other neuropsychiatric symptoms due to the brain injury, in general, the sample of patients had a wide range of chronic symptoms including impaired cognition, impaired mood, impaired sleep and wakefulness, posttraumatic stress disorder and pain, which caused them to seek treatment at a TBI specialty outpatient clinic.

Normal control subjects

CorTechs Labs normal control subjects were not used in the current study. NeuroQuant® software, produced by CorTechs Labs, Inc., was used to analyze MRI brain volume in this study (see the NeuroQuant® software was used for brain volume measurement section). The NeuroQuant® program is associated with its own normal control database developed by CorTechs Labs. However, although the standard NeuroQuant® computer-automated analysis provided volume data on over 20 brain regions (http://www.cortechs.net/products/neuroquant.php and Brewer, 2009), it provided comparisons to the CorTechs normal control group for only 3 brain regions. Otherwise, the normal control data in the CorTechs Labs database were not publicly available and were not made available for the current study.

ADNI normal control subjects were used in the current study. Therefore, in order to assess NeuroQuant®'s ability to detect changes in other brain regions, this study used a group of normal controls different from the CorTechs Labs normal controls. The normal control data for the current study were obtained from a larger group previously studied as part of the Alzheimer's Disease Neuroimaging Initiative (ADNI) (Jack et al., 2008; Petersen et al., 2010; Weiner et al., 2010). The ADNI normal control data were obtained from an online database which had been made publicly available (http://adni.loni.ucla.edu).

For information required in publications based on ADNI data, see Inline Supplementary Methods 2.

The ADNI normal control subjects were selected to be healthy and free of cognitive problems. For details, see Jack et al. (2008), Petersen et al. (2010), and Weiner et al. (2010).

Description of normal control sample. For the NeuroQuant® analyses reported herein, a subgroup of 20 normal control subjects (10 men, 10 women) was chosen from the ADNI database. The mean age at the time of the first MRI scan was 68.3 years (SD 3.6 years; range 60.0–71.5), the mean interval between the first and second MRI scans was 1.13 years, and the mean number of years of education was 16.0 (SD 3.1; range 9–20).

Comparing patients and normal control subjects

The groups of patients and ADNI normal controls did not differ significantly with respect to sex (Pearson Chi-Square = .27, df = 1, P = .60).

Distributions of age data were not normal for the normal controls (Shapiro–Wilk statistic = 0.80, df = 20, P = .001). Therefore, in order to compare the two groups with respect to age, a nonparametric test (Wilcoxon) was chosen. The normal control subjects were significantly older than the patients (Chi-Square = 32.8, P < .0001).

The two groups did not differ significantly with respect to years of education (independent t-test, t = −1.87, df = 44, P = .07).

Brain imaging

NeuroQuant® software was used for brain volume measurement

MRI brain volume was measured using NeuroQuant®, a computer-automated method (http://www.cortechs.net/products/neuroquant.php). The U.S. Food and Drug Administration (FDA) cleared NeuroQuant® for the routine clinical measurement of brain MRI volume in human subjects. NeuroQuant® has been reported to be reliable for measuring brain volume in normal subjects, patients with TBI, and other neuropsychiatric patients (Brewer, 2009; Huppertza et al., 2010; Kovacevic et al., 2009; Ross et al., 2012a, 2012b, 2012c).

Magnetic resonance imaging

Each patient had a 3.0 Tesla MRI of the brain performed at one of various radiology centers using the scanning protocol recommended for allowing later NeuroQuant® analysis; this protocol is described in detail on the NeuroQuant® website (http://www.cortechs.net/products/neuroquant.php). In addition to the general requirements for having an MRI (e.g. having no magnetic metal in the head), the NeuroQuant® protocol required, at a minimum, the following:

- Supported MRI scanner (GE, Siemens, or Phillips)
- MRI scanning protocol based on the ADNI scanning protocol
- T1 timing sequence
- Non-contrast
- Sagittal
- 3D
- Scan included nose, ears and vertex without wrap around.

For further details regarding scanning parameters, see the CorTechs Labs website http://www.cortechslabs.com/wp-content/uploads/2014/03/ScannerSetup_3-5-14.pdf.

NeuroQuant® automated brain MRI segmentation

The brain MRI data for each patient or ADNI normal control was uploaded to the NeuroQuant® server, which processed and analyzed the brain imaging data. This computer-automated analysis involved several steps, including stripping the brain of scalp, skull and meninges; inflating the brain to a spherical shape; mapping the spherical brain to a common spherical space shared with the Talairach atlas brain (Talairach and Tournoux, 1988); identification of brain segments (that is, regions); and deflation of the patient's brain back to its original shape while retaining the identifying information for brain segments. The output of the NeuroQuant® computer-automated analysis included a report which contained volumetric information, and a set of DICOM-formatted brain images which were segmented, with each region identified by a distinctive color.

NeuroQuant® brain volume measures used for the current study included whole brain parenchyma (WBP), cortical gray matter (GM), cerebral white matter (CWM), brainstem and cerebellum. In addition, a combined region including subcortical nuclei (SCN) and infratentorial regions (IFT) was calculated (see the Calculation of volume of subcortical nuclei and infratentorial regions (SCN + IFT) section).

The NeuroQuant® segmented DICOM images were inspected for errors, a step recommended by the makers of NeuroQuant® in order to ensure accurate identification of brain regions by the software. The segmentation results for each region were visually inspected by two of the authors. If a region was identified inaccurately by the NeuroQuant program, it was omitted from the subsequent analyses. For the current study, no errors were found for whole brain parenchyma or cerebral white matter. One patient had data for cortical gray matter omitted due to segmentation errors.

NeuroQuant® brain volume analysis

Brain volume change (% change per year) was calculated as follows. For each brain region, the percentage volume difference was determined by subtracting the volume at time 1 from the volume at time 2, dividing the result by the volume at time 1, and expressing the resulting proportion as a percentage. The annual rate of volume change was calculated by dividing the percentage volume difference by the duration between scans (measured in years).

In the current study, the patterns of volume change among brain regions were tracked over time (see the Application of the volume estimation model to the TBI patients section). To assist in understanding these patterns, brain volume acceleration (% volume change per year per year) was calculated, as follows. For each brain region, the volume change (% change per year) at time 1 was subtracted from the volume change at time 2, and the result was divided by the volume change at time 1. This result was annualized by dividing it by the duration between scans (measured in years).

Estimation of brain volume across the life span

Replicating the volume change vs. age model(s) of Hedman et al.

To estimate brain volume just before the accident, data from Hedman et al. (2012) were used (with permission kindly given by Dr. Hugo Schnack). Specifically, the brain volume growth/atrophy curves (plotted over the life span) which can be seen in Hedman Fig. 2 (WBP % volume change per year vs. age), Fig. 4a (GM % volume change per year vs. age) and Fig. 4b (CWM % volume change per year vs. age) were used.

The first step in the creation of the volume estimation model(s) used in the current study involved exactly replicating Hedman et al.'s growth/atrophy curves. Data below age 22 were not used because Hedman et al. reported uncertainty about the curve fit/regression lines for data below that age.

Integrating the volume change vs. age model(s) to produce volume vs. age model(s)

The next step was integration of the volume change vs. age data to produce volume vs. age data. This step followed the lead of Hedman et al., who used starting data (WBP volume) and integration of their volume change vs. age model (shown in their Fig. 2; thick line) to produce volume vs. age data (Hedman Fig. 3), with the curve in Fig. 3 being the integral of Fig. 2.

For the current study, the same approach was used for each of the 3 brain volume estimation models (WBP % volume change per year vs. age; GM % volume change per year vs. age; and CWM % volume change per year vs. age). For each model, the mean volume and mean age of the normal controls (68 years old) were entered initially, and brain volume at other ages throughout the life span was calculated by integrating (summing) over time.

For example, for the WBP volume model, the mean WBP volume for our sample of normal control subjects was 1102 cm^3 (Fig. 1). This was entered into the model at age 68 years, the mean age of the sample. The model then calculated (estimated) WBP volume from age 22 to 88 years (Fig. 1). For details, see Inline Supplementary Methods 3.

Integration of Hedman and Tate models to map %ICV onto volume (cc)

Another goal of the current study was to estimate brain volume based on subjects' total intracranial volume (ICV), following the lead of Blatter et al. (1995) and Tate et al. (2011). This approach was necessary for estimating the brain volume of the TBI patients just before the date of injury (see below).

Blatter, Tate and colleagues showed that, because ICV does not change during adulthood, and because brain volume changes in predictable ways, there is a predictable relationship between ICV and brain volume across the life span. For example, if total ICV is known during later adult life, maximal brain volume can be predicted, or estimated, at an earlier point in life. In the current study, this idea was expanded by combining the growth/atrophy curves of Hedman et al. to allow prediction, or estimation, of brain volume at any point in the adult lifespan. This integration of the Hedman and Tate models is described below.

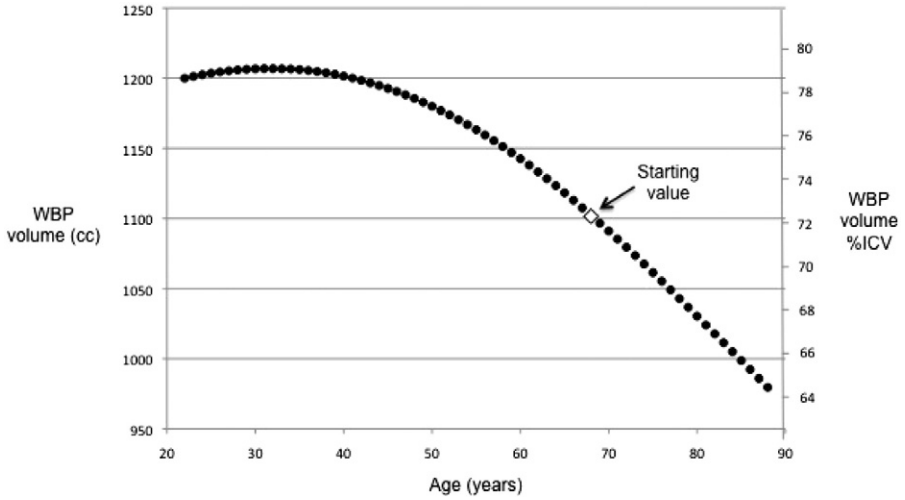

Fig. 1. Graph of whole brain parenchymal (WBP) volume across the life span, using the volume estimation model. The starting value was the mean WBP volume for the sample of 20 normal control subjects (1102 cm^3) at the mean age of 68 years. The mean total ICV (right axis) of 72.2%. The model then calculated by integration of WBP volume and %ICV across the life span.

For each volume vs. age model, the relationship between ICV and brain volume was determined for our sample of normal control subjects as follows. For each year of age, brain volume as a percentage of ICV was calculated by dividing the brain volume by ICV and expressing the result as a percentage. This approach was used to map %ICV (Fig. 1, right axis) onto brain volume measured in cm^3 (left axis) across the range of brain volumes. For details, see Inline Supplementary Methods 4.

Regarding the other two volume change models, starting values were 459 cm^3 for GM, and 436 cm^3 for CWM, both also entered at the age of 68.3 years.

Thus established, the volume vs. age model could be applied to new normal subjects (including TBI patients before injury; see below) by entering age and total ICV. The model would use age to determine the expected brain volume expressed as %ICV; this would be multiplied by total ICV to calculate (estimate) brain volume in cc.

Testing the reliability of the integrated volume estimation method

Because not all our normal control subjects were age 68 (their ages varied from 60 to 72 years), the reliability of the above approach could be tested. Each model was tested by considering the subjects as individuals. Thus, for each brain volume measure (WBP, GM and CWM), the respective volume estimation model was used by entering the ICV and age data for each normal control subject. Brain volume data were estimated across the life span, but data of primary interest at this point were the estimated values at the age at which MRI data were actually obtained on the normal control. These estimated volume data were compared to the actual (measured) volume data at the same age. The tests showed high reliability between estimated and measured brain volume for WBP (ICC = .95, df = 19, P < .001), GM (ICC = .81, df = 19, P < .001) and CWM (ICC = .89, df = 19, P < .001) (Figs. 2, 3 and 4).

Application of the volume estimation model to the TBI patients

Estimating brain volume in patients. Each model then was used for each TBI patient to estimate brain volume on the date of injury (that is, just prior to injury) by entering into the model the patient's age on that date, and ICV (which presumably did not change across the adult lifespan), then calculating (estimating) brain volume in cm^3. An

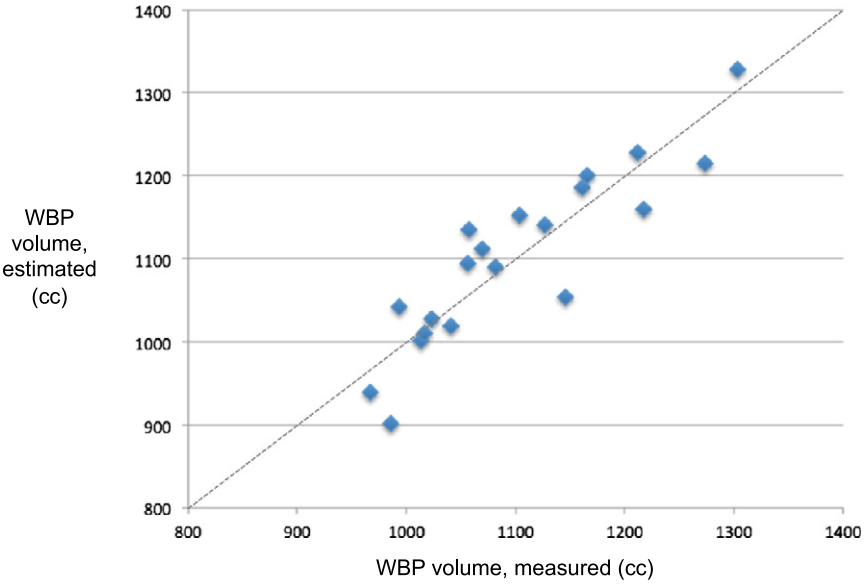

Fig. 2. Graph of measured whole brain parenchymal (WBP) volume vs. estimated WBP volume for the normal control subjects. Using the estimation model of WBP volume change over the lifespan, and its relationship to total intracranial volume (ICV), WBP volume was estimated for each normal control based on ICV. The estimated values correlated highly with measured values (ICC = .95), supporting the reliability of the model.

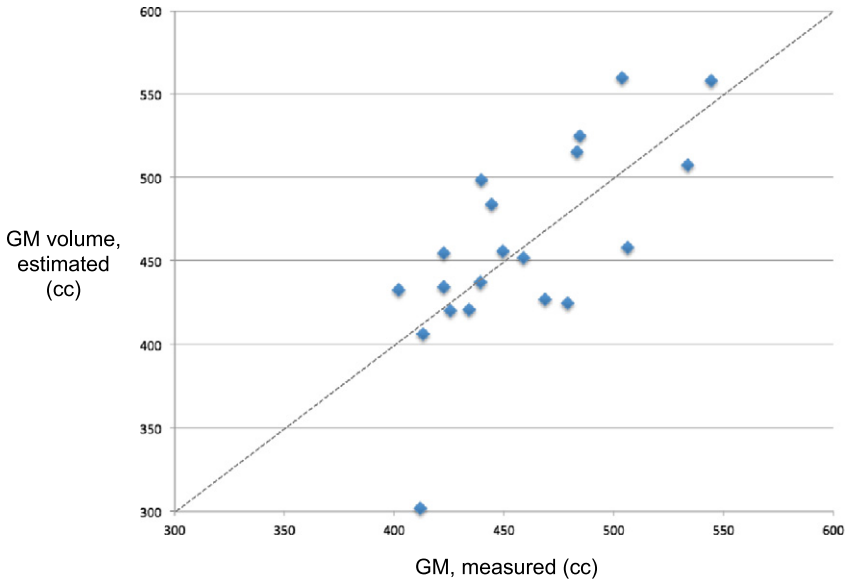

Fig. 3. Graph of measured cortical gray matter (GM) volume vs. estimated GM volume for the normal control subjects. Using the estimation model of brain volume change over the lifespan, and its relationship to total intracranial volume (ICV), cortical gray matter (GM) volume was estimated for each normal control based on TICV. The estimated values correlated highly with measured values (ICC = .81).

example of the application of the volume estimation model to a TBI patient is shown in Fig. 5. For details, see legend for Fig. 5 and Inline Supplementary Methods 5.

Estimating change of brain volume in patients. The next step was to estimate change in brain volume from before to after the injury. For each patient, change in brain volume from the date of injury (t0) to the first MRI after injury (t1) was calculated in the usual manner, that is, by subtracting the volume at t1 from the volume at t0, dividing the result by the volume at t0, expressing the result as a percentage, and annualizing the result by dividing by the interscan duration (in years).

Creation of age-adjusted normal data for comparison with TBI patients

Rationale. The volume estimation models also provided the opportunity to adjust the brain volume data of the normal controls to match the age of the patients. Since the results of Hedman et al. were based on brain imaging studies in 2211 healthy subjects, using sophisticated techniques, the results reflected essentially the population values for normal brain volume change over the life span. As such, the models could be used to adjust the brain volume of our normal controls to any desired age, at least for ages greater than 22 years. Therefore, for comparisons between patients and normal control subjects, the brain volume data

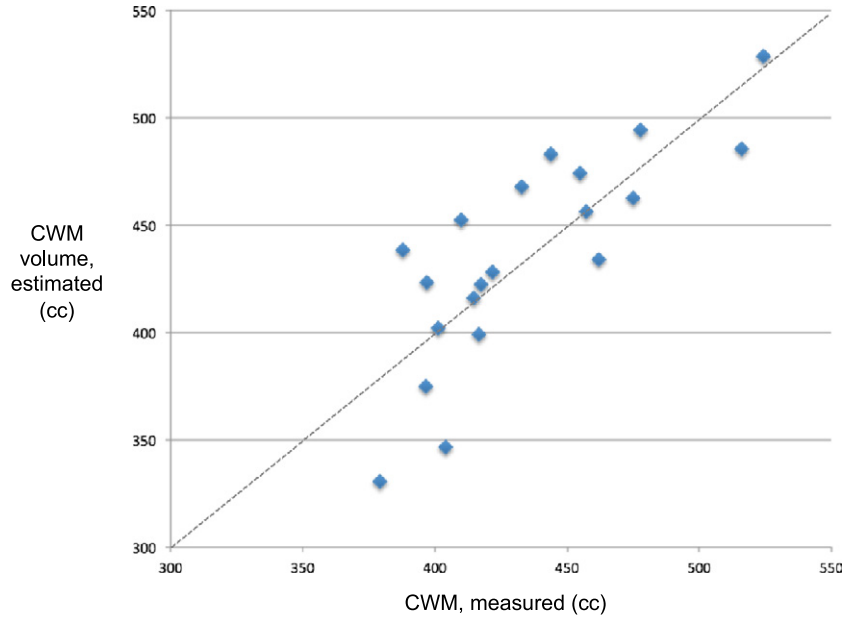

Fig. 4. Graph of measured cerebral white matter (CWM) volume vs. estimated CWM volume for the normal control subjects. Using the estimation model of CWM volume change over the lifespan, and its relationship to intracranial volume (ICV), CWM volume was estimated for each normal control based on ICV. The estimated values correlated highly with measured values (ICC = .89).

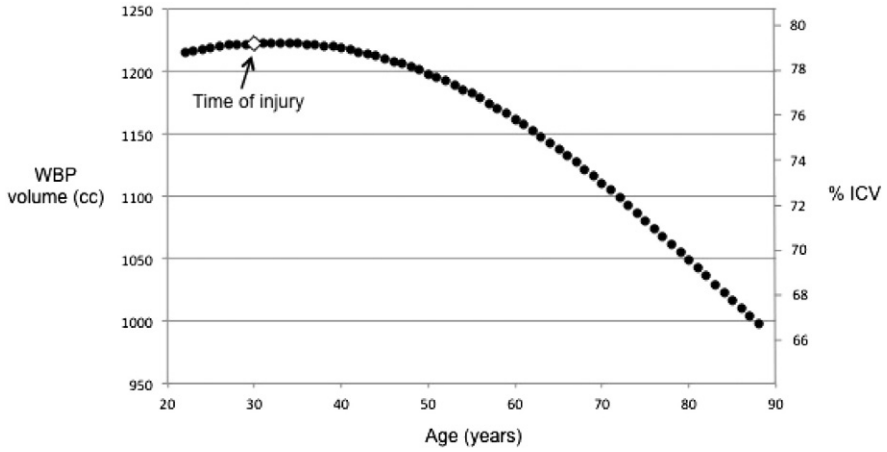

Fig. 5. Application of the volume estimation model to a patient with mild traumatic brain injury. The patient's ICV was measured to be 1546 cm^3 (which happened to be larger than the mean ICV of the normal controls (1527 cm^3)). The patient's ICV was entered into the WBP volume vs. age model, maintaining the same relationship between %ICV and age across the lifespan as was the case for the normal controls (in other words, the graphed data points are the same with respect to the right axes in this figure and Fig. 1). However, because the patient's ICV was larger than the mean ICV of the normal controls, the WBP volume of the patient was calculated (estimated) to be larger than that of the normal controls at each age (in other words, with respect to the left axes, the graphed data points in Fig. 1 were shifted up in this figure).

of the normal controls was age-adjusted accordingly. This approach was analogous to the more commonly used method of co-varying out age. However, the commonly used approach is more limited than the Hedman approach because it uses a smaller sample of normal controls (in our case, 20 subjects vs. 2211 subjects) and a more limited age range (ages 60–72 vs. ages 22–88 years) to estimate the relationship between age and brain volume for the population over the life span.

Method for age-adjusting %ICV in normals. For the normal control subjects, the age-adjustment method was applied to brain volume expressed as a percentage of ICV. This measure of brain volume was used because it corrects for interindividual differences in cranial size and sex (Bigler, 2011). For example, at t1 (the time of the first MRI after injury), the mean age of the patients was 47.0 years, so brain volume was adjusted for each normal control to age 47.0 years. For example, normal GM volume was 33.2% of ICV at that age. For our sample of normal controls, mean GM volume at that age was 507.6 cm^3 (mean) with a SD of 42.1 cm^3. However, although there was variance between normal controls with respect to age-adjusted volume measured in cm^3, there was no variance with respect to %ICV because, per the volume estimation model, each of the 20 normal controls was estimated to have a %ICV = 33.2% at that age. This raised an issue because lack of variance between normal control subjects prevented statistical tests of inference.

Therefore, it was desirable to estimate variance with respect to %ICV within the group of normal controls in order to be able to conduct statistical tests of inference. An approach was developed which used variance in %ICV in the normal controls (mean age 68.3 years) to estimate variance in the age-adjusted data (age 47.0 years in this example). For details, see Inline Supplementary Methods 6.

Because a reliably estimated SD, in addition to a mean, was available, these data could be used in statistical tests of inference.

Method for age-adjusting volume change data in normal control subjects. The age-adjustment method next was applied to brain volume change data. As noted above, each normal control subject had two MRI scans performed, at time points nt1 and nt2, with a mean interscan interval of 1.1 years. The change data from the nt1–nt2 scans were used for comparisons with the patients' t0–t1 data and t1–t2 data. For details, see Inline Supplementary Methods 7.

Although the analyses described above supported the reliability of the brain volume estimation method, because the normal control subjects were older than the patients, it was possible that the between-group differences noted in the Results section (see below) were due artifactually to the older ages of the normal controls. This potential limitation was tested by examining the correlations between the ages of the patients and the t0–t1 brain volume measures. All correlations were small and non-significant, suggesting that artifactual effects of age did not affect the t0 brain volume estimates. For details, including rationale and results of the correlation analyses, see Inline Supplementary Methods 8.

Calculation of volume of subcortical nuclei and infratentorial regions (SCN + IFT)

From a volume perspective, the Hedman models covered all major brain subregions except subcortical nuclei (SCN) and infratentorial regions (IFT) (Fig. 6). However, the volume for this set of brain regions (SCN + IFT) could be calculated by using the following formula: SCN +-IFT = WBP − GM − CWM.

Statistical analyses

Comparisons between groups

For between-group comparisons (TBI patients vs. normal controls) independent samples t-tests were used. For each comparison, Levene's test was used to test for equality of variances between groups. If variances differed significantly, then an unequal-variance t-test was used. For cases in which there were more severe violations of the assumptions necessary for parametric tests, the Wilcoxon Rank Sum (nonparametric) test was used to compare groups.

A nominal logistic regression analysis was done to attempt to use brain volume change measures to predict group membership (TBI patients vs. normal controls). This model was chosen over a discriminant function analysis because the data were not normally distributed.

Analyses within the patient group

For correlations within the patient group, the brain volume data violated assumptions necessary to perform parametric tests. Therefore, Spearman's rank-order (nonparametric) tests were used.

Comparisons of individual patients to the group of normal control subjects for t0–t1

Rationale. The large majority of peer-reviewed published medical studies are group studies which do not apply the results to individual patients. This focus on groups has the advantage of maximizing power and helping to understand the nature and causes of disease. However,

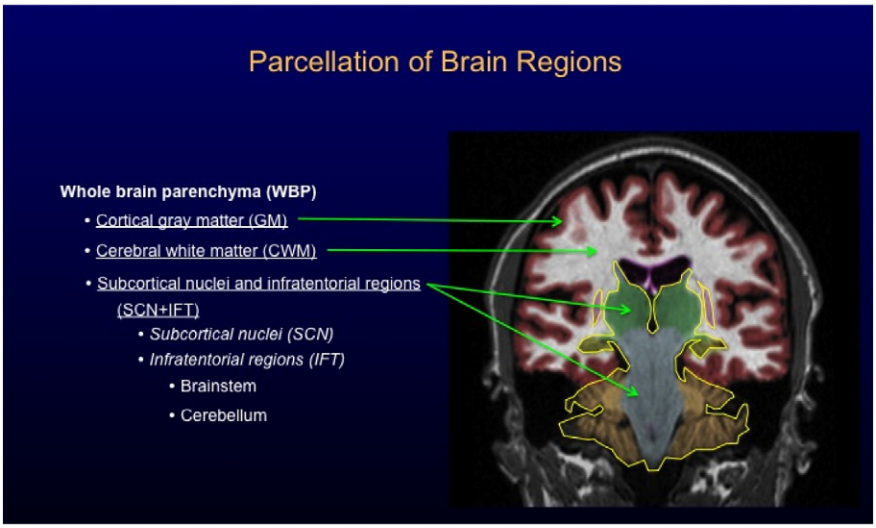

Fig. 6. Parcellation of brain regions used in this study. The Hedman models included most of the brain but did not include subcortical nuclei (SCN) or infratentorial regions (IFT). However, volume for this set of brain regions (SCN + IFT) could be calculated by using the following formula: SCN + IFT = WBP − GM − CWM. The anatomic relationship between brain regions is shown above in a coronal section of the head with NeuroQuant® segmentation (colored brain regions).

failing to apply the results to individual patients limits the ability of clinicians to apply the results in their everyday practice, which consists of working with a series of individual patients. Therefore, in the current study, individual patients were compared to the group of normal controls with respect to several brain volume measures.

Age adjustment and determination of cutoff points for abnormality at 5th percentile and 95th percentile. For each comparison between a single patient and the group of normal controls, the group of normal controls had their ages adjusted (using the method described above) to match the age of the patient, thus minimizing the effect of age on the brain volume analysis. In the next step, the mean and SD of the brain measures were determined for the normal controls at that age. Cutoffs between normal and abnormal values were defined as the lower 5th percentile rank (= 1.6449 SD below the mean) and upper 95th percentile rank (= 1.6449 SD above the mean), as described previously (Ross et al., 2012a, 2012b, 2012c, 2013).

Volume change data were annualized unless interscan interval was too short. The mean interscan interval for the normal controls was 1.1 years. Therefore, for patients whose interscan interval was greater than 1.1 years, the percentage change in brain volume was annualized by dividing by the duration of the interscan interval, and it was compared to the normal controls' annualized data.

However, for patients whose interscan interval was less than 1.1 years, the percentage change in brain volume was not annualized because it would have amplified measurement error. Therefore, the nonannualized data was used in those instances in order to reduce the rate of false positive findings. For details, see Inline Supplementary Methods 9.

Best estimate vs. conservative estimate regarding cutoff points for abnormality. The method described above produced a best estimate of whether a single patient's brain volume measure was abnormally large or small. However, for t0 data, since the brain volume was estimated and not actually measured, a question could be raised about the reliability of the estimate, because the actual brain volume, if measured, might have been smaller or larger than the best estimate.

This issue was addressed by using the normal control data to determine the accuracy (or error rate) of the volume estimate and adjusting the patient's brain volume measure accordingly. This produced a conservative estimate, designed to minimize the risk of false positive statistical findings, but increasing the risk of false negative findings. For details, see Inline Supplementary Methods 10.

Results

Findings for the interval between date of injury (t0) and first MRI scan after injury (t1)

t0–t1: comparisons between the groups of patients and normal controls

For the t0–t1 time interval, the group of TBI patients was compared to the group of normal control subjects with respect to brain % volume change per year. Wilcoxon Rank Sum tests revealed that the patients had significantly greater atrophy of the whole brain parenchyma

Table 1
Comparisons between patients and normal controls with respect to % volume change. Measurement interval for patients was t0–t1, and for normal controls it was nt1–nt2 (age adjusted). Key: SCN—subcortical nuclei. IFT—infratentorial.

Brain region	Group	(% volume change/year)		Wilcoxon Rank Sum tests			
		Mean	SD	Chi-Square	df	Sig. (2-tailed)	Effect size d
Whole brain parenchyma	Patient	−4.03	6.68	10.32	1	0.001*	−1.0
	Normal	−0.20	0.83				
Cortical gray matter	Patient	1.94	10.05	0.08	1	.78	0.4
	Normal	−0.26	2.01				
Cerebral white matter	Patient	−17.90	28.62	22.07	1	<.0001*	−1.2
	Normal	−0.11	2.27				
SCN + IFT	Patient	14.56	34.13	15.17	1	.0001*	0.8
	Normal	−0.07	1.57				

* Indicates statistically significant findings (P<.05).

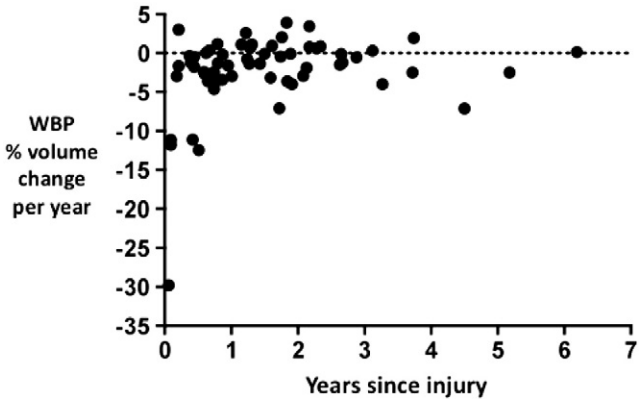

Fig. 7. Graph of WBP volume change per year vs. time after injury. Each point represents a patient's volume change data at the midpoint of the t0–t1, t1–t2 or t2–t3 time interval. Sixty data points were available for 26 patients.

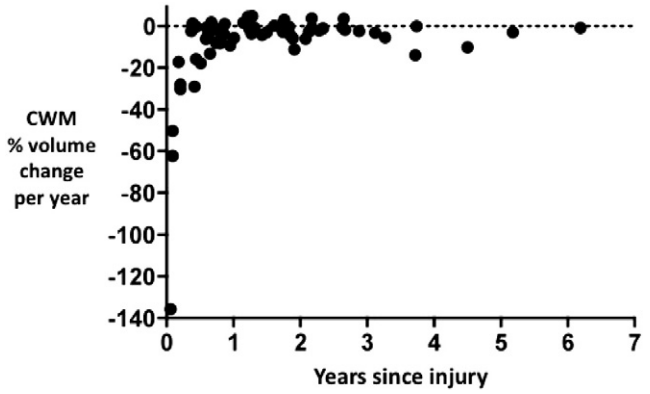

Fig. 9. Graph of CWM volume change per year vs. time after injury. Each point represents a patient's volume change data at the midpoint of the t0–t1, t1–t2 or t2–t3 time interval. Fifty-nine data points were available for 26 patients.

(WBP) and cerebral white matter (CWM), and significantly greater enlargement of subcortical nuclei + infratentorial (SCN + IFT) volume (Table 1). The associated effect sizes were large.

The rates of changes in WBP, GM, CWM and SCN + IFT were very large within the first few months after injury, and they decreased rapidly (approaching zero) over the following months and years (Figs. 7–10). Changes in GM were unique in that some patients initially had rapid enlargement of GM, while other patients initially had rapid atrophy (Fig. 8).

t0–t1: correlations within the patient group

Results of Spearman rank-order correlations between rates of brain volume change (t0–t1) are shown in Table 2. Atrophy of WBP correlated significantly with atrophy of GM and with atrophy of CWM.

Results of Spearman rank-order correlations between rates of brain volume change (t0–t1) and brain volume expressed as a percentage of ICV (%ICV) (t1) are shown in Table 3. Rates of change for each of the 4 brain regions (WBP, GM, CWM and SCN + IFT) correlated significantly with their %ICV counterpart. Enlargement of SCN + IFT also correlated significantly with large brainstem and large cerebellum.

t0–t1: comparisons of individual patients to the group of normal controls

Each patient was compared to the group of normal control subjects with respect to brain volume change (t0–t1) (Table 4). Not unexpectedly, the pattern of findings was similar to that of the group comparisons for the same time interval, with the patients having significantly more progressive atrophy of the WBP and CWM, and significantly more progressive enlargement of SCN + IFT volume. However, the findings for GM were mixed: a significant subgroup of patients had abnormally rapid decrease of GM volume, while another subgroup of patients had abnormally rapid increase of GM volume.

t1: comparisons between groups

For t1, the group of TBI patients was compared to the group of normal control subjects with respect to brain volume (%ICV). A series of independent samples t-tests revealed that the patients had significantly smaller WBP and CWM, and significantly larger SCN + IFT (Table 5). The associated effect sizes were large.

t1: comparisons of individual patients to the group of normal control subjects

Each patient individually was compared to the group of normal control subjects with respect to brain volume (%ICV) at t1 (Table 6). The pattern of results was similar to that for the group comparisons, described just above. The largest "hit rate" occurred for abnormally small CWM (61.5% of the patients were identified as having abnormally small CWM).

t0–t1, and t1: hit rate for a single MRI

It was of interest to explore the overall hit rate using volume and volume change measures based on a single MRI after injury. To accomplish this goal, the t1 volume data (Table 6) and t0–t1 volume change data (Table 3) were combined using the data most relevant to TBI (for

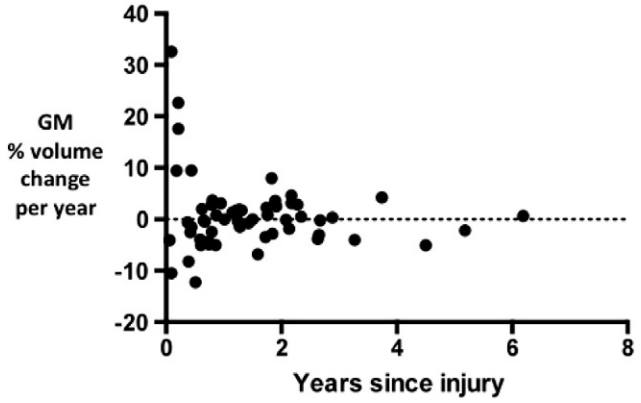

Fig. 8. Graph of GM volume change per year vs. time after injury. Each point represents a patient's volume change data at the midpoint of the t0–t1, t1–t2, and t2–t3 time intervals. Fifty-nine data points were available for 25 patients.

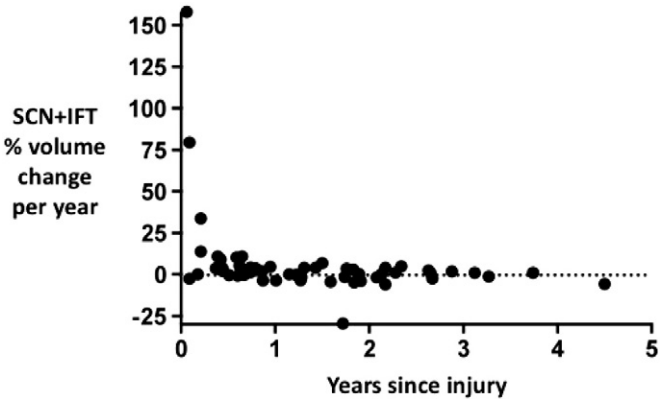

Fig. 10. Graph of SCN + IFT volume change per year vs. time after injury. Each point represents a patient's volume change data at the midpoint of the t0–t1, t1–t2 or t2–t3 time interval. Fifty-six data points were available for 26 patients.

Table 2
Spearman correlations within the patient group regarding volume change measures during the t0–t1 interval. N = 25 or 26 patients. Key: CWM—cerebral white matter. GM—gray matter. IFT—infratentorial regions. SCN—subcortical nuclei. WBP—whole brain parenchyma.

% volume change/year	% volume change/year					
	GM		CWM		SCN + IFT	
	R	P	R	P	R	P
WBP	.48	.02*	.64	.0005*	.10	.65
GM	–	–	−.14	.49	−.15	.48
CWM	–	–	–	–	−.25	.23

* Indicates statistically significant findings (P<.05).

example, tests of CWM atrophy, not enlargement). For volume change data, only conservative estimates and not best estimates were used. The combined set of data was tabulated and the overall hit rate was 88.0% (Table 7).

Findings for the interval (t1–t2) between the first and second MRI scans after injury

t1–t2: comparisons between the groups of patients and normal control subjects

For the t1–t2 time interval, the group of TBI patients was compared to the group of normal control subjects with respect to % brain volume change per year. A series of independent samples t-tests revealed that the patients had significantly greater atrophy of the whole brain parenchyma (WBP) and cerebral white matter (CWM) (Table 8). The associated effect sizes were large.

Unlike the findings for the t0–t1 interval, SCN + IFT did not continue to enlarge. In contrast, it decreased with a medium effect size (R = −0.6) albeit nonsignificantly (P = .13).

t1–t2: correlations within the patient group

Results of Spearman rank-order correlations between rates of brain volume change (t1–t2) are shown in Table 9. Atrophy of WBP correlated significantly with atrophy of GM, CWM and SCN + IFT. The correlation between atrophy of WBP and atrophy of SCN + IFT contrasted with the finding during the t0–t1 time interval in which WBP atrophy was weakly associated with SCN + IFT enlargement (R = .10, P = .65).

The data from t0–t1 and t1–t2 suggested an apparent bidirectional nature of brain volume change for SCN + IFT. Therefore, volume acceleration values were compared within the patient group. A series of Sign tests (a nonparametric counterpart to the paired t-test) showed that volume acceleration for SCN + IFT differed significantly from that of GM and CWM (Table 10).

t2: comparisons between groups

For t2, the group of TBI patients was compared to the group of normal control subjects with respect to brain volume (%ICV). A series of independent samples t-tests revealed that the patients had significantly smaller WBP and CWM, and significantly larger SCN + IFT (Table 11). The associated effect sizes were medium to large.

Summary graph (t0–t1 and t1–t2)

The primary data of the current study were summarized graphically by plotting brain volume (%ICV), % volume change per year, and volume acceleration (% change per year per year) versus years after injury (Figs. 11, 12 and 13). For reference, normative data derived from the volume estimation model were included, with the normative ages matched to the mean ages of the patients at each time point.

Nominal logistic regression

Rationale and techniques regarding logistic regression

It was of interest to attempt to predict group membership (TBI patient vs. normal control) with a single test based on a group of brain volume measures. A nominal logistic regression model was chosen because it could accommodate the nonnormal distribution of the data. Various combinations of data were tried with the goal of attempting to attain a clear separation between groups while using a small number of brain volume measures.

Optimal model and resulting equation for predicting group membership

The optimal model appeared to be one which used t0–t1 annualized rates of change of brain subregions (GM, CWM and SCN + IFT). The model showed significance overall (Chi-Square = 61.83, df = 3, P < .0001) and significant contributions by CWM (Chi-Square = 39.62, df = 1, P < .0001), SCN + IFT (Chi-Square = 23.69, df = 1, P < .0001) and GM (Chi-Square = 8.96, df = 1, P = .003). The model predicted group membership (20 normal controls and 25 patients) with 100% accuracy. The equation used to predict group membership was as follows:

$$y = 168.38 + (59.63 * \text{CWM}) - (42.29 * \text{SCN} + \text{IFT}) + (14.26 * \text{GM})$$

expressed in percentage change in brain volume per year. Lower values of y predicted membership in the TBI patient group. In other words, TBI patients had lower logistic regression scores, due to CWM atrophy, GM atrophy and SCN + IFT enlargement. The cutoff point was zero, so that subjects with logistic predictor values < 0 were predicted(or classified) as patients, and subjects with values > 0 were classified as normal controls. The mean value for normal controls was 161.2 (SD = 115.3, range = 14.3–432.0).

Testing cross-validity of logistic regression model

The cross-validity of the logistic regression model was tested using a leave-one-out design. Accordingly, the logistic regression was repeated on the group of 45 subjects (25 patients, 20 normal controls) 45 times, each time leaving one subject out, repeating the logistic regression using the same input variables, and then using the results to test the

Table 3
Spearman correlations within the patient group between volume change measures (t0–t1 interval) and %ICV (t1). N = 25 or 26 patients. Key: BS—brainstem. Cerebell.—cerebellum. CWM—cerebral white matter. GM—gray matter. ICV—intracranial volume. IFT—infratentorial regions. SCN—subcortical nuclei. WBP—whole brain parenchyma.

Brain region	% of ICV	% volume change per year							
		WBP		GM		CWM		SCN + IFT	
		R	P	R	P	R	P	R	P
Primary regions	WBP	.71	<.0001*	.45	.03*	.30	.14	.26	.21
	GM	.46	.02*	.82	<.0001*	−.21	.32	.06	.77
	CWM	.48	.01*	.01	.95	.58	0.002*	−.03	.87
	SCN + IFT	.20	.35	−.17	.41	−.05	.83	.82	<.0001*
SCN + IFT sub regions	SCN	−.08	.72	−.17	.41	.02	.94	.21	.31
	Cerebell.	.14	.50	−.17	.43	−.11	.59	.82	<.0001*
	BS	.15	.47	−.29	.15	.15	.46	.57	.003*

* Indicates statistically significant findings (P<.05).

Table 4
Comparisons between individual patients and the group of normal control subjects with respect to rates of brain volume change during t0–t1. Values represent the percentage of patients who had values beyond the cutoff point for abnormality. N = 25 or 26 patients. Key: CWM—cerebral white matter. GM—gray matter. IFT—infratentorial regions. SCN—subcortical nuclei. WBP—whole brain parenchyma.

	Percentage of patients with abnormal rates of volume change					
	<5 percentile		>95 percentile		Conservative estimates of changes most relevant to TBI	
Brain region	Best estimate	Conservative estimate	Best estimate	Conservative estimate		
WBP	50%[a]	30.8%[a]	7.7%	0.0%	WBP atrophy	30.8%[a]
GM	20%[a]	4.0%	24%[a]	12.0%	GM atrophy	4.0%
CWM	73.1%[a]	42.3%[a]	0.0%	0.0%	CWM atrophy	42.3%[a]
SCN + IFT	4.0%	0.0%	72.0%[a]	44.0%[a]	SCN + IFT enlargement	44.0%[a]
Overall hit rates	84.0%[a]	60%[a]	80.0%[a]	48.0%[a]	Overall hit rate	80.0%[a]

[a] Associated with P < .05 regarding a Chi-Square test of the hypothesis that the distribution of the data differed significantly from the distribution expected by chance alone, i.e. 5% abnormal and 95% normal.

classification of the excluded subject. The results showed that 25 out of 25 patients were predicted to be patients, and 18 out of 20 normal controls were predicted to be normal controls; i.e. two normal controls were mistakenly predicted to be patients. Thus, the test had a sensitivity of 100% and specificity of 90%.

Comparisons of individual patients to normal controls

The logistic regression equation was used to calculate predictor values for each of the TBI patients, based on their t0–t1 volume change data. Each patient was compared individually to the group of normal control subjects with respect to the predictor values. The approach used was the same as those described above for comparisons of individual patients to normal controls. Accordingly, the cutoff point for abnormality was defined as the lower 5th normative percentile, and this was calculated as an age-adjusted value according to the age of each patient being tested. For details, see Inline Supplementary Results 2.

The analyses showed that 84.0% of patients (21 out of 25) were correctly classified in the patient subgroup using the best estimate, and 80.0% of patients (20 out of 25) were classified in the patient subgroup using the conservative estimate. When the same methods were applied to the normal control group, using a leave-one-out analysis, 100% of the normal control subjects were identified in the normal control group using best estimates and conservative estimates. Thus, the best estimate method had a sensitivity of 84% and specificity of 100%, and the conservative estimate method had a sensitivity of 80% and specificity of 100%.

Examination of the relationship between logistic regression predictor values and time after injury showed that predictor values were much more negative (that is, predictive of membership in the TBI group) closer to the time of injury. In this sample of patients, all data collected with a midpoint time interval of less than 0.79 years (midpoint between t0 and MRI done at 1.58 years) were abnormal with respect to the conservative cutoff. In other words, all patients with an MRI obtained less than 1.58 years after injury were classified correctly.

Discussion

Main findings

This study was, to our knowledge, the first to examine brain volume before and after injury in patients with TBI, using modern volumetric methods, and powered with enough subjects to find abnormalities in patients with mild or moderate TBI. Brain volume before injury was estimated (not measured) using a novel technique which was tested and found to be reliable and valid.

The results showed that brain volume decreased rapidly after injury, with the decrease being driven by a rapid decrease in cerebral white matter (CWM) volume.

Surprisingly, at the same time, deeper and smaller brain regions (collectively referred to herein as subcortical nuclei + infratentorial regions, designated SCN + IFT) enlarged rapidly. The main regions driving the enlargement probably were the cerebellum and brainstem.

Over the months and years after injury, the direction of volume change in SCN + IFT reversed, switching from enlargement to diminution.

To our knowledge, this pattern of findings (rapid atrophy of CWM, initial rapid enlargement of SCN + IFT, and eventual reversal from enlargement to diminution of SCN + IFT) has not been reported previously for any brain disorder. A nominal logistic regression analysis using the main measures of volume change was able to predict group membership (TBI patients vs. normal control subjects) with perfect accuracy. These findings suggest an approach for assisting in the diagnosis of TBI using MRI brain scanning.

Development of the brain volume estimation method

The brain volume estimation method, which was used for estimating pre-injury brain volume, was tested with respect to its reliability. For the normal control subjects, estimates of brain volume were compared to actual measurements of brain volume, and the results were found to be highly reliable. This approach was limited in that the age range of the

Table 5
Comparisons between groups with respect to brain volume measures (%ICV) at t1. Key: CWM—cerebral white matter. GM—gray matter. IFT—infratentorial regions. SCN—subcortical nuclei. WBP—whole brain parenchyma.

Brain region	Group	% intracranial volume		Independent samples t-tests			
		Mean	SD	t	df	Sig. (2-tailed)	Effect size d
Whole brain parenchyma	Patient	75.31	2.98	−3.19	44	0.003*	−0.9
	Normal	77.84	2.38				
Cortical gray matter	Patient	33.28	2.02	0.08	43	.94	0.0
	Normal	33.23	2.56				
Cerebral white matter	Patient	28.00	1.94	−6.31	44	<.0001*	−1.9
	Normal	31.60	1.88				
SCN + IFT	Patient	14.02	0.84	3.95	43	0.003*	1.2
	Normal	13.01	0.86				

* Indicates statistically significant findings (P<.05).

Table 6
Comparisons between individual patients and the group of normal control subjects for brain volume measures (%ICV) at t1. Values represent the percentage of patients who fell beyond the cutoff for abnormality. N = 25 or 26 patients. Key: IFT—infratentorial regions. SCN—subcortical nuclei.

Brain region	Percentage intracranial volume	
	<5 percentile	>95 percentile
Whole brain parenchyma	26.9%[a]	0.0%
Cortical gray matter	0.0%	0.0%
Cerebral white matter	61.5%[a]	0.0%
SCN + IFT	0.0%	16.0%[a]

[a] Associated with P < .05 regarding a Chi-Square test of the hypothesis that the distribution of the data differed from that expected by chance alone, i.e. 5% abnormal and 95% normal.

Table 7
Comparisons between individual patients and the group of normal control subjects for brain volume measures (%ICV at t1, and rates of volume change from t0 to t1) most relevant to TBI. For the patients, these data were based on a single MRI after injury. Values represent the percentage of patients who fell beyond the cutoff for abnormality. N = 25 or 26 patients. Key: CWM—cerebral white matter. GM—gray matter. IFT—infratentorial regions. SCN—subcortical nuclei. WBP—whole brain parenchyma.

Brain region	Percentage intracranial volume	Rates of volume change	Overall
WBP atrophy	26.9%[a]	30.8%[a]	34.6%[a]
GM atrophy	0.0%	4.0%	4.0%
CWM atrophy	61.5%[a]	42.3%[a]	65.4%[a]
SCN + IFT enlargement	16.0%[a]	44.0%[a]	44.0%[a]
Overall hit rates	76.0%[a]	80.0%[a]	88.0%[a]

[a] Associated with P < .05 regarding a Chi-Square test of the hypothesis that the distribution of the data differed from that expected by chance alone, i.e. 5% abnormal and 95% normal.

normal controls was 60–72 years, hence not covering most of the life span. However, because the ability to use intracranial volume to predict brain volume probably would worsen as subjects age, the high reliability in our older normal controls made it likely that the procedure also would be reliable with younger normal subjects.

Additional evidence supporting the reliability, or internal consistency, of the model came from the finding that rates of volume change from t0 to t1 correlated in expected ways with cross-sectional volume measures at t1 (Table 3). For example, greater rates of whole brain parenchymal (WBP) atrophy from t0 to t1 correlated with decreased WBP volume at t1. These findings supported the volume estimation model because the estimates correlated in expected ways with actual volume measures.

Findings for the interval between time of injury (t0) and first MRI scan after injury (t1)

The set of t0–t1 analyses showed a consistent pattern of results with respect to WBP, CWM and SCN + IFT. After injury, WBP decreased rapidly, consistent with many previous studies which have found that WBP volume decreases after TBI (for review, see Bigler, 2013; Ross, 2011).

Most of the decrease in WBP volume was driven by a rapid decrease in CWM. This finding generally is consistent with a large number of studies which have found that TBI is characterized by white matter injury (for review, see Bigler, 2013). The rates of decrease were extremely large near the time of injury, with the CWM volume of several patients decreasing at the rate of 20% per year or more. Such rapid rates of atrophy are rarely observed in other brain disorders and, if they persisted for more than a few months, would be fatal within a couple or few years. Fortunately, these rates did not persist beyond a few months; instead they decayed rapidly.

Surprisingly, at the same time, SCN + IFT increased rapidly. The growth/atrophy curves of Hedman et al.—and therefore the volume estimation model of the current study—did not provide information about subregions of SCN + IFT, so the volumes of those subregions could not be estimated at t0. However, correlations between SCN + IFT volume change and the volume of its subregions (subcortical nuclei, brainstem and cerebellum) at t1 showed significant correlations with the brainstem and cerebellum, but not the subcortical nuclei. Those findings suggested that the progressive enlargement of SCN + IFT after injury was due to enlargement of the brainstem and cerebellum, but this conclusion should be tested in the future with more direct measurement techniques. The rates of increase were extremely large near the time of injury, with the SCN + IFT volume of several patients increasing at the rate of 10% per year or more. Similar to changes in CWM volume, such rapid rates of volume change are rarely observed in other brain disorders. To our knowledge, this is the first study to find enlargement of SCN + IFT in the months following traumatic brain injury. The reason for enlargement of SCN + IFT was unclear. Possible causes include inflammation or swelling of tissue (Bigler, 2013) or attempts by the brainstem and cerebellum to compensate for damage in other brain regions (Buckner, 2013). Alternatively, it is possible that the measurement of SCN + IFT volume was affected by contrast variations, which may influence volumetric measures based on tissue contrast. This effect is particularly true with respect to deep gray matter nuclei. It is possible

Table 9
Spearman correlations within the patient group regarding volume change measures during the t1–t2 interval. N = 19 to 21 patients. Key: CWM—cerebral white matter. GM—gray matter. IFT—infratentorial regions. SCN—subcortical nuclei. WBP—whole brain parenchyma.

% volume change per year	% volume change per year					
	GM		CWM		SCN + IFT	
	R	P	R	P	R	P
WBP	.66	0.002*	.62	0.004*	.59	0.008*
GM	–	–	−.12	.62	.22	.37
CWM	–	–	–	–	.39	.11

* Indicates statistically significant findings (P<.05).

Table 8
Comparisons between patients and normal controls with respect to % volume change. Measurement interval for patients was t1–t2, and for normal controls it was nt1–nt2 (age adjusted) N = 19 to 21 patients. Key: SCN—subcortical nuclei. IFT—infratentorial.

Brain region	Group	(% volume change/year)		Independent t-tests			
		Mean	SD	t	df	Sig (2-tailed)	Effect size d
Whole brain parenchyma	Patient	−1.54	2.87	−2.02	23.5	.05*	−0.7
	Normal	−0.22	0.83				
Cortical gray matter	Patient	−0.68	3.80	−0.40	27.1	.69	−0.1
	Normal	−0.29	2.02				
Cerebral white matter	Patient	−2.86	4.72	−2.30	27.3	0.03*	−0.8
	Normal	−0.17	2.27				
SCN + IFT	Patient	−2.70	7.19	−1.60	19.6	0.13	−0.6
	Normal	0.00	1.57				

* Indicates statistically significant findings (P<.05).

Table 10
Comparisons between rates of volume acceleration within the patient group. N = 18 patients. Key: CWM—cerebral white matter. GM—gray matter. IFT—infratentorial regions. SCN—subcortical nuclei.

Brain region	Volume acceleration (t0–t1) to (t1–t2)					
					Sign test for comparisons between brain regions within patient group	
	Normal controls (for reference only)		TBI patients		Cortical gray matter	Cerebral white matter
	Mean	SD	Mean	SD	Prob > abs (M)	
Cortical gray matter	−0.02	Not available	−10.42	27.69	–	–
Cerebral white matter	−0.05	Not available	41.11	101.10	0.24	–
SCN + IFT	0.06	Not available	−35.46	113.61	0.03*	.001*

* Indicates statistically significant findings (P<.05).

that diffusion alterations influenced contrast between deep gray and white matter, leading to volume estimation errors that might explain the unexpected increase of deep gray matter structure volume.

The changes in WBP, CWM and SCN + IFT were possibly exponential, but further studies will be needed to test this hypothesis.

The findings for cortical gray matter (GM) were not as clear as for CWM or SCN + IFT. When compared to the normal controls, the group of patients showed no significant volume change in GM during the t0–t1 interval. On the other hand, WBP atrophy correlated significantly with GM atrophy. Furthermore, the results of the comparisons between individual patients and the group of normal controls were mixed, with a subgroup of patients having GM atrophy and another subgroup having GM enlargement. It appeared that TBI caused different pathophysiological changes in these subgroups of patients for reasons which were unclear. It was possible that GM atrophy was due to death of neurons, or that enlargement was due to inflammation or swelling. Another possibility was that, since GM covered a large amount of the cerebrum, that different subregions of GM were affected differently.

Findings for the interval between the first (t1) and second (t2) MRI scans after injury

The set of t1–t2 analyses continued to show progressive atrophy of WBP and CWM, but the rates of atrophy became much smaller than during the earlier t0–t1 interval.

However, unlike the findings for the t0–t1 interval, SCN + IFT did not continue to enlarge. In contrast, it decreased with a medium effect size (R = −0.6) albeit nonsignificantly (P = .13), and it contributed significantly to WBP atrophy during the t1–t2 interval. Furthermore, volume acceleration for SCN + IFT differed significantly from that of GM and CWM. To our knowledge, there are no other brain disorders which show a similar pattern of reversal of the direction of volume change in SCN + IFT.

Nominal logistic regression

Nominal logistic regression analysis produced a model which resulted in the ability to predict group membership (TBI patients vs. normal controls) with 100% accuracy.

Based on comparisons between individual patients and the group of normal controls with respect to the logistic regression predictor values, the conservative method for predicting group membership had a sensitivity of 80% and specificity of 100%. This hit rate (80%) was the highest for any single individual test examined in this study, which was not unexpected, since that was the purpose of the logistic regression analysis.

Examination of the relationship between logistic regression predictor values and time after injury (including t0, t1 and t2 data) suggested that all or almost all patients would test positive if they had a single MRI done within 1 year after injury. However, by 2 years after injury, the hit rate probably would drop to about half of that because the acute effects of the brain injury would be more difficult to detect. Therefore, although longitudinal studies with data actually measured at both time points are ideal, the conservative estimate (based on a single MRI performed within one year of injury) appeared capable of predicting group membership (TBI vs. normal control) with close to 100% accuracy.

The set of individual tests related to t0–t1 also produced a high overall hit rate for abnormality (88.0%; see Table 7).

Limitations

A limitation of this study was that the normal control subjects were significantly older than the patients. Age is well-known to affect brain volume, causing (in our sample, beyond 60 years) two main effects: (1) atrophy of most brain regions, and (2) increasing variability of brain volume. The between-groups differences in brain volumes were corrected for differences in age by using the volume estimation method to adjust the brain volumes of the normal control subjects to ages which

Table 11
Comparisons between groups with respect to brain volume (%ICV) at t2. Key: CWM—cerebral white matter. GM—gray matter. IFT—infratentorial regions. SCN—subcortical nuclei. WBP—whole brain parenchyma.

Brain region	Group	% intracranial volume		Independent samples t-tests			
		Mean	SD	t	df	Sig. (2-tailed)	Effect size d
WBP	Patient	74.60	3.27	-3.29	36.59	.002*	-1.0
	Normal	77.54	2.39				
GM	Patient	33.33	2.30	0.43	38.03	.67	0.1
	Normal	33.01	2.57				
CWM	Patient	27.51	1.30	-7.92	33.51	<.0001*	-2.5
	Normal	31.53	1.89				
SCN + IFT	Patient	13.76	1.32	2.20	34.71	.03*	0.6
	Normal	13.00	0.87				

* Indicates statistically significant findings (P<.05).

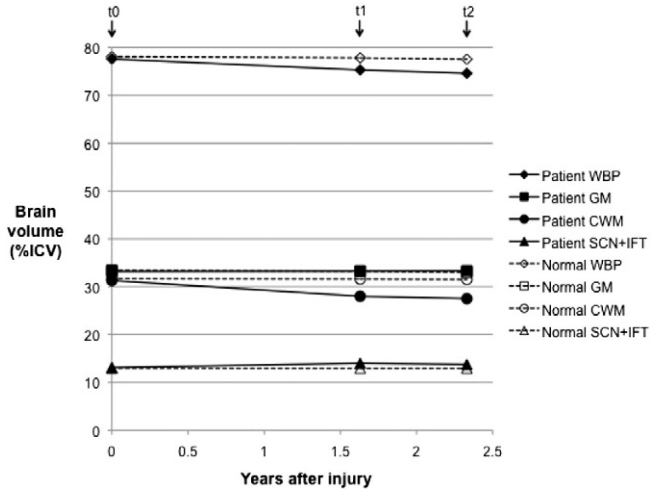

Fig. 11. Graph of brain volume (%ICV) vs. years after injury. The mean age of the patients on the date of injury (t0) was 45.3 years. The duration between t0 and t1 was 1.63 years, and the duration between t0 and t2 was 2.33 years. For reference, normative data derived from the volume estimation model were plotted for ages matching the mean patient ages at each point. Key: WBP—whole brain parenchyma. GM—cortical gray matter. CWM—cerebral white matter. SCN + IFT—subcortical nuclei and infratentorial regions.

matched the patients' ages. As mentioned in the Methods section, this technique is analogous to, but stronger than, the traditional technique of covarying out age, and it probably eliminated most differences in brain volume due to differences in age. In addition, within the patient group, lack of significant correlations between age and the brain volume change measures associated with the use of age-adjusted normal data (i.e. volume change from t0 to t1) supported the conclusion that the older age of the normal controls did not artifactually influence the main findings. Nevertheless, in future studies, it will be important to test the brain volume estimation method and the central findings of this study using age-matched normal control subjects.

Another limitation of this study was that the patients did not have their brain volume measured at the date of injury (t0); it was merely estimated. However, despite decades of research in this area, there are very few studies of brain volume before and after injury. The reasons for this limitation include the following: (1) No one knows when the injury will occur, generally making it impossible to obtain an MRI just before injury; (2) It is impractical to obtain MRIs on large groups of healthy subjects

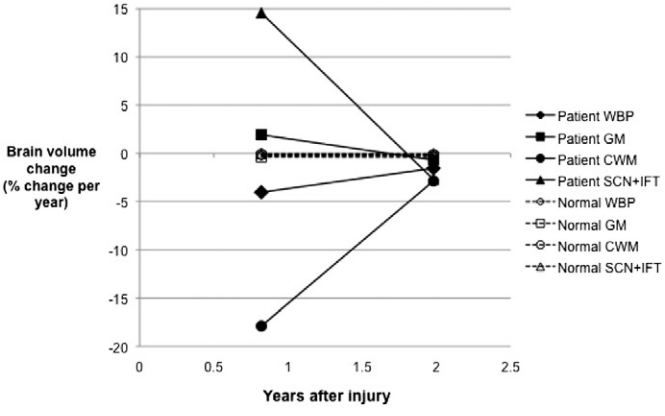

Fig. 12. Graph of brain volume change (% change per year) vs. years after injury. The first set of data was plotted at the midpoint between t0 and t1 (0.82 years after injury, mean age of patients = 46.2 years) and the second set of data was plotted at the midpoint between t1 and t2 (1.98 years after injury, mean age of patients = 47.9 years). For reference, normative data derived from the volume estimation model were plotted for the ages matching the mean patient ages at each time point. Key: WBP—whole brain parenchyma. GM—cortical gray matter. CWM—cerebral white matter. SCN + IFT—subcortical nuclei and infratentorial regions.

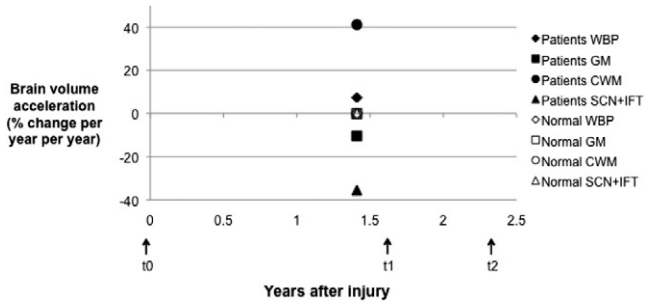

Fig. 13. Graph of brain volume acceleration (% change per year per year) vs. years after injury. The data were plotted at the midpoint (1.41 years after injury, mean age of patients was 46.0) between the t0–t1 midpoint and the t1–t2 midpoint. For reference, normative data derived from the volume estimation model were plotted for the age matching the mean patient. Key: WBP—whole brain parenchyma. GM—cortical gray matter. CWM—cerebral white matter. SCN + IFT—subcortical nuclei and infratentorial regions.

each year in order to capture the approximately 1% per year who will suffer a traumatic brain injury which leads to persistent symptoms; (3) Although a sports model—in which pre-injury testing is performed on a group of athletes prior to the season, anticipating that a significant number of them will suffer concussions during the season—is an excellent model, it is limited in the following ways: (a) it is still only a minority of athletes who will suffer concussions leading to persistent symptoms; and (b) sports concussion may be different in important ways from other situations involving TBI, for example, victims of motor vehicle accidents, who often suffer multiple bodily injuries in addition to the concussion. Given the extraordinary difficulties involved in actually measuring brain volume just before an accident, it would be very helpful to have a reliable way of estimating the brain volume.

Another limitation of this study included the fact that the brain volume estimation technique was developed and tested, in part, on the same sample of normal control subjects. However, parts of the model were developed independently (for example, the brain volume growth/atrophy curve over the lifespan by Hedman et al. (2012); and the earlier work by Blatter, Tate and colleagues (Blatter et al., 1995; Tate et al., 2011)). Furthermore, data in the current study not involving the normal controls (that is, data involving the TBI patients) supported the validity of the approach. It will be important to attempt to replicate the findings of this study using independent samples.

The current study selected only patients who did not have pre-accident brain disorders which would have affected their brain volume. This approach was necessary to ensure that the estimated brain volume changes occurred after the time of injury. This assumption could not be made for patients who had pre-accident brain disorders which affected brain volume.

This study used NeuroQuant® software to measure brain volume. Other studies using NeuroQuant® or software found to be highly reliable with NeuroQuant would be expected to find similar results and could use similar cutoff scores for abnormality. However, other systems for analyzing brain volume may or may not find similar results and the validity and reliability of those systems would need to be established.

The current study examined patients with mild or moderate TBI who had symptoms which persisted for months to years after the injury and sought treatment at a TBI specialty clinic. Therefore, these results may not apply to other TBI patients, for example, patients with mild TBI who have complete resolution of symptoms within hours to days.

Finally, we did not correct for multiple statistical tests of inference because we judged that it was more important not to increase the rate of Type II errors (false negative findings) than to decrease the rate of Type I errors (false positive findings). There were 102 inferential tests performed, each with an alpha level set at 0.05. Fifty-four tests were significant. The number of tests expected to be significant due to chance alone was $0.05 \times 102 = 5.1$. Because the number of positive tests (54) was much higher than the number expected by chance alone (5.1),

we can be confident that the vast majority of positive findings were not due merely to chance. The best way to deal with the possibility of increased Type 1 error will be for future studies to attempt to replicate or extend the findings of the current study.

Conclusions

The approach used in the current study deserves further consideration as a means for providing diagnostic information regarding TBI, based on the following considerations: (1) the rates of CWM atrophy near the time of injury were very high, much higher than for most other brain disorders; (2) the rates of enlargement of SCN + IFT (probably reflecting enlargement of the brainstem and cerebellum) near the time of injury were very high, much higher than for most other brain disorders; (3) the opposite pattern of CWM atrophy and SCN + IFT enlargement near the time of injury may be unique among brain disorders; and (4) the opposite directions of volume accelerations of GM and CWM compared with SCN + IFT during the later stages after injury (from t0–t1 to t1–t2 interval in this study) may be unique among brain disorders.

In contrast to current methods for diagnosing TBI, which are based mostly on the patient's subjective report, the approach used in the current study is based on objective, quantitative data. Although brain volume measurement will not replace the clinically based diagnosis of TBI any time soon, we propose that MRI-based methods such as this one be tested further, especially with respect to its sensitivity and specificity, as a tool for assisting in the diagnosis of TBI.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2014.07.043.

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Conflict of interest

The authors report no financial or other conflict of interest related to the work reported herein.

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