Prospective longitudinal MRI study of brain volumes and diffusion changes during the first year after moderate to severe traumatic brain injury

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Abstract

The objectives of this prospective study in 62 moderate-severe TBI patients were to investigate volume change in cortical grey matter (GM), hippocampus, lenticular nucleus, lobar white matter (WM), brainstem and ventricles using a within subject design and repeated MRI in the early phase (1-26 days), 3 and 12 months postinjury, and to investigate the impact clinical TBI subtypes based on Glasgow coma scale (GCS) score, duration of post-traumatic amnesia (PTA), and diffusion axonal injury (DAI) grade. Changes in local diffusion properties in GM were studied to identify any associations between tissue microstructure, clinical TBI subtypes and brain volumes. Lastly we determined if MRI-derived brain volumes from the early phase and after 3 months provided additional, significant predictive value to 12-month outcome classification after adjusting for GCS and PTA.

Cortical GM loss was rapid, largely finished by 3 months, but the loss was not associated with clinical subtype. However, cortical GM volume at 3 months was the best independent predictor of 12-month outcome. The hippocampus and lenticular nucleus underwent protracted volume loss statistically significant at 12 months, with a distinct trajectory of volume decline compared to cortical GM. Hippocampal volume loss was most pronounced and rapid in individuals with PTA>2 weeks. The 3-month volumes of hippocampus and lentiform nucleus were independent predictors of 12-month outcome after adjusting for established outcome predictors. In the brainstem, volume loss was significant at both 3 and 12 months, and all time points, even the early, were associated with outcome at 12 months. Brainstem volume was associated with GCS score and the presence of DAI. Lobar WM volume was decreased first at 12 months, but still followed a similar trajectory of volume loss as cortical GM. Surprisingly DAI grade had no impact on WM volume. Ventricular dilation developed predominantly during the first 3 months, and was strongly associated with changes in brainstem and cortical GM volumes, but not lobar WM volume.

Higher ADC values were detected in cortex in individuals with severe TBI, DAI and PTA>2 weeks, from 3 months. There were no associations between ADC values and volumes, and ADC values did not predict outcome.

Keywords: Post traumatic amnesia, diffuse axonal injury, Glasgow coma scale, ADC, outcome

Introduction

General cerebral atrophy is a common consequence of moderate to severe traumatic brain injury (TBI).[1–4] This general atrophy is not predicted by focal lesion volume,[5] and develops over at least 3 years with the bulk loss occurring in the first year.[3,6] However, the trajectories of volume changes for different brain structures remain unknown. There is evidence from rodent models of closed head injury that brain region and injury type determine the histopathological response, and hence degree of tissue loss and outcome.[7–9] Longitudinal studies of structural changes following TBI in humans have so far only contained two, often poorly defined, time points of MRI,[10] which is insufficient to describe trajectories of volume loss in different brain structures. Furthermore, the impact of different clinical characteristics related to TBI subtype classification, e.g. injury severity and presence of diffuse axonal injury (DAI)/traumatic axonal injury, on the different brain structures has not been explored systematically. Hence the primary objectives of this prospective study in moderate and severe TBI patients were to investigate the trajectories of volume change in total cortical GM, hippocampus, lenticular nucleus, lobar WM, brainstem and ventricles using a within subject design and repeated MRI in the early phase, 3 and 12 months after injury, and relate the volume changes to different TBI subtypes from the early phase. Changes in local diffusion properties in GM were studied to identify any associations between tissue microstructure as described with diffusion weighted imaging (DWI), TBI subgroups and brain volumes.

Our final aim was to determine if MRI-derived brain volumes obtained in the early phase and after 3 months provided additional, significant predictive value to 12-month outcome after adjusting for established outcome predictors.

Based on the animal literature we predicted that cortical volume declined first, mainly between the early and 3-month scan. Rodent TBI studies consistently show that the

histopathological response including neuronal necrosis and apoptosis is largely completed in the cortex 3 months after injury,[11–13] although volume loss may continue for 12 months.[14] In the hippocampus, on the other hand, protracted neuronal loss lasting for at least 12 months is described in animals.[11,14,15] In addition prominent changes in synapse morphology and reduced hippocampal neurogenesis are reported.[16–18] Hippocampal volume loss is reported to be greatest during the first weeks, but continues for at least 12 months in rodents.[8,14] We predicted that hippocampal volume loss in humans following moderate-severe TBI would be protracted, and continue during the entire observation period.

For the lenticular nucleus less information is available, but quite disparate histopathological results are reported for other subcortical GM nuclei. In animals and humans both very early and slowly evolving histopathological changes have been shown.[19–21] Patient studies have documented both volume loss[22–24] and no volume change in the lenticular nucleus.[25] Taken together the data suggest that lenticular nucleus volume most likely declines slowly, and we expected to see a significant reduction at 12 months.

Lobar WM changes are described as the slowest and take place over a very protracted time period due to Wallerian/Wallerian-like degradation being lengthy and incomplete in the brain compared to the peripheral nervous system.[26–28] Significant lobar WM loss was therefore expected to develop between 3 and 12 months after injury.

Human studies have shown that the brainstem volume is reduced in the chronic phase after TBI,[3,29] but animal studies have not focused on this region. We anticipated that brainstem volumes decreased slowly, similar to the lobar WM.

TBI leads to ventricular dilation in both humans and animals. One patient study reports that the period of ventricular dilation lasts until 2-7 months postinjury with no significant increase thereafter.[6] In rodents ventricular enlargement is described to take place until 6 months post-injury.[30,31] We therefore expected the ventricular volume increase to take place mainly

between the early and 3-month scan. Ventricular dilation is considered to result primarily from WM volume loss,[32] but if ventricular dilation develops early after TBI, GM volume loss may also contribute significantly. We therefore investigated the relationship between ventricular volume change over time and the volume changes in cortical GM, lobar WM and brainstem to establish the relative importance of volume loss in these structures to ventricular dilation.

The impact of TBI subgroups on volume loss in different brain structures was assessed. Lower Glasgow coma scale (GCS) scores were expected to lead to more severe loss of lobar WM and brainstem volumes, as unconsciousness postinjury is associated with deeper lesions[33] and increasing mechanical forces converging in the midbrain.[9] Longer duration of post traumatic amnesia (PTA) (>2 weeks) was predicted to decrease hippocampal volumes significantly more than short PTA (≤2 weeks) since PTA is a manifestation of hippocampal dysfunction and hence likely degree of injury. DAI was predicted to result in increased loss of lobar WM volumes, and with increasing DAI severity brainstem volume loss. Based on postmortem evidence, DAI also gives rise to increased cortical thinning and could affect cortical volume.[34]

To investigate the impact of injury mechanisms on GM diffusion properties we used apparent diffusion coefficient (ADC) values from DWI. It has previously been demonstrated that TBI patients with lesions visible on conventional MRI have increased ADC values in the brainstem the first month after TBI,[35] while ADC values in cortex and subcortical GM increased between ~2 and ~13 months in moderate-severe TBI.[23] Thus we expected the severe TBI patients to have increased ADC values in the midbrain at 3 months while cortical ADC values would increase more slowly in both severe and moderate TBI. Since DAI leads to changes in cortical architecture with loss of neurons, neuronal soma shrinkage, axonal changes and myelin loss,[20,28,34] we expected that cortical ADC values would be higher in DAI

than non-DAI patients after 3 and 12 months. Moreover, we predicted that longer PTA would be associated with increased hippocampal ADC values due to increased cell loss.

Previous MRI studies in TBI patients have shown that localization of traumatic brain lesions is significantly associated with outcome.[36–39] Here we wanted to investigate if MRI-derived brain volumes from the early or 3-month scans have predictive power above that obtained with the established outcome predictors GCS score and duration of PTA.[40,41] Identification of biomarkers in the early phase after TBI is important to optimize rehabilitation endeavors for all levels of outcome.

Materials and methods

The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services. Written consent was obtained from the patient or, for individuals under aged or incapacitated, their next of kin. Permission was obtained from the Norwegian Directorate of Health to use data from the deceased without consent from their next of kin.

Subjects

124 moderate to severe (GCS score ≤13) TBI patients aged 14-65 years were admitted to the Neurosurgical Department, St. Olav's Hospital, Trondheim University Hospital, Norway, in the period October 10, 2004–October 10, 2007. St. Olav's Hospital is the only level 1 trauma center in a region of 680 000 inhabitants. 17 of these patients did not participate in MRI study (did not consent, could not be examined with MRI or did not participate for other reasons). An additional 20 patients died before acquisition of follow-up MRI scans. Longitudinal MRI data was thus available for 87 TBI patients. Only patients with at least 2 successful

segmentations of the T1-weighted 3d brain volumes were included in the study, therefore another 25 patients were excluded.

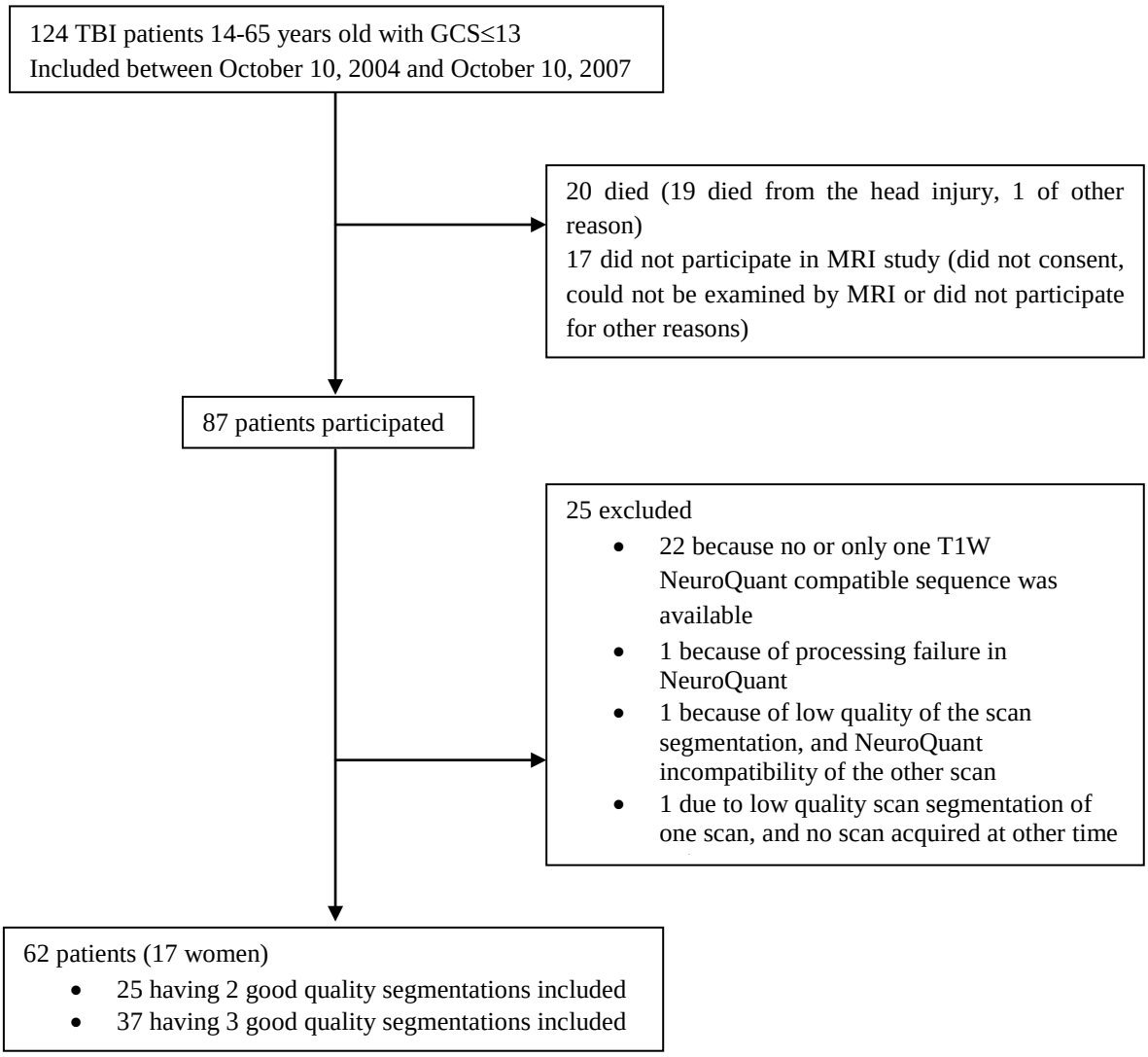
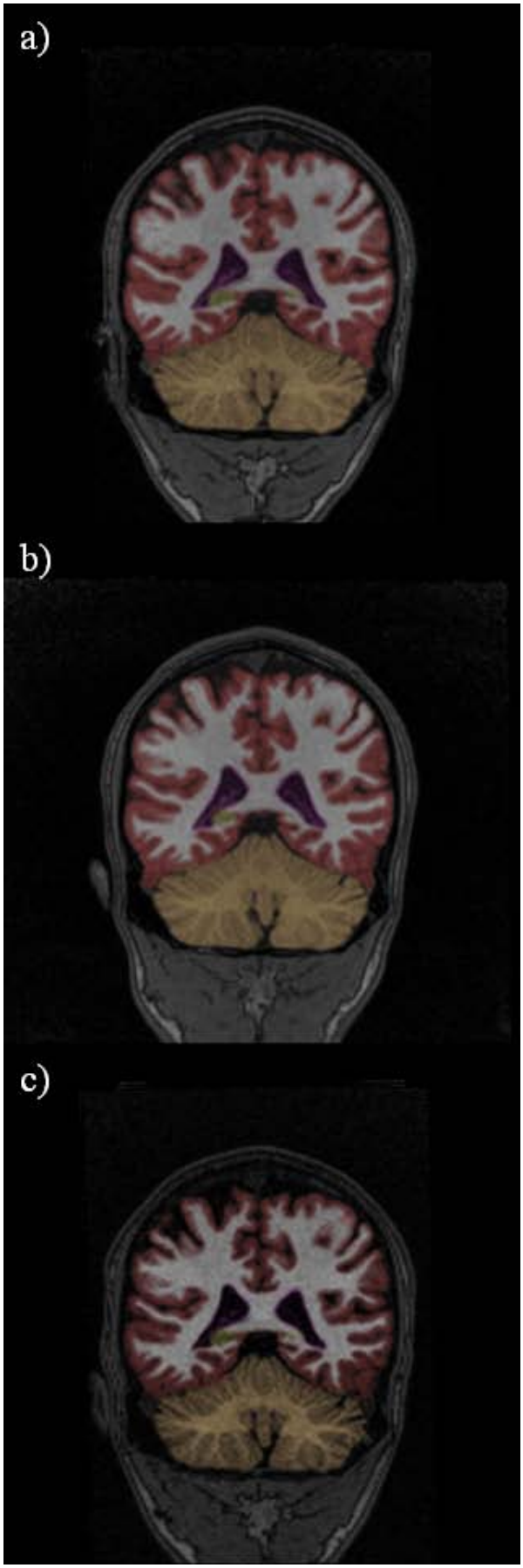


Figure 1. *TBI patients with GCS≤13 that could not be explained by other factors than traumatic head injury, admitted to Neurosurgical Department, St. Olavs Hospital, Trondheim University Hospital, Norway in the period 2004-2007. Flow chart of the inclusion and exclusion criteria, and the final number of patients participating in the current study.*



For each T1-weighted 3d scan the segmentation quality was assessed visually and scans were excluded if hematomas were included as brain tissue, or GM, WM or ventricles were incorrectly delineated.

See Figure 1 for details about patient inclusion and exclusion and Figure 2 for an example of successful NeuroQuant segmentation. Six scans were excluded due to insufficient segmentation quality. Five of these were acquired in the early phase, and segmentation failed due to hematomas segmented as brain tissue (n=3), incorrect delineation of WM and GM of injured frontal lobe (n=1), and excessive motion (n=1). One 3-month scan failed due to a MRI technical mistake.

Figure 2. *Successful NeuroQuant segmentation of repeated MRIs obtained in a 21-year-old patient with moderate traumatic brain injury at a) 9 days postinjury, b) 3 months and c) 12 months postinjury.*

Unsuccessful segmentations resulted in exclusion of two TBI patients. Furthermore, in four TBI patients the number of scans was reduced from three to two.

In total 161 segmented T1-weighted 3d scans from 62 moderate to severe TBI patients were included in the final sample. 37 patients had three and 25 patients had two scans successfully segmented.

The longitudinal TBI MRI study was originally not a case-control study. However, findings in the ADC analysis in the TBI group made us acquire diffusion-weighted images post-hoc in a small control group (n=9, 4 females) aged 20-29 years. Since this was not a planned comparison, the control data serves purely as a reference of normal ADC values, and all statistical comparisons of DWI data were performed within the TBI group.

MRI and image processing

Scanning was performed at three different time points: early phase (0-26 days post- injury), after 3 months and after 12 months. The early phase scan was acquired as soon as the clinical condition of the patients allowed it.

Scan protocol

MRI acquisition was performed on a 1.5 T Siemens Symphony Sonata scanner (Siemens, Erlangen, Germany) with an eight-channel head coil. A T1-weighted 3d magnetization prepared rapid acquisition gradient echo sequence (MPRAGE) was obtained in the sagittal plane with TR=7.1 ms; TE=3.45 ms; flip angle=7°; TI=1000 ms; FOV=256×256; acquisition matrix of 256×192×128, reconstructed to 256×256×128, giving a reconstructed voxel resolution of 1.00×1.00×1.33 mm. For diffusion-weighted imaging a single-shot spin echo planar imaging sequence with 19 slices, slice thickness 5 mm, TR=3300ms; TE=110 ms;

NEX=4; acquisition matrix size 256×256; FoV=230; in-plane resolution 0.9×0.9 mm and baseline images (b=0 s/mm^2) plus varying diffusion gradient strength along each of three orthogonal directions with b=500 and 1000 s/mm^2 was used. Diffusion trace maps were computed from the isotropic diffusion images and used to estimate ADC values. FLAIR, T2, and T2*-weighted hemosequence were also acquired.

For the post-hoc control group MRI acquisition was performed on a 1.5 T Siemens Avanto scanner (Siemens, Erlangen, Germany) with a twenty-channel head coil, which had replaced the MRI system used in the patient group. Otherwise the scan protocol used the imaging parameters described above.

Segmentation of the T1-weighted 3d brain scans

Fully automated segmentation was performed using NeuroQuant (CorTechs Labs, La Jolla, CA, US) which is a FDA-cleared tool for clinical evaluation of hippocampal atrophy in mild cognitive impairment and Alzheimer's disease.[42,43] The output from NeuroQuant segmentation contains volumes of the total lobar WM, total cortical GM, lateral, third, fourth, and inferior lateral ventricle, cerebellum, hippocampus, amygdala, caudate, putamen, pallidum, thalamus and brainstem given as both absolute volume in cm^3 and relative to the intracranial volume (ICV). In this study only volumes relative to ICV (%ICV) are used. The ICV-corrected volumes of the segmented structures from the left and right hemispheres were combined. The ICVs in the early phase and 12-month scan were significantly different (linear mixed model; mean ± standard error: early phase 1740.06±19.98 cm^3; at 12 months 1706.10±19.74 cm^3; $p<0.001$). ICV is estimated from the total brain volume in NeuroQuant, and may therefore be influenced by brain edema in the early phase and brain atrophy in the chronic phase. Differences between ICV measurements based on total brain volume in the

early and chronic are not surprising in TBI patients.[2,3] Therefore, volumes of segmented structures were normalized using the ICV estimated from the 3-month scan.

Because of the large number of segmented brain structures, we chose to analyze the brain structures with low frequency of segmentation mistakes. The ICV-corrected volumes of cortical GM, hippocampus, brainstem, lenticular nucleus, lobar WM, and ventricles were compared between the early, 3-month and 12-month scans. Putamen and pallidum were evaluated together as they form the lenticular nucleus. The lenticular nucleus was used since it was better delineated than the thalamus in the TBI patients. Total ventricular volume was calculated as the sum of third, fourth and lateral ventricular volumes. We evaluated the ventricular volumes together since all ventricles have been shown to dilate in TBI.[22,29]

In addition the volume change over time was calculated for all structures. For the solid structures the following was used: early phase volume minus volume at 3 months, and early phase volume minus volume at 12 months. For the ventricles: volume at 3 months minus early phase volume, and volume at 12 months minus early phase volume. This was done in order for all mean changes to be positive.

Total brain atrophy from the early phase to 3 and 12 months was estimated using linear mixed model based on the sum of the volumes of the solid structures (cortical GM, hippocampus, lenticular nucleus, lobar WM, and brainstem) subtracting the ventricular volume.

ADC measurements in GM

ADC measurements were performed in PACS using Spectra Workstation IDS5 11.4.1 in all 62 patients. Ten circle-shaped ROIs with radius of 2.7 mm were positioned in apparently healthy tissue bilaterally in cortex, hippocampus and the brainstem (Figure 3). The ROI was moved to the closest apparently healthy tissue within the structure of interest in patients with visible focal pathology on MRI. The cortical ROIs in the superior frontal sulcus and

postcentral sulcus were positioned at the sulcal depth in order to avoid measuring ADC in GM close to the skull with possible minor contusions. The cortical ROIs were placed on the same slice, just above the most dorsal slice displaying the lateral ventricles. In the posterior insular gyrus ROIs were set on the slice where the head of the caudate nucleus had its greatest extent. ROIs in the hippocampus and brainstem were positioned on the most caudal slice where mesencephalon was still visible. In the hippocampus the ROIs were located as laterally as possible in the anterior part of the hippocampal body in order to avoid placing the ROIs within the hippocampal head which frequently suffers from contusions in TBI. For all ROIs, the ADC values from the left and right hemisphere were averaged. The image analyst (V.B.) was blinded to clinical information. 14 scans were reassessed and test-retest reliability calculated.

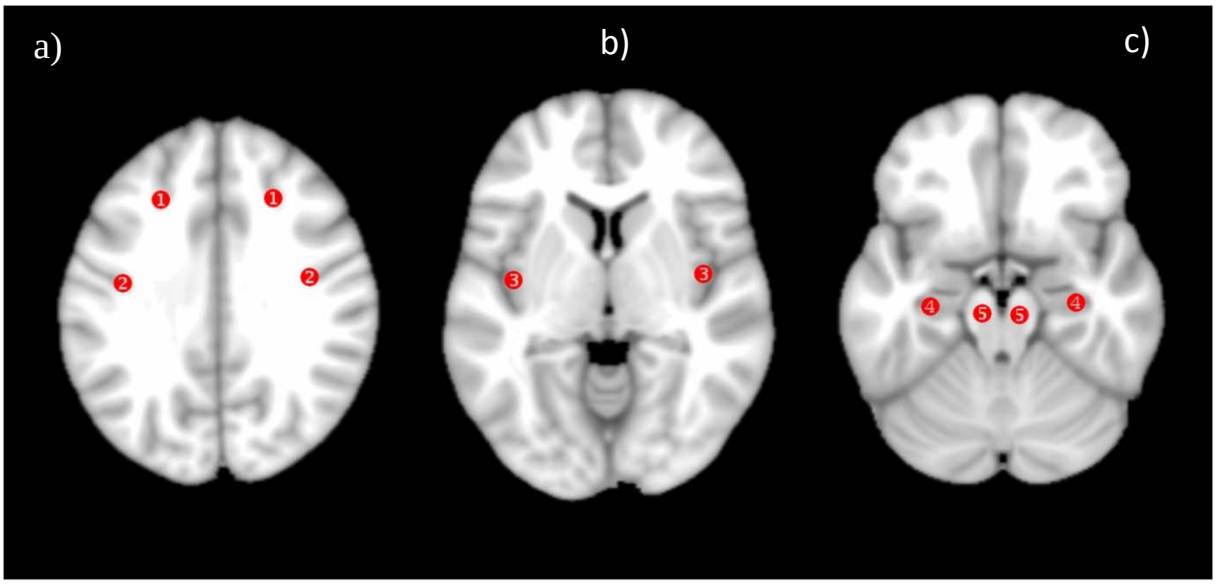


Figure 3. *ROI placement for ADC measurements in*

a) ❶ *Superior Frontal Sulcus ROI,* ❷ *Postcentral Sulcus ROI;*

b) ❸ *Insular Gyrus ROI;*

c) ❹ *Hippocampal ROI,* ❺ *Brainstem ROI.*

Circle-shaped ROIs with radius of 2.7 mm were positioned in apparently healthy tissue. In TBI patients with visible focal pathology, the ROI was moved to the closest apparently healthy GM tissue within the structure of interest.

Injury-related variables

GCS score was recorded at hospital admittance, or before intubation in case of a pre-hospital intubation. All patients included had GCS≤13 that could not be explained by other factors than head injury, as explained in previous publications from this cohort.[39,44] The head injury severity scale (HISS) is based on GCS, where GCS≤8 is considered severe while GCS 9-13 as moderate head injury.[45]

GCS scores were available for all included patients.

PTA was recorded and dichotomized into PTA>2 weeks and PTA≤2 weeks which corresponds to moderate and moderate-severe Mississippi PTA intervals, respectively. Mississippi PTA intervals have been shown to be more accurate predictors of TBI outcome than the traditional Russell intervals.[40] Data on PTA were missing in 4 patients. These were excluded from analyses including PTA.

DAI classification was performed by experienced senior neuroradiologists based on the early FLAIR, T2- and T2*-weighted scans, for details see.[39] DAI was classified into Grade 1; traumatic lesions confined to lobar WM, Grade 2; lesions also detected in the corpus callosum, and in Grade 3; presence of brainstem lesions.[46] DAI classification was available for all included patients.

Outcome assessment

Global outcome was assessed by telephone interview 12 months after injury using the structured interview for Glasgow Outcome Scale-Extended (GOSE).[47,48] To reduce the

potential error associated with the telephone setting,[49] relatives or caregivers also provided information, for details see.[39] Outcome data were available for all included individuals, but missing in 5 non-included patients. Patients were dichotomized into disability (GOSE<7) and good recovery (GOSE≥7) after 12 months.

Statistical analyses

Characteristics of included and excluded TBI patients.

The Mann-Whitney *U*-test, χ^2-test, independent samples *T*-test and the Wilcoxon *U*-test were used to compare demographic and clinical data.

Longitudinal brain volume changes

Significant changes in the volume of each of the brain structures at each time point was calculated with a linear mixed effects model. Significant differences in the slope of volume loss over time between the different brain structures were calculated with the linear mixed model with random intercept.

The relationships between ventricular volume change and volume changes of lobar WM, cortical GM and brainstem were explored using linear regression separately for the periods early to 3 months and early to 12 months. There were separate regression models for lobar WM, cortical GM, and brainstem volume changes as the volume changes were expected to be correlated.

Impact of TBI subgroup on brain volumes was investigated in the following dichotomized groups; moderate vs. severe TBI, PTA≤ 2 weeks vs. >2 weeks, and DAI vs. non-DAI. The volumes were compared over time within each group and between the dichotomized groups.

For DAI a subdivision into four DAI groups, i.e. non-DAI=0, and DAI grade 1, 2, and 3 were performed and the effect of degree of DAI was assessed with one-way ANOVA.
A significant *F*-value was followed by a paired Student's *T*-tests within the group, and with independent samples *T*-test between the dichotomized groups. The resulting *p*-values were Bonferroni corrected for number of time points evaluated for each structure separately.

The following analyses of volume differences between the different injury mechanism groups were performed:
Moderate vs. severe TBI: differences between cortical GM, hippocampal, lenticular nucleus, lobar WM, brainstem and ventricular volumes in early phase, at 3 and 12 months were investigated. Since DAI was more frequent in severe TBI, the presence of DAI was included in the analysis of lobar WM volume differences between moderate and severe TBI.
PTA≤2 weeks vs.>2 weeks: differences between cortical GM, hippocampal, and ventricular volumes in early phase, at 3 and 12 months.
DAI vs. non-DAI: differences in cortical GM, lobar WM, brainstem, and ventricular volumes in early phase, at 3 and 12 months.
DAI grade 0-3: the effect of degree of DAI (no DAI, DAI grade 1, 2 or 3) as a continuous variable on brainstem and total ventricular volumes was assessed in the early phase, at 3 and 12 months.

Outcome prediction
Hierarchical regression analysis was performed to determine if early and 3-month MRI-derived volumes contributed significantly to 12-month GOSE outcome after taking into account the established outcome predictors GCS score and PTA (PTA≤2 weeks=0, PTA>2 weeks=1) which were entered in the first step as the base model with which all subsequent

models were compared. The MRI measures were brainstem volume in early phase, and cortical GM, hippocampal, lenticular nucleus and ventricular volumes at 3 months. In addition we used the Rotterdam CT scores[50] obtained from the worst CT scan, to see if this neuroimaging modality provided additional predictive value.[51] Overall variance explained (R^2) and statistical significance were calculated for every model. R^2-change and significance of *F*-value change were calculated as differences between the base model and all subsequent models.

Longitudinal analysis of ADC changes in GM

For test-retest reliability of ADC measures, we calculated intraclass correlation coefficients (ICC) using two way random single measures.[52]

A linear mixed effects model was used in the statistical comparisons of the ADC values in the different ROIs over time in the entire TBI group, and between the TBI subgroups; moderate vs. severe TBI, PTA≤ 2 weeks vs. >2 weeks, DAI vs. non-DAI. The effect of degree of DAI (non-DAI=0, DAI 1, 2, and 3) was assessed with ANOVA. Significant *F*-values were followed by paired Student's *T*-test or independent sample T-test. The resulting *p*-values were Bonferroni corrected as described earlier. Association between ADC values and corresponding volumes at 3 and 12 months were examined using Pearson correlation.

Clinical and demographic data (age, GCS and GOSE scores) are given as mean ± standard deviation, and volumes and ADC values as mean ± standard error. The threshold for statistical significance was $p<0.05$ for all analyses, including analyses applying Bonferroni correction for multiple comparisons.

Results

TBI patient characteristics (Table 1)

There were no significant differences with regard to the clinical variables between included and excluded TBI patients who survived for 12 months. Of the included patients 58% suffered moderate and 42% severe TBI. See Table 1 for details.

Comparison between the included males (n=45) and females (n=17) showed that GCS score, HISS, and PTA and GOSE score at 12 months were similar between the sexes.

Injury mechanism, age and number of days between the head injury and the MRI scans were similar in the moderate and severe TBI patients. There were significantly more patients with PTA>2weeks, any grade of DAI and poor 12-month outcome in the severe than in the moderate TBI group.

Table 1. *TBI patient characteristics*

		All (n=62)	*Moderate[a] (n=36)*	*Severe[b] (n=26)*
Age (years)		*32.5 ± 15.2*	*34.3 ± 15.8*	*30.1 ± 14.4*
GCS		*9.1 ± 3.4*	*11.5 ± 1.6**	*5.7 ± 1.8**
Mechanism of injury	*Road accident*	*34 (55%)*	*19 (53%)*	*15 (58%)*
	Fall injury	*23 (37%)*	*13 (36%)*	*10 (38%)*
	Other	*4 (6%)*	*3 (8%)*	*1 (4%)*
	Unknown	*1 (2%)*	*1 (3%)*	*0*
Post-Traumatic Amnesia	*>2 weeks*	*19 (31%)*	*5 (14%)**	*14 (54%)**
	≤2 weeks	*39 (63%)*	*30 (83%)**	*9 (35%)**
Diffuse Axonal Injury	*No*	*19 (31%)*	*17 (47%)**	*2 (8%)**
	Yes	*41 (66%)*	*19 (53%)**	*22(85%)**
	Stage 1	*13 (21%)*	*5 (14%)**	*8 (31%)**
	Stage 2	*17 (27%)*	*11 (31%)**	*6 (23%)**
	Stage 3	*11 (18%)*	*3 (8%)**	*8 (31%)**
With disability (GOSE <7)		*27 (44%)*	*12 (33%)**	*15 (58%)**
Good recovery (GOSE 7-8)		*35 (56%)*	*24 (67%)**	*11 (42%)**
Days between injury and MRI[c]	*Scan 1*	*10.2 ± 6.7*	*9.0 ± 6.2*	*12.1 ± 7.3*
	Scan 2	*98.4 ± 15.2*	*98.2 ± 14.3*	*98.6 ± 16.7*
	Scan 3	*373.5 ± 26.3*	*377.1 ± 30.1*	*369.0 ± 20.3*

[a]GCS 9-13.

[b]GCS 3-8.

[c]in days.

** Significant difference between Moderate and Severe TBI.*

Numbers of patients (percentages) or means ± standard deviations are shown. Percentages were calculated considering the total number of patients in each column.

GCS, Glasgow Coma Scale; GOSE, Glasgow Outcome Scale Extended; MR, Magnetic Resonance.

Longitudinal brain structure volume changes in the entire TBI group (Figures 4 and 5)

During the first 12 months after TBI significant volume differences were found for all analyzed structures, and the trajectories of volume change differed between the structures (Figure 4). Cortical GM experienced a rapid volume reduction and was significantly reduced at 3 months, but did not change significantly after that. For the subcortical GM structures, hippocampal and lenticular nucleus volumes decreased more slowly and the volume loss was not significant before 12 months. Likewise, the lobar WM volume was significantly reduced first at 12 months compared to both the early and the 3-month scan. Brainstem volumes decreased significantly throughout the 12 months. The ventricular volume expanded from the early phase to the 3 month time point but was not significantly enlarged before the 12-month time point. Statistical comparison of the slope of the volume loss over time between the different brain structures demonstrated that cortical GM loss followed a significantly different trajectory compared to the hippocampus ($p<0.0001$) and lenticular nucleus ($p<0.0001$). The hippocampus and lenticular nucleus had similar trajectories. The trajectory of WM loss was significantly different from each of the other brain structures ($p<0.0001$), except for cortical GM. The comparisons between the volume loss trajectories for subcortical GM nuclei and WM and brainstem volume, and between WM and brainstem revealed that they were all significantly different ($p<0.001$).

Ventricular dilation was strongly associated with change in brainstem volumes at 3 months ($R^2=0.246$, $p=0.001$) and 12 months ($R^2=0.488$, $p<0.001$), and total cortical GM volume at 3 months ($R^2=0.143$, $p=0.012$) and 12 months ($R^2=0.331$, $p<0.001$). Ventricular dilation was not significantly associated with lobar WM volume changes at any time point.

The total brain volume decline was 3.8±0.8% of ICV between the early and 3-month scan, and 5.7±0.8% of ICV between early and 12-month scan.

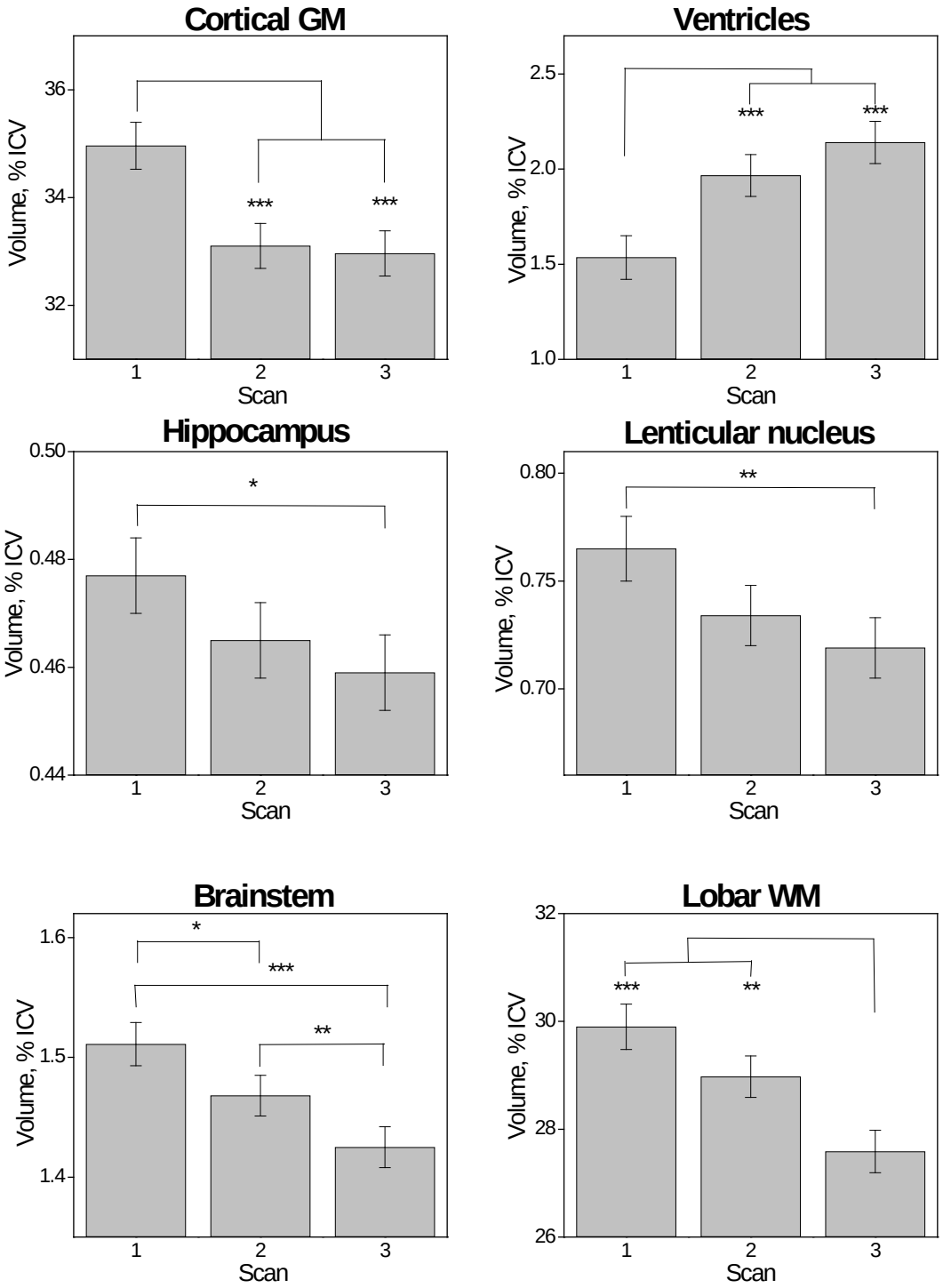


Figure 4. *Longitudinal changes in mean ICV-corrected brain volumes with standard errors in the early phase (0-26 days post-injury, scan 1), 3 months (scan 2), and 12 months post injury (scan 3). Volumes of left and right side of the structures were summed and evaluated together.*

**** p<0.001, ** p<0.01, * p<0.05.*

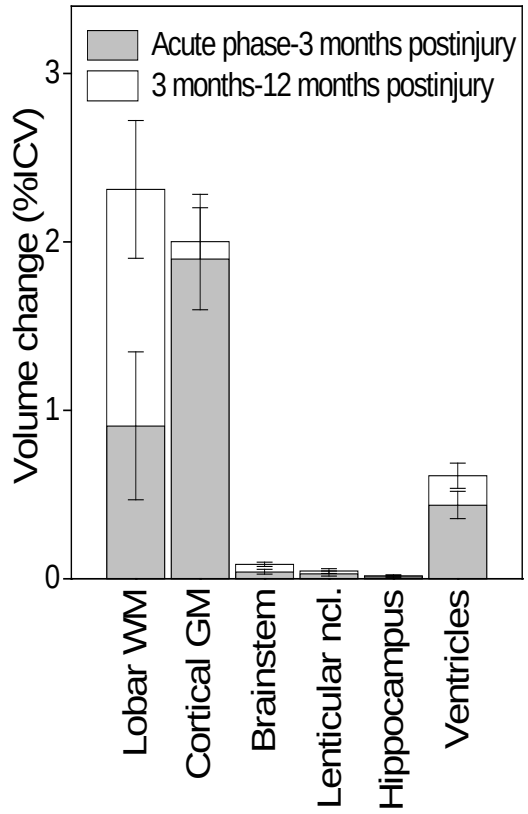


Figure 5. *Illustration of the volume changes over time.*

Volume changes (as %ICV) early phase-3 months and 3 months-12 months postinjury. The differences between different time points are calculated using linear mixed model. Means ± error bars are plotted.

The impact of clinical TBI subtypes on brain volumes (Figures 6 and 7)

TBI severity. Lobar WM and brainstem volume at 12 months were smaller in the severe compared to the moderate TBI group (lobar WM: 26.34±0.58% ICV vs. 28.60±0.51% ICV, p=0.012; brainstem: 1.36±0.03% ICV vs. 1.47±0.02% ICV, p=0.005) while cortical GM, ventricular, hippocampal and lenticular nucleus volumes were similar. The difference in lobar WM volume at 12 months was significant also after correcting for the presence of DAI (p=0.012).

Moreover, hippocampal volume change between the early and 3-month scan was larger in the severe group compared to the moderate group (0.03±0.01 %ICV vs. 0.00±0.01%ICV, p=0.046). For the other structures, there were no differences in volume changes over time between moderate and severe TBI.

PTA duration (Figure 6a). Patients with PTA>2 weeks had smaller hippocampi at 3 and 12 months, and larger ventricles at 3 and 12 months compare to patients with PTA≤2 weeks. Cortical GM volumes did not differ significantly between the PTA groups.

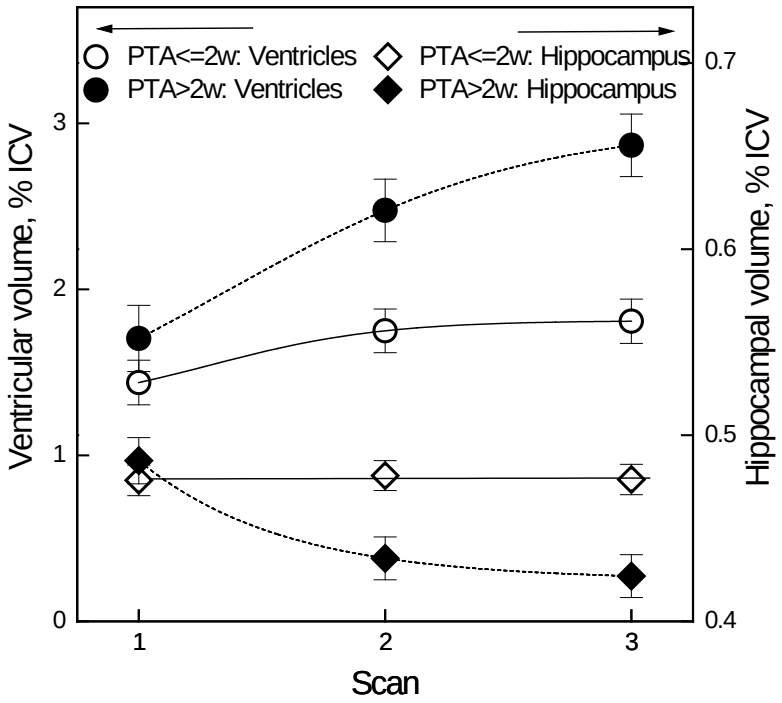
Hippocampal volume change over time was larger in the PTA>2 weeks group than in the PTA≤2 weeks between the early and 3-month scan (0.05±0.01 %ICV vs. 0.00±0.01 %ICV, p=0.003). Cortical GM and ventricular volume changes over time did not differ between the PTA groups.

DAI groups (Figure 6b and 7). Smaller brainstem volumes were present at all time points in the DAI group compared to the non-DAI group. No differences in cortical GM, lobar WM or ventricular volumes were present between the DAI and non-DAI groups.

The non-DAI group had larger brainstem volumes than DAI grade 2 in early phase; than the DAI grade 2 and 3 at 3 months, and DAI grade 1, 2 and 3 at 12 months. In DAI 3 brainstem volumes decreased significantly between the early and 3 months scan, and further till 12 months. In DAI 1 and 2 significantly decreased brainstem volumes were present first after 12 months. In the non-DAI group brainstem volumes did not change with time.

No significant differences between the DAI groups were observed in ventricular volumes.

a)



b)

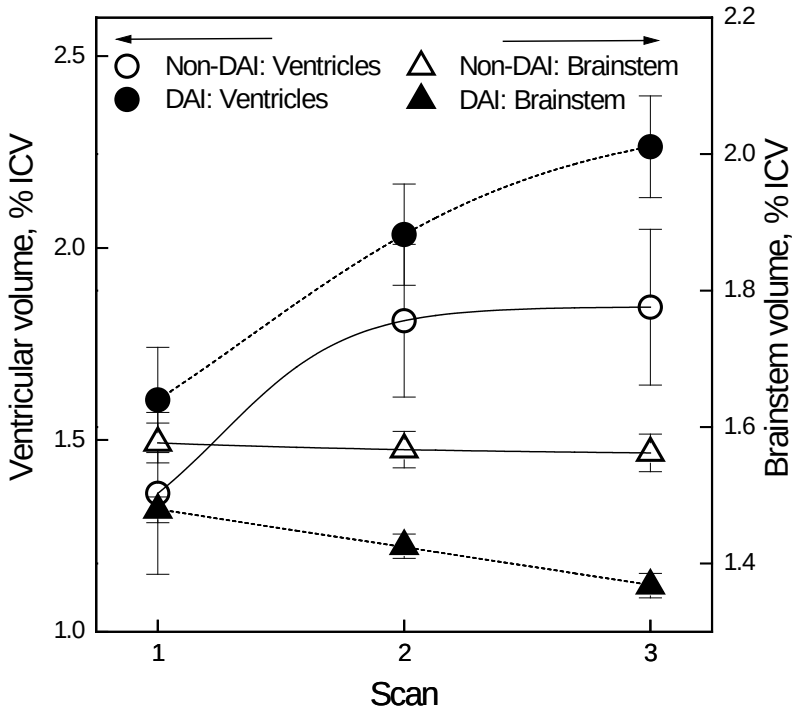


Figure 6. *Mean volumes of different brain structures plotted for:*

a) patients with PTA > and ≤ 2 weeks.

b) DAI and non-DAI patients.

For statistical evaluation of volume differences see Materials and Methods, and Results.

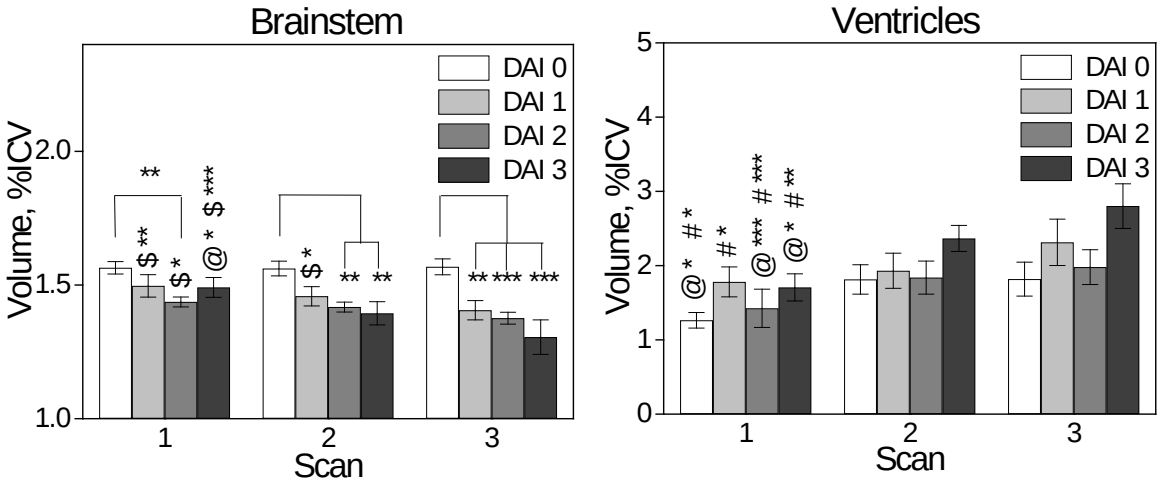


Figure 7. *The association between the longitudinal changes in mean brainstem and ventricular ICV-corrected volumes for the non-DAI (non-DAI=DAI 0) and the three DAI groups (DAI grade 1, 2, 3). Figures show means and standard errors.*

Left and right side volumes were summed and evaluated together.

Significant differences in brainstem and ventricular volumes between the non-DAI and the three DAI groups were found at all time points:

*Significant differences between different scanning points within each DAI group were confirmed and marked as: @ - significantly different from scan 2; $ - significantly different from scan 3 (*** p<0.001, ** p<0.01, * p<0.05).*

Please note that the units on the Y axis differ between the plots, and that the X axis does not always cross the Y axis at 0.

Outcome prediction based on early and 3-month MRI volumes (Table 2)

The hierarchical regression analyses with PTA and GCS scores in the base model demonstrated that the MRI-derived brain volumes of the brainstem in the early phase, and cortical GM, hippocampus, and lenticular nucleus at 3 months explained significantly more of the final outcome than the base model (Table 2). The ventricular 3-month volume did not add to the 12-month outcome prediction, and neither did the Rotterdam CT score.

Table 2. *Hierarchical regression analysis of 12-month outcome prediction using early and 3-month brain structure volumes with GCS and PTA as base model*

Models	R^2	R^2*-change*	*Significance of F change*
Base model	*0.385*		
Brainstem early model	*0.449*	*0.064*	*0.027*
CortGM 3-month model	*0.509*	*0.124*	*0.001*
Ventricular model 3-month model	*0.428*	*0.042*	*0.058*
Hippocampal 3-month model	*0.466*	*0.081*	*0.008*
Lenticular nucleus 3-month model	*0.497*	*0.112*	*0.001*
Rotterdam CT score model	*0.402*	*0.017*	*0.215*

Base predictors: GCS, PTA duration (0=PTA≤2 weeks; 1=PTA>2 weeks)

Brainstem early model: GCS, PTA duration, brainstem volume 0-26 days postinjury

CortGM predictors: GCS, PTA duration, cortical GM volume at 3 months

Ventricular predictors: GCS, PTA duration, ventricular volume at 3 months

Hippocampal predictors: GCS, PTA duration, hippocampal volume at 3 months

Lenticular nucleus predictors: GCS, PTA duration, lenticular nucleus volume at 3 months

Rotterdam CT score predictors: GCS, PTA duration, Rotterdam CT score

CortGM, cortical grey matter; GCS, Glasgow Coma Scale; PTA, posttraumatic amnesia duration.

Analysis of ADC value changes in GM

Test-retest reliability of ADC measurements

ICC of repeated measurements was 0.74 for superior frontal sulcus, 0.86 for postcentral sulcus, 0.69 for insular gyrus, 0.88 for hippocampus and 0.89 for brainstem.

Overall changes in entire TBI group. No ADC value changes over time were found in any ROI in the entire TBI group.

The impact of clinical TBI subtypes on ADC value changes

TBI severity (Table 3a). The severe TBI group had higher ADC values in the brainstem at 3 months, the superior frontal sulcus at 3 and 12 months, and the postcentral sulcus at 12 months. No significant differences in ADC values between moderate and severe TBI groups were found at any time point for insula or hippocampus.

In the severe TBI group the ADC values increased over time in the superior frontal sulcus from the early phase to 3 months ($p=0.043$), and postcentral sulcus from early phase to both 3 and 12 months (early phase<3months, $p=0.028$; early phase<12 months, $p=0.027$). There were no differences in brainstem, insula or hippocampal ADC values between any time points. There were no differences in ADC values over time in any ROI in the moderate TBI group.

PTA duration (Table 3b). At 12 months the ADC values in the superior frontal sulcus were higher in patients with PTA>2 weeks than in PTA≤2 weeks, while no differences were found in the other ROIs. In the PTA>2 weeks group ADC values in insula increased between 3 and 12 months ($p=0.046$). In the group with PTA≤2 weeks no differences in ADC values overtime were found in any ROI.

Table 3. *Longitudinal ADC values of TBI subgroups and comparison between the clinical TBI subtypes.*

a) Moderate versus severe TBI

		Moderate TBI	*Severe TBI*	*p-value*
Superior frontal sulcus				
	Early phase	*68.2±1.1*	*68.1±1.3*	*NS*
	3 months	*66.9±1.0*	*71.5±1.2*	*0.011*
	12 months	*65.0±1.1*	*70.7±1.2*	*0.005*
Postcentral sulcus				
	Early phase	*70.2±0.9*	*69.6±1.1*	*NS*
	3 months	*69.9±0.9*	*73.2±1.0*	*NS*
	12 months	*68.9±1.0*	*73.2±1.0*	*0.009*
Insular gyrus				
	Early phase	*81.5±1.1*	*81.9±1.3*	*NS*
	3 months	*80.7±1.0*	*81.9±1.2*	*NS*
	12 months	*81.4±1.1*	*83.9±1.2*	*NS*
Hippocampus				
	Early phase	*86.1±1.4*	*88.0±1.7*	*NS*
	3 months	*85.8±1.3*	*87.8±1.5*	*NS*
	12 months	*85.1±1.4*	*88.7±1.5*	*NS*
Brainstem				
	Early phase	*80.6±1.7*	*81.5±2.1*	*NS*
	3 months	*80.5±1.6*	*87.5±1.9*	*0.016*
	12 months	*80.3±1.7*	*83.5±1.9*	*NS*

b) Patients with PTA ≤2 weeks versus > 2 weeks

		PTA≤2 weeks	*PTA>2 weeks*	*p-value*
Superior frontal sulcus				
	Early phase	*68.2±1.0*	*67.9±1.5*	*NS*
	3 months	*68.0±1.0*	*70.4±1.5*	*NS*
	12 months	*66.3±1.1*	*70.8±1.5*	*0.037*
Postcentral sulcus				
	Early phase	*70.0±0.9*	*69.8±1.4*	*NS*
	3 months	*70.4±0.9*	*72.9±1.3*	*NS*
	12 months	*69.9±1.0*	*72.6±1.3*	*NS*
Insular gyrus				
	Early phase	*81.7±0.9*	*81.5±1.4*	*NS*
	3 months	*81.9±0.9*	*79.5±1.3*	*NS*
	12 months	*81.8±1.0*	*84.0±1.3*	*NS*
Hippocampus				
	Early phase	*87.0±1.1*	*86.2±1.6*	*NS*
	3 months	*86.0±1.1*	*85.0±1.5*	*NS*
	12 months	*86.1±1.1*	*87.8±1.5*	*NS*
Brainstem				
	Early phase	*80.8±1.5*	*81.3±2.2*	*NS*
	3 months	*81.8±1.4*	*83.8±2.1*	*NS*
	12 months	*81.2±1.5*	*82.6±2.1*	*NS*

ADC in 10^{-5} m^2/s. Values given as means ±standard deviations.

Early phase scans were acquired 0-26 days postinjury.

Statistical differences between moderate vs. severe or PTA≤2 vs. >2 weeks were Bonferroni-corrected for every structure separately.

ADC, apparent diffusion coefficient; NS, non-significant; TBI, traumatic brain injury.

DAI groups (Figures 8 and 9). In the superior frontal sulcus and postcentral sulcus ADC values were higher in TBI patients with DAI than in non-DAI patients at 3 and 12 months. The ADC values increased significantly from the early phase to 3 months in the superior frontal ($p=0.009$) and the postcentral sulcus ($p=0.005$) in the DAI group. No differences were found in ADC values or ADC value change over time in the insula, hippocampus or brainstem ROIs between the non-DAI and the DAI groups, or within the DAI grades.

The different DAI grades were associated with significant differences in cortical GM ADC values (Figure 9). In DAI 3, the superior frontal sulcus ADC values were higher than in the non-DAI group at 3 months, and compared to both non-DAI and DAI 1 at 12 months. In DAI 2 the ADC values were higher in the superior frontal sulcus at 3 and 12 months than in the non-DAI group. The DAI 3 group had higher ADC values in the postcentral sulcus at both 3 and 12 months than non-DAI and DAI 1 groups. In the superior frontal sulcus ADC values decreased between 3 and 12 months ($p=0.018$) in DAI 1, and increased between the early phase and both 3 and 12 months in DAI 2 (early phase<3 months, $p=0.036$; early phase<12 months, $p=0.018$). There were no significant changes over time within the non-DAI and the DAI 3 groups.

The ADC values increased between the early phase and 3 months in the DAI 2 group ($p=0.020$) in the postcentral sulcus. In DAI 3, the ADC values were significantly increased both at 3 and 12 months compare to the early phase (early phase<3 months, $p=0.003$; early phase<12 months, $p=0.003$). ADC values in postcentral sulcus did not change with time in the non-DAI and DAI 1 groups.

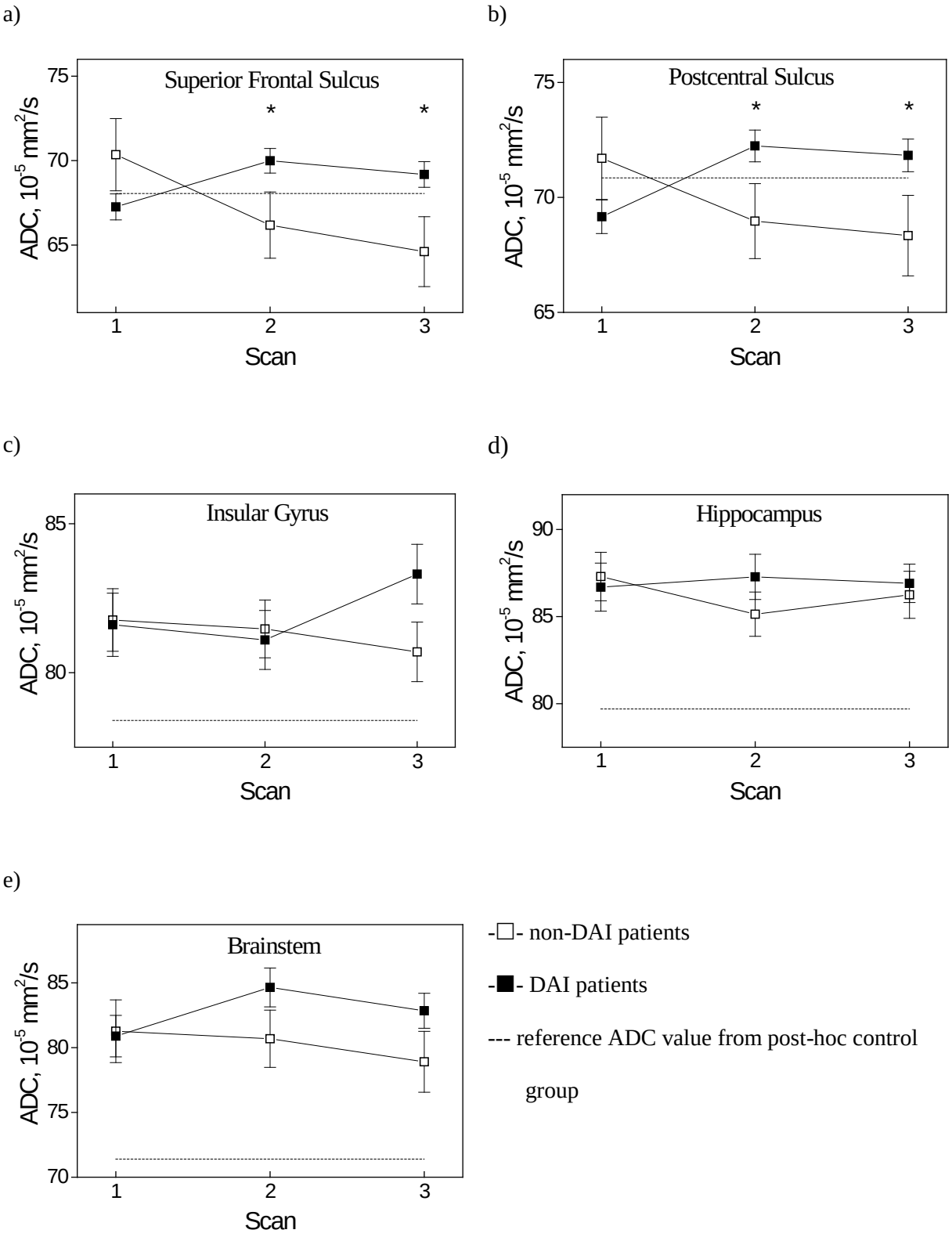


Figure 8. *Longitudinal changes in mean ADC values with standard deviations in non-DAI (□) and DAI (■) patients in the Superior Frontal Sulcus (a), Postcentral Sulcus (b), Insular Gyrus (c), Hippocampus (d) and Brainstem (e). Right and left side values were averaged*

*Statistical significant differences between Non-DAI and DAI groups are marked with *.*

Significant differences were found between:

a) DAI (1) and DAI (2), p=0.009; Non-DAI (2) and DAI (2), p=0.028; Non-DAI (3) and DAI (3), p=0.001.

b) DAI (1) and DAI (2), p=0.006; Non-DAI (2) and DAI (2), p=0.020; Non-DAI (3) and DAI (3), p=0.004.

The p-values were corrected for multiple comparisons. The mean ADC value for the controls is plotted with ----- as a reference.

Please note that the units on the Y axis differ between the plots, and that the X axis does not cross the Y axis at 0.

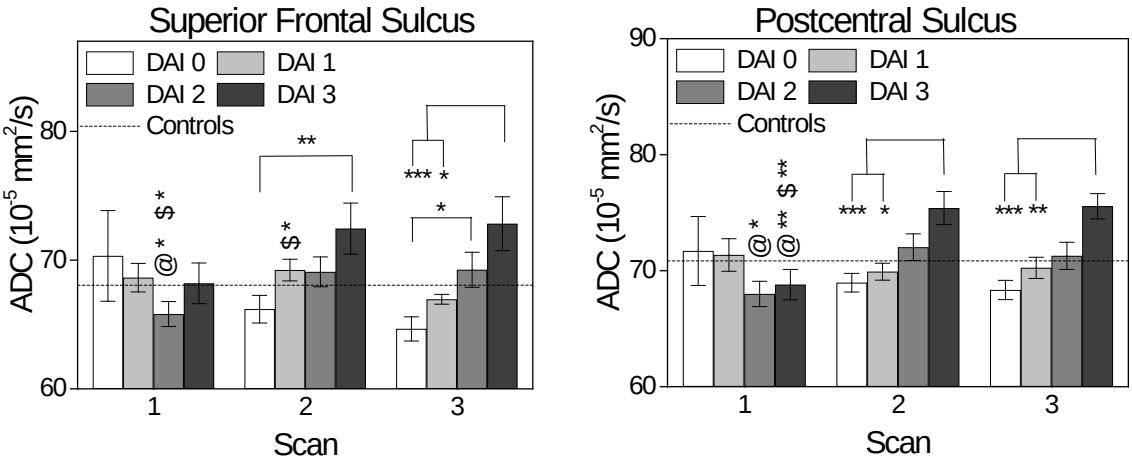


Figure 9*. The associations between longitudinal changes in mean ADC values for the non-DAI group (non-DAI =DAI 0) and the three DAI groups (DAI grade 1, 2, 3). The mean of the controls' ADC values were plotted as ------ as a reference value.*

Differences were evaluated using one-way ANOVA, and significant F-values were followed by independent samples T-tests, the resulting p-values were Bonferroni corrected for the three time points separately. The post-hoc control group was not used in the statistical analysis.

Longitudinal analysis within each DAI group was performed separately for both ROIs using a linear mixed model. Significant F-values were followed by the Student's T-test. The resulting p-values were Bonferroni corrected separately for every ROI.

*Significant differences between different time points for each DAI group were confirmed and marked as: @ - significantly different from scan 2; $ - significantly different from scan 3 (*** p<0.001, ** p<0.01, * p<0.05).*

ROI ADC values versus brain structure volumes.

There were no correlations between the ADC values in cortex, hippocampus or brainstem and the volumes of these structures at 3 and 12 months.

ADC values of superior frontal and postcentral sulci from 3 months did not add to the 12-month outcome prediction (Table 4).

Table 4. *Hierarchical regression analysis of 12-month outcome prediction using early 3-month ADC values with GCS and PTA as base model*

Models	R^2	R^2*-change*	*Significance of F change*
Base model	*0.385*		
Superior frontal sulcus model	*0.393*	*0.008*	*0.413*
Postcentral sulcus model	*0.402*	*0.017*	*0.234*

Base predictors: GCS, PTA duration

Superior frontal sulcus model: GCS, PTA duration, Superior frontal sulcus ADC value at 3 months

Postcentral sulcus model: GCS, PTA duration, Postcentral sulcus ADC value at 3 months

ADC, apparent diffusion coefficient; GCS, Glasgow Coma Scale; PTA, posttraumatic amnesia duration.

Discussion

This is to the best of our knowledge the largest number of moderate and severe TBI patients included in a prospective, longitudinal MRI study of brain volumes and diffusion changes where data has been systematically collected at 3 different time points, i.e. early phase, 3 and 12 months post-injury. Moreover, the patient group included in this study was representative for the entire group of TBI patients surviving for 1 year. The results confirmed most of our predictions, but also revealed new, unexpected findings.

Longitudinal volume changes

Significant cortical GM volume loss took place between the early and 3-month scan without any further significant decline at 12 months. This is the first direct evidence for cortical GM volume loss being an early occurrence after TBI in humans, similar to results in animal models.[12,14,31] The rapid cortical volume loss probably stems from a combination of neuronal necrosis and apoptosis initiated at time of injury and peaking early post-injury.[11] Supporting these observations in animals, human PET studies show early and widespread neurodegeneration in intact cortex following TBI.[53,54] We did not find any evidence for GCS scores, duration of PTA or presence of DAI being associated with cortical volume loss. The effect of DAI and PTA on cortical volumes may, however, be localized to specific cortical regions for instance connected to affected WM tracts or the medial temporal lobe, and hence not be reflected in total cortical volumes.

The volume loss in the hippocampus was slower than in the cortex and not significant before 12 months after injury. This continuous loss of hippocampal volume lasting for 12 months after TBI in humans concurs with animal studies.[8,14] However, both animal data[8,14] and autopsy data from humans[55] demonstrate significant early neuronal loss in the hippocampus.

The lack of a significant decline in hippocampal volume between the early and 3 month-scan in the current study may be due to the early scans being obtained at ~10.2 days after injury. At that time point some volume loss may already have taken place. Alternatively, MRI-based hippocampal volume loss may take longer to develop than histological loss. Still, our result concurs with findings in cross-sectional studies where decreased hippocampal volume is detected first after 3 months in moderate-severe TBI patients compared to matched controls.[24,56] The current data also agree with results from longitudinal studies with two time points, which show that hippocampal volumes decline over a protracted period from 1 week to 2.5 years.[22,57] The slowly evolving hippocampal volume loss is consistent with the protracted apoptotic neuronal death described in this region,[15] coupled with glial proliferation[58] which may counteract some of the volume change, and impaired neurogenesis[18] which may affect volume when accumulated over time. The hippocampus is critical for declarative memory,[59,60] and hippocampal damage is known to produce symptoms of anterograde and retrograde amnesia.[61] It was thus not surprising to find that patients with PTA>2 weeks had significantly lower hippocampal volumes at both 3 and 12 months than patients with PTA≤2 weeks. Moreover, hippocampal volume loss was markedly more pronounced from the early to 3 months in the PTA>2 weeks group. These results demonstrated that PTA reflects hippocampal neuronal injury and that more severe PTA is associated with more rapid and extensive neuronal death.

Significant volume loss developed over 12 months in the lenticular nucleus. Decreased lenticular nucleus volume has been reported in cross-sectional and longitudinal studies of TBI in children and adults ≥1 year after injury.[24,32,62] The histopathological changes in the lenticular nucleus include apoptosis, neuronal loss and/or shrinkage due to transneuronal degeneration and loss of myelinated axons as described in animal studies.[21,63] Based on the

current results these processes take place over a protracted period. The present data do not support the notion that basal ganglia respond more rapidly and briefly to TBI than the hippocampus.[19] Rather, the current study showed that the trajectory of volume loss was similar in the subcortical GM structures, i.e. the lenticular nucleus and hippocampus. Moreover, the subcortical GM structures were shown to differ significantly from the cortex with regard to the slopes of volume loss suggesting important differences in the histopathological responses between these GM structures.

As predicted lobar WM volume loss decreased mainly in the late phase, being significant first at 12 months. This concurs with the slowly evolving pathological response in WM.[26–28] The extended period of volume loss found in lobar WM in humans in the current study is most likely the macroscopic consequence of the protracted histological changes in WM. Previous longitudinal studies have only been able to show a significant decline in WM volumes between an early (1-2 months) and a later time point (6-12 months) after mild to severe TBI.[2,3,22] Surprisingly the trajectory of WM loss and cortical GM loss did not differ significantly although the slope of WM loss was significantly different from all other brain structures. This finding likely reflects a significant interaction between WM volume loss and cortical volume loss over time at the individual level.

Lobar WM volume loss was associated with injury severity as patients with severe TBI had significantly reduced lobar WM volumes compared to the moderate TBI group. This difference was present also after correcting for the presence of DAI. Unexpectedly, DAI was not associated with decreased lobar WM volume. This may be due to DAI classifications being based on visible lesions on conventional MRI which may not fully describe WM involvement in TBI.

The brainstem was the only structure in which volume loss was significant between every scan session, thus demonstrating particularly pervasive and enduring consequences of TBI to this region. Previous MRI studies have reported brainstem volume loss in cross-sectional and longitudinal studies with two time points in both children and adults with moderate and/or severe TBI.[3,22,64] Direct WM injury probably contributed to the early brainstem volume decline since patients with DAI grade 3 had the fastest and largest reduction of brainstem volume, present from 3 months. Also the group with severe TBI had smaller brainstem volumes, but this was detectable first after 12 months, appearing at the same time as reduced lobar WM volumes in this group. The WM tracts in the brain stem thus appear to be particularly sensitive to TBI.

Ventricular volumes were significantly increased at 3 months, followed by a non-significant increase at 12 months. This result is a refinment of the previously described timeframe of significant ventricular dilation which was shown to last until ~2-7 months postinjury.[6] Ventricular dilation at 3 and 12 months was associated with brainstem and cortical GM volumes. Neither lobar WM volumes nor the presence of DAI were associated with larger ventricular volumes. These results demonstrate the importance of deep WM loss in particular, followed by cortical GM to ventricular dilation in TBI, rather than hemispheric WM loss.

For the entire TBI group mean brain atrophy was ~4% of ICV after 3 months and ~6% of ICV after 12 months, demonstrating that the bulk volume loss occured within the first 3 months. Still, a notable protracted component to the atrophy was present. No directly comparable human studies exist, but endpoint brain volume atrophy in the chronic phase after TBI is reported to be from 1.4% to 8% in cross-sectional and longitudinal studies in mild to severe TBI.[1–3]

Longitudinal ADC changes

Increased ADC values were detected only in individuals with the most serious brain injuries, i.e. severe TBI, PTA>2 weeks, and DAI, and there were no associations between ADC values and volumes. Higher ADC values were located to deep, radiologically unaffected sulcal cortical GM, and increased significantly throughout the 12 month period, even when total cortical volume remained similar between 3 and 12 months. Thus it appears that small-scale remodeling of cortex after TBI persisted for a longer period than large-scale volumetric changes, and that these changes are independent processes. A significant contribution from DAI to the increase in cortical ADC values was present where more severe DAI grades gave rise to increased cortical ADC values developing at a more rapid timescale. In DAI the cortical ADC changes may be connected to delayed neuronal death, soma shrinkage, loss of cortical axons and myelination developing over an extended time period.[65,66]

As expected increased ADC values at 3 months was present in the brainstem in the severe TBI group, but not in moderate TBI. This finding lends further support to the notion that deep brain injury is significantly more pronounced in severe than moderate TBI. PTA>2 weeks was not associated with increased hippocampal ADC values. Both increased and decreased ADC values are reported after TBI in animals and humans.[23,67,68] We predicted that PTA>2 weeks would led to increased ADC values due to increased cell loss, but did not find support for this assumption in the data. It is possible that the pathophysiological mechanisms leading to PTA are more heterogeneous than we expected and/or that volume loss combined with glial proliferation counteracted any effect of neuronal loss on ADC values.

MRI-derived early and 3-month volumes for 12-month outcome prediction

The hierarchical regression analysis demonstrated that MRI-derived volumes at 3 months contributed independently to 12-month outcome prediction even adjusting for duration of

PTA and GCS scores. This finding demonstrates a significant clinical potential for MRI volumes obtained in at 3 months for outcome prediction. For the brainstem even the early phase volume was an independent outcome predictor. Rotterdam CT score, on the other hand, did not have additional predictive value.

Cortical ADC values at 3 months did not improve outcome prediction suggesting that these changes per se are of secondary importance, relative to that of cortical volume loss.

Methodological considerations

This is the first study using a fully automated method, NeuroQuant, for deriving brain volumes from MRI scans in patients with TBI. Our results show that NeuroQuant performed very well in moderate-severe TBI patients even in the presence of different types of injuries and postoperative changes. Its limitations were restricted to segmentation problems in the early phase due to hematomas and contusions, and excessive movement during scanning. For the 3-month scans no segmentations failed. Since several volumes derived from the 3-month scan were independent predictors of outcome at 12 months, MRI at 3 months appear to be of clinical value in the assessment of the brain injury and prediction of later function. However, for the individual patient the presence of focal injury (contusions, hemorrhages) also plays a significant role for outcome, but it was beyond the scope of the present paper to evaluate the interaction between focal injuries and the atrophy in different brain structures.

It should be noted that the volume change measures obtained using independent NeuroQuant segmentations would not be expected to have the spatial specificity and power to detect subtle change that many across time point registration methods might provide.[69–71] On the other hand, NeuroQuant has previously been shown to have results comparable to that of hand segmentation of subregional volumes by anatomical experts,[42] and thus provides an identifiable and translatable measure of structure volume. As an FDA-cleared tool used at

more than 100 clinical sites and applied to over 30,000 individuals in routine clinical practice, the tool yields standardized data that are directly comparable to results obtained in the clinic. Further, by providing independent segmentations of each time point, this approach is inverse-consistent and not subject to registration bias that may inflate measures of change provided by registration-based techniques.[72] Thus, the approach yields a conservative measure of the regional brain volume changes associated with TBI.

Test-retest variability of the ADC measurements was generally good, but not excellent for any of the ROIs (ICC 0.69 to 0.89). ADC measurements were performed by a single rater, therefore interrater variability could not be assessed as Ozturk and colleagues recommend.[73] ADC values in grey matter may change slightly during normal aging.[74] This should not affect the current data because of its longitudinal design with follow-up time over one year which probably would not be sufficient for significant ADC change in a healthy subject. As the controls were substantially younger than patients, their ADC values may not be directly comparable.

Conclusion

In summary, the effect of TBI on brain volumes differed between brain structures, and injury subgroups (GCS score, PTA, DAI) were associated with specific patterns of volume loss. Changes in cortical diffusion properties were detected in the more severe TBI subgroups in particular in patients with DAI grade 3, where diffusion properties increased in value and encompassed more cortical regions with time. Volume changes and diffusion changes were not associated, and thus appeared to be separate processes. Cortical GM, hippocampus and lenticular nucleus volumes at 3 months and early phase brainstem volumes were all very significant independent predictors of 12-month outcome even after adjusting for PTA duration

and GCS scores. Thus TBI induced early and widespread structural brain volume loss which has significant impact on outcome in the chronic phase in moderate and severe TBI patients.

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