Traumatic axonal injury - relationships between lesions in early phase and diffusion tensor imaging parameters in the chronic phase of traumatic brain injury.

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ABSTRACT

Background: This prospective study of traumatic brain injury (TBI) patients investigated fractional anisotropy (FA) from chronic diffusion tensor imaging (DTI) in areas corresponding to persistent and transient traumatic axonal injury (TAI) lesions detected in clinical MRI from early phase.

Materials and methods: 38 patients (mean 24.7 [range 13-63] years) with moderate-severe TBI and 42 age and sex matched healthy controls were included. Patients underwent 1.5 T clinical MRI in the early phase (median 7 days) including fluid-attenuated inversion recovery (FLAIR) and T2* gradient echo (T2*GRE) sequences. TAI lesions from early phase were characterized as non-hemorrhagic or microhemorrhagic. In the chronic phase (median 3 years) patients and controls were imaged at 3 T with FLAIR, T2*GRE, T1 and DTI sequences. TAI lesions were classified as transient or persistent. The FLAIR/T2*GRE images from the early phase were linearly registered to the FA images from the chronic phase, and lesions manually segmented on the FA-registered FLAIR/T2*GRE images.

Results: For region of interests (ROIs) from both non-hemorrhagic and microhemorrhagic lesion, we found a significant linear trend of lower mean FA from ROIs in healthy controls, to ROIs in patients without either non-hemorrhagic or microhemorrhagic lesions, and further to transient and finally persistent lesion ROIs ($p<0.001$). Histogram analyses showed lower FA in persistent than transient non-hemorrhagic lesion ROIs ($p<0.001$), but this was not found in microhemorrhagic lesion ROIs ($p=0.08$-0.55).

Conclusion: The demonstrated linear trend of lower FA values from healthy controls to persistent lesion ROIs was found in both non-hemorrhagic and microhemorrhagic lesions and indicates a gradual increasing disruption of the microstructure. Lower FA values in persistent compared to transient lesions were only found in non-hemorrhagic lesions. Thus, clinical

MRI techniques are able to depict important aspect of white matter pathology across the stages of TBI.

Significance Statement (Word count: 96, maximum 100)

Patients with TBI had an early MRI assessed for traumatic axonal injury (TAI) lesions and a subsequent diffusion tensor imaging (DTI) scan in the chronic phase. The fractional anisotropy (FA) values from the DTI were extracted from TAI lesion areas and compared with corresponding areas in patients without TAI lesions and healthy controls. The study demonstrated that white matter had quite different FA values depending on whether they comprised tissue affected by different early TAI lesions. Thus, an early MRI will provide information that also can be helpful when interpreting FA values from the chronic phase.

INTRODUCTION (wordcount 570, maximum 600)

Traumatic axonal injury (TAI), also called diffuse axonal injury, induced in white matter at the time of injury has been found in all severities of traumatic brain injury (TBI) (Inglese et al. 2005; Skandsen et al. 2009). TAI consist of both non-hemorrhagic and microhemorrhagic lesions which are classified on clinical MRI according to their MRI appearance and the severity staged based on the localization in hemispheric white matter (stage 1), corpus callosum (stage 2) and brain stem (stage 3) (Adams et al. 1989). Early clinical MRI is challenging in this patient group but is now increasingly used for more comprehensive evaluation of the extent and type of injuries resulting from TBI (Moen et al. 2012; Parizel et al. 2005).

Diffusion tensor imaging (DTI) has demonstrated more extensive damage to the white matter in patients with TBI than revealed by clinical MRI (Huisman et al. 2003; Inglese et al. 2005). Fractional anisotropy (FA) is a DTI parameter that quantifies the degree of diffusion restriction in white matter fibers ranging from 0, i.e. isotropic diffusion corresponding to the situation in cerebrospinal fluid, to 1, i.e. maximal anisotropic diffusion, which corresponds to diffusion along one axis with full restriction in the other directions (Mukherjee et al. 2008). FA is considered a more sensitive biomarker of white matter injury in TBI than other DTI parameters such as mean diffusivity (MD) (Cubon et al. 2011; Haberg et al. 2014; Newcombe et al. 2007), but the sensitivity of the different DTI parameters can be dependent on the type of pathology (Acosta-Cabronero et al. 2010). Most previous DTI studies have found decreased FA, indicating injured microstructure, in several white matter areas in the chronic stage following all severities of TBI (Bendlin et al. 2008; Inglese et al. 2005; Kumar et al. 2009; Xu et al. 2007).

A previous study has demonstrated that non-hemorrhagic and microhemorrhagic TAI lesions behave differently over time: A higher percentage of non-hemorrhagic lesions disappear during the initial three months after TBI, while microhemorrhagic lesions largely persist (Moen et al. 2012). Although both lesion types involve breakage of the blood brain barrier (Fazekas et al. 1999; Young et al. 2008), they differ histopathologically which could explain their different behavior over time. Non-hemorrhagic lesions, depicted as hyperintense lesions in T2 fluid-attenuation inversion recovery (FLAIR) sequences, are characterized by white matter edema in the acute stage and gliosis in chronic stage (Parizel et al. 2005). Microhemorrhagic lesions are depicted as hypointense foci in the T2* gradient echo (T2*GRE) or susceptibility weighted sequences, and represent ferromagnetic characteristics of blood break down products (Huisman et al. 2003). The histopathology of microhemorrhagic lesions has not to date been studied in TBI, but in cerebrovascular disorders (Tatsumi et al. 2008; Werring 2011) these lesions are assumed to be caused by direct injuries to the small vessels and thereby extravasation of blood (Fazekas et al. 1999).

No earlier MRI study has evaluated FA in persistent and transient non-hemorrhagic and microhemorrhagic TAI lesions in TBI. The aim of this study was to depict persistent and transient non-hemorrhagic and microhemorrhagic TAI lesions from early clinical MRI and characterize FA from the corresponding areas in chronic DTI. We hypothesized that FA from ROIs in TBI patients without TAI lesions would be lower than in healthy controls, but higher than FA in TAI lesion ROIs. We also assumed that persisting non-hemorrhagic and microhemorrhagic TAI lesions would be connected to more severe injury than transient lesions.

MATERIAL AND METHODS (wordcount 2397)

Patients and healthy controls

A total of 38 patients between 13-63 years admitted to St. Olavs Hospital, Trondheim University Hospital, Norway with moderate-severe TBI according to the Head Injury Severity Scale (HISS) classification (Stein and Spettell 1995) were included. The patients were admitted in the time period October 2004-July 2008, and were examined with a clinical MRI at 1.5 T in the early phase (median 7 [range 2-41] days), and a more extended MRI including DTI at 3 T in the chronic phase (median 3.0 [range 1.5-5.4] years). The methods used for prospective collection of demographic and injury-related variables are described in previous studies (Moen et al. 2012; Skandsen et al. 2010; Skandsen et al. 2009). Injury Severity Score (ISS) classified the general degree of multi trauma (Baker et al. 1974). Rotterdam CT score indicated the severity of the TBI in a six scale classification (Maas et al. 2005), where we used the worst CT scan obtained in the early phase.

MRI was acquired in 42 healthy volunteers matched to the TBI group for age (mean 28.1 [range 16.6 – 61.3, SD 9.0] years) and sex (76.2% male and 23.8% female). The control group was scanned in the same time period (2009-2010), and with the same 3 T scanner and protocol as used in the chronic phase for the patient group.

Image acquisition

Early phase clinical MRI

The MRI examinations in the early phase were performed at either the study hospital (St. Olavs Hospital) (n=35) or at two local hospitals (n=3). At the study hospital two different 1.5 T scanners equipped with a six-channel head coil were used (Siemens Symphony or Siemens

Avanto; Siemens Medical, Erlangen Germany), while the three patients scanned at two different local hospitals were scanned with a 1.5 T Philips Gyroscan NT Intera System. All patients underwent the same scanning protocol:

- sagittal, transverse, and coronal T2-weighted FLAIR imaging: 24 slices, TR 9000 msec, TE 109 msec, TI 2500 msec, number of averages 1, FOV 23 cm, flip angle 150°, acquisition matrix 256 × 256, slice thickness 5.0 mm, gap 1.0 mm, in plane resolution 0.9 × 0.9 mm.
- transverse T2*-weighted gradient echo imaging: 24 slices, TR 830 msec, TE 25.8 msec, number of averages 1, FOV 21 cm, flip angle 20°, acquisition matrix 410 × 512, slice thickness 5.0 mm, gap 1.0 mm, in plane resolution 0.5 × 0.4 mm.

Chronic phase MRI

All MRI examinations in the chronic phase were acquired on the same 3 T Siemens Trio scanner with Quantum gradients (30 mT/m) and a Head Matrix Coil (12 element receive only coil). The scanning protocol consisted of:

- A 3D T2-weighted FLAIR scan: 192 sagittal slices, TR 5000 msec, TE 388 msec, flip angle 120°, TI 1800 msec, FOV 256 x 256 mm, acquisition matrix 256 x 256 x 192, slice thickness 1.0 mm, in plane resolution 1.0 x 1.0 mm.
- A transverse T2*-GRE series: 40 slices, TR 511 msec, TE 20 msec, flip angle 20°, FOV 220 x 220 mm, acquisition matrix 192 x 256 mm, slice thickness 3.0 mm, gap 0.3 mm, in plane resolution 1.1 x 0.9 mm.
- A 3D MPRAGE sequence: 160 sagittal slices, TR 2300 msec, TE 2.88 msec, flip angle 9°, TI 900msec, FOV 256 x 256 mm, acquisition matrix 256 x 256 x 192, slice thickness

1.2 mm, in plane resolution 1.0 x 1.0 mm. The sequence was also reconstructed in coronal and transversal slices.

The DTI sequence was a single-shot balanced-echo EPI sequence acquired in 30 non-collinear directions with b = 1000 s/mm^2 using the following parameters: 55 transversal slices, TR 6800 ms, TE 84 ms, FOV 240 x 240 mm, acquisition matrix 96 x 96, slice thickness 2.5 mm, no gap, in plane resolution 2.5 x 2.5 mm. For each slice, six images without diffusion weighting (b=0), and 30 images with diffusion gradients were acquired. The DTI sequence was repeated twice for increased signal-to-noise-ratio. In order to correct for image distortion caused by magnetic susceptibility artifacts two additional b0 images were acquired with opposite phase-encode polarity (Holland et al. 2010b).

Image analyses

Lesion detection in early clinical MRI

The FLAIR/T2*GRE images from the early phase were used to delineate TAI lesions (Fig. 1). Non-hemorrhagic TAI lesions were defined as hyperintense white matter lesions identified in the FLAIR sequence, while microhemorrhagic TAI lesions were defined as hypointense white matter lesions identified in the T2*GRE sequence. TAI was classified into three stages according to a modified neuropathological staging based on the location of lesions (Gentry 1994; Kim and Gean 2011); TAI stage 1 included white matter lesions in the hemispheres and cerebellum. Lesions at the interface between grey and white matter were also considered as TAI stage 1 lesions. TAI stage 2 included lesions in corpus callosum and in TAI stage 3 lesions were present in the brain stem. All MRIs were analyzed by the first author in cooperation with two experienced neurologists blinded for patient ID and clinical information. Superficial lesions in the cerebral cortex were defined as contusions. Parenchymal changes following insertion of

external ventricle drainage or intracranial pressure device were not included as traumatic lesions. For more information regarding the analyses performed in the early phase MRI, we refer to two earlier studies (Moen et al. 2014; Moen et al. 2012).

Inter rater agreements analyses were performed where 30 of the T2*GRE sequences from early phase were reevaluated by an experienced neuroradiologist (M.F.) with an interclass correlation coefficient (ICC) of 0.95 (95% CI 0.90, 0.98) for detection of number of microhemorrhagic TAI lesions. For the FLAIR sequences V.B. and M.F. in cooperation reevaluated the estimation of total non-hemorrhagic TAI lesion volumes in 19 patients with an ICC of 0.95 (95% CI 0.88, 0.98).

The TAI lesions were followed longitudinally from the early to chronic phase and defined as either persistent or transient dependent on if they were detectable or not in the FLAIR/T2*GRE images from the chronic phase.

DTI analyses

DTI analyses were performed with tools from the FMRIB software library (FSL, Oxford Centre for Functional MRI of the Brain, UK; www.fmrib.ox.ac.uk/fsl). The two DTI acquisitions and extra b0 images were merged into a single 4D file, and image artifacts due to motion and eddy current distortions were minimized by registration of the DTI acquisitions to the b=0 image using affine registration. The gradient vectors were rotated to account for movement in the affine registrations. Image distortion caused by magnetic susceptibility artifacts was minimized with a nonlinear B0-unwarping method using paired images with opposite phase-encode polarities, resulting in opposite spatial distortion patterns, and alignment of the resulting images using a fast nonlinear registration procedure (Holland et al. 2010a). The brain was extracted using Brain Extraction Tool (BET, part of FSL). FMRIB's Diffusion Toolbox (FDT) was used

to fit a diffusion tensor model to the raw diffusion data in each voxel. Voxel wise maps of FA, MD and eigenvalues were calculated for the patients and healthy controls.

Region of interest (ROI) analyses

In order to compare FA values in persistent and transient lesion ROIs from the chronic phase, FLAIR/T2*GRE images from the early phase were linearly registered to the corresponding FA images from the chronic phase using FLIRT (FSL). Lesion ROIs were manually created in the FA registered FLAIR/T2*GRE images (Fig.1)

Persistent and transient non-hemorrhagic lesions

The sagittal FLAIR images from the early phase were registered via the sagittal T1 images from the chronic phase to their respective FA images (Fig. 2, A-C). However, four of the patients had missing or too poor quality of the sagittal FLAIR images from the early phase and the coronal FLAIR images were used. For these four patients a four step registration was used; coronal FLAIR images were registered via coronal T1 images from the chronic phase to sagittal T1 images from the chronic phase, and finally to the FA images. Further, three of the patients had pronounced cerebral atrophy in the chronic phase which made it necessary to use a four step registration also for these patients; sagittal FLAIR images from the early phase were registered via transverse T1 images from the early phase to transverse T1 images from the chronic phase to FA images.

Following the registration, K.G.M. in consensus with L.E. manually drew the outline around every persistent and transient non-hemorrhagic lesion on the FA-registered FLAIR images (Fig. 2), in this way defining the lesion ROI. To secure that the lesion ROI was within

the borders of the actual lesion, the outline was drawn smaller than the actual size of the lesion if this was possible in view of the size of the lesion. Also the non-registered early FLAIR scan was used as a visual control to the manual segmentation of the FA registered FLAIR image, to secure that only voxels located within the lesion were included in the lesion ROI. Because of this procedure and the fact that all lesion ROIs were located in white matter, we have chosen not to use cut off values for FA. Since the lesions varied in size, the number of voxels contained within each ROI differed. We analyzed both FA of all individual voxels within the ROIs and mean FA of the whole lesion ROI.

We performed histogram analyses where FA values from all the individual voxels in each ROI were compared between persistent and transient TAI lesions, while mean FA obtained from the lesion ROIs was used in the linear trend analyses where mean FA also in ROIs of patients without non-hemorrhagic lesions and healthy controls were compared. All FA analyses were done stratified on location (hemispheric white matter and corpus callosum) i.e. FA values from ROIs in corpus callosum were only compared to FA values from other ROIs in corpus callosum. All analyses were done ROI based independent of the individual subject, and thus one patient could contribute with either one, two or more lesion ROIs. If there were three lesion ROIs in one patient, these three ROIs were therefore not combined to one single mean lesion ROI.

Persistent and transient microhemorrhagic lesions

The transverse T2*GRE images from the early phase were registered via sagittal T1 images from the chronic phase to their respective FA images (Fig.3). ROIs were delineated and analyzed with histogram linear trend analyses in the same way as described for the non-hemorrhagic lesions in the section above. One patient had to be excluded from the

microhemorrhagic lesion analyses due to poor quality of the T2*GRE image from the early phase.

ROIs in TBI patients without either non-hemorrhagic or microhemorrhagic lesions and in healthy controls

In order to compare mean FA values from lesions with mean FA values from patients without TAI lesions in the early phase and in healthy controls, ROIs in typical TAI areas were established for the two latter groups. The analyses were performed separately for the FLAIR and the T2*GRE sequences. Thus, the patient group without lesions was different for the analyses of FLAIR and T2*GRE sequences, since this group was interpreted as patients without any non-hemorrhagic lesions in the FLAIR analyses and as patients without any microhemorrhagic lesions in the T2*GRE analyses.

A mean FA image from all TBI patients and healthy controls was created by using the first part of the tract based spatial statistics (TBSS) analysis in FSL (Smith et al. 2006). All subjects' FA data were aligned to each other, identifying the "most typical" subject in the study, which was used as a target image. This target image was affine-aligned to the MNI152 standard space using a nonlinear registration tool IRTK (part of FSL) (Rueckert et al. 1999), and all the FA images were transformed into 1x1x1mm MNI152 space by combining the nonlinear transform to the target FA image with the affine transform from that target to MNI152 space. A mean FA image was created from all the aligned FA images.

Further, to ensure that the ROIs in patients without either non-hemorrhagic or microhemorrhagic lesions and healthy controls were placed in the same white matter areas as for the lesion ROIs, all visible lesions from the early MRI scans were registered from the individual FA maps to this mean FA image. This was done by using the nonlinear transform

from the individual FA image to the mean FA image from the TBSS analyses. Thus, all persistent and transient lesion ROIs from the patients with non-hemorrhagic and microhemorrhagic lesions were combined to either a common non-hemorrhagic lesion mask (Fig. 4) or a common microhemorrhagic lesion mask (Fig. 5). In this way, the mean FA values were extracted from the same areas both in subjects with and without the corresponding TAI lesions. Six different ROIs were selected in the hemispheric white matter (one in each frontal lobe, one in each temporal lobe, and a total of two in parietal and/or occipital lobe depending on the location of lesions), and three in corpus callosum (genu, truncus and splenium), both for the common non-hemorrhagic and microhemorrhagic lesion masks.

Statistical analyses

Demographics and injury-related characteristics are presented as numbers with percentages, mean with standard deviations (SD) and median with ranges. Glasgow Coma Scale (GCS) scores, ISS and Rotterdam CT scores were all analyzed with Kruskal-Wallis Test and Mann-Whitney U test due to lack of normal distributions. Chi Square test was used for comparison of proportions. In the comparison between DTI parameters in transient and persistent lesion ROIs, the Mann Whitney U test was Bonferroni corrected due to multiple testing, according to this formula ($\alpha = 1 - (1-p)^{16}$) due to 16 independent tests.

Histograms with the distribution of individual voxel values of FA were tested with a non-parametric approach with Kolmogorov-Smirnov test for two independent samples (Siegel and Castellan Jr. 1988). To compare mean FA from persistent and transient non-hemorrhagic and microhemorrhagic lesions to ROIs in patients without detectable sequence-specific lesions and healthy controls, a non-parametric test for trend across ordered groups (Cuzick`s Test for

Trend) was used to evaluate the linear trend between mean FA in the different group analyses (Cuzick 1985).

The precision of estimates was assessed with 95% confidence intervals (CI). All tests were considered statistically significant at a probability value < 0.05. All statistical analyses were carried out using the IBM© Statistical Package for the Social Sciences (SPSS) © Statistics version 19 and STATA/SE version 11.2.

Ethics

The Regional Committee for Medical Research Ethics approved this study. Written consent was obtained either from participants or their next of kin if below the age of 16.

RESULTS

Patient characteristics, lesions in clinical MRI and DTI parameters

In table 1 the demographics, injury related characteristics and MRI findings of the 38 included patients are described in detail. The study population had a mean age of 24.7 (range 13.2-63.3) years, and consisted of 29 (76%) males. 17 (45%) patients were injured in a road traffic accident, and 17 (45%) patients in a fall accident. Mean ISS was 26 (range 9-45). Of the 38 included individuals, 18 (47%) patients suffered a severe TBI and 20 (53%) patients a moderate TBI. A total of 20 (53%) patients underwent neurosurgery, of which 9 had evacuation of a mass lesion.

In the early MRI examination, 33 (87%) patients had TAI lesions; 14 (37%) had TAI stage 1, 11 (29%) TAI stage 2 and 8 (21%) TAI stage 3. In 28 patients (74%) cortical contusions were detected in FLAIR and/or T2*GRE sequences. 25 patients had 61 visible non-hemorrhagic lesions in the early FLAIR sequences, of which 15 (25%) were persistent in the chronic phase FLAIR sequences (Table 2). 29 patients had 97 visible microhemorrhagic lesions in the early phase T2*GRE sequences, of which 86 (89%) were persistent in the chronic phase T2*GRE sequences. For the brain stem only 7 non-hemorrhagic lesions and 3 microhemorrhagic lesions were detected, and due to these low numbers the brain stem lesions were not included for further analyses (Table 2).

Table 3 shows the DTI parameters including FA, mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) for the transient and persistent lesion ROIs based on location. We found no significant differences between transient and persistent lesions after Bonferroni correction (Table 3).

Fractional anisotropy (FA) in non-hemorrhagic lesions

Histogram analyses of all the individual voxels in the ROIs showed reduced FA for persistent compared to transient non-hemorrhagic lesions both in the hemispheric white matter (Fig. 6A, $p<0.001$) and corpus callosum (Fig. 6B, $p<0.001$). A significant linear decrease in mean FA was found in both the hemispheric white matter and corpus callosum; the highest mean FA was found in ROIs among healthy controls followed by ROIs with no non-hemorrhagic lesions and then ROIs in transient non-hemorrhagic lesions. The lowest FA was found in persistent non-hemorrhagic lesion ROIs (Fig. 7, $p<0.001$). The difference in mean FA was most pronounced between ROIs placed in lesions and ROIs placed in areas without lesions (Fig. 7).

Fractional anisotropy (FA) in microhemorrhagic lesions

Histogram analyses of the individual FA voxel values did not show any statistically significant differences between persistent and transient lesion ROIs, neither in the hemispheric white matter ($D=0.13$, $n= 815$, $p=0.55$), nor in the corpus callosum ($D=0.23$, $n=194$, $p=0.08$). However, when comparing mean FA values in persistent and transient microhemorrhagic lesion ROIs with mean FA values in ROIs of patients without microhemorrhagic lesions and healthy controls, a significant linear decrease of mean FA was found in both hemispheric white matter and corpus callosum (Fig. 8, $p<0.001$). The highest mean FA was found in white matter ROIs in healthy controls, followed by ROIs in patients with no microhemorrhagic lesions. The lowest mean FA was found in persistent microhemorrhagic lesion ROIs in the hemispheric white matter, but for the corpus callosum the lowest mean FA was found in the transient lesion ROIs.

However there were only four patients in this group and the standard deviation was high, so the trend test still showed a highly significant linear decrease ($p<0.001$).

DISCUSSION (wordcount: 1536, maximum 1600)

In this prospective longitudinal study, white matter FA in the chronic phase of TBI was explored in areas with persistent and transient non-hemorrhagic and microhemorrhagic TAI lesions identified from early clinical MRI. Furthermore, FA values from equivalent areas in TBI patients without either non-hemorrhagic or microhemorrhagic lesions and in matched healthy controls were investigated and linear trends assessed. Lower FA values were found in TBI patients without either non-hemorrhagic or microhemorrhagic lesions compared to healthy controls. Even lower FA was found in transient TAI lesion areas, and lowest FA was found in persistent TAI lesion areas. This linear relationship in FA was found both for non-hemorrhagic and microhemorrhagic lesions, but statistical significant difference between FA in persistent and transient lesions was only found for non-hemorrhagic lesions.

Fractional anisotropy (FA) in non-hemorrhagic lesions

Histogram analyses of individual FA voxel values in the hemispheric white matter and corpus callosum demonstrated significantly reduced FA in persistent compared to transient non-hemorrhagic lesions. This indicates that brain areas with persistent lesions have more pronounced microstructural damage. A linear relationship was observed where white matter FA gradually decreased from ROIs in healthy controls to ROIs in TBI patients without non-hemorrhagic lesions and further to transient non-hemorrhagic lesion ROIs. The lowest FA was found in persistent non-hemorrhagic lesion ROIs. This finding show a gradually increasing severity of the white matter injury, with the most severe reduction in mean FA being associated with persistent lesions depicted in the FLAIR image in the chronic phase. This is the first DTI

study to compare FA in ROIs of persistent versus transient non-hemorrhagic lesions and to demonstrate such a linear decrease of mean FA values.

So far, only one other study has examined FA values in white matter lesions depicted in clinical MRI from the chronic phase (Gupta et al. 2005). In that study, FA in non-hemorrhagic lesions were compared to FA in regions of normal appearing white matter in the contralateral side, using a within subject design. There was no control group, and the non-hemorrhagic lesions were not subdivided into persistent and transient lesions. However, the results are still relevant and comparable to the results in our study, since they found lower FA values in non-hemorrhagic lesions in the chronic phase when compared to normal appearing white matter in TBI patients.

Several previous DTI studies from the chronic phase have reported lower FA in most parts of the brain after TBI when compared to healthy controls (Bendlin et al. 2008; Haberg et al. 2014; Newcombe et al. 2011), and an association between injury severity and degree of reduction in FA has also been found (Haberg et al. 2014; Rutgers et al. 2008). In patients with moderate and severe TBI, TAI lesions are often present in early clinical MRI (Lagares et al. 2009; Moen et al. 2014; Moen et al. 2012; Skandsen et al. 2009), and it is expected that lower FA values in these lesion areas reflect more severely injured white matter microstructure. However, in previous DTI studies FA has not directly been studied in lesion ROIs from the early phase, as we have done in this study.

Fractional anisotropy (FA) in microhemorrhagic lesions

Even though we found no statistical significant differences in mean FA between persistent and transient microhemorrhagic lesions, there was a significant linear trend of lower mean FA in both hemispheric and callosal white matter ROIs in healthy controls, to ROIs in patients without

microhemorrhagic lesions, and further to transient and finally persistent microhemorrhagic lesion ROIs.

No other study has directly compared FA in persistent versus transient microhemorrhagic lesions, and no other study has compared DTI parameters in microhemorrhagic lesions to controls. There are three other recent TBI studies that have shown a relationship between microhemorrhagic lesions from the early phase and reduced FA values in DTI from the chronic phase (Asano et al. 2012; Benson et al. 2012; Kumar et al. 2009). In one DTI study of 8 patients with microhemorrhagic lesions, lower FA values in corpus callosum in early MRI was found when compared to controls, and the reduced FA persisted at 6 months (Kumar et al. 2009). In 10 patients with mild-moderate TBI, DTI in the chronic phase showed reduced FA in regions which included most of the microhemorrhagic lesions detected in the T2*GRE sequence from the chronic phase (Asano et al. 2012). Also, Benson et al. reported that T2*GRE and FLAIR lesion areas depicted in MRI from the chronic phase were co-localized to areas with reduced FA in DTI indicating more severe injury to the visible lesion areas (Benson et al. 2012; Benson et al. 2007). However, all these studies differ from the present, since lesion areas were not directly studied and they also included few patients.

Common considerations for non-hemorrhagic and microhemorrhagic lesions

Despite the linear relationship shown in figure 7 and 8, we observed that the main difference in mean FA values was found between the ROIs of TBI patients with lesions versus ROIs in patients without either non-hemorrhagic or microhemorragic lesions. This could suggest that areas in the brain with detectable lesions in the early stage represent areas with more pronounced microstructural damage. Longitudinal TBI studies have demonstrated that many non-hemorrhagic lesions disappear early (Chung et al. 2012; Moen et al. 2012). In the present study, ROIs that appear normal and are thought to represent normal white matter in the chronic stage, can demonstrate quite different FA values depending on whether the ROIs comprise early lesions or not. This underscores that access to an early MRI scan is necessary for a proper evaluation of location, type, extent and duration of TAI lesions, such information would also be beneficial when interpreting the DTI.

It is known from previous studies that microhemorrhagic lesions develop differently over time compared to non-hemorrhagic lesions since the former more often persist, and microhemorrhagic lesions are more often depicted and more numerous in the hemispheres (Messori et al. 2003; Moen et al. 2012). Since microhemorrhagic lesions represent a paramagnetic phenomenon caused by microbleedings in the tissue, they probably affect the microstructure of axons to a less degree as compared to non-hemorrhagic lesions, which represents edema in the early phase and later gliosis. This could explain the findings that FA changes in microhemorrhagic lesions were more subtle compared to the FA changes in non-hemorrhagic lesions. The differences in diffusion may also be more easily detected in non-hemorrhagic lesions since they are more extensive.

Strengths and limitations of the study

One of the main strengths of this study is the prospective data collection and the comprehensive MRI protocol both in early and chronic phase. Furthermore, all image analyses from the early phase were performed blinded for patient identification and clinical information. A large healthy control group is also an important strength.

There are limitations in the study; time from injury to the early MRI scan should ideally have been shorter and more standardized to avoid missing already disappeared lesions, which particularly applies to the non-hemorrhagic lesions (Moen et al. 2012). The sample size should preferably have been larger. Since 3 T MRI is more sensitive in detecting TBI lesions, the use of 1.5 T MRI in the early phase could lead to non-detection (Scheid et al. 2007) and introduce partial volume effects, especially for microhemorrhagic lesions due to their small size. Since only lesion detection was done in FLAIR or T2*GRE sequences from these early 1.5 T MRI scans (all DTI data are from the same 3T scanner), we consider this limitation as minor. An earlier study also concluded that the use of different MRI scanners only had limited influence on the measured brain volumes in patients with multiple sclerosis (Gasperini et al. 2001).

Differences in contrast in the FLAIR sequence could introduce errors, since we had 3D acquisition in the chronic phase and 2D in the early phase. Microhemorrhagic lesions are also prone to distort DTI data due to the susceptibility effect that could cause potentially misleading FA values. Some DTI studies use cut-off values for FA for excluding voxels below a predetermined level since these could be outside the target area. It is, however, difficult to precisely determine a FA cut-off value, since the values could be substantially reduced due to injury in itself and not caused by being outside the white matter.

CONCLUSION

The most important finding in this study was a linear trend with gradually lower mean FA values from ROIs in white matter in healthy controls to ROIs in persistent TAI lesions in TBI patients. The mean FA values in ROIs of TBI patients without either non-hemorrhagic or microhemorrhagic lesions were lower than in ROIs of healthy controls, which again were even lower in transient lesion ROIs. The lowest mean FA values were found in persistent lesion ROIs. This linear relationship in mean FA was found both for non-hemorrhagic and microhemorrhagic lesions, but statistical significant difference between persistent and transient lesions was only found for non-hemorrhagic lesions.

The study demonstrates that white matter ROIs have quite different FA values depending on whether they comprise tissue affected by early TAI lesions as well as the type of the lesion. An early MRI would be essential to derive this information that could be helpful when interpreting the DTI parameters in TBI research.

Conflict of interest statement:

I declare on behalf of all authors, there are no financial, personal or professional interests that could influence this paper.

Roles of authors:

I declare that all authors have contributed sufficiently for being an author of this paper:

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Study design: K.G.M., A.V., A.K.H., L.E.

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FIGURE LEGENDS

Figure 1: The steps from the image analyses illustrated in a flow-chart.

Figure 2: The sagittal FLAIR image (A) from the early phase was linearly registered to the transverse FA image (C) from the chronic phase. The lesion, in this case a non-hemorrhagic lesion (red arrow) in the corpus callosum, was then manually drawn in the FA registered FLAIR image (B) and applied (red-yellow color) to the chronic FA image (C).

Figure 3: The transverse T2*GRE image (A) from the early phase was linearly registered to the transverse FA image (B) from the chronic phase. The lesions, in this case a microhemorrhagic lesion in corpus callosum (red unbroken arrow) and a microhemorrhagic lesion in the right frontal hemisphere (broken green arrow), were manually drawn on the FA-registered T2*GRE from the early phase and applied to the chronic FA image (B).

Figure 4: The mean FA image from all TBI patients and healthy controls were used to compute regions of interest (ROIs) to obtain mean FA values from areas with frequent non-hemorrhagic lesions. In image A two of the frontal ROIs in the hemispheric white matter are indicated (green color), and in image B two of the parietal ROIs are shown (green color). In image C all three ROIs with frequent non-hemorrhagic lesions in corpus callosum are indicated in genu, truncus and splenium (red color), respectively.

Figure 5: The mean FA image from all the TBI patients and healthy controls were used to compute regions of interest (ROIs) to obtain mean FA values from areas with frequent microhemorrhagic lesions. In image A one frontal ROI in the hemisphere is indicated (green

color), and in image B two of the hemispheric white matter (one parietal and one occipital) ROIs are shown (green color). In image C two of the three ROIs with frequent microhemorrhagic lesions in corpus callosum are indicated in genu and splenium (red color), respectively.

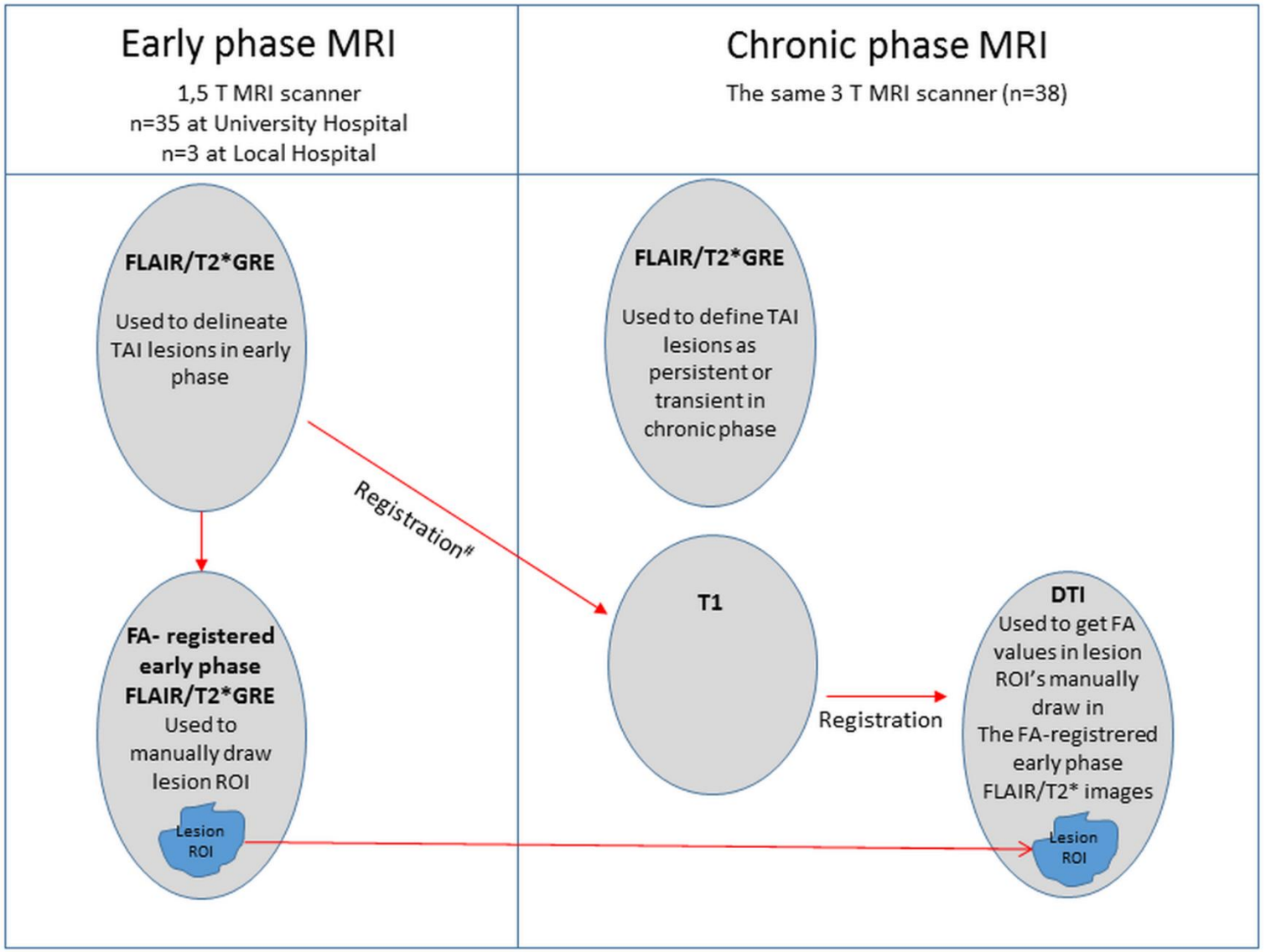
Figure 6A: Histograms of individual FA voxel values in persistent (histogram I) and transient (histogram II) non-hemorrhagic lesions in the hemispheric white matter. A two-sample Kolomogorov-Smirnov test determined significant different distributions ($p<0.001$).

Figure 6B: Histograms of individual FA voxel values in persistent (histogram I) and transient (histogram II) non-hemorrhagic lesions in corpus callosum. A two-sample Kolomogorov-Smirnov test determined significant different distributions ($p<0.001$).

Figure 7: Box-whisker plot indicating median (both numeric and as a thick black line) with the box indicating 25 and 75 percentiles of the mean FA in ROIs from healthy controls, patients with no non-hemorrhagic lesion (NHL), transient non-hemorrhagic lesions and persistent non-hemorrhagic lesions. Cuzick`s Test for Trend showed a p-value of < 0.001 for both the hemispheric white matter and corpus callosum.

Figure 8: Box-whisker plot indicating median (both numeric and as a thick black line) with the box indicating 25 and 75 percentiles of the mean FA in ROIs from healthy controls, patients with no microhemorhagic lesion (MHL), transient microhemorrhagic lesion and persistent microhemorrhagic lesion. Cuzick`s Test for Trend showed a p-value of < 0.001 for both the hemispheric white matter and corpus callosum.

FIG 1



For n=7 patients the registration method was slightly different due to a better registration result. We refer to the Region of interest (ROI) analyses section in the Materials and Methods for further details.

FIG 2

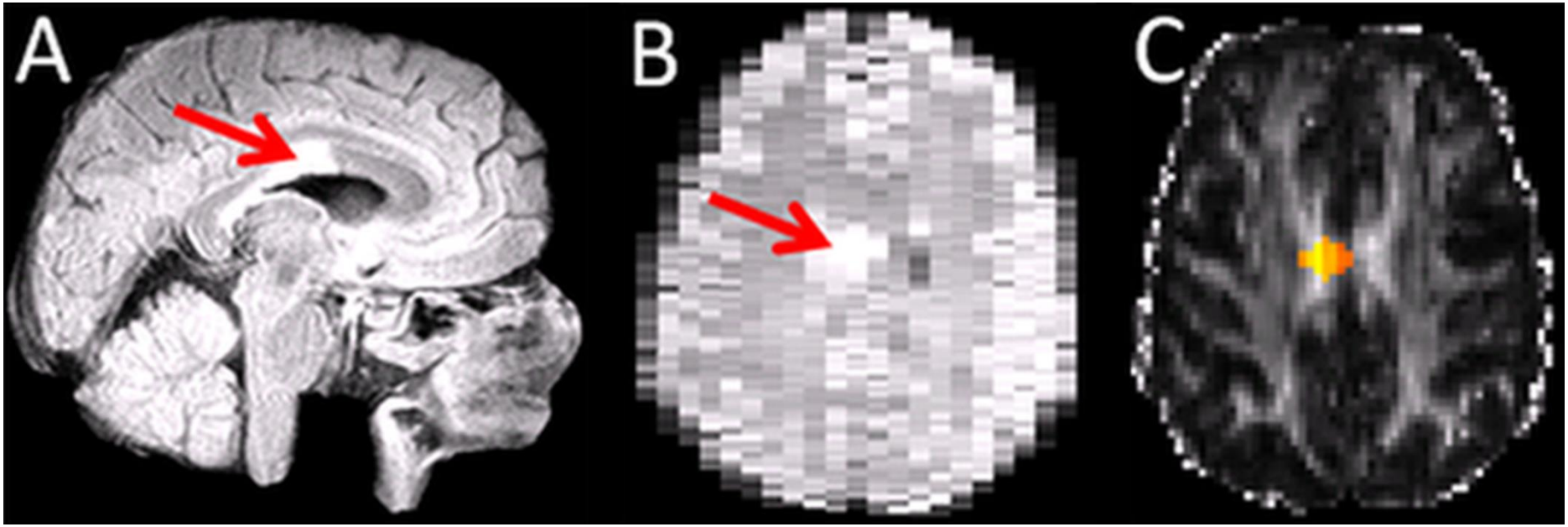


FIG 3

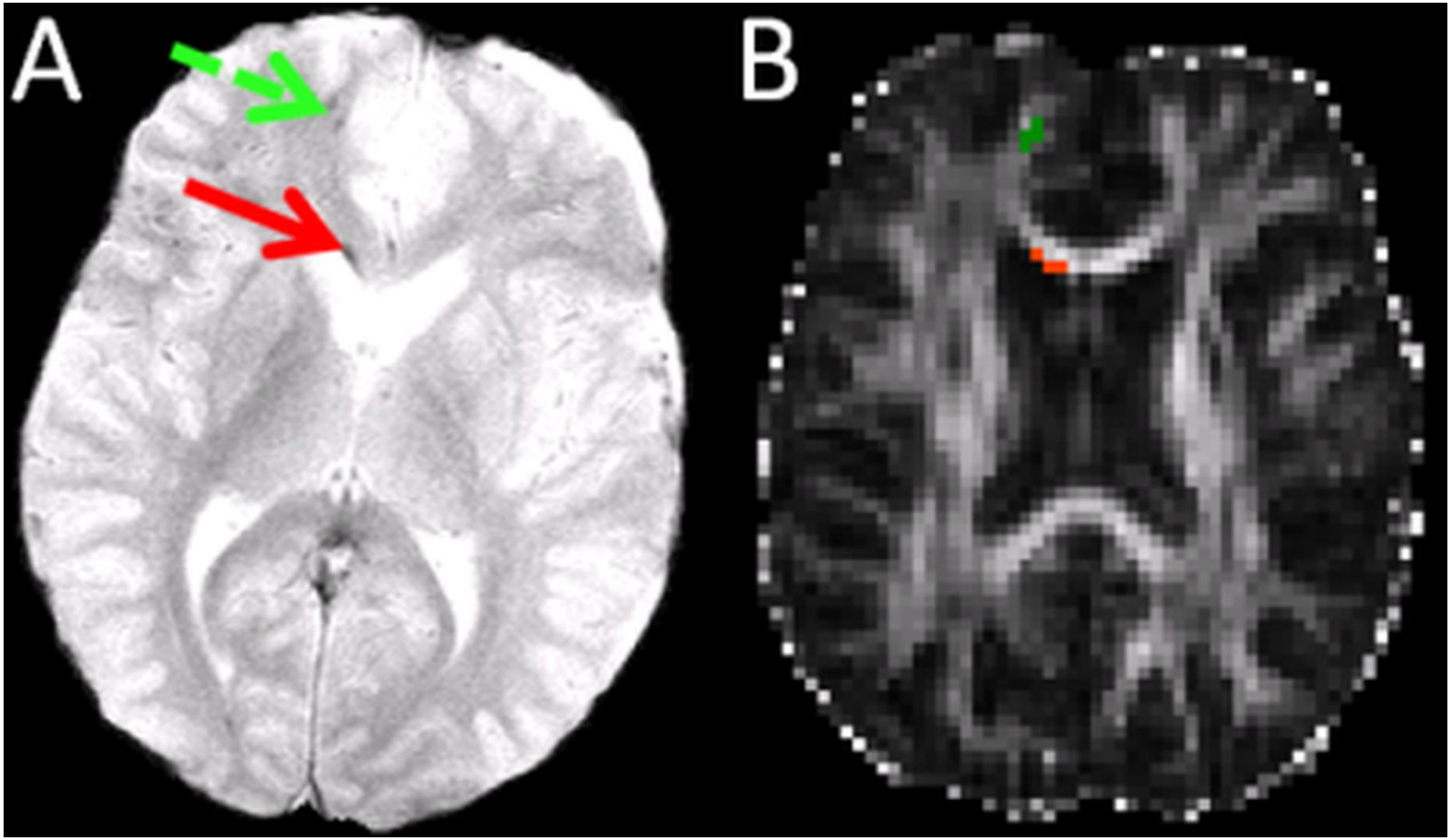


FIG 4

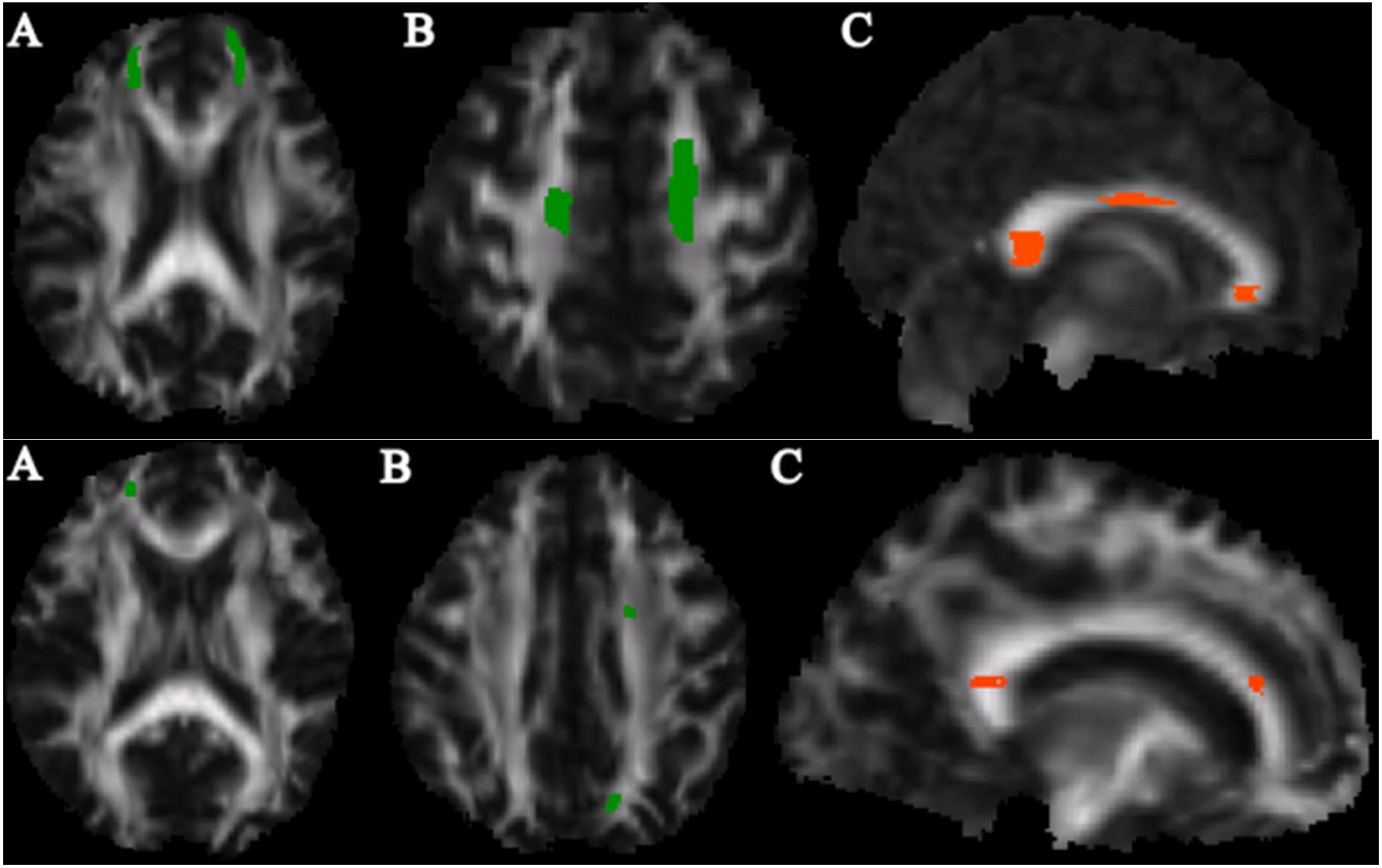


FIG 5

FIG 6A

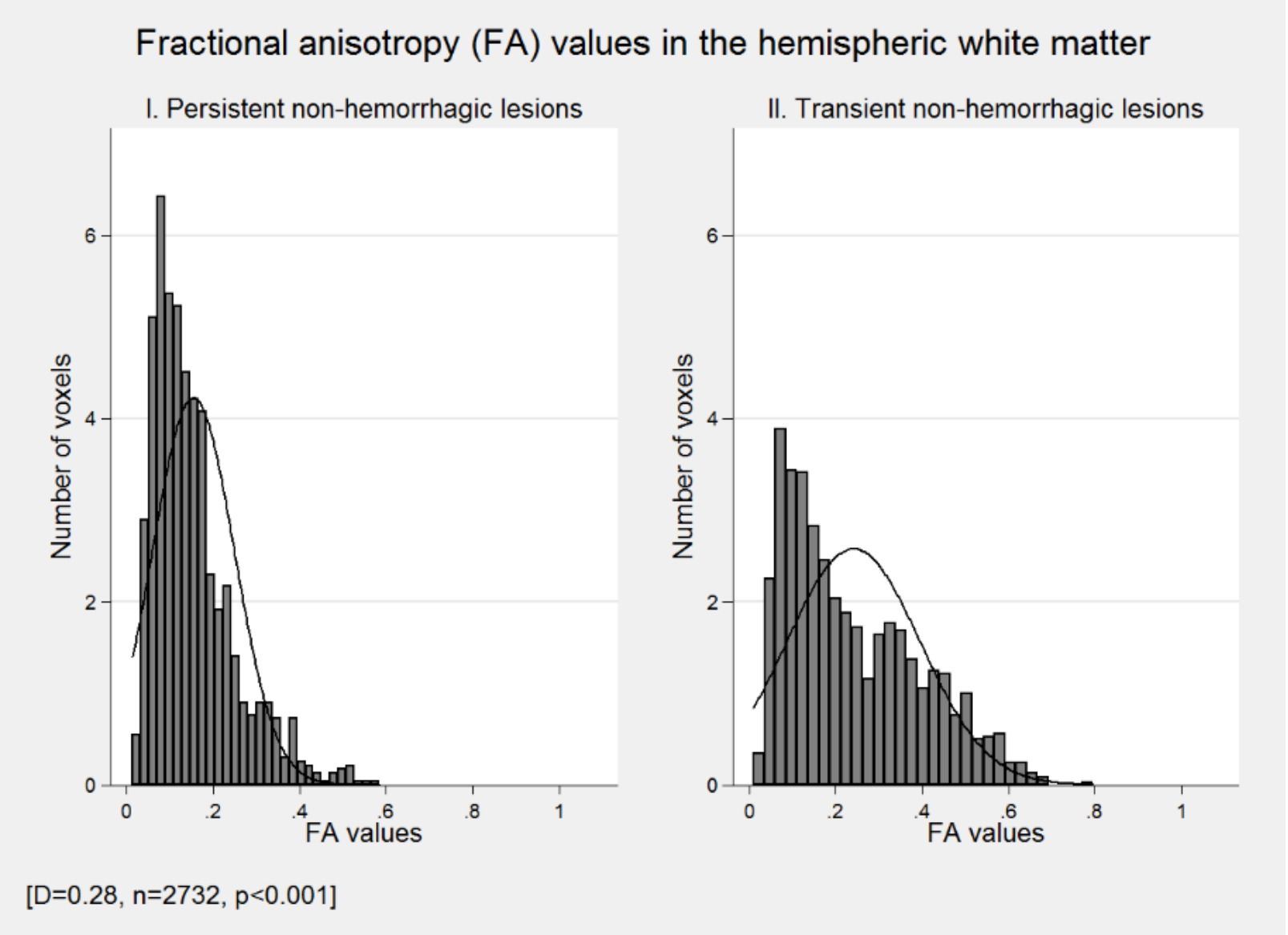

Fractional anisotropy (FA) values in the hemispheric white matter
I. Persistent non-hemorrhagic lesions
II. Transient non-hemorrhagic lesions
Number of voxels
0
2
4
6
0
.2
.4
.6
.8
1
FA values
[D=0.28, n=2732, p<0.001]

FIG 6B

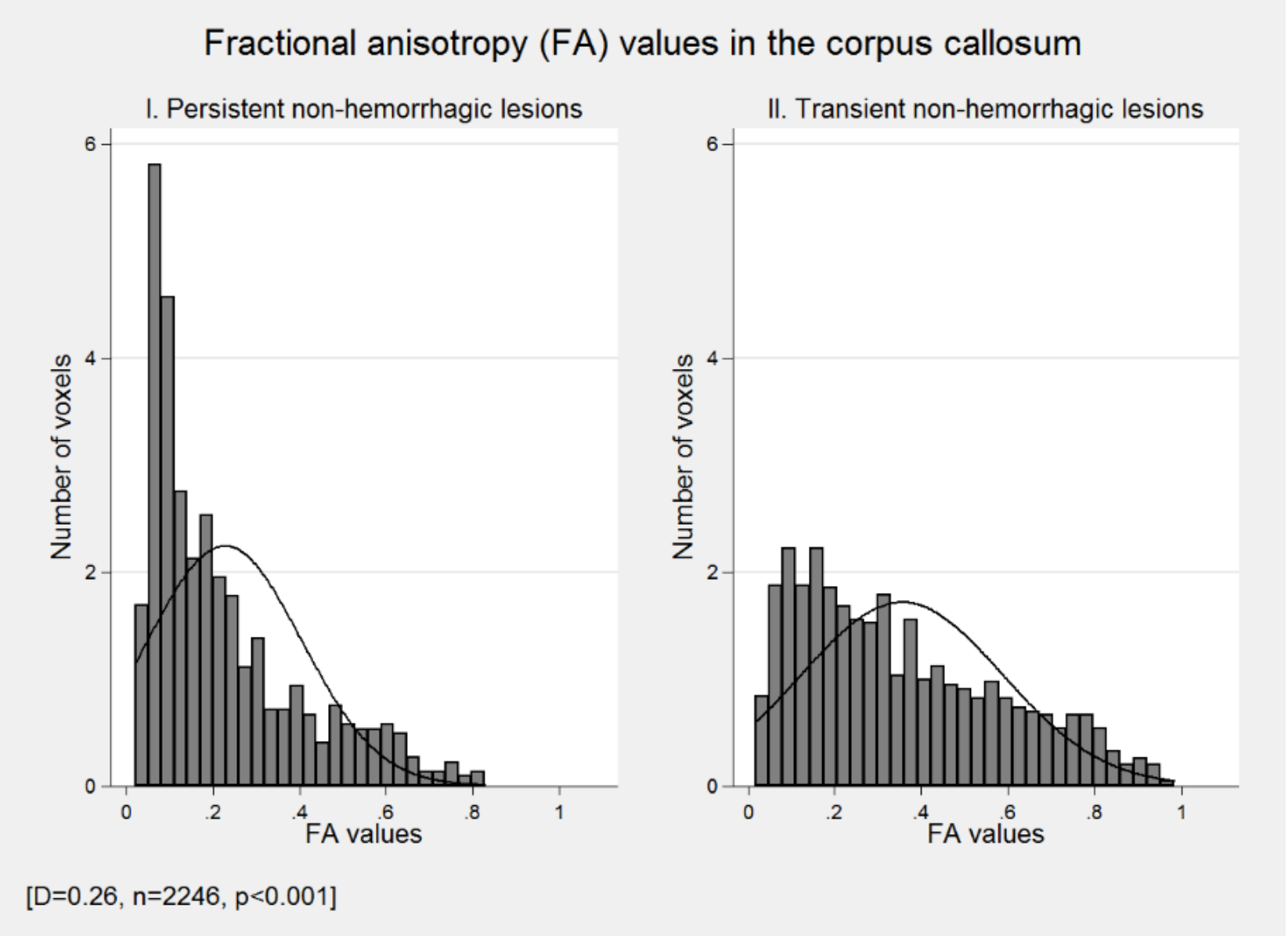

Fractional anisotropy (FA) values in the corpus callosum
I. Persistent non-hemorrhagic lesions
II. Transient non-hemorrhagic lesions
Number of voxels
0
2
4
6
0
.2
.4
.6
.8
1
FA values
[D=0.26, n=2246, p<0.001]

FIG 7

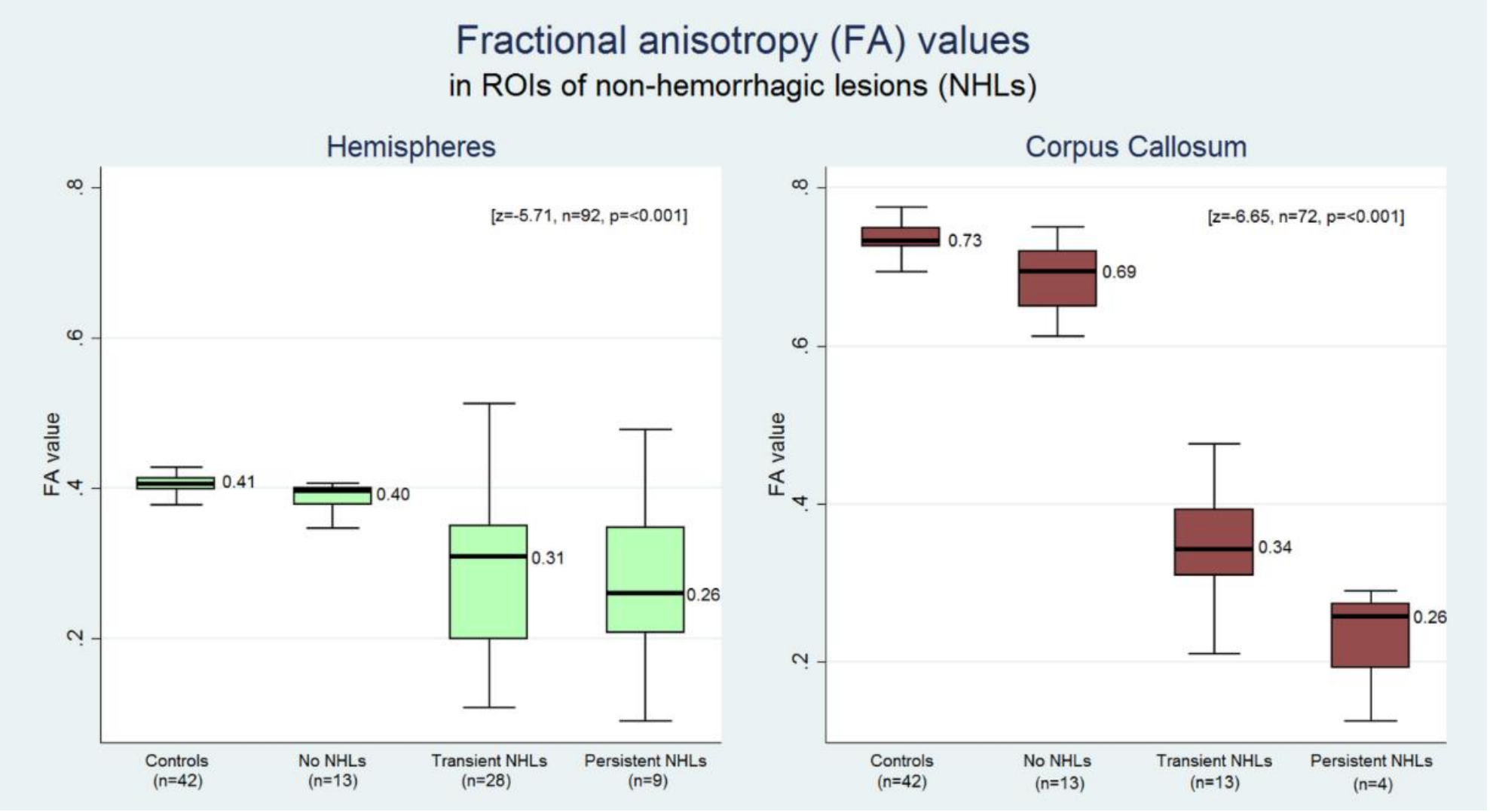

Fractional anisotropy (FA) values
in ROIs of non-hemorrhagic lesions (NHLs)
Hemispheres
[z=-5.71, n=92, p=<0.001]
FA value
.2
.4
.6
.8
0.41
0.40
0.31
0.26
Controls (n=42)
No NHLs (n=13)
Transient NHLs (n=28)
Persistent NHLs (n=9)
Corpus Callosum
[z=-6.65, n=72, p=<0.001]
FA value
.2
.4
.6
.8
0.73
0.69
0.34
0.26
Controls (n=42)
No NHLs (n=13)
Transient NHLs (n=13)
Persistent NHLs (n=4)

Fig 8

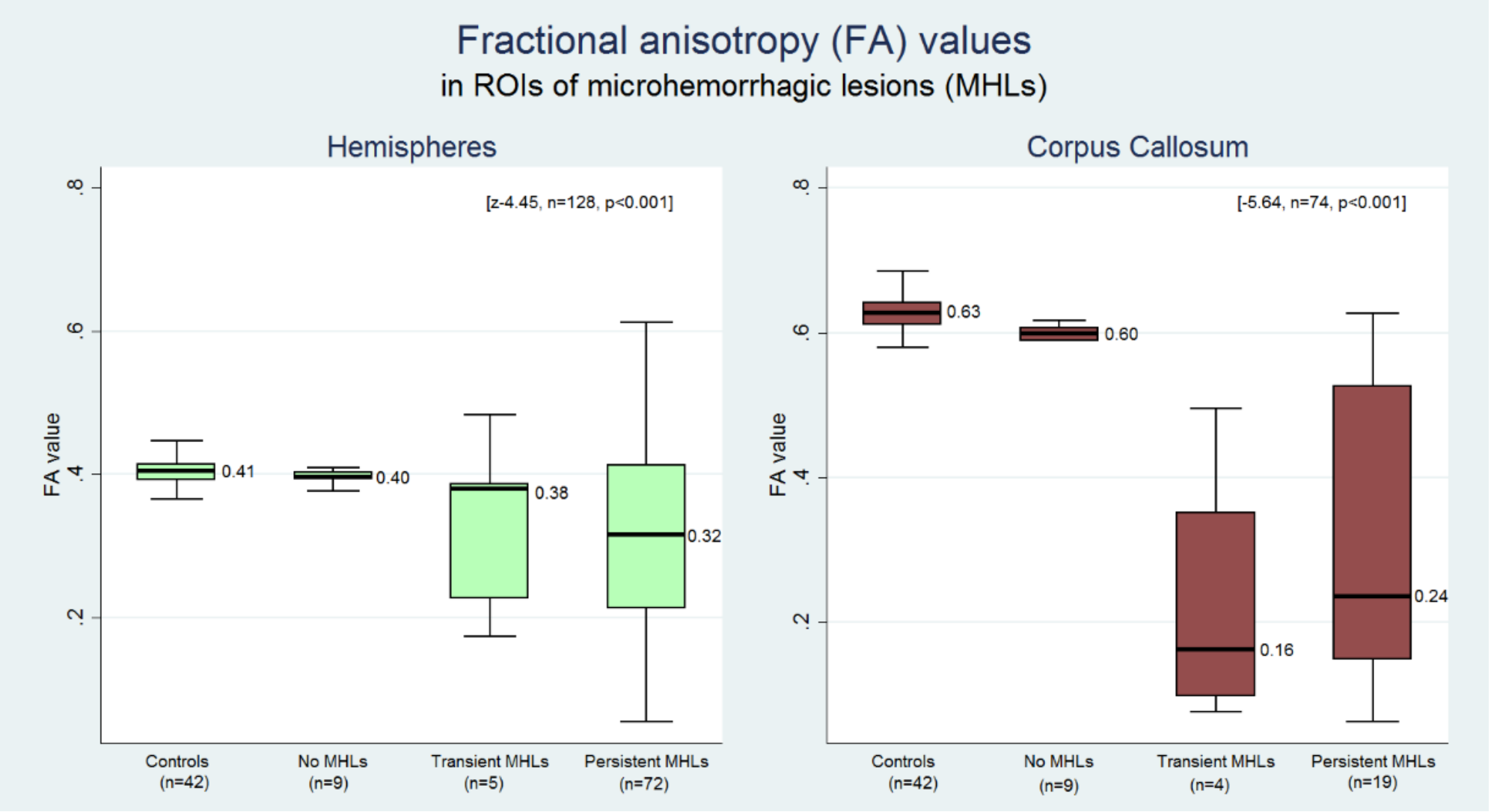

Fractional anisotropy (FA) values
in ROIs of microhemorrhagic lesions (MHLs)
Hemispheres
[z-4.45, n=128, p<0.001]
FA value
.2
.4
.6
.8
0.41
0.40
0.38
0.32
Controls (n=42)
No MHLs (n=9)
Transient MHLs (n=5)
Persistent MHLs (n=72)
Corpus Callosum
[-5.64, n=74, p<0.001]
FA value
.2
.4
.6
.8
0.63
0.60
0.16
0.24
Controls (n=42)
No MHLs (n=9)
Transient MHLs (n=4)
Persistent MHLs (n=19)

Table 1 Demographic and injury related variables of each individual TBI patient based on Glasgow Coma Scale score

HISS class	GCS score*	Sex	Age**	Injury mechanism	Pupil-size	CT#	GOSE score##	MRI time§	TAI stage	Visible injury to CC	Number of lesions in the early MRI				DTI time§§
											MHLs		NHLs		
											Hem	CC	Hem	CC	
Mod	-	M	23	Fall	N	3	7	41	1	No			3		3,9
Mod	13	M	31	Fall	N	3	7	14	No TAI	No					4,7
Mod	13	M	16	Fall	N	1	8	2	No TAI	No					4,5
Mod	13	F	25	Fall	N	2	7	4	No TAI	No					2,3
Mod	13	F	18	Other	N	1	8	13	No TAI	No					3,0
Mod	13	M	30	RTA	N	3	6	21	1	No	3				2,1
Mod	13	F	63	Fall	N	5	5	6	2	Yes	1	1		2	2,0
Mod	13	M	22	Fall	N	2	8	6	2	Yes	4	1			2,1
Mod	13	M	24	Other	N	2	7	3	2	Yes	2			1	1,8
Mod	12	M	55	Fall	N	5	5	26	1	No	1		2		3,8
Mod	12	M	29	Fall	N	3	8	14	No TAI	No					3,1
Mod	12	M	19	RTA	N	3	8	5	2	Yes	1	1	1	1	1,5
Mod	11	M	16	Other	N	2	7	2	1	No	3				3,3
Mod	11	M	34	RTA	N	4	5	7	3	Yes	1		3		2,5
Mod	11	M	32	Fall	N	3	8	5	1	No			1		1,5
Mod	11	M	15	Fall	N	3	8	4	3	Yes	5	1	1		1,7
Mod	9	F	22	RTA	N	2	8	11	3	No	3		3		5,4
Mod	9	F	14	Other	N	2	7	6	2	Yes	2		2	1	3,2
Mod	9	F	41	RTA	N	3	8	2	3	Yes	4	1	1	1	3,0
Mod	9	M	13	Fall	N	2	8	3	1	No					3,3
Sev	8	M	25	Fall	N	4	8	4	1	No	4				4,3
Sev	8	F	16	RTA	N	2	7	11	1	No	3		1		3,8
Sev	8	M	15	RTA	N	4	7	17	1	No	4		2		3,4
Sev	7	M	22	Fall	N	2	6	7	2	Yes	2	2	3	1	3,0
Sev	7	F	30	RTA	N	2	7	8	2	Yes	3	1	2	1	1,5
Sev	6	M	18	RTA	N	1	8	6	1	No	2		3		4,1
Sev	6	M	16	Fall	N	3	6	25	3	Yes	4	1			2,2
Sev	6	M	24	RTA	N	4	5	23	2	Yes				1	2,5
Sev	5	M	21	RTA	N	2	7	3	3	Yes	2	2		1	4,1
Sev	5	M	20	RTA	N	2	7	15	1	No	1				3,2
Sev	5	M	24	Fall	N	3	7	2	2	Yes	4	1	2	1	3,1
Sev	5	M	19	RTA	N	5	5	14	2	Yes	1			1	2,0
Sev	5	F	17	RTA	N	2	8	11	1	No	1				1,7
Sev	5	M	20	RTA	N	2	6	5	2	Yes	3		1	1	1,5
Sev	3	M	20	Fall	N	2	8	4	2	Yes	1	1	3	1	5,4
Sev	3	M	19	RTA	U.D	4	3	35	3	Yes	5	3	1	2	3,5
Sev	3	M	47	RTA	N	3	6	11	2	Yes	2	1	1	1	3,0
Sev	3	M	26	Fall	B.D.	2	8	29	1	No	4				1,5
Mean	*8,6*		*24,7*			*2,7*	*6,9*	*11,2*							*3,0*

*At admission to univeristy hospital or before intubation, in one patient the exact GCS is missing ** At injury time (in years) # CT Rotterdam score ## At 12 months post-injury

§ Time between injury and early conventional MRI aquisition (in days) §§ Time between injury time and chronic DTI aquisition (in years)

TBI, traumatic brain injury; HISS, head injury severity scale; GCS, glasgow coma scale; GOSE, glasgow outcome scale extended; MRI, magnetic resonance imaging; DTI, diffusion tensor imaging; TAI, traumatic axonal injury; CC, corpus callosum; Hem, hemispheres; MHLs, microhemorrhagic lesions; NHLs, non-hemorrhagic lesions; Mod, moderate TBI; Sev, severe TBI; F, female; M, male; RTA, road traffic accident; N, normal; U.D., unilateral dilatation; B.D., bilateral dilatation

Table 2 Evolution of TAI lesions in the clinical MRI from the early to chronic phase

	Non-hemorrhagic lesions (in 25 patients)			**Microhemorrhagic lesions (in 29 patients)**		
	Total	Persistent	Transient	*Total*	Persistent	Transient
Hemispheres	*37 (61%)*	9 (15%)	28 (46%)	*77 (79%)*	72 (74%)	5 (5%)
Corpus callosum	*17 (28%)*	4 (7%)	13 (21%)	*17 (18%)*	13 (13%)	4 (4%)
Brain stem	*7 (11%)*	2 (3%)	5 (8%)	*3 (3%)*	1 (1%)	2 (2%)
Total	*61 (100%)*	15 (25%)	46 (75%)	*97 (100%)*	86 (89%)	11 (11%)

Values are lesion number (%). TAI, traumatic axonal injury.

Table 3 DTI parameters from ROIs in persistent and transient TAI lesions

	Non-hemorrhagic lesions						Microhemorrhagic lesions					
	Persistent			*Transient*			*Persistent*			*Transient*		
	Mean	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*
Hemispheres												
FA	0.28	0.13	9	0.29	0.11	28	0.31	0.14	72	0.33	0.13	5
MD (10^{-3} mm^2/sec)	0.91	0.29	9	0.85	0.13	28	0.90	0.24	72	0.79	0.07	5
λ_1 (10^{-3} mm^2/sec)	1.14	0.24	9	1.09	0.11	28	1.18	0.23	72	1.07	0.12	5
λ_2 (10^{-3} mm^2/sec)	0.90	0.29	9	0.81	0.14	28	0.84	0.26	72	0.73	0.08	5
λ_3 (10^{-3} mm^2/sec)	0.69	0.35	9	0.65	0.18	28	0.67	0.28	72	0.55	0.14	5
Corpus callosum												
FA	0.23	0.07	4	0.36	0.10	13	0.32	0.21	13	0.22	0.19	4
MD (x10^{-3} mm^2/sec)	1.63	0.20	4	1.48	0.42	13	1.70	0.72	13	1.84	1.21	4
λ_1 (10^{-3} mm^2/sec)	1.98	0.30	4	2.01	0.47	13	2.17	0.61	13	2.15	1.19	4
λ_2 (10^{-3} mm^2/sec)	1.53	0.17	4	1.29	0.42	13	1.54	0.80	13	1.77	1.23	4
λ_3 (10^{-3} mm^2/sec)	1.36	0.14	4	1.14	0.41	13	1.39	0.79	13	1.62	1.21	4

The table shows the mean DTI parameters for all lesion ROIs, divided into locations.

DTI; diffusion tensor imaging, ROI; region of interest, TAI; traumatic axonal injury, SD; standard deviation.

FA; fractional anisotropy, MD; mean diffusivity, λ_1 equals the axial diffusivity.