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Análise longitudinal por imagens de tensor de difusão do encéfalo de pacientes com lesão axonial difusa traumática moderada e severa

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutora em Ciências

Programa de Radiologia

Orientadora: Profa. Dra. Celi Santos Andrade

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Longitudinal analysis of brain diffusion tensor imaging in patients with moderate and severe traumatic diffuse axonal injury

Thesis presented to the Faculty of Medicine of the Universidade de São Paulo to obtain the title of Doctor of Science

Concentration area: Radiology

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DEDICATORY

Dedicated to my family, in special my parents, who have supported me along my personal and academic trajectory.

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To Lia, our tireless secretary, always helpful and available.

“If human life were long enough to find the ultimate theory, every thing would have been solved by previous generations. Nothing would be left to be discovered.”

Stephen Hawking

SUMMARY

LIST OF ABBREVIATIONS

AD	Axial diffusivity
ADC	Apparent diffusion coefficient
BET	Brain extraction tool
CC	Corpus callosum
CSF	Cerebrospinal fluid
CST	Cortical spinal tract
CT	Computed tomography
DAI	Diffuse axonal injury
DKI	Diffusion kurtosis imaging
DS	Digital span
DTI	Diffusion tensor imaging
DWI	Diffusion weigthed imaging
FA	Fractional anisotropy
FACT	Fiber assignment by continuous tracking
FFE	Fast field echo
FLAIR	Fluid attenuated inversion recovery
FMRIB	Functional MRI brain
FOV	Field of view
FWE	Family-wise error
GCS	Glasgow Coma Scale
GEE	Generalized estimating equation
GOS	Glasgow Outcome Scale
GOS-E	Glasgow Outcome Scale Extended
HARDI	High angular resolution diffusion imaging
HVLT	Hopkins verbal learning test
IFOF	Inferior fronto-occipital fascicle
ILF	Inferior longitudinal fascicle
IQ	Intelligence quotient
IT	Inversion time
JHU	Johns Hopkins University

L	Left
MD	Mean diffusivity
MR	Magnetic resonance
MRI	Magnetic resonance imaging
NP	Neuropsychological
PRESTO	Principles of echo shifting with a train of observations
R	Right
RD	Radial diffusivity
ROI	Region of interest
SLF	Superior longitudinal fascicle
TBI	Traumatic brain injury
TBSS	Tract-based spatial statistics
TFCE	Threshold-free cluster enhancement
TRACULA	Tracts constrained by underlying anatomy

RESUMO

Grassi DC. *Análise longitudinal por imagens de tensor de difusão do encéfalo de pacientes com lesão axonial difusa traumática moderada e severa* [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2021.

Introdução: Atualmente, o traumatismo cranioencefálico (TCE) é um problema de saúde pública mundial devido à sua alta prevalência, morbidade e mortalidade. Os mecanismos envolvidos no trauma são complexos e diferentes tipos de lesões podem estar presentes, dentre elas a lesão axonial difusa (LAD). Sabe-se que a LAD é altamente prevalente em vítimas de trauma moderado e grave, sendo caracterizada por diferentes processos fisiopatológicos complexos e prolongados que determinam um processo neurodegenerativo encefálico. **Objetivo:** Avaliar longitudinalmente com imagens de tensor de difusão (DTI) a integridade da substância branca cerebral de pacientes com LAD moderada e severa em três momentos após o trauma e comparativamente com indivíduos controles normais. Além disso, verificar se há correlação entre os parâmetros quantitativos de DTI e dados dos exames neuropsicológicos dos pacientes. **Métodos:** Foram selecionadas 20 vítimas de TCE moderado e grave e 20 controles, pareados para idade e sexo. Foi realizado exame de RM em três diferentes tempos: 2 meses (tempo 1), 6 meses (tempo 2) e 12 meses (tempo 3) do TCE. No grupo controle, o exame de RM foi realizado em um único momento. Utilizamos métodos de análise de tratografia para a avaliação do fascículo longitudinal superior e do corpo caloso, bem como a técnica de análise voxel a voxel para a avaliação do encéfalo total para extrair os parâmetros quantitativos de DTI. Além disso, diferentes domínios cognitivos foram avaliados, bem como análise do desfecho clínico dos pacientes. Testes de correlação foram feitos usando os parâmetros de DTI com os domínios cognitivos examinados. Foram considerados positivos os resultados com $p< 0,05$. **Resultados:** O grupo de pacientes apresentou em todos os segmentos estudados valores médios de FA menores e valores médios de DM maiores em relação ao grupo controle. A análise baseada em voxel indicou mudanças significativas ao longo do tempo nos parâmetros de DTI no grupo dos pacientes. Foram observadas melhora na atenção e memória dos pacientes. Identificamos diferentes correlações entre os parâmetros de DTI e as diferentes funções cognitivas ao longo do tempo. **Discussão e conclusões:** Observamos alterações quantitativas nos valores de DTI nos tratos estudados. A FA está relacionada com a integridade axonial e valores reduzidos estão presentes em estados de perda da coesão da substância branca. Além disso, altos valores de DM podem estar relacionados com a perda da organização tecidual

microestrutural. A diversidade dos achados de correlação dos índices cognitivos avaliados e as respectivas áreas estudadas demonstram quão heterogêneas e extensas são as alterações microestruturais no grupo dos pacientes estudados. A mudança destas relações ao longo do tempo confirma o caráter dinâmico e prolongado dos mecanismos biológicos envolvidos na LAD. Os resultados deste estudo demonstram que mesmo após 12 meses do evento traumático, há alterações microestruturais na substância branca e estas são detectáveis por DTI. Acreditamos que, no futuro, os parâmetros quantitativos de DTI poderão ser úteis como biomarcadores na estimativa da gravidade de lesão e guiar processos de reabilitação.

Descritores: Traumatismos craniocerebrais; Lesão axonal difusa; Imagem por tensor de difusão; Testes neuropsicológicos; Degeneração neural; Neuroimagem.

ABSTRACT

Grassi DC. Longitudinal analysis of brain diffusion tensor imaging in patients with moderate and severe traumatic diffuse axonal injury [thesis]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2021.

Introduction: Currently, traumatic brain injury (TBI) is a worldwide public health problem due to its high prevalence, morbidity, and mortality. The mechanisms involved in trauma are complex, and different types of injuries may be present, including diffuse axonal injury (DAI). DAI is highly prevalent in victims of moderate and severe trauma, being responsible for most individuals who are in a vegetative state or with severe loss of ability after injury. It involves different and prolonged pathophysiological processes which lead to neurodegeneration. **Objective**: To evaluate longitudinally using diffusion tensor imaging (DTI) the integrity of the cerebral white matter in patients with moderate and severe DAI at three moments after trauma, and in comparison with healthy control subjects. In addition, we aimed to test correlations between the DTI parameters and patients' neuropsychological data. **Methods**: Twenty victims of moderate and severe TBI and 20 controls, matched for age and sex, were selected. MRI exam was performed at three different times: 2 months (time 1), 6 months (time 2) and 12 months (time 3) of the TBI. In the control group, the MR examination was performed in a single moment. We evaluated the superior longitudinal fascicle and the corpus callosum with tractography analysis, as well as the the whole brain with voxelwise analysis to extract DTI quantitative parameters. In addition, different cognitive domains were tested, and patients' clinical outcome was assessed one year after trauma. Correlation tests were performed using the DTI parameters with the cognitive domains examined. Results with $p < 0.05$ were considered significant. **Results**: The patients group had lower mean FA values and higher mean MD values in all sites when compared to the control group. Voxelwise analysis indicated significant changes over time in DTI parameters in the patients group. We observed improvements in patients' attention and memory. We identified different correlations between the DTI parameters and the different cognitive functions over time. **Discussion and Conclusions:** We observed quantitative changes in the DTI values in the studied segments. FA is related to axonal integrity and reduced values are present in states of loss of white matter cohesion. Moreover, high MD values may be related to the loss of the microstructural organization of neuronal tissues. The various correlations between cognitive indexes and DTI parameters in different brain areas demonstrate how heterogeneous and extensive the microstructural changes are

present in trauma victims. Changes in the correlations over time confirms the dynamic and prolonged nature of the biological mechanisms involved in DAI. The results of this study demonstrate that even after 12 months of the traumatic event, there are microstructural changes in the white matter, and these are detectable by DTI. We believe that, in the future, the quantitative parameters of DTI may be useful as biomarkers in estimating the severity of the injury and guiding rehabilitation processes.

Descriptors: Craniocerebral trauma; Diffuse axonal injury; Diffusion tensor imaging; Neuropsychological tests; Nerve degeneration, Neuroimaging.

1 INTRODUCTION AND LITERATURE REVIEW

1.1 The introduction and literature review of this thesis are based on the following two articles

Publication 1 is a literature review of diffusion tensor imaging (DTI) in the evaluation of diffuse axonal injury (DAI). This article was commented in a Letter to the Editor by Santos et al. (http://dx.doi.org/10.1590/0004-282x20180113). Therefore, we have also published a Reply Letter (Publication 2).

a) Publication 1

1. Title: Current contribution of diffusion tensor imaging in the evaluation of diffuse axonal injury
2. Literature review
3. Journal: Arquivos de Neuropsiquiatria
4. Status: published in March 2018 (https://doi.org/10.1590/0004-282x20180007)

Current contribution of diffusion tensor imaging in the evaluation of diffuse axonal injury

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Abstract:

Traumatic brain injury (TBI) is the number one cause of death and morbidity among young adults. Moreover, survivors are frequently left with functional disabilities during the most productive years of their lives. One main aspect of TBI pathology is diffuse axonal injury (DAI), which is increasingly recognized due to its presence in 40 to 50% of the cases that requires hospital admission. DAI is defined as widespread axonal damage, characterized by complete axotomy and secondary reactions due to overall axonopathy. These changes can be seen in neuroimaging studies as hemorrhagic focal areas and diffuse edema. However, DAI findings are frequently under-recognized in conventional neuroimaging studies. In this scenario, diffusion tensor imaging (DTI) plays an important role because it provides further information on white matter integrity that is not obtained with standard magnetic resonance imaging (MRI) sequences. There are extensive reviews concerning the physics of DTI and its use in the context of TBI patients, but these issues are still hazy for many health-allied professionals. Herein, we aim to review the current contribution of diverse state-of-the-art DTI analytical methods to the understanding of the pathophysiology and prognosis of DAI, to serve as a quick reference for those interested in planning new studies and who are involved in the care of TBI victims. For this purpose, a comprehensive search in Pubmed was performed using the following keywords: “traumatic brain injury”, “diffuse axonal injury”, and “diffusion tensor imaging”.

Key Words: Magnetic resonance imaging; diffusion tensor imaging; tractography; diffuse axonal injury, traumatic brain injury

Traumatic brain injury

Currently, traumatic brain injury (TBI) is a worldwide public health problem due to its high prevalence, morbidity and number of deaths. The most affected individuals are young males, who are more likely to engage in risk-taking behaviors. These traumatic events may result in several disabilities, loss of productivity and impaired quality of life. Therefore, understanding the mechanisms of trauma, grading it, and providing adequate medical care to the victims are essential to minimize the large social and economic consequences of TBIs[1,2].

One of the most important aspects for guaranteeing an optimized approach for tretating a TBI victim is understanding how the trauma occurred and providing a detailed and prompt

clinical examination of the victim. Neurotrauma mechanisms are complex, and multiple types of injuries over the CNS may coexist, such as skull fractures, contusions, hematomas, and diffuse axonal injury (DAI)[3,4]. The Glasglow Coma Scale (GCS) is the most widely used clinical classification and depends on the best eye, verbal and motor responses. According to GCS, a TBI can be graded as mild, moderate or severe. This scoring system was created in 1975 and lacks the sensitivity to predict subtle but meaningful residual dysfunction, such as physical, cognitive, psychological and behavioral deficits[5].

More comprehensive clinical algorithms have been developed to determine mortality and persistent disabilities in TBI victims. Two of the most used and well-known are the IMPACT and CRASH algorithms. The IMPACT algorithm was developed during clinical trials of severe TBI victims, and CRASH algorithm was developed with mild and moderate TBI patients. Both algortithms use clinical predictors such as age, GCS score and pupillary reactivity at hospital admission. The addition of biomarkers, such as protein S100-beta, microtubule associated protein 2 and myelin basic protein, as well as imaging data from head computed tomography (CT) scans, has been shown to improve the reliability of these prognostic models[5]. However, the clinical manifestations present after traumatic events vary, and predicting prognosis in an individual patient remains challenging in daily practice[5-7].

In particular, patients with DAI frequently exhibit an apparent discrepancy between clinical status (usually moderately to severely compromised) and early imaging findings (often normal or minimally abnormal)[3,4]. Moreover, it is not well established why some survivors regain complete function while others remain severely disabled. Therefore, it is critical to understand the pathophysiology of DAI and to foster non-invasive neuroimaging tools to reveal the damage to the central nervous system and provide guidance for therapeutic decisions and counseling for TBI patients and their families.

Pathophysiology of diffuse axonal injury

DAI is defined as wide axonal injury with microscopic and macroscopic components, which may only be visible in severe cases upon CT and magnetic resonance imaging (MRI). The mechanism of injury is based on the inertia of the brain: when fast and strong accelerations and decelerations occur, different structures with distinct densities (such as gray and white matter) suffer shearing and straining forces which stretch and damage axons, leading to diffuse axonal injury[2,4]. Commonly affected brain sites are the corpus callosum, fornices, subcortical

white matter and cerebellum[7]. Once the victim presents in a comatose state with a GCS less than 8, the probability of brainstem involvement becomes significant and the prognosis worsens[8].

It is very important to understand that DAI does not only consist of the axonal injury itself. Complex neuropathological processes ensue, such as an inflammatory response secondary to the traumatic event associated with protein deposition. After direct axonal injury, multiple changes occur microscopically, including cellular death, synaptic dysfunction, activation of glial cells and anomalous protein deposition (Tau and Aβ proteins)[9-11].

Damage to an axon does not always means its disconnection: connections may occasionally still be present, but it is not well known if they remain functional[12]. It is well established that neuronal death *per se* evolves into Wallerian degeneration, which is defined as the progressive anterograde disintegration of axons and accompanying demyelination that occurs after injury to the proximal axon or cell body[13]. Furthermore, it has been increasingly recognized that anomalous protein deposition secondary to axonal injury might be related to the future manifestation of Alzheimer's disease in some patients[14,15].

Histopathologically, DAI can be divided into three degrees: grade I - microscopically widespread axonal injury in any location; grade II: grade I findings plus focal lesions in the corpus callosum; and grade III: findings of grade II plus focal lesions in rostral portion of the brainstem[16].

Neuroimaging

Neuroimaging evaluation of a TBI patient is based on CT and MRI studies. During emergency care, TBI victims are usually first evaluated with CT scans, which are fast and accurate in identifying life-threatening conditions that may require prompt intervention, such as extra-axial hematomas[7].

There is a mismatch between the CT findings and clinical presentation in TBI patients. For instance, punctate microhemorrhages on the corpus callosum and gray-white matter junction are shown in only 10% of all TBI patients. Within two weeks after a traumatic event, neuronal loss can be inferred on CT as a discrete ventricular enlargement in some patients[7,14].

An MRI, despite being less widely available and requiring longer scan times than a CT, is the best modality to assess brain injuries because it provides a better identification of anatomic features and higher spatial resolution[3]. Hemorrhages are represented by loss of signal

in gradient echo and susceptibility-weighted sequences (Figure 1), whereas areas of edema present as high signals in T2-weighted and fluid attenuation inversion recovery (FLAIR) sequences (Figure 2).

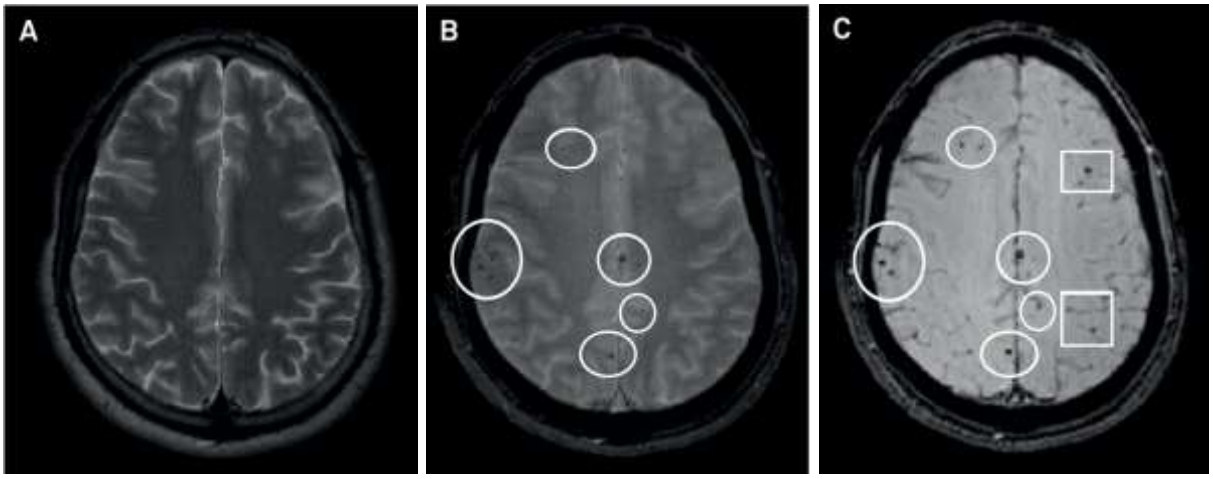

Figure 1: A 19-year-old male patient with DAI and GCS of 8 after a motorcycle accident. While T2 conventional sequences (A) are rather insensitive for hemorrhagic lesions, T2*-GRE (B) shows numerous foci of signal loss (circles) in the subcortical white matter corresponding to areas of extravascular blood. SWI (C) is even more sensitive than the previous two sequences, exhibiting more conspicuous (circles) and numerous lesions (squares) on both cerebral hemispheres. The images were performed sequentially during the same examination at a 3 Tesla scanner.

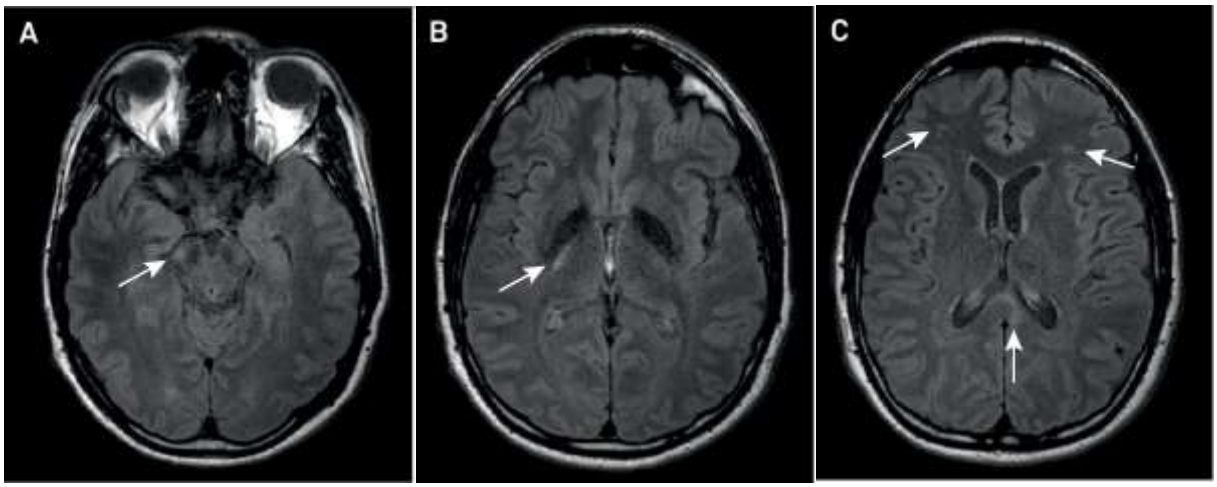

Figure 2: A 27-year-old male patient with moderate TBI due to a motorcycle accident. At admission, Glasgow Coma Scale score was 10 and he presented with left hemiparesis and left third nerve palsy. Axial FLAIR images show hyperintense lesions (arrows) in the lateral aspect of the right cerebral peduncle (A), right internal capsule (B), splenium of the corpus callosum, as well as subtle lesions in the subcortical bilateral frontal white matter (C).

Nevertheless, more advanced techniques, such as diffusion tensor imaging (DTI), is more sensitive to neuronal lesions in areas that appear normal on conventional MRI sequences, especially in patients with DAI[14,17-19].

Diffusion weighted imaging

Since its medical debut, diffusion weighted imaging (DWI) has largely been used in routine clinical practice in applications ranging from diagnosing stroke to helping physicians determine tumor cellularity. Diffusion weighted imaging is an integral part of any MRI brain examination and is of paramount importance in radiological diagnosis.

Diffusion weighted imaging is based on the random motion of water molecules, which was first described by the botanist Robert Brown in 1827. In a homogeneous liquid environment without any barriers, water diffusion is free (isotropic); in other words, it has no preferred direction. In contrast, when there are surrounding structures restricting water molecule movement, a preferred diffusion direction becomes apparent (anisotropic diffusion)[20].

In the brain, several components hinder the water diffusion in all directions, making the diffusion anisotropic. This anisotropic diffusion depends on the geometry and composition of natural barriers, such as cell membranes, myelin sheaths and primary microstructural components (neurofilaments and microtubules). For this reason, anisotropy is markedly high in well-organized white matter tracts and is lower in cerebrospinal fluids[21].

Diffusion weigthed imaging shows hyperintensities in pathological conditions, such as in high cellularity density (tumors), abnormal cellular uptake of water (cytotoxic edema) or entrapment of water between myelin membranes (intramyelinic edema). However, other increases in water concentration, such as observed in vasogenic edema or gliosis, also cause nonspecific hyperintensity on DWI, which is a T2-weighted sequence[22].

To distinguish between true restriction of water molecules diffusion and artifacts (T2 shine-through effects), apparent diffusion coefficient (ADC) maps were developed. When

analyzing each voxel from the image with two different *b* values, it is possible to quantify water diffusivity and convert this information into a visual map (ADC maps). The term "apparent" concerns the fact that calculated ADCs in tissues vary according to the previously attributed *b* values. Moreover, the ADC information corresponds to each voxel (in the order of millimeters) but not at the microscopic level of cell structures (in the order of a few micrometers). On ADC maps, restricted water diffusion is confirmed as a low signal intensity[23,24].

In patients with traumatic injury, cytotoxic edema usually occurs in cases of cortical contusion and DAI. Cortical contusions usually affect both superficial cortical gray matter and subcortical white matter but do not typically follow a vascular distribution. In acute DAI, it is possible to detect multifocal areas with restricted diffusion, which appear bright on DWI and dark on ADC maps. Predilection sites are the corpus callosum, especially the splenium, cerebral peduncles, deep white matter structures and gray-white matter interface (Figure 3). This restricted water diffusion may vanish in a few days or may evolve into residual lesions with a persistent high signal intensity on FLAIR and T2-weighted images[25].

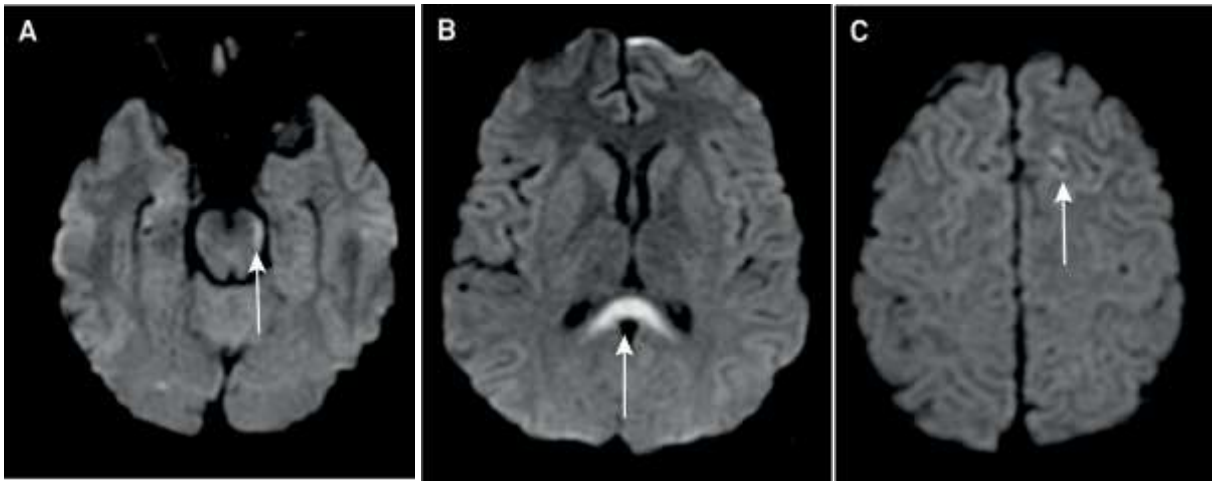

Figure 3: Diffusion-weighted images in the axial plane of a young patient four days after a head injury demonstrate bright lesions (arrows) in areas typically affected in DAI: left cerebral peduncle (A), splenium of the corpus callosum (B), and subcortical frontal white matter (C). The lesions were dark on ADC maps (not shown).

Diffusion Tensor Imaging

The tensor model was proposed to characterize and quantify diffusion anisotropy. By measuring diffusion in at least six different gradient directions applied to the three different axes (X- horizontal, Y- vertical and Z- perpendicular to X), it is possible to determine an average water diffusion according to the distance and intensity for each voxel[26,27].

Fractional anisotropy (FA) is a scalar measure that reflects the microstructural geometry and is very high (close to 1) in normal white matter but is usually lower in damaged white matter and is close to zero in the cerebrospinal fluid[27]. Colormaps can be created based on the first eingenvector that composes FA calculation in which each color represents the main diffusion direction of WM tracts (conventionally, red is used for left-right, green for anteroposterior, and blue for superior-inferior directions), and the degree of brightness is proportional to the magnitude of anisotropy[28,29] (Figure 4).

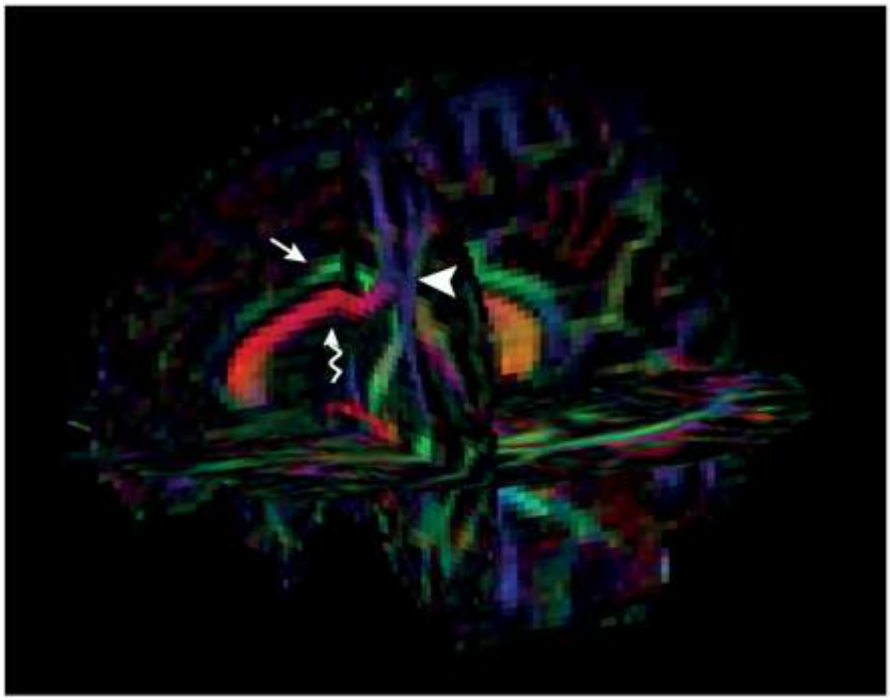

Figure 4: Three-dimensional FA colormap of an 18-year-old TBI victim. The patient was admitted with a GCS score of 8 after a motorcycle accident, despite the use of a helmet. Conventionally, long association fibers with anteroposterior direction such as the cingulum are represented in green (straight arrow), inter-hemispheric commissural fibers (e.g., corpus callosum, curved arrow) with left-right direction are represented in red, while projection tracts with superior-inferior route such as the costicospinal tract are represented in blue (arrowhead).

The mean diffusivity (MD) reflects the overall degree of water diffusion in all directions, regardless of its orientation dependence[30]. Radial (RD) and axial diffusivities (AD) are other quantitative DTI parameters. AD in the principal tensor direction (first eigenvector), with its associated magnitude (first eigenvalue), and RD represents the other two directions perpendicular to the principal direction of the diffusion tensor. Investigations with animal models have indicated that RD correlates with demyelination, whereas AD seems to be related to more profound tissue damage and axonal loss[31,32]. Still, translational characteristics of these studies remain to be proven.

Several acquisition parameters influence the quality of diffusion data. The ability to obtain a valuable dataset is strongly related to the strength of the gradient coils and the maximum *b* values that can be achieved. For most brain studies, a *b* value of 1000 s/mm^2 is usually adequate and results in an effective compromise between sensitivity and the signal-to-noise ratio (SNR). Ideally, images should be acquired with high and isotropic spatial resolution (1 – 2 mm^3). Many gradient directions, optimally at least 32, should be applied. However, there is a trade-off between the optimal acquisition parameters and the scanning time. Head motion should be minimized as much as possible. Preprocessing steps may, however, partly attenuate motion artifacts and the eddy current artifacts[33,34].

Distinct methods are available to analyze diffusion tensor images. Herein, we aim to briefly discuss the basic principles, advantages and caveats of each main DTI analytical method, namely, region-of-interest analysis, tractography and voxelwise analysis, along with the relevant findings of recent DTI studies of TBI patients.

Region-of-interest analysis

In region-of-interest (ROI) analysis, diffusion parameters are obtained from a pre-determined area of the brain or around a specific anatomic structure. The area-of-interest can be manually drawn and there is no requirement to use anatomical atlas. This method is also suitable for studies in patients with large brain lesions in different sites or great anatomic distortions that could bias other analytical methods that require registration steps. Once the ROI is determined, the mean values of the water diffusion parameters are obtained[33,34] (Figure 5).

One advantage of this method is that even small brain regions, such as subcortical areas and deep basal ganglia, van be assessed. Another benefit is that multiple comparisons errors are less prominent if the investigator predefines an *a priori* hypothesis and fewer areas are

examined. Region-of-interest analysis identifies even subtle changes and is thus one of the most sensible and straightforward analytical methods available[33].

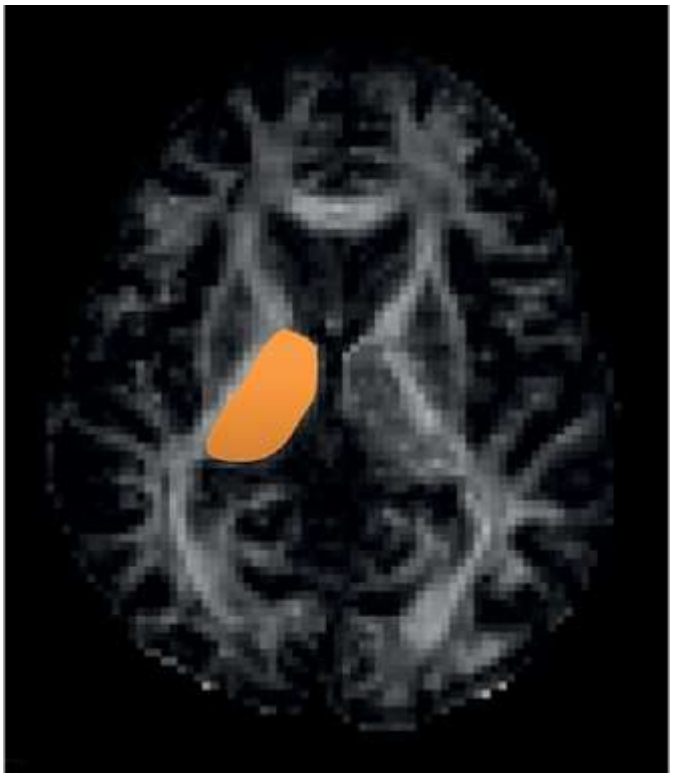

Figure 5: Diffusion parameters can be extracted from a selected ROI. In this case of a 42-year-old woman who suffered physical aggression and presented with severe TBI, the right thalamus (orange shape) was manually delineated in the FA map.

However, certain considerations must be underscored: some information may be missed whne only one area is being studied; it may be difficult to compare the same ROIs between individuals due to intrinsic variability; and the process of delimitating the ROI can be laborious if it appears in multi-slice images or in the case of big data with multisubject comparisons. It is extremely important to know where to look for the changes; ROI analysis of nonaffected areas can identify normal parameters, but truly true compromised areas can be missed. The manual nature of the ROI drawing may lead to the low reproducibility of intra- and intersubject ROI correspondence; hence, anatomic atlas tools or semi-automatic methods are advisable. For this reason, it is possible to standardize ROI-studies by using segmentation and registration steps; segmentation helps determine the area of interest, whereas registration matches the corresponding points among all images in different subjects[33-35].

Mac Donald et al. demonstrated that DTI could detect white matter injury in a mouse model of DAI and could determine the approximate timing of injury. These authors extracted diffusion parameters with ROI analyses from the corpus callosum and the external capsule and compared the findings with histological and electron microscopy characteristics. In comparison with uninjured mice, anisotropy measures were lower in the injured group in all stages. During the early acute phase (less than one day), axial diffusivity was reduced and axonal injury was present histologically. In the subacute phase (one week to one month after injury), a reduction of anisotropy was accompanied by increases in AD, RD and MD, which reflected the dominance of demyelination and edema at histological evaluation. The authors proposed that if similar mechanics are present in human TBI, these DTI changes could be used for novel clinical and forensic applications. However, the authors did not find any correlation between severity of histological damage and the DTI parameters[36].

Another study evaluated 10 healthy individuals and five patients with mild TBI within 24 hours of injury and one month later. The authores extracted diffusion parameters with ROIs positioned in the corpus callosum and internal and external capsules. Soon after the injury, TBI patients demonstrated significant reductions in anisotropy compared with controls. In the 30-day-control, two patients showed slight increases in FA values when compared with their initial results, which was considered a possible sign of recovery[37].

According to Huisman et al.[18], changes in water diffusion anisotropy do occur in TBI, and these changes may be biomarkers for severity of tissue injury and predictors for outcome. Patients were analyzed within seven days of the event, and the data were compared with those from a control (healthy) group. The studied regions included the internal capsule, splenium, thalamus and putamen. This study showed that FA was significantly decreased in the posterior limb of the internal capsule and splenium of the corpus callosum. Furthermore, there was a statistically significant correlation between FA values in the DAI predilection sites and the severity of head injury, as measured BY acute and subacute neurologic assessments (acute GCS and Rankin scores)[18].

In addition, another longitudinal study analyzed 11 TBI victims in acute (less than seven days) and subacute (from eight days to rehabilitation discharge) stages and correlated DTI findings with a disability rating scale. Eleven ROIs were chosen based on previous studies that demonstrated an association between FA and functional or cognitive outcomes in TBI. It was apparent that the FA values varied according to the pathophysiologic processes of TBI. During the acute phase, FA values were lower than they were in the subacute stage, possibly because

of brain edema. Consequently, subacute values were more likely to reflect the axonal structural integrity. Therefore, these authors suggested that the optimal timing of DTI data acquisition for TBI prognostication might be during the subacute stage of injury[38].

Tractography

Three-dimensionional visualization of DTI information is also possible. Tensor information allows the trajectories to be estimated by inferring WM fiber orientations. Tractography allows the parcellation of white matter, and this information may be particularly useful in anatomofunctional studies because white matter bundles are linked to specific cognitive, language, behavioral, and motor functions (Figure 6)[39].

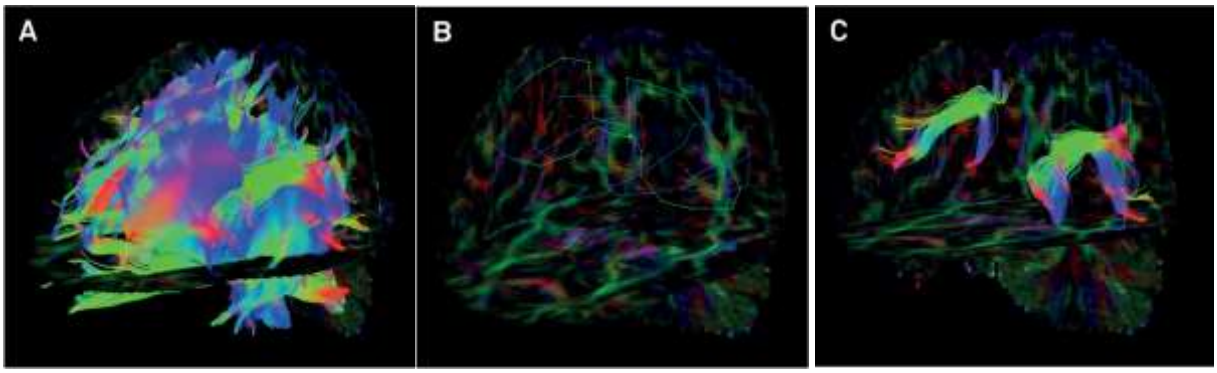

Figure 6: A young female patient had a motorcycle accident and presented at the emergency room with a Glasgow Coma Score of 3. She persisted with language impairment one year after the traumatic event. It is possible to evaluate DTI parameters from any tract related to a specific cognitive domain. The superior longitudinal fasciculus is linked to language skills because it interconnects Broca's area (responsible for speech production in the frontal lobe), Geschwind's area (semantic processing in the parietal lobe) and Wernicke's territory (speech comprehension in the temporal lobe). First, whole-brain tractography was obtained with a brute-force approach from the full tensor data (A). A set of "AND", "SEED" and "NOT" ROIs was placed on both cerebral hemispheres based upon *a priori* anatomical knowledge (B). The superior longitudinal fasciculi were then virtually dissected with a deterministic streamline approach and displayed on a FA color-encoded map (C).

Tractography consists of three processing steps: seeding, propagation and termination. Seeding involves determining the area from which the fiber bundles will be drawn, and the most common approach is to define an ROI and placing one or more seeds in the expected trajectory of the tract. Another possible way is to use automatic seeding for the whole brain from a full set of tensor data[29,39].

Propagation is how visual schemes of fibers are generated, and different algorithms are developed to link intervoxel information. Algorithms are based on deterministic or probabilistic approaches. The first one is based on a streamline principle: from the seed location (ROI) nearby voxels will be linked to the same streamline if their principal eigenvectors have congruous orientations or similar FA values. Probabilistic tractography, in constrast, represents an estimation based on multiple possible fiber directions in each seed. For this reason, probabilistic tractography tends to disperse trajectories more than deterministic methods and has the potential to delineate a greater proportion of the WM tract[28,29,39,40].

Finally, termination is the last step of fiber tracking procedure with well-defined criteria. Tipically, FA thresholds (usually higher than 0.25 to avoid contamination by CSF and grey matter) and turning angle thresholds (generally 30º or 60º depending on the known curvature of the tract of interest). This method of "virtual dissection" allows the isolation of specific anatomic fibers pathways from DTI datasets and has been proven useful in several adult and pediatric conditions (Figure 7)[39].

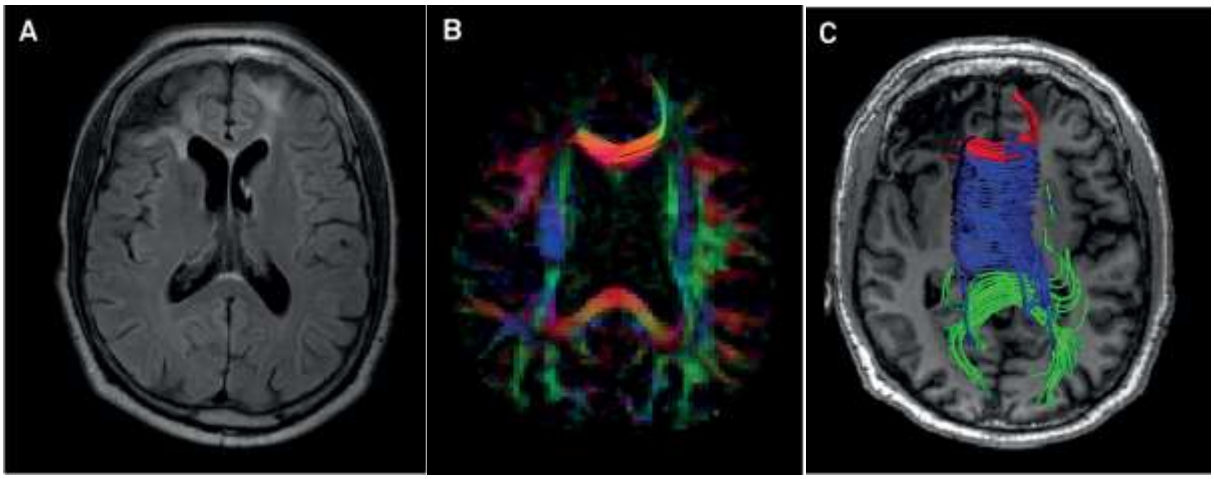

Figure 7: Axial FLAIR image of an adult male patient with chronic post-traumatic sequelae shows gliosis in the frontal lobes, mainly in the right side (A). FA colormap demonstrates paucity of WM fibers and reduction of brightness due to reduction of FA values in the frontal lobes, more pronounced in the right side (B). DTI-based tractography of the splenium, body

and genu of the corpus callosum (represented in green, blue, and red colors, respectively) shows premature termination of streamlines of peripheral fibers of the genu projecting to or from the prefrontal regions coincidental to the areas of low signal intensity on the axial T1-weighted image (C).

Nevertheless, there are some important shortcomings that must be taken into account while analyzing tractography. This method estimates fibers tract anatomy on a macro scale. Voxels are in the order of millimeters, whereas the axonal diameter is in the order of microns. It is also not possible to differentiate afferent from efferent bundles. Moreover, assumption of homogeneous unidirectional tensors is unrealistic because many brain regions comprise more than one fiber bundle. Crossing, diverging or kissing fibers result in incorrect direction estimations and pathways and may lead to abrupt tract termination. Diffusion spectrum imaging (DSI), q-space imaging (QSI), q-ball imaging (QBI), and high angular resolution diffusion imaging (HARDI) are more sophisticated approaches that may overcome some of these limitations[40].

Wang et al. studied 12 patients who suffered from severe TBIs within seven days and nine moths after injury. They analyzed the corpus callosum, fornix and peduncular projections with deterministic tractography. The authors could identify at least one DTI parameter demonstrating DAI-associated alterations in each region. Furthermore, they demonstrated a good correlation between DTI findings and long-term prediction outcome, as measured with Glasgow Outcome Scale-Extended scores[41].

Another group demonstrated that patients with mild TBI have reduced FA values in various white matter locations and various fiber bundles within 5.5 months after trauma. In comparison with healthy-matched controls, the authors demonstrated lower anisotropy in multiple white matter regions, predominantly in the cerebral lobar white matter, cingulum and corpus callosum, using deterministic tractography. A minority of fibers showed premature discontinuation on fiber tracking, and the authors presumed that this may have been caused by the presence of sharply angulated fibers or by small areas of hemosiderin that were not visible on MRI[42].

A large study evaluated 106 TBI chronic patients who had no abnormalities on conventional MRI in comparison with 62 healthy controls. The investigators applied a deterministic approach to extract the volume and FA measures of long association tracts from the uncinated fasciculus, superior cingulum, temporal cingulum, superior longitudinal

fasciculus, arcuate fasciculus, inferior fronto-occipital fasciculus and inferior longitudinal fasciculus. Injured patients demonstrated reduced FA values in both uncinated fasciculi, both inferior fronto-occipital fasciculi and in the right inferior longitudinal fasciculus. However, the tract volumes were not significantly decreased in injured patients[43].

A recent study evaluated trauma-exposed police officers with and without post-traumatic stress disorder using a 3T system. The authors applied an automated and unbiased reconstruction of WM tracts using a global probabilistic tractography method known as TRACULA (TRActs Constrained by UnderLying Anatomy)[44] and found significantly higher mean diffusivity in the right uncinate fasciculus in the affected group. The uncinate fasciculus is the major white matter tract connecting the amygdala to the prefrontal cortex. These authors also found that the MD of the right uncinate fasciculus was positively associated with anxiety symptoms in patients with post-traumatica disorder[45].

Head injury survivors usually present with persistent cognitive symptoms that impair their quality of life, such as deficits in language performance and executive function. In the future, tractography studies could be used to monitor the benefits of target therapies in these patients by evaluating DTI metrics in specific white matter tracts linked to specific functional domains.

Voxelwise analysis

Voxelwise analysis has become more popular because its automatic approach requires minimal intervention and less user dependence. Voxelwise analysis is suitable for global analyses of brain parenchyma and is particularly useful for large group comparisons of individuals with no significant distortions in brain anatomy[33,46].

Primarily, the images must be standardized into a template to make sure that each voxel corresponds to the same anatomic location in all subjects. Thus, a critical step is to define the best way to register and compare multiple images from different individuals in an accurate manner[33].

Previously, voxelwise analyses were performed with voxel-based morphometry using T1 weighted-images. Although some constraints related to image registration, segmentation and smoothing are present, voxel-based morphometry can still be used to explore diffusion images in particular research scenarios.

Tract-based spatial statistics is the current leading method of voxelwise analysis using nonlinear image transformation of FA images across subjects[46]. Tract-based spatial statistics uses the mean FA values from individual subjects to create a skeletonized map with the local maximal FA values for each tract. With this approach, the differences in all the voxels of the brain, except those from the skeleton voxels are ignored. Another challenge is registration inaccuracies of areas with high contrast of FA values, such as adjacent to ventricles[33,47]. A more recent alternative approach for the registration step of tract-based spatial statistics is to use the full tensor information with a complementary tool, known as DTI-ToolKit, which has been shown to reduce the number of misassigned voxels by a total of seven[47,48].

In voxelwise analysis, all the voxels in the image are compared with each other in a local manner, and hence statistical procedures to control for multiple comparisons errors might be carried out. These may, in turn, reduce the sensitivity for more subtle findings in particular regions[33].

Our group evaluated twenty adults with moderate to severe TBI at a 3.0T MRI scanner in the acute ($t_1 < 3$ months), subacute ($6 < t_2 < 9$ months) and chronic stages ($12 < t_3 < 15$ months) following trauma. According to tract-based spatial statistics analysis, the patients exhibited one large cluster with statistically significant lower FA values ($p < 0.001$) at all times (t_1, t_2, t_3) compared to the controls (Figure 8), but the number of affected voxels decreased over time by 2% at t_2 and by 7.3% at t_3. During the chronic stage (t_3), patients recovered white matter damage in comparison with the acute stage (t_1) with significant increases of FA in the bilateral anterior thalamic radiations, forceps major and minor, corticospinal tracts, cingulum, uncinate, inferior fronto-occipital, superior and inferior longitudinal fasciculi. Patient performances on cognitive measures was suboptimal at all three stages, but also improved over time in the same fashion as white matter recovery[49].

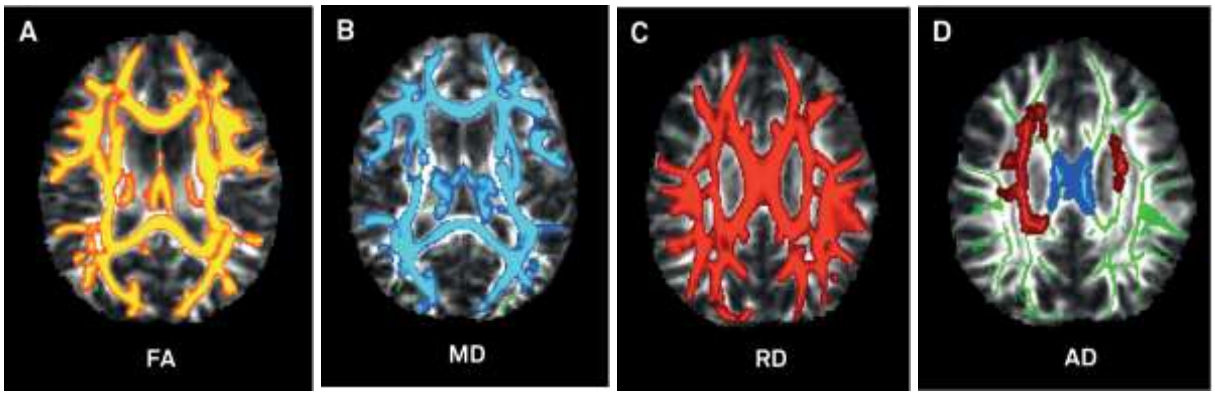

Figure 8: Twenty patients with acute moderate-to-severe TBI were compared with 20 age- and sex-matched controls with tract-based spatial statistics (TBSS). The patients exhibited one large cluster with statistically significant lower FA values (A). The TBI patients also demonstrated significant increases in mean diffusivity (B) and radial diffusivity (C) in extensive areas of the brain, as well as increased axial diffusivity in a less extensive area (shown in red), except for the corpus callosum, which showed increased axial diffusivity (depicted in blue) (D).

One study applied voxel-based analysis and ROI analysis in 10 adolescent patients with mild TBI who were assessed within one week after injury and compared with a paired-control group. The results indicated increased FA, decreased radial diffusivity and unchanged axial diffusivity in multiple brain regions, which may be related with axonal cytotoxic edema and reflect acute injury. Moreover, the alterations in DTI metrics were highly correlated with postconcussive symptoms severity and emotional distress[50].

Lipton et al.[51] retrospectively analyzed 17 cognitively-impaired mild TBI victims who underwent neuroimaging studies between eight months and three years after the trauma event and compared these patients with a healthy cohort of 10 individuals. Voxel-based analysis showed multiple areas of lower FA and high mean diffusivity in the white matter bilaterally, especially in the corpus callosum, subcortical white matter and internal capsules[51].

Another study indicated extensive changes in major intra- and interhemispheric white matter tracts in patients with diffuse axonal injury. The authors carried out both ROI analysis and voxel-based analysis in nine chronic TBI patients (approximately four years after the event) and in 11 healthy individuals. The results indicated significant lower FA values in the corpus callosum, internal and external capsules, superior and inferior longitudinal fascicles and in the fornix in the TBI group. Furthermore, ADC values were increased not only where the FA values were lower, but also in otherwise-normal regions, possibly indicating that ADC may be an even more sensitive measurement than FA in detecting widespread white matter damage[52].

Conclusion

TBI remains as a major public health problem worldwide. Computed tomography and MRI have played a crucial role in the acute setting, but several challenges arise when applying neuroimaging methods to predict clinical outcome in patients with a broad range of injury

severity, especially in individuals who develop persistent symptoms despite minor findings on standard imaging.

Diffusion tensor imaging takes advantage of the intrinsic property of anisotropic diffusion of water molecules in brain tissues to probe tissue integrity and organization. Although increasing publications have reported applying this technique in various clinical and research scenarios, DTI principles and its various methodological approaches remain unfamiliar for some allied-health professionals. We reviewed some advantages and shortcomings of the most commonly applied analytical methods (ROI, tractography and voxelwise analyses) along with their applications in the investigation of TBI.

Particularly in patients with diffuse axonal injury, natural barriers to the free water diffusion such as the cytoskeleton, axons and myelin sheath may be damaged, leading to the ubiquitous finding of reduced FA in distinct brain areas. Increased radial diffusivity and increased mean diffusivity usually accompany these FA changes. It seems that abnormalities in DTI metrics may correlate with the timing of head injury, severity biomarkers and long-term prognosis. In the future, DWI and DTI may also aid in selection of TBI patients for targeted therapies and in monitoring the effectiveness of treatments.

Upcoming investigations should try to select more homogeneous groups of patients and clearly state inclusion and exclusion criteria. Longitudinal studies with a combined quantification of DTI metrics, instead of transversal studies with putative evaluation of isolated indices, should enhance comprehension of TBI pathophysiology. These studies might foster the goal of alleviating the burden associated with TBI.

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Diffuse Axonal Injury: Diffusion tensor imaging parameters and correlation with clinical outcome

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Dear Editor,

We appreciate the effort of Santos and colleagues for writing the letter entitled "Diffuse axonal injury: diffusion tensor imaging and cognitive outcome"[1] about the published article by Grassi et al.[2]. We would like to thank for all their comments on our paper and we acknowledge the opportunity to reply to their considerations.

Traumatic brain injury remains a major public health concern, directly affecting millions of otherwise healthy individuals, as well as, indirectly their household members, who usually have to deal with long-term sequelae, including psychiatric symptoms and cognitive deficits. During the last years, advanced magnetic resonance (MR) techniques have played an important

role in detecting abnormalities that were once under-recognized when using conventional MR technology. In particular, diffusion tensor imaging represents an important advanced MR tool in the context of traumatic brain injury and diffuse axonal injury[3]. There are already extensive compendiums concerning the physics of diffusion tensor imaging, however, instead, in our work we aimed to briefly review its basic principles and main analytical methods (region-of-interest, tractography and voxelwise analyses), along with the main relevant findings in the context of traumatic brain injury and diffuse axonal injury[2].

Taking into account the advantages of diffusion tensor imaging in the noninvasive exploration of brain microstructure and networks, one should not be surprised by the striking number of recent publications using this technique in the evaluation of patients at different stages after a traumatic episode, ranging from mild to moderate and severe injuries[2,3]. However, there is still an urge to associate diffusion tensor imaging findings with clinical aspects and to correlate the scores with cognitive outcomes, making it valuable and accessible as a prognostic tool in a daily clinical practice.

Fortunately, new scientific studies are evolving steadly and, soon after our recently-published paper[2], new evidences have strengthened the relationship between diffusion tensor imaging abnormalities and diffuse axonal injury outcomes. As pointed by Santos et al., the work conducted by Hellstrom and colleagues[4] indicated robust associations between self-reported cognitive, somatic and emotional symptoms, 12 months after mild traumatic brain injury with white matter diffusion tensor imaging parameters, extracted with a vowelwise analysis, dubbed as tract-based spatial statistics. This work also reinforced physiologic effects of aging on brain white matter structures, leaving the older brain more vulnerable to subtle injury-related processes[3]. This also emphasizes the need to control the age as a potential confounding variable on case-control diffusion tensor imaging studies.

A work by Leon et al.[5] assessed 217 victims of moderate to severe TBI 19 days after the traumatic episode. Twenty-eight white matter fiber bundles were chosen because of their susceptibility to trauma and were evaluated by region-of-interest-analysis. Diffusion tensor imaging metrics were highly associated with unfavorable clinical outcome after six months to one year after the trauma.

Furthermore, a recent meta-analysis of 20 studies investigated correlations between diffusion tensor imaging measures and seven cognitive domains in mild to severe TBI victims. All studies pointed a concordance between diffusion tensor imaging parameters and cognition:

increased fractional anisotropy values were associated with higher cognitive performance, especially regarding memory and attention functions[5].

It is expected that diffusion tensor imaging evaluation will have potential clinical application in head injury survivors in the near future. Nevertheless, most findings heretofore were based on single works and hence upcoming studies are awaited to highlight the prognostic value of diffusion tensor imaging. There is still much work to be done. Larger scale, longitudinal analyses with homogeneous TBI groups might play a decisive role in how this technique will prove helpful in predicting a patient´s prognosis and also aiding in selection of patients who might benefit from targeted therapies.

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2 OBJECTIVES

To evaluate longitudinally with diffusion tensor imaging (DTI) the integrity of the cerebral white matter in patients with moderate and severe diffuse axonal injury (DAI) at three moments after trauma, by using two distinct analytical methods:

a) DTI-based tractography of the corpus callosum (CC) and the bilateral superior longitudinal fascicles (SLF);

b) Whole-brain voxelwise analysis.

We also aimed to correlate the DTI scalar indices with neuropsychological tests assessing different cognitive domains.

3 SYSTEMATIZED TEXT

3.1 The systematized text refers to the following original articles, according to the objectives of the thesis

c) Publication 3

1. Title: Dynamic changes in white matter following traumatic brain injury and how diffuse axonal injury relates to cognitive domain
2. Objectives covered: to evaluate longitudinally with DTI-based tractography the integrity of the corpus callosum and the superior longitudinal fascicles of patients with moderate and severe diffuse axonal injury (DAI) up to one year after trauma and to correlate the DTI findings with cognitive functions
3. Journal: Brain Injury
4. Status: published in January 2021 (https://doi.org/10.1080/02699052.2020.1859615)

Dynamic changes in the white matter in TBI victims and how diffuse axonal injury relates to cognitive domains after trauma

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Abstract

Objective: The goal is to evaluate longitudinally with diffusion tensor imaging (DTI) the integrity of cerebral white matter in patients with moderate and severe DAI and to correlate the DTI findings with cognitive deficits.

Methods: DAI victims (n=20) were scanned at three timepoints (2, 6 and 12 months) after trauma. A healthy control group (n=20) was evaluated once with the same high-field MRI scanner. The corpus callosum (CC) and the bilateral superior longitudinal fascicles (SLFs) were assessed by deterministic tractography with ExploreDTI. A neuropschychological evaluation was also performed.

Results: The CC and both SLFs demonstrated various microstructural abnormalities in between-groups comparisons. All DTI parameters demonstrated changes across time in the body of the CC, while FA (fractional anisotropy) increases were seen on both SLFs. In the splenium of the CC, progressive changes in the mean diffusivity (MD) and axial diffusivity (AD) were also observed. There was an improvement in attention and memory along time. Remarkably, DTI parameters demonstrated several correlations with the cognitive domains.

Conclusions: Our findings suggest that microstructural changes in the white matter are dynamic and may be detectable by DTI throughout the first year after trauma. Likewise, patients also demonstrated improvement in some cognitive skills.

Keywords: brain injury, white matter, MRI, DTI, tractography, cognition

Introduction

Traumatic brain injury (TBI) is a complex public health issue worldwide because of its high prevalence, morbidity and mortality(1,2). In the last decades, studies have demonstrated the vulnerability of cerebral parenchyma in the trauma scenario and also its importance when correlating with cognitive and psychological impairments in survivors(3,4).

Diffuse axonal injury (DAI) plays an essential role in TBI since it is present in almost half of the victims who need hospitalization and because it is related to brain dysfunctions(5–8). The widespread axonal injury leads to a loss of the brain connectiveness, causing cognitive, motor, and sensory deficits(9–11). Also, DAI is associated with the development of the chronic neurodegenerative traumatic disorder(12,13).

There are different and complex mechanisms involved in the pathophysiology of DAI. Histopathological studies have shown primary and secondary axonal lesions, inflammatory and regeneration processes accompanied by Wallerian degeneration and neuroplasticity that may be the related with the clinical and cognitive outcomes in TBI survivors(7,14). Computed tomography (CT) and conventional magnetic resonance imaging (MRI) are relatively insensitive for these microstructural changes. Nevertheless, diffusion tensor imaging (DTI) is an advanced MRI technique that is able to probe microstructural integrity by exploring the diffusion of water molecules in brain tissues(15–17). Therefore, we hypothesized that these abnormalities would be detectable by DTI metrics along the first year after trauma.

In the last decades, DTI has demonstrated its capability to study brain architecture, geometry and microstructure, and it has been used in the evaluation of several neurological conditions, including brain trauma(18,19). Among different DTI methods of analysis, tractography has been commonly used to parcellate and to assess the white matter tracts in TBI victims, ranging from mild to severe trauma, and even in those without associated findings in conventional MRI(20–23).

Fiber tracts connect different regions of the brain in order to module neuronal impulses. The greatest white matter bundle in the human brain is the corpus callosum (CC), responsible to link homologous regions of both cerebral hemispheres, and also involved in motor, psychological and cognitive activities(24). The genu, body and splenium are the main CC subdivisions connecting the orbitofrontal regions, the frontoparietal lobes and the occipital cortices, respectively. Another critical tract involved in different cognitive processes such as language, memory, emotions and attention is the superior longitudinal fascicle (SLF), which connects Broca´s area (frontal lobe), Geshwind´s area (parietal lobe) and Wernicke´s territory (temporal lobe) in the same brain hemisphere(25). Several studies have demonstrated the vulnerability of these brain tracts in the context of traumatic brain injury victims(20,22,26,27). However, longitudinal studies assessing the dynamic changes of white matter in DAI are scant in the literature.

This study aims to longitudinally evaluate with DTI the integrity of the CC and the SLFs in patients with moderate and severe DAI at three moments during the first year after the traumatic event, and also in comparison with a matched healthy control group. In addition, correlations between DTI quantitative parameters and neuropsychological data will also be scrutinized.

Materials and methods

Patients

Selection criteria

This study was approved by the Institutional Review Board, and all individuals agreed to be in the study and signed the informed consent form. Patients included in this study were adult outpatients (ages between 18 and 55 years old) admitted to the Emergency Room of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil, due to moderate and severe head trauma according to Glasgow Coma Scale (GCS) scores of 3–12 at initial evaluation, and who met the following criteria: (1) clinical and tomographic diagnosis of DAI, (2) a Marshall score(28) of I, II or III based on CT evaluation, (3) had no focal lesions greater than $10cm^3$, (4) had no midline shift greater than 0.5cm, (5), had no epidural hematomas that determined compression of the brain parenchyma, (6) had no previous head injury history with hospitalization.

Description of patient's sample

Initially, two hundred and twenty-five head trauma patients were evaluated, twenty patients (11.25% of those) were included in the final analysis and two hundred and five (88.75%) were excluded based on the following reasons:186 patients did not meet clinical and/or tomographic criteria for DAI; seven losses of follow-up; five individuals had exclusion safety criteria for MRI examination; five DTI artifacts; one patient deceased; and one patient developed epidural compressive hematoma.

For the 20 patients who met the selection criteria, demographic characteristics were as follows: 17 men and 3 women; mean age was 29.6 years (SD$\pm$6.8), 14 patients presented with moderate head trauma (GCS of 8–12) and 10 suffered motorcycle accidents.

Brain imaging

Magnetic resonance imaging

Each patient had a 3.0 Tesla MRI of the brain performed on the same scanner (Intera Achieva, Philips Medical System, Best, The Netherlands) with an eight-channel head coil (Philips Medical System) at three timepoints: (1) 2 months after the trauma, (2) 6 months after the trauma and (3) 12 months after the trauma. A healthy age- and sex-matched control group of 20 individuals was also scanned once.

Anatomical imaging protocol was acquired in the sagittal plane with a 3D T1-weighted Fast Field Echo (3DT1-FFE) sequence covering the entire brain (180 slices), and the following parameters: inversion time (IT) = 700 ms; TR/TE = 6.2 ms/2.7 ms; flip angle = 8°; acquisition matrix = 240 x 240; field of view (FOV) = 240 x 240 x 180 mm; voxel resolution = 1 mm^3 (isotropic); slice thickness = 1.0 mm; completion time = 4 minutes. The susceptibility weighted image protocol consisted of principles of Echo Shifting with a Train of Observations (PRESTO) 3D-T1FFE sequence, axially acquired (a total of 230 slices - 1mm thick), according to these specifications: TR/TE = 22/29 ms; flip angle = 10°; FOV= 220 x 182 mm; matrix = 224 x 224; voxel size = 0.98 x 0.98 x 1.0 mm; completion time = 3 minutes.

DTI data acquisition

DTI images were collected in the axial plane with gradients applied in 32 non- collinear directions. The entire brain was covered within 70 slices, 2 mm-thick each, with no gaps in between. One image with no diffusion weighting was obtained (b = 0 s/mm^2). Other parameters used were: TR/TE = 8500 /61ms; b value = 1000 s/mm2; matrix = 128 x 128; FOV ("field-of-view") = 256 x 256 mm; $2mm^3$ isotropic voxel; NEX = 1; completion time = 7 minutes.

Pre-processing

All data were pre-processed using the functional MRI brain (FMRIB) software library (FSL), version 5.0 (available at http://www.fmrib.ox.ac.uk/fsl/), following this sequence: brain extraction tool (BET), FMRIB's linear image registration tool (FLIRT) and correction of eddy current induced distortions(29,30). Motion correction was completed using the free toolbox ExploreDTI (A. Leemans, University Medical Center, Utretch, The Netherlands), by rotating the B-matrix in order to keep the orientation input accurate. Investigation for residuals and outliers of the diffusion tensor fit was done with the same software, ending on residual maps similar on all groups. Moreover, the same software was used for tensor calculation and fiber tracking (31,32).

Tractography

A whole-brain tractography was first automatically obtained in the native space using a brute-force approach of every pixel. Deterministic tractography technique was then achieved following a predesigned combination of specific procedures, which included positioning of multiple regions-of-interest (ROIs) on different planes, based on prior anatomical knowledge

and previous studies(33,34). The FACT (fiber assignment by continuous tracking) algorithm was calculated with a fractional anisotropy threshold of 0.25 and maximum angles of 30º for the CC and 60º for the SLF, equally applied to all subjects(35).

The CC was segmented in three parts: genu, body and splenium. According to the Hofer and Frahm's representation(36), the genu was defined as the one-sixth part of the anterior CC, while the splenium the last one-fourth and the body the residual part. The CC was virtually dissected following these steps: first, "SEED" ROIs were marked in the paramedian plane along the CC. To securely track fibers from left and right hemispheres, "AND" ROIs were traced in the midsagittal portion, and, finally, "NOT" ROIs were drawn in the axial and coronal planes in order to eliminate horizontally and vertically oriented fibers (e.g. cingulum and corticospinal tracts, respectively). To dichotomize the SLF fibers, two "SEED" ROIs were delineated in the coronal plane, and two "NOT" ROIS were used to the axial plane to exclude any tracks that had a vertical orientation (towards the corona radiata or the corticospinal fibers) (Figure 1).

The following average quantitative DTI parameters were extracted from each tract: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD).

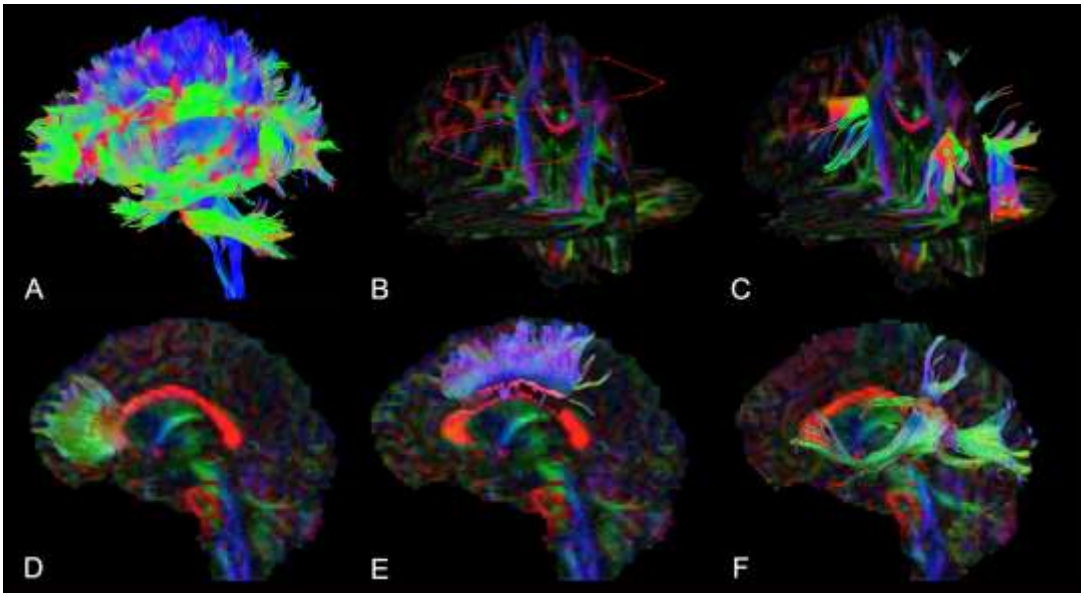

Figure 1. DTI-based tractography post-processing with ExploreDTI. A, whole-brain tractography is first obtained with a brute-force approach. B, SEED (in blue) and NOT (in red) ROIs placed in multiple planes of FA maps to virtually dissect the bilateral SLFs. C. Three-

dimensional representation of both SLFs on oblique view. D, E and F show the final results of segmentation of the CC in the lateral plane: genu, body, and splenium, respectively.

Neuropsychological tests

An experienced neuropsychologist (ALCZ) performed specific tests to assess different cognitive domains. The patients were submitted to neuropsychological (NP) assessment only at timepoints 2 and 3 because of comprehension difficulties, mental confusion and agitation typically seen in the early post-trauma stage. The results of each test were converted into a Z-score according to age and years of education. On timepoint 2, three patients were not able to complete the NP tests. On timepoint 3, one patient had missing information concerning the IQ estimation.

The Hopkins verbal learning test (HVLT) evaluates the episodic verbal memory. It consists on immediate, late recall and later recognition of a list containing 12 words(37). The examiner reads the list and the patient is asked to repeat as many words as possible. This procedure is repeated two more times and then 25 minutes after that (later recognition).

The Victoria Stroop test assesses selective attention and inhibition control. It consists of three cards, one of them with colors, the other with random words colored and the last with color names with mismatched colors(38). The patient has to say aloud the colors´ names as fast as possible.

Both semantic verbal and phonologic fluency were assessed by the FAS test – where the patient is asked to say as many words as possible beginning with each letter F, A and S. For the semantic verbal assessment – using animals as a category, the patient has to say as many different animals as possible in one-minute interval(38).

One subtest present in the Wechsler Memory Scale (WAIS – III) evaluates the working memory in forward and reverse recall of a digit sequence(39). A digit sequence is presented at one digit per second rate and after that the patient has to recall it in forward and in backward sequences.

IQ estimation was calculated by combining the performance on both vocabulary and matrix reasoning tests present in the WAIS – III(39,40). The vocabulary test consists on the presentation of words and the patient is asked to define them. In the matrix reasoning test, a matrix of abstract pictures in which there is one picture missing is presented, and the patient has to choose one option that better suits the missing picture.

Statistical analyses

Statistical analysis was performed using IBM – SPSS statistics for Windows, version 25 (International Business Machines Statistical Package for the Social Sciences Inc., Chicago, IL, USA). A professional statistical expert was consulted for all analyses.

Initially, the data was inspected for outliers and distributional characteristics. There were no considerable asymmetries in all DTI quantitative samples or NP tests results.

Comparisons between the patients and the control group were performed with Student´s t-test. A generalized linear function test with robust standard error and unstructured correlation matrix was performed to evaluate changes over time of DTI parameters and neuropsychological tests. After that, the Benjamin - Hochberg procedure for repeated measures was performed. To calculate correlation coefficients, Pearson and Spearman's tests were used. Results were considered significant with p-value <0.05.

Results

Comparisons of the DTI parameters between patients and healthy controls

The DTI parameters (FA, MD, AD and RD) extracted from the CC and both SLFs in the control group and in the patients group at all three timepoints can be seen in the supplementary Table S1.

In order to examine the early microstructural abnormalities after moderate and severe TBI, we compared the patients group at timepoint 1 with the healthy controls (Figure 2). We found significant differences in all DTI parameters at all segments of the CC with lower FA values and higher MD and RD values in the patients group (p<0.001). There were no significant differences in AD in any CC segment when comparing both groups. For both SLFs, we found similar results as those found in the corpus callosum, except for AD in the left SLF, which demonstrated significant higher values in the patient's group (p=0.04).

Supplementary Table S1. DTI quantitative results for each white matter tract.

Tract		Controls (n=20)	PT1 (n=20)	PT2 (n=20)	PT3 (n=20)
Genu	FA	0.516 ± 0.015	0.467 ± 0.045	0.468 ± 0.048	0.467 ± 0.051
	MD	0.777 ± 0.032	0.829 ± 0.046	0.837 ± 0.052	0.841 ± 0.060
	AD	1.288 ± 0.037	1.308 ± 0.041	1.320 ± 0.044	1.325 ± 0.050
	RD	0.522 ± 0.031	0.589 ± 0.059	0.593 ± 0.065	0.599 / 0.075
Body	FA	0.535 ± 0.010	0.486 ± 0.028	0.481 ± 0.027	0.485 ± 0.029
	MD	0.756 ± 0.019	0.795 ± 0.034	0.807 ± 0.040	0.809 ± 0.043
	AD	1.279 ± 0.029	1.278 ± 0.040	1.293 ± 0.051	1.296 ± 0.054
	RD	0.494 ± 0.017	0.552 ± 0.037	0.562 ± 0.039	0.563 ± 0.047
Splenium	FA	0.578 ± 0.016	0.530 ± 0.030	0.529 ± 0.031	0.530 ± 0.029
	MD	0.792 ± 0.020	0.842 ± 0.039	0.851 ± 0.039	0.859 ± 0.048
	AD	1.398 ± 0.031	1.409 ± 0.053	1.425 ± 0.047	1.442 ± 0.059
	RD	0.489 ± 0.022	0.558 ± 0.044	0.565 ± 0.047	0.567 ± 0.049
Right SLF	FA	0.466 ± 0.017	0.428 ± 0.034	0.427 ± 0.033	0.435 ± 0.030
	MD	0.712 ± 0.024	0.744 ± 0.032	0.746 ± 0.039	0.744 ± 0.043
	AD	1.107 ± 0.033	1.115 ± 0.032	1.116 ± 0.038	1.122 ± 0.047
	RD	0.514 ± 0.023	0.559 ± 0.039	0.562 ± 0.045	0.555 ± 0.046
Left SLF	FA	0.491 ± 0.017	0.454 ± 0.033	0.456 ± 0.032	0.461 ± 0.027
	MD	0.700 ± 0.019	0.739 ± 0.030	0.745 ± 0.034	0.741 ± 0.036
	AD	1.112 ± 0.033	1.133 ± 0.028	1.144 ± 0.030	1.143 ± 0.031
	RD	0.494 ± 0.0174	0.542 ± 0.039	0.545 ± 0.0421	0.540 ± 0.041

The results are shown in mean values ± standard deviations. MD, AD and RD are presented in x 10^{-3} mm^2/s. n = number. PT1, PT2 and PT3 accounts for the patients groups at timepoints 1, 2 and 3, respectively.

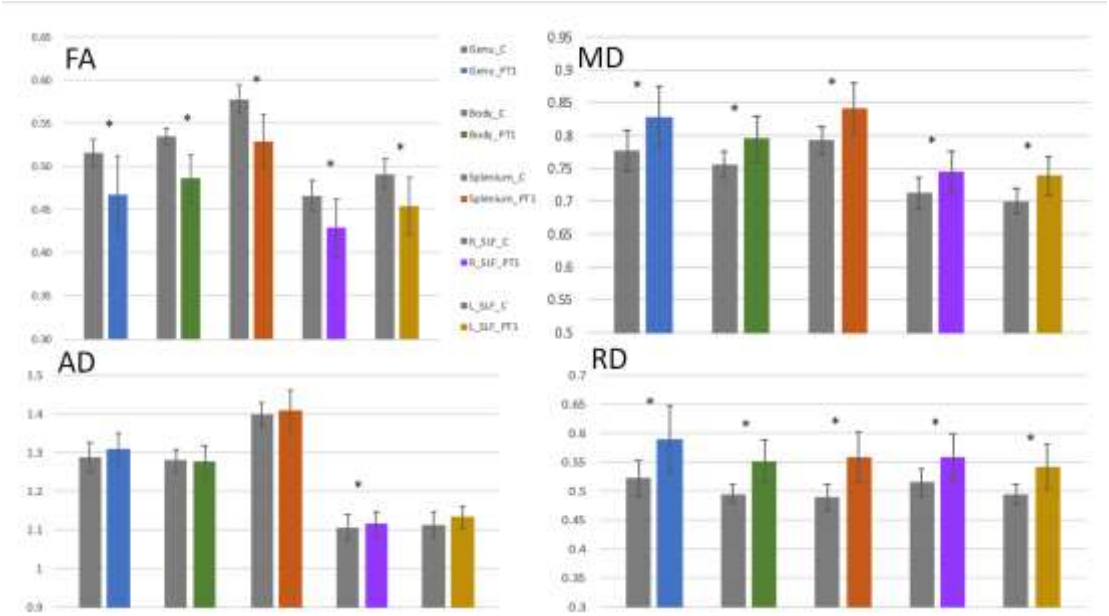

Figure 2. Column bar graphics exhibit the mean values with 95% confidence intervals (error bars) of DTI metrics (FA, MD, AD, RD) for the patients group at timepoint 1 (PT1) in each CC segment (genu in blue, body in green, splenium in orange) and both SLFs (right SLF in purple, left SLF in yellow). The corresponding parameters in the control group are shown in grey color. MD, AD and RD are expressed in x 10^{-3} mm^2/s. The corresponding values for the control group are exhibited in grey color. Statistically significant differences ($p<0.05$) obtained with Student´s t-test are indicated in the graphics with asterisks (*).

Changes in the DTI parameters along time

Table 1 summarizes the results from the generalized estimating equation (GEE) for the comparison of all quantitative DTI metrics in the patients group considering the three timepoints.

In the body of the CC, FA demonstrated a decrease between timepoints 1 and 2, and then a significant increase at timepoint 3 ($p=0.02$). For both MD and AD, we also observed the same pattern of increasing values along time in the body ($p=0.003$, $p=0.025$), and splenium ($p<0.001$, $p<0.001$), respectively. For RD, we found increasing values in the body of the CC ($p=0.016$). There were no other significant changes in the DTI parameters in the genu of the CC (Figure 3).

Table 1. Results obtained with generalized estimation equation to evaluate of DTI parameters in the patients group (n=20) along timepoints 1, 2 and 3.

Tract	**DTI metric**	***p*-value**
Genu	FA	0.835
	MD	0.063
	AD	0.061
	RD	0.181
Body	FA	*0.020*
	MD	*0.003*
	AD	*0.025*
	RD	*0.016*
Splenium	FA	0.992
	MD	*<0.001*
	AD	*<0.001*
	RD	0.070
Right SFL	FA	*0.003*
	MD	0.764
	AD	0.575
	RD	*0.035*
Left SLF	FA	*0.035*
	MD	0.231
	AD	0.088
	RD	0.180

Significant *p*-values are shown in italics.

We also found significant FA increases along time in both right and left SLFs (p=0.003, p=0.035, respectively), accompanied by a significant decrease for RD values in the right SLF (p=0.035). No other significant changes over time for the other DTI parameters were observed in the SLFs (Figure 3).

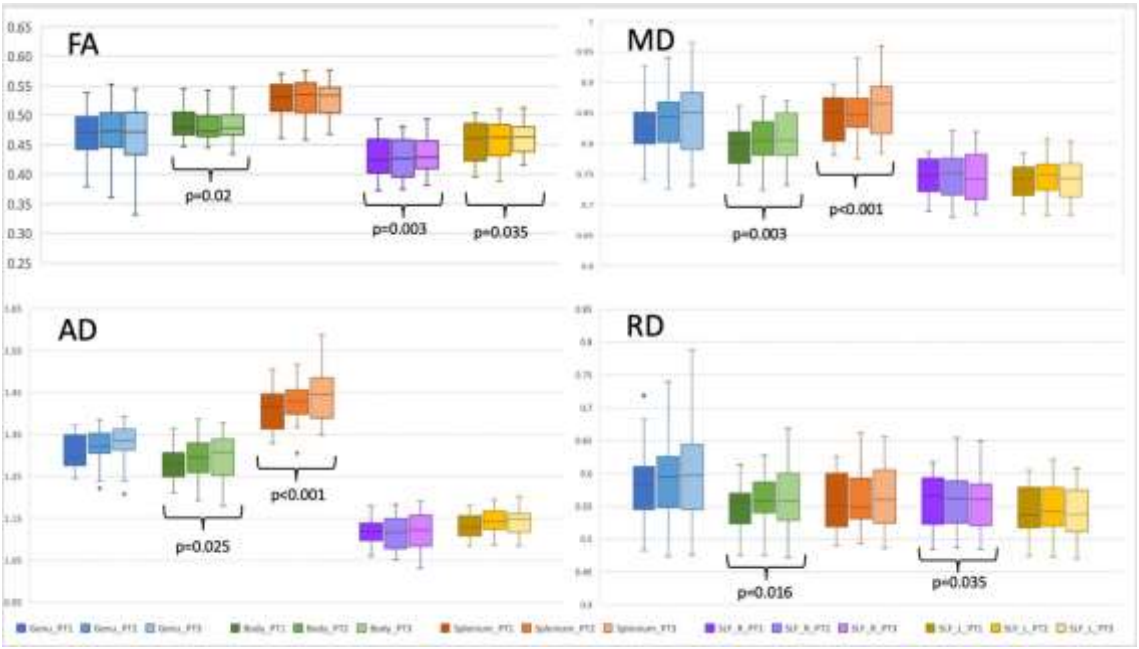

Figure 3. Box-plot graphics exhibit comparisons of DTI metrics (FA, MD, AD, RD) along time for the patients group in each CC segment and in both SLFs (genu in blue shades, body in green shades, splenium in orange shades, right SLF in purple shades, and left SLF in yellow shades). MD, AD and RD are expressed in x 10^{-3} mm^2/s. Statistically significant differences along time found with generalized linear function tests are indicated in the graphics with the corresponding *p*-values.

Neuropsychological evaluation

Neuropsychological tests indicated deficits in all cognitive domains in the patients group as indicated by negative Z-scores at both timepoints 2 and 3. Along time, however, patients presented improvement of the performances on memory (p=0.004) and attention (p=0.001). Other domains such as verbal fluency, working memory and IQ estimation did not demonstrate significant modifications throughout time (Table 2).

Table 2. Z-scores computed for each cognitive domain and the statistical results after generalized estimated equation using time as a model of effect.

Cognitive domain	PT2	PT3	*p*-value
Memory	-2.478 ± 0.171	-1.980 ± 0.205	*0.004*
Attention	-1.941 ± 0.249	-1.113 ± 0.251	*0.001*
Verbal fluency	-1.348 ± 0.190	-1.239 ± 0.132	0.473
Working memory	-0.424 ± 0.158	-0.425 ± 0.155	0.993
IQ	-1.006 ± 0.141	-0.885 ± 0.144	0.101

The results are shown in mean values ± standard deviations. PT2 and PT3 accounts for the patients groups at timepoints 2 and 3, respectively. Significant *p*-values are shown in italics.

Correlations between DTI parameters and neuropsychological tests

There were several significant correlations between DTI parameters and the results of the neuropsychological tests at both timepoints 2 and 3. These results are summarized in the supplementary Table S2.

At timepoint 2, we found positive correlations of FA values in the genu of the corpus callosum with attention (p=0.031), and in the splenium with attention (p=0.036) and working memory (p=0.003). There were also positive correlations of FA values in the right (p=0,039) and left (p=0,009) SLFs with IQ. In parallel, MD values in the genu correlated negatively with working memory (p=0,05), as well as in the splenium with attention (p=0.009), verbal fluency (p=0.002), working memory (p=0,032) and IQ (p=0,024). MD values also correlated negatively with verbal fluency on left SLF (p=0.037). Moreover, RD values in the genu were correlated negatively with verbal fluency (p=0.049); in the splenium with attention (p=0.018), verbal fluency (p=0.02), working memory (p=0.032) and IQ (p= 0.009); and in the left SLF with IQ (p=0.044). There was no evidence of correlations between AD at any regions studied and the neuropsychological results at timepoint 2.

At timepoint 3, FA values at the genu also demonstrated a positive correlation with the attention index (p=0.02). Correspondingly, RD values in the same site showed a negative correlation with the same cognitive domain (p=0.036). AD values in the left SLF showed a negative correlation with attention index (p=0.004). There were no other significant correlations between DTI metrics and the cognitive domains at timepoint 3.

Supplementary Table S2. Spearman correlation tests with significant correlations between DTI metrics and neuropsychological results

Tract	DTI parameter	Timepoint	Attention	Verbal fluency	Working memory	IQ
Genu	FA	PT2	R=0.508 *p*=0.031			
	FA	PT3	R=0.514 *p*=0.020			
	MD	PT2			R=-0.456 *p*=0.050	
	RD	PT3	R=-0,445 *p*=0.049			
Splenium	FA	PT2	R=0.496 *p*=0.036		R=0.645 *p*=0,003	R=0.636 *p*=0.005
	MD	PT2	R=-0.594 *p*=0.009	R=-0.529 *p*=0.020	R=-0.494 *p*=0.032	R=-0.530 *p*=0.024
	RD	PT2	R=-0.549 *p*=0.018	R=-0.465 *p*=0.045	R=-0.511 *p*=0,025	R=-0.597 *p*=0.009
Right SLF	FA	PT2				R=0.489 *p*=0.039
	AD	PT2		R=-0.557 *p*=0.013		
Left SLF	FA	PT2				R=0.600 *p*=0.009
	MD	PT2		R=-0.481 *p*=0.037		
	AD	PT2		R=-0.553 *p*=0.014		
	RD	PT2				R=-0.479 *p*=0.044
	AD	PT3	R=-0.620 *p*=0.004			

PT2 and PT3 accounts for the patients groups at timepoints 2 and 3, respectively.

Discussion

Our study demonstrates extensive diffusion abnormalities in the white matter, characterized by lower FA and higher MD, in all the evaluated segments in DAI patients in comparison to healthy controls. This is coherent to the widespread feature of DAI following moderate and severe trauma and is in line with several previous works conducted in animal models and in humans(9,18–20,41–50).

FA is the most commonly used parameter in DTI studies to assess integrity and geometry of axonal fibers. High FA values (close to one) are observed in brain regions containing well-organized parallel axon arrays. On the other hand, brain regions with no internal directional organization are associated with low FA (close to zero). In contrast to FA, MD represents the overall water diffusion, regardless its direction, and is affected by both radial and axial diffusivities(51,52). It has been largely discussed which processes underlie the changes in RD and AD and how they should be interpreted(51–55). Herein, we found more pronounced increments in RD, which are possibly associated with demyelination and neuroinflammation, specially the water accumulation within the myelin sheath (intramyelinic oedema). There were also higher AD values in the patients group, but this difference was significant only in the right SLF, which most likely reflects abnormalities in cell density and increase in extracellular space. Kinnunen et al. also found the same combination of abnormalities in DTI-derived scalar metrics using a voxelwise approach in TBI patients in several brain regions, including the CC and SLFs(9). Our work reinforces the utility of both FA and MD as sensitive DTI biomarkers of microstructural damage in DAI patients, even in otherwise normal-appearing parenchyma on conventional MRI.

The longitudinal evaluation of DTI parameters in our study demonstrated that the diffusion abnormalities are not stationary, but also change into some extent along time after trauma. This was particularly evident in the body of the CC, that showed an initial decrease in FA values followed by an increase in the second phase post-injury, accompanied by an increase in MD, AD and RD in the overall study interval. There were also progressive increments in MD and AD values in the splenium. In the SLFs, there were progressive increases in FA mean values, accompanied by significant changes in RD in the right side. Indeed, several works demonstrated that, in addition to the primary axonotmesis directly caused by rotational forces at the moment of the impact, other pathological processes ensue afterwards. There is evidence of a late secondary pro-inflammatory response associated with deposition of myelin debris, overexpression of cytokines, synaptic dysfunction, activation of glial cells, and also deposition of anomalous proteins, such as Tau and beta-amyloid(56–59). On the other hand, in a search for homeostasis and regeneration, a continuous process of debris clearance and anti-inflammatory response is also triggered, with reparative mechanisms that contribute to a neurological recovery(60,61).

One study using a mouse model showed early isolated axonal injury, followed by demyelination, oedema, and persistent axonal damage up to one month after the experiment,

which were accompanied by progressive changes in scalar indices, suggesting that DTI may indicate approximate timing of injury(43). Tissue reorganization have been detected to start as early as days after trauma and within 4 weeks, along with an increase in fiber density in affected regions in a rodent model(62). Other investigations conducted in humans that analyzed TBI victims from mild to severe trauma also demonstrated FA changes from early phases up to several years after trauma(42,45,48). In addition to the continuing process of debris clearance and neuronal regeneration, another reasonable explanation for the progressive increment in FA may be related to the vascular injury associated with DAI that causes local bleeding with hemoglobin degradation and iron deposition, which may also determine dynamic changes in FA values (63,64). Non-invasive methods such as DTI might be helpful to foster the understanding of the underlying complex pathophysiologic abnormalities and the microstructural anatomical substrates of the commonly observed cognitive deficits in TBI victims.

Following recovery from transient loss of consciousness and partial or complete recuperation of acute neurological deficits caused by head trauma, DAI survivors may present persistent disabilities, loss of productivity and impaired quality of life(1,2). Cognitive impairments depend on multiple variables, such as the trauma severity, rehabilitation and even genetic factors(65–67). A neuropsychological assessment indicated compromise of all cognitive functions in the patients group in our study up to one year after trauma, but there was significant improvement of episodic verbal memory and attention domains along time. This is in agreement with a meta-analysis review of 39 cross-sectional TBI studies, which indicated that cognitive functions improve after moderate to severe TBI but remains markedly impaired up to two years post-injury(67).

Moreover, we found significant correlations between DTI metrics and cognitive performances. There were positive correlations between FA values in the genu of the CC with attention at both evaluated phases, as well as negative correlation between MD values and working memory at timepoint 2. The mechanics of head trauma places the ventral and lateral surfaces of the frontal lobes in particular vulnerability for damage(68,69). Given the frontal projections of the genu, it is not surprising that executive functions mediated by these areas could be correlated with microstructural abnormalities as detected by DTI in our study. The splenium is also frequently injured in head trauma due to specific anatomical features such as its close proximity to the fixed falx that determines how the shearing forces propagates in this region. There were significant correlations between DTI indices extracted from the splenium

and several cognitive domains, including attention, verbal fluency, working memory and IQ at six months post-trauma. Our results also indicated positive correlations between FA values in both SLFs and IQ at the same timepoint, in addition to negative correlations between the other DTI metrics and verbal fluency and IQ. The correlations were more pronounced in the left SLF, what may be related to the by far more prevalent functional language dominance in the left cerebral hemisphere(70). Furthermore, there were more pronounced correlations in our study at six-moths post-injury, suggesting this interval as the optimal timing of DTI data acquisition for evaluation of cognitive outcomes.

Others authors have also found correlations between DTI parameters and neurocognition. Hashim et al. evaluated 19 subacute (up to one year post-trauma) and chronic (from one up to five years post-trauma) patients with a voxelwise approach and found persistent functional loss in chronic TBI, and also correlations between diffusion indices with memory and visuomotor coordination test scores, but not with executive function(71). Another group conducted a longitudinal study with region-of-interest based analysis at specific brain sites and demonstrated significant correlations with clinical outcomes up to 15 months after severe trauma(72). There is also evidence of associations between DTI indices and self-reported cognitive and emotional symptoms at 12-months post-injury in mild TBI(4). This study also pointed strongest effects in frontal regions including the forceps minor and the genu of the CC(4).

Lack of correlations between some DTI metrics and cognitive domains at specific sites in our study, especially in the body of the CC, may be related to the relatively low number of participants that are ideally required for correlational studies(73). Indeed, we have applied strict exclusion criteria in order to evaluate a very homogeneous group of moderate and severe TBI patients with a pure presentation of DAI rather than evaluating patients with a broader spectrum of traumatic injuries, such as large intra-axial and extra-axial hematomas. Furthermore, another possible explanation is that distant rewiring and behavioral compensation may mediate spontaneous improvement of cognitive deficits after TBI. Although these mechanisms are not completely understood and do not represent true pathological recovery, it is supposed that second behavior in intact circuits overtake the original cognitive with associated shifts in anatomofunctional maps topography(74,75).

There are several methodological approaches to analyze DTI datasets. Region-of-interest analysis allows straightforward extraction of diffusion parameters from a predetermined area of the brain, but only a limited part of the cerebral structure is evaluated in

two-dimensional images. Voxelwise analysis is broadly applied on research scenarios because it is suitable for global analyses of brain parenchyma and allows semi-automated comparison of large groups of patients. Some shortcomings of this approach, however, are the need for alignment and registration of brain volumes to a standard space, with its associated inaccuracies(18). Herein, rather than applying an exploratory evaluation prone to multiple error biases, we chose to evaluate with tractography the greatest inter-hemispheric commissure bundle and one long association tract that are known to link critical cortical regions and to modulate several cognitive functions.

Still, some caveats of the deterministic streamline tractography approach should be mentioned. This technique indirectly estimates fiber tract anatomy based on the main direction of water molecules diffusion in each voxel (in the order of millimeters), by far much bigger than the axonal diameter (in the order of microns). This assumption of homogeneous unidirectional vectors is unrealistic and gives erroneous estimations of fiber pathways in areas of crossing fibers. Furthermore, longer acquisition times and motion artifacts limit increases in spatial resolution. Other robust diffusion analysis techniques that soothe some of these limitations are evolving steadfastly, such as global probabilistic tractography, high angular resolution diffusion imaging (HARDI), q-ball imaging and diffusion kurtosis analysis (DKI)(18,75–77). So far, however, these approaches require more sophisticated processing algorithms and are less feasible for implementation in clinical sets to evaluate individual TBI patients.

Finally, this study emphasizes the utility of DTI to obtain quantitative information about the white matter microstructure in patients with moderate and severe brain injury. Our results showed extensive and dynamical changes in DTI parameters throughout the first year after trauma. In parallel, patients also demonstrated better performance scores in different neuropsychological domains over time, which could be correlated with DTI metrics at particular brain sites, indicating the potential role of microstructural reorganization and neuroplasticity. DTI is a noninvasive method that could be helpful in monitoring progression of DAI and to select cognitively compromised patients for targeted therapies in the future.

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Declaration of interest

The authors have no conflicts of interest to declare.

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Longitudinal whole-brain analysis of multi-subject diffusion data in diffuse axonal injury

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ABSTRACT

Background: Diffuse axonal injury occurs in high acceleration and deceleration forces in traumatic brain injuries (TBI). This lesion leads to disarrangement of the neural network, which can result in some grade of deficiency. The Extended Glasgow Outcome Scale (GOS-E) is the primary outcome instrument to evaluate TBI victims. Diffusion tensor imaging (DTI) assesses the white matter (WM) microstructure based on the displacement distribution of water molecules.

Objective: Study the WM microstructure along the first year after trauma using DTI, check the patient's clinical outcome, and test for associations.

Methods: We scanned 20 TBI victims of moderate and severe trauma at 2 months and 1 year after the event. Imaging processing was done with the FMRIB software library; we used the tract-based spatial statistics software yielding fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) for statistical analyses. We computed the average difference between the two measures across subjects and performed a one-sample t-test and threshold-free cluster enhancement, p-value < 0.05, corrected. The GOS-E evaluated clinical outcomes. We tested for associations between outcome measures and significant mean FA clusters.

Results: Significant clusters of altered FA were identified anatomically using the JHU WM atlas. We found spotted areas of FA increment along time, in the right brain hemisphere and the left cerebellum. Extensive regions of increased MD, RD, and AD were observed. Patients presented an excellent overall recuperation. There were no associations between FA and outcome scores, but we cannot exclude a small to moderate association.

Keywords: Craniocerebral Trauma; Diffuse Axonal Injury; Diffusion Tensor Imaging; Glasgow Outcome Scale; Regeneration.

INTRODUCTION

Traumatic brain injury (TBI) causes different complex brain lesions such as hematomas, contusions, vascular injuries, and diffuse axonal injury (DAI). DAI results from high-energy acceleration and deceleration forces, determining shearing strains in the white matter, leading to disconnection or dysfunction of the neural network[1].

Head injuries, particularly DAI, result in distinct functional deficits, such as physical, cognitive, and behavioral impairments, which dramatically affect life quality, return to daily activities, and social reintegration of survivors[2]. In 1975, Jennett and Bond developed the Glasgow Outcome Scale (GOS), and it was used as a primary outcome measure in phase III trials in TBI[3,4]. Afterward, acknowledging some limitations of the GOS, the Glasgow Outcome Scale – Extended (GOS-E) was developed. Since its definition in 1981, it has been primarily used and recommended as the primary outcome measurement in TBI studies[5,6].

DAI is not only restricted to mechanical forces at the moment of the trauma. Many different processes are triggered, such as inflammatory responses, molecular changes, apoptosis, and Wallerian degeneration. Therefore, the pathophysiology of DAI can be divided into primary and secondary lesions. The primary axonal lesion is the complete disconnection related to the kinetic energy in the trauma moment. In contrast, secondary axonal injuries are indirect and progressive lesions to neurons that ensue late after the initial shock[7]. The impact sparks molecular and cellular events that disturb the homeostasis, leading to changes in neurons and the regional microglia that can persist for years[8].

Traditional imaging modalities such as computed tomography and standard magnetic resonance (MR) sequences, such as T1 and T2 weighted sequences, are not sensitive to show the white matter (WM) damage related to DAI. Diffusion tensor imaging (DTI) is an advanced MR modality based on water molecules diffusion that measures the preferential displacement along the white matter tracts and has been used to access the brain microstructure in different pathologies, including head injuries[9]. There are diverse methods available to analyze DTI images, such as region-of-interest analysis and tractography. One of the most commonly used is the whole-brain approach to test for group-comparisons, for which tract-based spatial statistics (TBSS) is particularly recommended for voxel wise and cluster-based analyses, constraining statistical analysis to the center of the tracts[9]. It is a semi-automated method, with minimal user dependence, that allows a whole-brain evaluation and is notably suitable for evaluating diffuse lesions in the brain parenchyma such as DAI[10,11].

Other groups have used this approach to assess white matter changes in head injury victims in different stages after trauma[12,13]. Lipton and colleagues conducted a study on patients with mild TBI who presented with persistent cognitive impairment eight months to three years after the trauma. They found decreased fractional anisotropy (FA) and increased mean diffusivity (MD) in the corpus callosum, subcortical white matter, and internal capsules compared to healthy controls[13]. Another group investigated adolescents with mild TBI in the

acute phase (from 1 to 6 days after the trauma event) compared to age-matched controls[14]. They found significantly decreased apparent diffusion coefficient (ADC) and radial diffusivity (RD) and increased FA in several white matter regions and the left thalamus, consistent with axonal cytotoxic edema in the acute phase post-injury. However, few published works analyzed the progressive changes in the white matter in DAI, particularly in moderate and severe trauma victims.

This study aims to evaluate longitudinally the white matter of patients with severe and moderate DAI at two moments defined as the subacute (two months) and early chronic phases (one year) following the trauma event. We also assessed patients´ clinical outcome one year after trauma, using the GOS-E scale[6]. Our central hypothesis is that DTI parameters change along time and can have a degree of correlation with functional outcome.

METHODS

Standard protocol approvals

The protocol was reviewed and approved by the institutional review board, the local ethics committee, and all participants gave written informed consent.

Study design and subjects

A prospective study was conducted throughout one year. Adult outpatients admitted at the Emergency Room of Clinics Hospital, Faculdade de Medicina da Universidade de São Paulo, victims of moderate and severe TBI (Glasgow Coma Scale scores between 3 and 12 at initial evaluation), presenting clinical and tomographic findings exclusively of DAI were eligible to be admitted in the study. Exclusion criteria were the presence of contusions greater than $10cm^3$, midline shift greater than 0.5 cm, extra-axial collection determining compression of the brain parenchyma, or any indication for surgical intervention. Patients with poor quality imaging studies that limited analysis, clinical contra-indications that precluded MR scanning, or loss of follow-up were also excluded.

Data acquisition

All data were acquired on a 3T system (Intera Achieva, Philips Healthcare, Best, The Netherlands). Patients were scanned using an 8-channel head proton coil (Philips Healthcare, Best, The Netherlands) at two time-points: two months (subacute phase) and one year (early

chronic phase) after the trauma. The routine protocol included fluid-attenuated inversion recovery (FLAIR), diffusion-weighted imaging (DWI), and susceptibility-weighted imaging (SWI) sequences. For the data analysis in this study, we used a volumetric T1-weighted and DTI sequences.

The 3D-T1 fast field echo, acquired in the sagittal plane, was obtained using the following parameters: FOV 240 x 240 x 180 mm^3; matrix 240 x 240mm; isotropic resolution; TR/TE 6,2/2,7ms; and acquisition time 4.13min.

The DTI sequence was acquired in the axial plane, using 32 directions and one b0 using the following parameters: 70 slices; slice thickness 2mm; no gap; field of view 256 x 256 mm; voxel resolution = $2mm^3$ (isotropic); TR/TE 8.500/61ms; b = 1000 s/mm^2; matrix 128 x 128; number of excitations (NEX) = 1; and acquisition time of 7 minutes.

Imaging processing and analysis

Initially, all diffusion images were pre-processed for eddy current corrections and extraction of non-brain voxels, using FMRIB's Diffusion Toolbox (FSL) software, version 5.0.11[9,15]. For motion correction, the free toolbox Explore DTI (A. Leemans, University Medical Center, Utrecht, The Netherlands) was used, which rotates the B-matrix while keeping the exact initial orientation. With this same software, visual quality inspection for residuals and outliers was performed in each data set[16,17].

Thereafter, FA maps were analyzed using TBSS[9]. All individual´s FA images were non-linearly registered to the most typical subject of the sample (using -n command), and then the aligned dataset was transformed into the MNI152 standard space ($1mm^3$). The mean aligned FA images were merged into a single four-dimensional (4D) average FA image. A mean FA skeleton was extracted from the generalized 4D image and the tracts were projected into the skeleton, using a 0.2 threshold[18]. To extract mean, axial, and radial diffusivities (MD, AD, and RD, respectively), non-linear warps and skeleton projections were applied to each DTI scalar parameter.

Statistical analysis

To assess differences in FA, MD, RD, and AD along time, we performed one-sample t-tests, using the average difference between the two measures across subjects. Initially, it was first computed the difference between the subacute minus the early chronic phases, and then the early chronic minus the subacute phases. Permutation-based nonparametric inferences were

made on unsmoothed statistical maps, using 5000 permutations, and the cluster-like structures were enhanced using the threshold-free cluster enhancement (TFCE) algorithm[19]. This approach was similarly also applied to the MD, AD, and RD maps. Data were corrected for multiple comparisons, using the family-wise error (FWE) rate, setting the significance level at $p < 0.05$.

Thenceforth, the cluster tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Cluster) was applied to extract the exact clusters, followed by the Atlasquery tool to obtain the coordinates (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlasquery) according to the Johns Hopkins University (JHU) white matter tractography atlas.

Outcome measure

We used the GOS-E at 12 months post-injury obtained at the medical appointment follow-up, which has been recommended as the main outcome measurement in TBI studies[7]. It consists in an eight-scale global measure of function, used to estimate physical disability grading[6]. It classifies patients into upper and lower levels of good recovery (GOS-E = 7,8), moderate disability (GOS-E = 5,6), severe disability (GOS-E = 3,4), vegetative state (GOS-E =2) and death (GOS-E =1).

Association analysis

The WM areas with FA differences along time were defined as ROIs and the mean FA values of each one was calculated. Then, to test for association of mean FA values of each ROI with GOS-E grading, we used Cohen's d effect size test. We segmented patients into two different groups: sub-optimal (GOS-E= 5 or 6) and optimal (GOS-E = 7 or 8) performance. We tested for associations of each ROI at two months and one year after trauma.

Taking into account the relatively small patient sample, we also estimated Cohen's d effect size test considering a bigger sample size (4 times our sample, with the same distribution).

RESULTS

In the initial screening, 225 patients with head trauma were evaluated, and the final analysis included twenty patients of those. Demographics of the final sample are described in Table 1. Two hundred and five subjects were excluded for the following reasons:

- 186 had no clinical and/or tomographic criteria for DAI;
- 7 follow-up losses;
- 5 were not eligible for MRI;
- 5 had low-quality DTI studies;
- 1 developed epidural compressive hematoma;
- 1 deceased.

Table 1. Demographics of the 20 patients included in the study.

Gender	Male = 16; Female = 4
Handedness	Right-handed = 16, left-handed = 4
Traumatic event	Motorcycle = 10 Car accident = 6 Run over = 3 Agression = 1
GCS at hospital admission	Moderate (GCS 9-12) = 14 Severe (GCS < 8) = 6
Interval between trauma and hospital admission	39 minutes (15 to 77 minutes)
One-year outcome	GOS-E 5 = 1 GOS-E 6 = 7 GOS-E 7 = 11 GOS-E 8 = 1

Table 1. Demographics.

GCS = Glasgow coma scale, GOS-E = Glasgow outcome scale extended

Evaluation of changes along time at two months and one year after trauma (chronic minus subacute volumes) with voxel based TFCE analysis indicated brain regions with FA increment along time, predominantly in the right hemisphere and in the left cerebellum. The significant brain clusters (Table 2) were found in the right superior longitudinal fascicle, the temporal part of the right superior longitudinal fascicle, right inferior fronto-occipital fascicle, right superior and inferior longitudinal fascicles, the body of corpus callosum, forceps major and left corticospinal tract (Figure 1). Moreover, we found extensive areas of increases in MD, RD, and AD ($p < 0.05$, FWE corrected) (Figure 2).

Table 2. The most significant clusters found in FA analysis.

Cluster Index	Voxels	p	X (mm)	Y (mm)	Z (mm)	Location
7	7	0.007	25	-44	36	R SLF, R IFOF
6	4	0.025	3	3	26	body corpus callosum
5	4	0.024	32	-41	30	R SLF, R SLF (temporal part), R ILF
4	2	0.027	-28	-47	-38	L CST
3	1	0.04	29	-68	11	forceps major, R IFOF, R ILF
2	1	0.038	30	-43	32	R SLF and R ILF
1	1	0.031	33	-35	32	R SLF, R SLF (temporal part)

R SLF = right superior longitudinal fascicle, R IFOF = right inferior fronto-occipital fascicle, R ILF = right inferior longitudinal fascicle, L CST = left cortical spinal tract

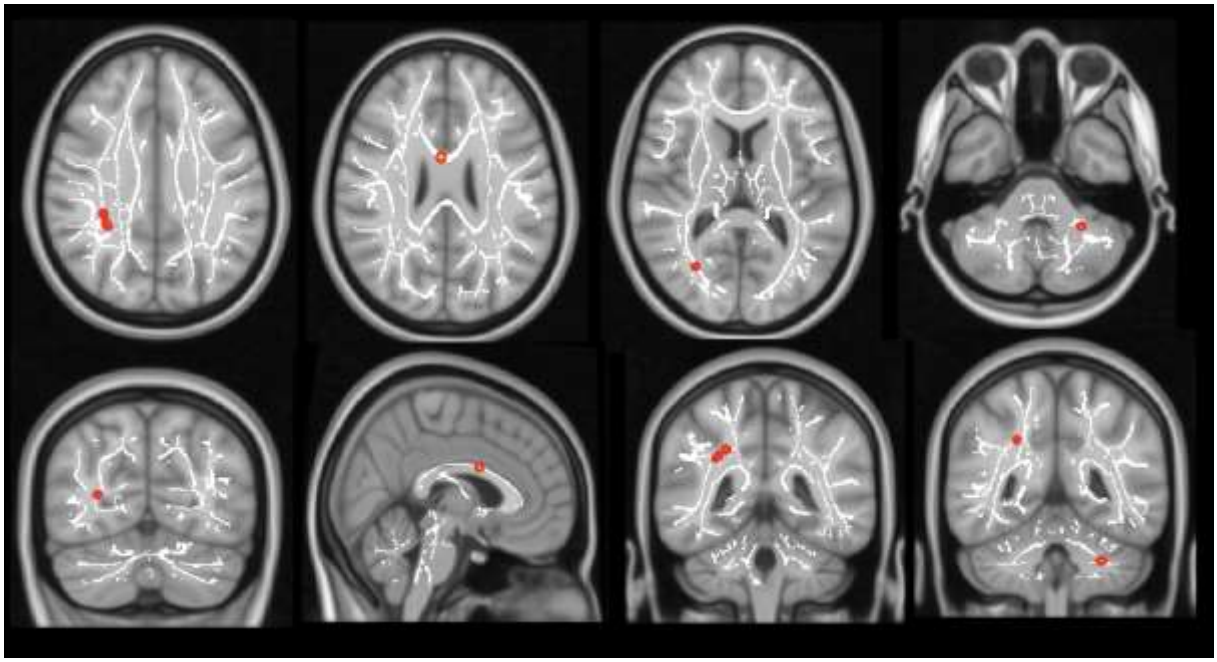


Figure 1. The most significant clusters found with increments in FA (early chronic minus subacute phase) are represented in red, TFCE ($p < 0.05$, FWE corrected). The mean FA skeleton is indicated in white. FA, fractional anisotropy.

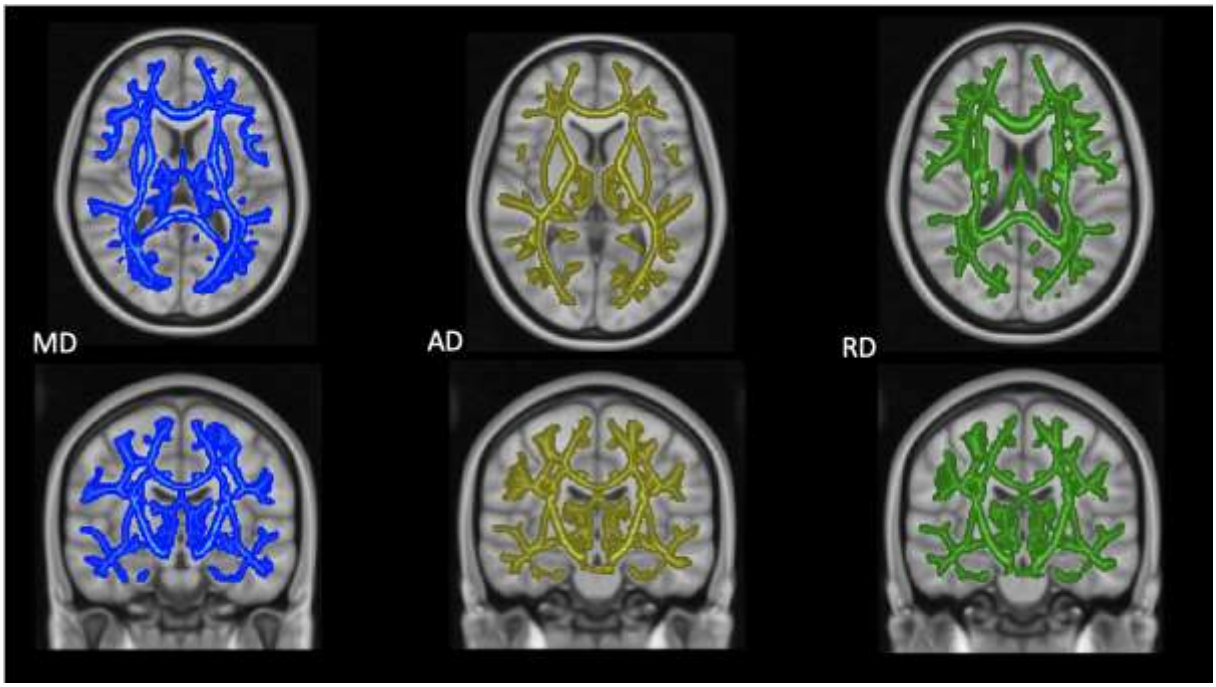
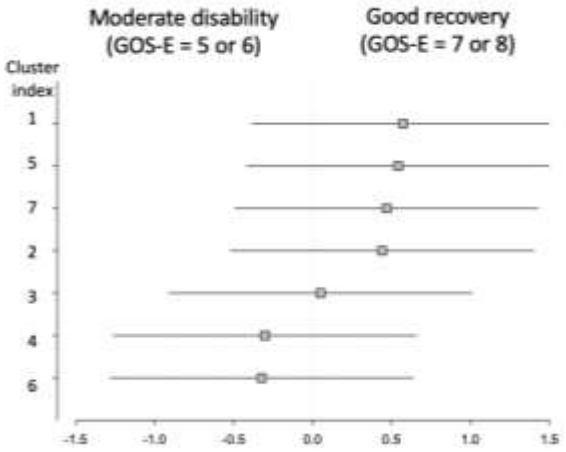


Figure 2. White matter differences between early chronic and subacute phases. Significant clusters ($p < 0.05$, FWE corrected). Blue depicts MD, yellow AD and green RD increases in the chronic phase. FWE, family-wise error; MD, mean diffusivity; AD, axial diffusivity; RD, radial diffusivity.

Of note, the one-sample t-test used to assess the difference between subacute minus the early chronic volumes did not demonstrate significant differences for any DTI parameter.

Correlations between the different FA ROIs and the one-year GOS-E grades were tested with different ROIs at 2-months and 1-year post-trauma (Figures 3 and 4). We did not find any correlations on either moment.

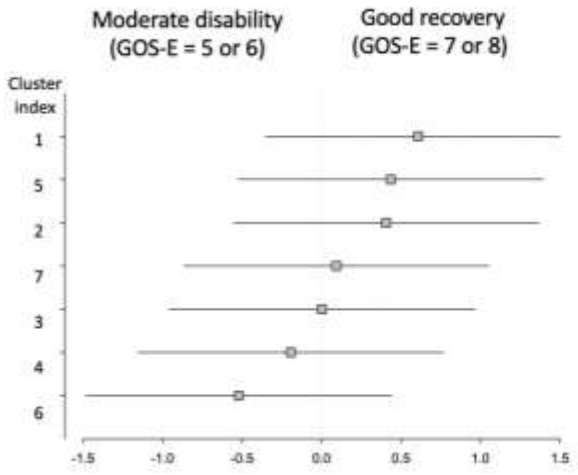


Cluster index	GOS-E Moderate disability	GOS-E Good recovery	d (IC95%)	*p* value
1	0.52±0.05	0.55 ±0.06	0.57 (-0.39 to 1.54)	0.225
5	0.45±0.06	0.48±0.06	0.54 (-0.42 to 1.51)	0.25
7	0.37±0.06	0.39±0.04	0.47 (-0.49 to 1.43)	0.317
2	0.43±0.04	0.45±0.06	0.44 (-0,52 –o 1.4)	0.347
3	0.53±0.08	0.53±0.06	0.05 (-0.91 to 1.01)	0.912
4	0.68±0.06	0.68±0.05	-0.3 (-1.26 to 0.66)	0.517
6	0.68 ±0.05	0.65±0.07	-0.32 (-1.29 to 0.64)	0.49

Figure 3. Subgroup analysis

The forest plot shows the effect size in the outcome variable across the prespecified subgroup according to GOS-E outcome stratification (moderate disability *vs* good recovery). Association analysis between different ROIs at 2 months after trauma with sub-optimal and optimal 1-year post-trauma GOS-E scores. Horizontal axis demonstrates differences between the groups of recovery according to each cluster.

Effect sizes values displayed along with respectively confidence interval of 95% and statically significance (*p*) obtained by Cohen's d test (squares).



Cluster index	GOS-E Moderate disability	GOS-E Good recovery	d (CI95%)	*p* value
1	0.54±0.04	0.58±0.07	0.61 (-0.36 to 1.57)	0.2
5	0.49±0.07	0.51±0.05	0.44 (-0.53 to 1.4)	0.353
2	0.46±0.4	0.48±0.07	0.41 (-0.56 to 1.37)	0.386
7	0.42±0.05	0.42±0.04	0.09 (-0.87 to 1.06)	0.841
3	0.58±0.09	0.58±0.07	0.00 (-0.96 to 0.96)	0.997
4	0.74±0.04	0739±0.4	-0.19 (-1.16 to 0.77)	0.676
6	0.72±0.03	0.69±0.06	-0.52 (-1.48 to 0.44)	0.268

Figure 4. Subgroup analysis

The forest plot shows the effect size in the outcome variable across the prespecified subgroup according to GOS-E outcome stratification (moderate disability *vs* good recovery). Association

analysis between different ROIs at one year after trauma with sub-optimal and optimal 1-year post-trauma GOS-E scores. Horizontal axis demonstrates differences between the groups of recovery according to each cluster.
Effect sizes values displayed along with respectively confidence interval of 95% and statically significance (*p*) obtained by Cohen's d test.

In addition, hypothetically increasing 4 times our sample, we found some associations the one-year GOS-E and the specific ROIs of FA increase at 2 months and 1 year after trauma (Table 3).

Table 3. Correlation analysis supposedly increasing by 4 times the sample size.

Cluster index	*p* value (2 months)	*p* value (1 year)
1	0.014	0.009
2	0.056	0.078
3	0.823	0.994
4	0.190	0.398
5	0.020	0.061
6	0.161	0.024
7	0.044	0.685

- *p* value obtained by Cohen's d test.

DISCUSSION

In our investigation, we did a whole-brain analysis using a semi-automated method to explore white matter changes over time in moderate and severe TBI victims. DTI has mainly been used to study white matter in the trauma scenario. However, most published articles are related to mild trauma and with different follow-up periods[12,13,20]. It is important to emphasize that our patient sample is very homogeneous, consisting of victims with moderate and severe trauma, explicitly diagnosed and exclusively with DAI, and followed throughout one year after the event.

We found some scattered areas of FA increase, notably in the right brain hemisphere, accompanied by vast regions in the brain and the cerebellum demonstrating an increase in MD, RD, and AD along time. Interestingly, the patients showed relatively good clinical outcomes, according to the GOS-E scale. We also found different associations between each brain region with increased FA and the late clinical outcome (GOS-E) two months and one year after trauma, which were more prominent when tested for a larger sample size. Our results are aligned with previous studies that have described white matter changes on DTI parameters along with time in victims of head trauma[21,22]. These ongoing DTI parameters are related to different pathophysiological processes such as inflammation, degeneration, and regeneration – which have already been described in experimental studies[23,24].

We identified a general area of increase in MD, AD, and RD in brain tracts one year after trauma. We consider that MD increase is mainly a result of high RD values and, in a lower degree to AD increment. MD represents the overall diffusivity of water molecules, which can be related to the increasing content of isotropic tissue with water content (gliosis)[25]. Although the biological basis for the anisotropy and diffusivity changes in tissues revealed by DTI data is still largely debated, studies using animal models have demonstrated that axonal injury itself is represented by AD changes and demyelination is associated with an increase in RD values[26]. Considering that increases in both AD and RD contribute positively to augments in MD values, it is reasonable to assume MD as a more sensitive parameter when compared to FA in our observation.

Moreover, in addition to axonal injury, other important and specific pathophysiological processes are also present in the trauma scenario, such as neuroinflammation, afferent degeneration, and debris clearance, and the magnitude of each one at different stages may imply distinct changes in DTI scalar values. Animal models' studies play an essential role in characterizing these other effects ensued by the trauma event and how they change over time. However, most of the articles published to date describe the changes seen in the early acute time after trauma, and only a limited number of articles evaluate long-term consequences[27]. It is already well established that the overall axonal injury in trauma survivors is a consequence of the secondary axonal injury, which is the indirect damage to neurons related to neuroinflammation and microglial activation, triggered by the initial impact and that can persist for years[23]. These processes are responsible for biochemical changes leading to local edema and changes in the microvascular circulation, leading to ischemia and demyelination, which can be illustrated by the RD increase over time[28]. Moreover, AD increase has been associated with

an increment of the extracellular water content, such as debris clearance, that would ease the water molecule movement in an axis parallel to the axons[29]. Thereby, we suppose that our results can be explained by the Wallerian or Wallerian-like degeneration process due to DAI or related to a secondary pathological process, such as regional ischemia and neuronal death may ultimately lead to brain atrophy[29].

We also found some spotted areas of FA increase in the right brain hemisphere and the left cerebellum over time. Different causes can be associated with FA increase, such as local fibrosis, hemorrhage areas, and neuronal sprouting[30]. FA is related to the microstructural organization, with high values (close to one) related to most anisotropic tissues. Microstructural organization after the trauma has been reported to start as early as days and can persist for years, which is linked to neuroplasticity[31]. The functional recovery accompanied by FA increment may be related somehow to neuroplasticity. Interestingly, we found areas of FA increase in the right brain hemisphere and in the left cerebellum, which may illustrate the involvement of the contralateral cerebellar hemisphere in functional and compensatory changes after trauma, as it has been already reported[32]. An interesting functional study evaluated children with moderate and severe trauma in comparison to controls, and it demonstrated that children with TBI demonstrated changes in functional cerebral activity and demonstrated increased recruitment of neural resources such as the cerebellum[33].

We tested for correlations between mean FA values at the subacute and early chronic phases of the specific regions that presented significant changes overtime and the GOS-E scores. We couldn't find any significant correlations; however, the lack of significance may be related to our sample size, relatively small when considering the optimal number of individuals required for correlational studies[35]. Still, some specific regions as the right SLF and the body of the corpus callosum demonstrated, at the early chronic phase, promising effect sizes in functional stratification between optimal and sub-optimal GOS-E scores and mean FA values by using a theoretical larger sample size.

Whole-brain voxel-wise analysis has been increasingly used to study DAI since the widespread nature of the disorder, as well as the advantages of minimal intervention for multi-subject group evaluation provided by this analytical method. However, with this technique, it is mandatory the use strict statistical procedures for correcting for multiple comparison errors, which reduces the sensitivity for detecting subtle changes[12].

One limitation of our study is the relatively small sample size. However, we included a particular and homogeneous group of patients with a minimum one-year survival after the

traumatic event, especially considering that victims of moderate and severe head trauma have high mortality rates in the first six months[34,35]. Moreover, we did have a particular group in terms of one-year survival, but these patients also presented an excellent recovery with high one-year GOS-E scores. This may be related to the exclusion of other conditions commonly associated with a head injury, such as contusions and hematomas that can imply a poorer outcome[2].

Concerning the methodology and imaging acquisition, we need to emphasize that more gradient encoding directions and more robust DTI acquisition and analytical methods such as high angular resolution diffusion imaging (HARDI), diffusion kurtosis imaging (DKI), and q-ball imaging are available and could have enhanced the power of the data analysis[11,36]. However, these approaches require longer acquisition times, more sophisticated algorithms, and are still less feasible to implement in clinical and research scenarios.

CONCLUSION

In conclusion, our work indicated changes in all DTI scalar metrics in the brain and cerebellum white matter in a homogeneous group of DAI victims along the first year following moderate and severe head trauma. This study can be important to guide future research in understanding the different pathophysiological processes that ensue at different stages during patient recovery. Upcoming studies are awaited to indicate DTI as a tool in signaling functional outcome, as well as an auspicious method to be used to guide therapies and rehabilitation procedures in trauma survivors.

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4 CONCLUSION

Our results showed extensive changes in the brain white matter microstructure in patients with moderate and severe DAI throughout the first year after trauma, by using DTI-based tractography and vowelwise analysis.

In addition, patients also demonstrated better performance scores in different neuropsychological domains over time, which could be correlated with DTI metrics in particular brain sites.

APPENDICES

APPENDIX A – Publication 1

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VIEWS AND REVIEWS

Current contribution of diffusion tensor imaging in the evaluation of diffuse axonal injury

Contribuição atual da imagem por tensor de difusão na avaliação da lesão axonal difusa

Daphine Centola Grassi[1], David Macedo da Conceição[1], Claudia da Costa Leite[1], Celi Santos Andrade[1]

ABSTRACT

Traumatic brain injury (TBI) is the number one cause of death and morbidity among young adults. Moreover, survivors are frequently left with functional disabilities during the most productive years of their lives. One main aspect of TBI pathology is diffuse axonal injury, which is increasingly recognized due to its presence in 40% to 50% of all cases that require hospital admission. Diffuse axonal injury is defined as widespread axonal damage and is characterized by complete axotomy and secondary reactions due to overall axonopathy. These changes can be seen in neuroimaging studies as hemorrhagic focal areas and diffuse edema. However, the diffuse axonal injury findings are frequently under-recognized in conventional neuroimaging studies. In such scenarios, diffuse tensor imaging (DTI) plays an important role because it provides further information on white matter integrity that is not obtained with standard magnetic resonance imaging sequences. Extensive reviews concerning the physics of DTI and its use in the context of TBI patients have been published, but these issues are still hazy for many allied-health professionals. Herein, we aim to review the current contribution of diverse state-of-the-art DTI analytical methods to the understanding of diffuse axonal injury pathophysiology and prognosis, to serve as a quick reference for those interested in planning new studies and who are involved in the care of TBI victims. For this purpose, a comprehensive search in Pubmed was performed using the following keywords: "traumatic brain injury", "diffuse axonal injury", and "diffusion tensor imaging".

Keywords: magnetic resonance imaging; diffusion tensor imaging; diffuse axonal injury; brain injuries, traumatic.

RESUMO

O traumatismo cranioencefálico (TCE) é a principal causa de morbimortalidade entre adultos jovens. Aqueles que sobrevivem são frequentemente deixados com sequelas funcionais nos anos mais produtivos de suas vidas. O principal aspecto fisiopatológico do TCE é a lesão axonial difusa (LAD), cada vez mais destacada pois está presente em 40 a 50% dos casos que necessitam de internação hospitalar. LAD é definida como a injúria axonial extensa caracterizada pela axoniotomia completa assim como pelas reações secundárias a axoniopatia, que são demonstradas por métodos de neuroimagem como áreas de edema e micro-hemorragia. Entretanto, os achados da LAD são frequentemente subestimados em estudos de neuroimagem convencional. É neste contexto que imagens por tensor de difusão (DTI) ganharam ênfase, já que permitem obter informações sobre a integridade da substância branca que não eram obtidas por sequências convencionais de ressonância magnética (RM). Existem artigos extensos sobre os fundamentos físicos e as aplicações de DTI em pacientes vítimas de TCE, no entanto, estes assuntos permanecem ainda nebulosos a alguns profissionais da área de saúde. Deste modo, propomos uma revisão didática sobre a contribuição do estado da arte de diferentes métodos analíticos de DTI no entendimento do processo da fisiopatologia e prognóstico da LAD, servindo assim como uma ferramenta acessível para aqueles interessados em planejamento de novos estudos e aqueles envolvidos no tratamento de vítimas de TCE. Uma pesquisa abrangente foi realizada no Pubmed com as seguintes palavras-chave: "traumatismo cranioencefálico", "lesão axonial difusa", "imagem por tensor de difusão".

Palavras-chave: imagem por ressonância magnética; imagem de tensor de difusão; lesão axonal difusa; lesões encefálicas traumáticas

TRAUMATIC BRAIN INJURY

Currently, traumatic brain injury (TBI) is a worldwide public health problem due to its high prevalence, morbidity and number of deaths. The most affected individuals are young males, who are more likely to engage in risk-taking behaviors. These traumatic events may result in several disabilities, loss of productivity and impaired quality of life. Therefore, understanding the mechanisms of trauma, grading the injury, and providing adequate medical care to the victims are essential to minimize the large social and economic consequences of TBIs[1,2].

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One of the most important aspects for guaranteeing an optimized approach for treating a TBI victim is understanding how the trauma occurred and providing a detailed and prompt clinical examination of the victim. Neurotrauma mechanisms are complex, and multiple types of injuries over the CNS may coexist, such as skull fractures, contusions, hematomas, and diffuse axonal injury[3,4]. The Glasgow Coma Scale (GCS) is the most widely used clinical classification system and depends on the best eye, verbal and motor responses. According to the GCS, a TBI can be graded as mild, moderate or severe. This scoring system was created in 1975 and lacks the sensitivity to predict subtle but meaningful residual dysfunctions, such as physical, cognitive, psychological and behavioral deficits[5].

More comprehensive clinical algorithms have been developed to determine the mortality and persistent disabilities in TBI victims. Two of the most used and well-known are the IMPACT and CRASH algorithms. The IMPACT algorithm was developed during clinical trials of severe TBI victims, and the CRASH algorithm was developed with mild and moderate TBI patients. Both algorithms use clinical predictors such as age, GCS score and pupillary reactivity at hospital admission. The addition of biomarkers, such as protein S100-beta, microtubule associated protein 2 and myelin basic protein, as well as imaging data from head computed tomography (CT) scans, has been shown to improve the reliability of these prognostic models[6]. However, the clinical manifestations present after traumatic events vary, and predicting prognosis in an individual patient remains challenging in daily practice[3,4,7].

In particular, patients with diffuse axonal injury frequently exhibit an apparent discrepancy between clinical status (usually moderately to severely compromised) and early imaging findings (often normal or minimally abnormal)[3,4]. Moreover, it is not well established why some survivors regain complete function while others remain severely disabled. Therefore, it is critical to understand the pathophysiology of diffuse axonal injury and to foster the development of noninvasive neuroimaging tools to reveal the damage to the central nervous system and provide guidance for therapeutic decisions and counseling for TBI patients and their families.

PATHOPHYSIOLOGY OF DIFFUSE AXONAL INJURY

Diffuse axonal injury is defined as wide axonal injury with microscopic and macroscopic components, which may only be visible in severe cases upon CT and magnetic resonance imaging (MRI). The mechanism of injury is based on the inertia of the brain: when fast and strong accelerations and decelerations occur, different structures with distinct densities (such as gray and white matter) suffer shearing and straining forces that stretch and damage axons, leading to diffuse axonal injury[3,4]. Commonly affected brain sites include the corpus callosum, fornices, subcortical white matter and cerebellum[7]. Once the victim presents in a comatose state with a GCS less than 8, the probability of brainstem involvement becomes significant and the prognosis worsens[8].

It is very important to understand that diffuse axonal injury does not only consist of the axonal injury itself. Complex neuropathological processes ensue, such as an inflammatory response secondary to the traumatic event associated with protein deposition. After direct axonal injury, multiple changes occur microscopically, including cellular death, synaptic dysfunction, activation of glial cells and anomalous protein deposition (Tau and Aβ proteins)[9,10,11].

Damage to an axon does not always lead to disconnection: connections may occasionally still be present, but it is not well known if they remain functional[12]. It is well established that neuronal death *per se* evolves into Wallerian degeneration, which is defined as the progressive anterograde disintegration of axons and accompanying demyelination that occurs after injury to the proximal axon or cell body[13]. Furthermore, it has been increasingly recognized that anomalous protein deposition secondary to axonal injury might be related to the future manifestation of Alzheimer's disease in some patients[14,15].

Histopathologically, diffuse axonal injury can be divided into three degrees: grade I: microscopically widespread axonal injury in any location; grade II: grade I findings plus focal lesions in the corpus callosum; and grade III: grade II findings plus focal lesions in the rostral portion of the brainstem[16].

NEUROIMAGING

Neuroimaging evaluation of a TBI patient is based on CT and MRI studies. During emergency care, TBI victims are usually first evaluated with CT scans, which are fast and accurate for identifying life-threatening conditions that may require prompt intervention, such as extra-axial hematomas[7].

There is a mismatch between the CT findings and clinical presentation in TBI patients. For instance, punctate microhemorrhages on the corpus callosum and gray-white matter junction are shown in only 10% of all TBI patients. Within two weeks after a traumatic event, neuronal loss can be inferred on CT as a discrete ventricular enlargement in some patients[7,16].

An MRI, despite being less widely available and requiring longer scan times than a CT, is the best modality to assess brain injuries because it provides a better identification of anatomic features and higher spatial resolution[3]. Hemorrhages are represented by a loss of signal in gradient echo and susceptibility-weighted sequences (Figure 1), whereas areas of edema present as high signals in T2-weighted and fluid attenuation inversion recovery (FLAIR) sequences (Figure 2).

Nevertheless, more advanced techniques, such as diffuse tensor imaging (DTI), are more sensitive to neuronal lesions in areas that appear normal on conventional MRI sequences, especially in patients with diffuse axonal injury[14,17,18,19].

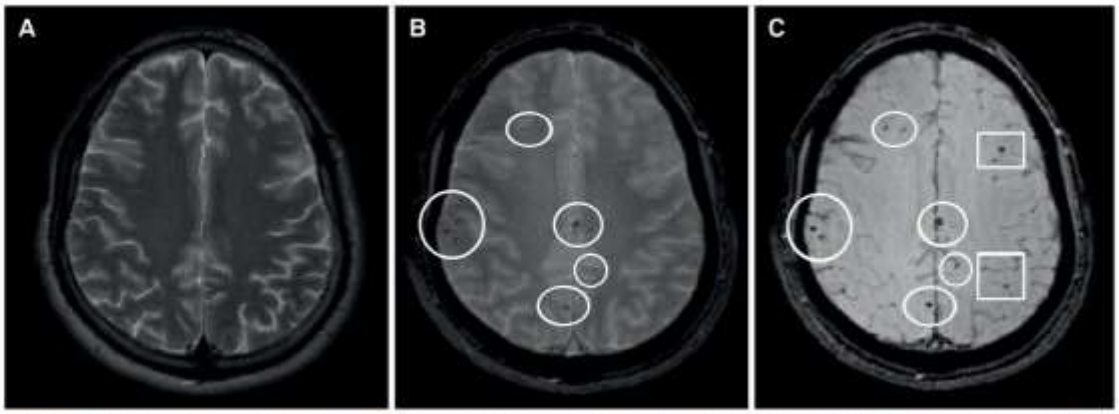

Figure 1. A 19-year-old male patient with diffuse axonal injury and Glasgow Coma Scale score of 8 after a motorcycle accident. While T2 conventional sequences (A) are rather insensitive for hemorrhagic lesions, T2*-GRE (B) shows numerous foci of signal loss (circles) in the subcortical white matter corresponding to areas of extravascular blood. Susceptibility weighted imaging (C) is even more sensitive than the previous two sequences, exhibiting more conspicuous (circles) and numerous lesions (squares) on both cerebral hemispheres. The images were performed sequentially during the same examination at a 3 Tesla scanner.

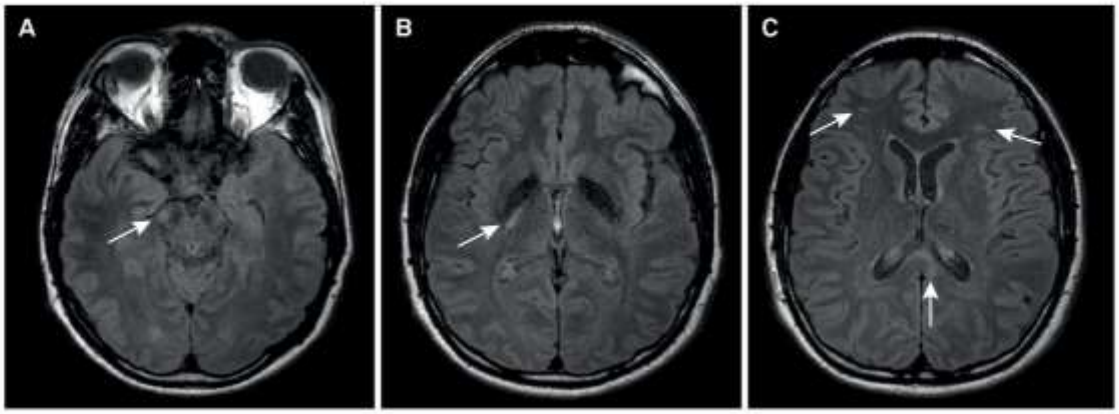

Figure 2. A 27-year-old male patient with moderate TBI due to a motorcycle accident. At admission, his Glasgow Coma Scale score was 10 and he presented with left hemiparesis and left third nerve palsy. Axial FLAIR images show hyperintense lesions (arrows) in the lateral aspect of the right cerebral peduncle (A), right internal capsule (B), splenium of the corpus callosum, as well as subtle lesions in the subcortical bilateral frontal white matter (C).

DIFFUSION WEIGHTED IMAGING

Since its medical debut, diffusion weighted imaging (DWI) has largely been used in routine clinical practice in applications ranging from diagnosing stroke to helping physicians determine tumor cellularity. Diffusion weighted imaging is an integral part of any MRI brain examination and is of paramount importance in radiological diagnosis.

Diffusion weighted imaging is based on the random motion of water molecules, which was first described by the botanist Robert Brown in 1827. In a homogeneous liquid environment without any barriers, water diffusion is free (isotropic); in other words, it has no preferred direction. In contrast, when there are surrounding structures restricting water molecule movement, a preferred diffusion direction becomes apparent (anisotropic diffusion)[20].

In the brain, several components hinder water diffusion in all directions, making the diffusion anisotropic. This anisotropic diffusion depends on the geometry and composition of natural barriers, such as cell membranes, myelin sheaths and primary microstructural components (neurofilaments and microtubules). For this reason, anisotropy is markedly high in well-organized white matter tracts and is lower in cerebrospinal fluids[21].

Diffusion weighted imaging shows hyperintensities in pathological conditions, such as in areas of high cellularity density (tumors), abnormal cellular uptake of water (cytotoxic edema) or entrapment of water between myelin membranes (intramyelinic edema). However, other increases in water concentration, such as observed in vasogenic edema or gliosis, also cause nonspecific hyperintensity on DWI, which is a T2-weighted sequence[22].

To distinguish between true restriction of water molecule diffusion and artifacts (T2 shine-through effects), apparent diffusion coefficient (ADC) maps were developed. When analyzing each voxel from the image with two different *b* values, it is possible to quantify water diffusivity and convert this information into a visual map (ADC map). The term "apparent" reflects the fact that calculated ADCs in tissues vary according to the previously attributed *b* values. Moreover, the ADC information corresponds to each voxel (in the order of millimeters) but not at the microscopic level of cell structures (in the order of a few micrometers). On ADC maps, restricted water diffusion is confirmed as a low signal intensity[23,24].

In patients with traumatic injury, cytotoxic edema usually occurs in cases of cortical contusion and diffuse axonal injury. Cortical contusions usually affect both superficial cortical gray matter and subcortical white matter but do not typically follow a vascular distribution. In acute diffuse axonal injury, it is possible to detect multifocal areas with restricted diffusion, which appear bright on DWI and dark on ADC maps. Predilection sites are the corpus callosum, especially the splenium, cerebral peduncles, deep white matter structures and gray-white matter interface (Figure 3). This restricted water diffusion may vanish in a few days or may evolve into residual lesions with a persistent high signal intensity on FLAIR and T2-weighted images[25].

DIFFUSION TENSOR IMAGING

The tensor model was proposed to characterize and quantify diffusion anisotropy. By measuring diffusion in at least six different gradient directions applied to the three different axes (X - horizontal, Y - vertical and Z - perpendicular to X), it is possible to determine an average water diffusion according to the distance and intensity for each voxel[26,27].

Fractional anisotropy (FA) is a scalar measure that reflects microstructural geometry and is very high (close to 1) in normal white matter but is usually lower in damaged white matter and is close to zero in the cerebrospinal fluid[27]. Colormaps can be created based on the first eigenvector that composes the FA calculation in which each color represents the main diffusion direction of white matter tracts (conventionally, red is used for left-right, green for anteroposterior, and blue for superior-inferior directions), and the degree of brightness is proportional to the magnitude of anisotropy[28,29] (Figure 4).

The mean diffusivity reflects the overall degree of water diffusion in all directions, regardless of its orientation dependence[30]. Radial and axial diffusivity are other quantitative DTI parameters. Axial diffusivity is the principal tensor direction (first eigenvector) with its associated magnitude (first eigenvalue), and radial diffusivity represents the other two directions perpendicular to the principal direction of the diffusion tensor. Investigations with animal models have indicated that radial diffusivity correlates with demyelination, whereas axial diffusivity seems to be related to more profound tissue damage and axonal loss[31,32]. Still, translational characteristics of these studies remain to be proven.

Several acquisition parameters influence the quality of diffusion data. The ability to obtain a valuable dataset is strongly related to the strength of the gradient coils and the maximum *b* values that can be achieved. For most brain studies, a *b* value

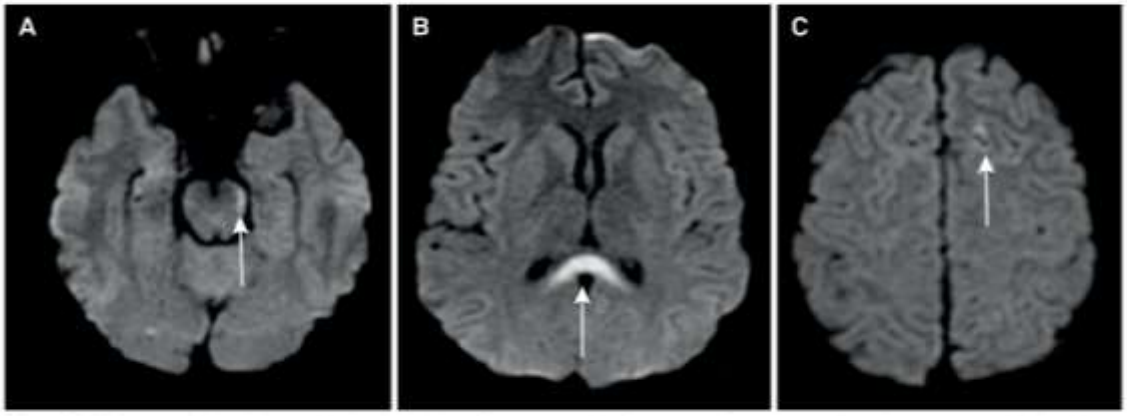

Figure 3. Diffusion-weighted images in the axial plane of a young patient four days after a head injury demonstrate bright lesions (arrows) in areas typically affected in diffuse axonal injury: left cerebral peduncle (A), splenium of the corpus callosum (B), and subcortical frontal white matter (C). The lesions were dark on ADC maps (not shown).

of 1000 s/mm² is usually adequate and results in an effective compromise between sensitivity and the signal-to-noise ratio. Ideally, images should be acquired with high isotropic spatial resolution (1–2 mm³). Many gradient directions, optimally at least 32, should be applied. However, there is a trade-off between the optimal acquisition parameters and the scanning time. Head motion should be minimized as much as possible. Preprocessing steps may, however, partly attenuate motion artifacts and the eddy current artifacts[33,34].

Distinct methods are available to analyze diffusion tensor images. Herein, we aim to briefly discuss the basic principles, advantages and caveats of each main DTI analytical method, namely, region-of-interest analysis, tractography and voxel-wise analysis, along with the relevant findings of recent DTI studies of TBI patients.

REGION-OF-INTEREST ANALYSIS

In region-of-interest (ROI) analysis, diffusion parameters are obtained from a predetermined area of the brain or around a specific anatomic structure. The area-of-interest can be manually drawn, and there is no requirement to use an anatomic atlas. This method is also suitable for studies in patients with large brain lesions in different sites or great anatomic distortions that could bias other analytical methods that require registration steps. Once the ROI is determined, the mean values of the water diffusion parameters are obtained[33,34] (Figure 5).

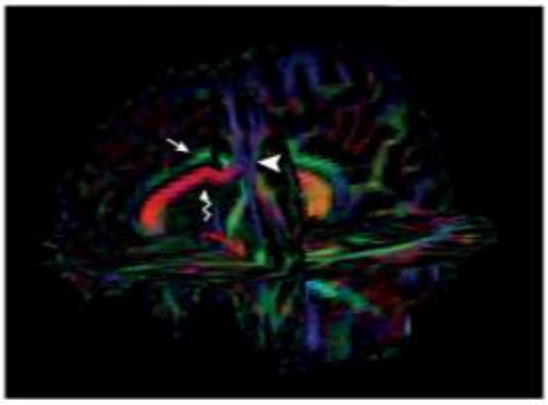

Figure 4. Three-dimensional FA colormap of an 18-year-old TBI victim. The patient was admitted with a Glasgow Coma Scale score of 8 after a motorcycle accident, despite the use of a helmet. Conventionally, long association fibers with anteroposterior direction such as the cingulum are represented in green (straight arrow), inter-hemispheric commissural fibers (e.g., corpus callosum, curved arrow) with left-right direction are represented in red, while projection tracts with superior-inferior route such as the corticospinal tract are represented in blue (arrowhead).

One advantage of this method is that even small brain regions, such as subcortical areas and deep basal ganglia, can be assessed. Another benefit is that multiple comparison errors are less prominent if the investigator predefines an *a priori* hypothesis and fewer areas are examined. Region-of-interest analysis identifies even subtle changes and is thus one of the most sensible and straightforward analytical methods available[35].

However, certain considerations for ROI analysis must be underscored: some information may be missed when only one area is being studied; it may be difficult to compare the same ROIs among individuals due to intrinsic variability; and the process of delimitation of the ROI can be laborious if it appears in multislice images or in the case of big data with multisubject comparisons. It is extremely important to know where to look for the changes; ROI analysis of nonaffected areas can identify normal parameters, but truly compromised areas can be missed. The manual nature of the ROI drawing may lead to the low reproducibility of intra- and intersubject ROI correspondence; hence, anatomic atlas tools or semi-automatic methods are advisable. For this reason, it is possible to standardize ROI studies by using segmentation and registration steps; segmentation helps determine the area of interest, whereas registration matches the corresponding points among all images in different subjects[33,34,35].

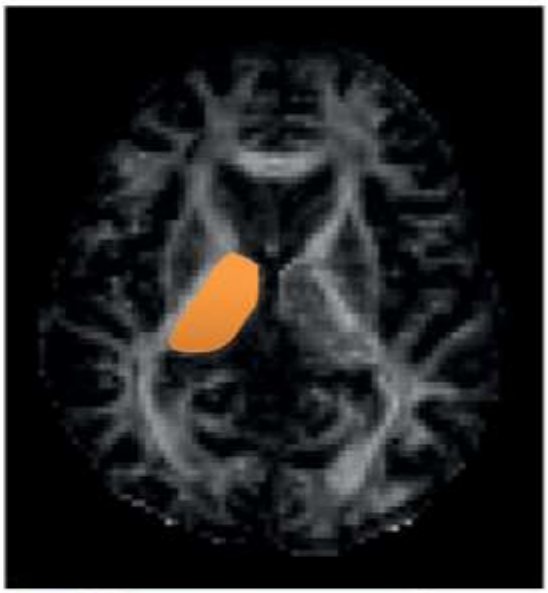

Figure 5. Diffusion parameters can be extracted from a selected ROI. In this case of a 42-year-old woman who suffered physical aggression and presented with severe TBI, the right thalamus (orange shape) was manually delineated in the FA map.

Mac Donald et al.[36] demonstrated that DTI could detect white matter injury in a mouse model of diffuse axonal injury and could determine the approximate timing of injury. These authors extracted diffusion parameters with ROI analyses from the corpus callosum and the external capsule and compared the findings with histological and electron microscopy results. In comparison with uninjured mice, anisotropy measures were lower in the injured group at all stages. During the early acute phase (less than one day), axial diffusivity was reduced and axonal injury was present histologically. During the subacute phase (one week to one month after injury), a reduction of anisotropy was accompanied by increases in axial diffusivity, radial diffusivity and mean diffusivity, which reflected the dominance of demyelination and edema at histological evaluation. The authors proposed that if similar mechanics are present in human TBI, these DTI changes could be used for novel clinical and forensic applications. However, the authors did not find any correlation between the severity of histological damage and the DTI parameters[36].

Another study evaluated 10 healthy individuals and five patients with mild TBI within 24 hours of injury and one month later. The authors extracted diffusion parameters with ROIs positioned in the corpus callosum and internal and external capsules. Soon after the injury, TBI patients demonstrated significant reductions in anisotropy compared with controls. In the 30-day-control, two patients showed slight increases in FA values when compared with their initial results, which was considered a possible sign of recovery[37].

According to Huisman et al.[38], changes in water diffusion anisotropy do occur in TBI, and these changes may be biomarkers for severity of tissue injury and predictors for outcome. Patients were analyzed within seven days of the event, and the data were compared with those from a control (healthy) group. The studied regions included the internal capsule, splenium, thalamus and putamen. This study showed that FA was significantly decreased in the posterior limb of the internal capsule and splenium of the corpus callosum. Furthermore, there was a statistically significant correlation between the FA values in the diffuse axonal injury predilection sites and the severity of head injury, as measured by acute and subacute neurologic assessments (acute GCS and Rankin scores)[38].

In addition, another longitudinal study analyzed 11 TBI victims in acute (less than seven days) and subacute (from eight days to rehabilitation discharge) stages and correlated DTI findings with a disability rating scale. Eleven ROIs were chosen based on previous studies that demonstrated an association between FA and functional or cognitive outcomes in TBI. It was apparent that the FA values varied according to the pathophysiologic processes of TBI. During the acute phase, the FA values were lower than they were in the subacute stage, possibly because of brain edema. Consequently, subacute values were more likely to reflect the axonal structural integrity. Therefore, these authors suggested that the optimal timing of DTI data acquisition for TBI prognostication might be during the subacute stage of injury[39].

TRACTOGRAPHY

Three-dimensional visualization of DTI information is also possible. Tensor information allows the trajectories to be estimated by inferring white matter fiber orientations. Tractography allows the parcellation of white matter, and this information may be particularly useful in anatomo-functional studies because white matter bundles are linked to specific cognitive, language, behavioral, and motor functions (Figure 6)[39].

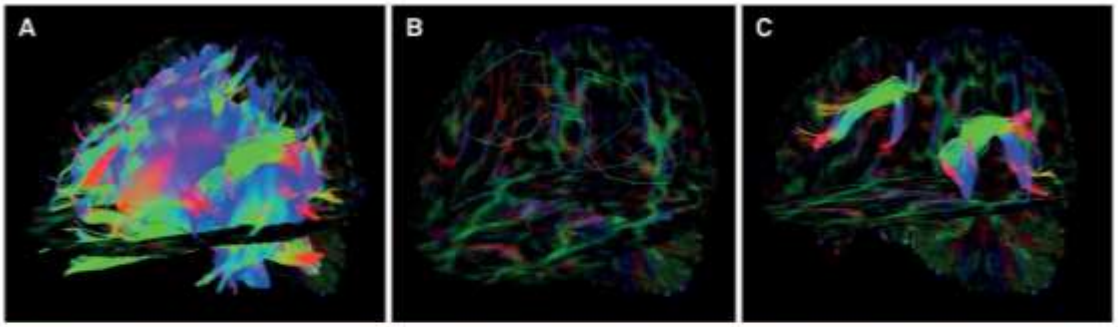

Figure 6. A young female patient had a motorcycle accident and presented at the emergency room with a Glasgow Coma Score of 3. She persisted with language impairment one year after the traumatic event. It is possible to evaluate DTI parameters from any tract related to a specific cognitive domain. The superior longitudinal fasciculus is linked to language skills because it interconnects Broca's area (responsible for speech production in the frontal lobe), Geschwind's area (semantic processing in the parietal lobe) and Wernicke's territory (speech comprehension in the temporal lobe). First, whole-brain tractography was obtained with a brute-force approach from the full tensor data (A). A set of "AND", "SEED" and "NOT" ROIs was placed on both cerebral hemispheres based upon *a priori* anatomical knowledge (B). The superior longitudinal fasciculi were then virtually dissected with a deterministic streamline approach and displayed on a FA color-encoded map (C).

Tractography consists of three processing steps: seeding, propagation and termination. Seeding involves determining the area from which the fiber bundles will be drawn, and the most common approach is to define an ROI and placing one or more seeds in the expected trajectory of the tract. Another possible method is to use automatic seeding for the whole brain from a full set of tensor data[29,39].

Propagation is how visual schemes of fibers are generated, and different algorithms are developed to link inter-voxel information. Algorithms are based on deterministic or probabilistic approaches. The first approach is based on a streamline principle: from the seed location (ROI) nearby voxels will be linked to the same streamline if their principal eigenvectors have congruous orientations or similar FA values. Probabilistic tractography, in contrast, represents an estimation based on multiple possible fiber directions in each seed. For this reason, probabilistic tractography tends to disperse trajectories more than deterministic methods and has the potential to delineate a greater proportion of the white matter tract[28,29,39-40].

Finally, termination is the last step of the fiber tracking procedure with well-defined criteria. Typically, FA thresholds (usually higher than 0.25 to avoid contamination by CSF and grey matter) and turning angle thresholds (generally 30° or 60° depending on the known curvature of the tract of interest) are used. This method of "virtual dissection" allows the isolation of specific anatomic fiber pathways from DTI datasets and has been proven useful in several adult and pediatric conditions (Figure 7)[28].

Nevertheless, there are some important shortcomings that must be taken into account when analyzing tractography. This method estimates fiber tract anatomy on a macro scale. Voxels are in the order of millimeters, whereas the axonal diameter is in the order of microns. It is also not possible to differentiate afferent from efferent bundles. Moreover, the assumption of homogeneous unidirectional tensors is unrealistic because many brain regions comprise more than one fiber bundle. Crossing, diverging or kissing fibers result in incorrect direction estimations and pathways and may lead to abrupt tract termination. Diffusion spectrum imaging, q-space imaging, q-ball imaging, and high angular resolution diffusion imaging are more sophisticated approaches that may overcome some of these limitations[40].

Wang et al.[41] studied 12 patients who suffered from severe TBIs within seven days and nine months after injury. They analyzed the corpus callosum, fornix and peduncular projections with deterministic tractography. The authors could identify at least one DTI parameter that demonstrated diffuse axonal injury-associated alterations in each region. Furthermore, they demonstrated a good correlation between the DTI findings and long-term prediction outcome, as measured with Glasgow Outcome Scale-Extended scores[41].

Another group demonstrated that patients with mild TBI have reduced FA values in various white matter locations and various fiber bundles within 5.5 months after trauma. In comparison with healthy-matched controls, the authors demonstrated lower anisotropy in multiple white matter regions, predominantly in the cerebral lobar white matter, cingulum and corpus callosum, using deterministic tractography. A minority of fibers showed premature discontinuation on fiber tracking, and the authors presumed that this may have been caused by the presence of sharply angulated fibers or by small areas of hemosiderin that were not visible on MRI[42].

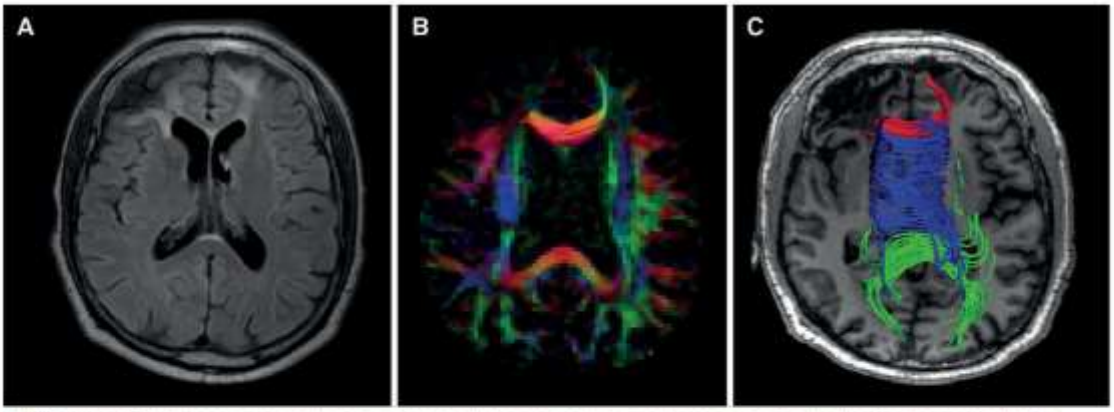

Figure 7. Axial FLAIR image of an adult male patient with chronic post-traumatic sequelae shows gliosis in the frontal lobes, mainly in the right side (A). FA colormap demonstrates paucity of white matter fibers and reduction of brightness due to reduction of FA values in the frontal lobes, more pronounced in the right side (B). DTI-based tractography of the splenium, body and genu of the corpus callosum (represented in green, blue, and red colors, respectively) shows premature termination of streamlines of peripheral fibers of the genu projecting to or from the prefrontal regions coincidental to the areas of low signal intensity on the axial T1-weighted image (C).

A large study evaluated 106 chronic TBI patients who had no abnormalities on conventional MRI in comparison with 62 healthy controls. The investigators applied a deterministic approach to extract the volume and FA measures of long association tracts from the uncinated fasciculus, superior cingulum, temporal cingulum, superior longitudinal fasciculus, arcuate fasciculus, inferior fronto-occipital fasciculus and inferior longitudinal fasciculus. Injured patients demonstrated reduced FA values in both uncinated fasciculi, both inferior fronto-occipital fasciculi and in the right inferior longitudinal fasciculus. However, the tract volumes were not significantly decreased in injured patients[42].

A recent study evaluated trauma-exposed police officers with and without post-traumatic stress disorder using a 3T system. The authors applied an automated and unbiased reconstruction of white matter tracts using a global probabilistic tractography method known as TRACULA (TRActs Constrained by UnderLying Anatomy)[44] and found significantly higher mean diffusivity in the right uncinate fasciculus in the affected group. The uncinate fasciculus is the major white matter tract that connects the amygdala to the prefrontal cortex. These authors also found that the mean diffusivity of the right uncinate fasciculus was positively associated with anxiety symptoms in patients with post-traumatic stress disorder[45].

Head injury survivors usually present with persistent cognitive symptoms that impair their quality of life, such as deficits in language performance and executive function. In the future, tractography studies could be used to monitor the benefits of target therapies in these patients by evaluating DTI metrics in specific white matter tracts linked to specific functional domains.

VOXELWISE ANALYSIS

Voxelwise analysis has become more popular because its automatic approach requires minimal intervention and less user dependence. Voxelwise analysis is suitable for global analyses of brain parenchyma and is particularly useful for large group comparisons of individuals with no significant distortions in brain anatomy[23,46].

Primarily, the images must be standardized into a template to ensure that each voxel corresponds to the same anatomic location in all subjects. Thus, a critical step is to define the best way to register and compare multiple images from different individuals in an accurate manner[23].

Previously, voxelwise analyses were performed with voxel-based morphometry using T1 weighted-images. Although some constraints related to image registration, segmentation and smoothing are present, voxel-based morphometry can still be used to explore diffusion images in particular research scenarios.

Tract-based spatial statistics is the current leading method for voxelwise analysis using nonlinear image transformation of FA images across subjects[46]. Tract-based spatial statistics uses the mean FA values from individual subjects to create a skeletonized map with the local maximal FA values for each tract. With this approach, the differences in all the voxels of the brain, except those from the skeleton voxels are ignored. Another challenge is registration inaccuracies of areas with high contrast FA values, such as those areas adjacent to ventricles[23,47]. A more recent alternative approach for the registration step of tract-based spatial statistics is to use the full tensor information with a complementary tool, known as the DTI-ToolKit, which has been shown to reduce the number of misassigned voxels by a total of seven[47,48].

In voxelwise analysis, all voxels in the image are compared with each other in a local manner, and hence statistical procedures to control for multiple comparison errors might be carried out. These may, in turn, reduce the sensitivity for more subtle findings in particular regions[23].

Our group evaluated 20 adults with moderate to severe TBI using a 3.0T MRI scanner during the acute ($t_1 < 3$ months), subacute ($6 < t_2 < 9$ months) and chronic stages ($12 < t_3 < 15$ months) following trauma. According to tract-based spatial statistics analysis, the patients exhibited one large cluster with statistically significant lower FA values ($p < 0.001$) at all times (t_1, t_2, t_3) compared with the controls (Figure 8), but the number of affected voxels decreased over time by 2% at t_2 and by 7.3% at t_3. During the chronic stage (t_3), patients recovered from white matter damage in comparison with the acute stage (t_1) with significant increases in FA in the bilateral anterior thalamic radiations, forceps major and minor, corticospinal tracts, cingulum, uncinate, inferior fronto-occipital, superior and inferior longitudinal fasciculi. Patient performance on cognitive measures was suboptimal at all three stages but also improved over time in the same fashion as white matter recovery[49].

One study applied voxel-based analysis and ROI analysis in 10 adolescent patients with mild TBI who were assessed within one week after injury and compared with a paired-control group. The results indicated increased FA, decreased radial diffusivity and unchanged axial diffusivity in multiple brain regions, which may be related to axonal cytotoxic edema and reflect acute injury. Moreover, the alterations in DTI metrics were highly correlated with postconcussive symptoms severity and emotional distress[50].

Lipton et al.[51] retrospectively analyzed 17 cognitively-impaired mild TBI victims who underwent neuroimaging studies between eight months and three years after the trauma event and compared these patients with a healthy cohort of 10 individuals. Voxel-based analysis showed multiple areas of lower FA and high mean diffusivity in the white matter bilaterally, especially in the corpus callosum, subcortical white matter and internal capsules[51].

Another study indicated extensive changes in major intra- and interhemispheric white matter tracts in patients with diffuse axonal injury. The authors carried out both ROI analysis and voxel-based analysis in nine chronic TBI patients

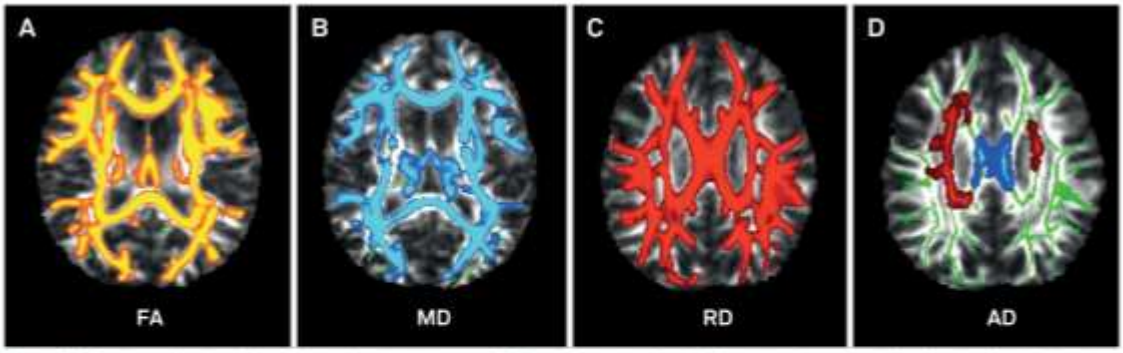

Figure 8. Twenty patients with acute moderate-to-severe TBI were compared with 20 age- and sex-matched controls with tract-based spatial statistics. The patients exhibited one large cluster with statistically significant lower FA values (A). The TBI patients also demonstrated significant increases in mean diffusivity (B) and radial diffusivity (C) in extensive areas of the brain, as well as increased acute diffusivity in a less extensive area (shown in red), except for the corpus callosum, which showed increased acute diffusivity (depicted in blue) (D). All comparisons were statistically significant.

(approximately four years after the event) and in 11 healthy individuals. The results indicated significantly lower FA values in the corpus callosum, internal and external capsules, superior and inferior longitudinal fascicles and in the fornix in the TBI group. Furthermore, ADC values were increased not only where the FA values were lower but also in otherwise-normal regions, possibly indicating that ADC may be an even more sensitive measurement than FA for detecting widespread white matter damage[10].

CONCLUSION

Traumatic brain injury remains a major public health problem worldwide. Computed tomography and MRI have played a crucial role in the acute setting, but several challenges arise when applying neuroimaging methods to predict clinical outcomes in patients with a broad range of injury severity, especially in individuals who develop persistent symptoms despite minor findings on standard imaging.

Diffuse tensor imaging takes advantage of the intrinsic property of anisotropic diffusion of water molecules in brain tissues to probe tissue integrity and organization. Although increasing publications have reported applying this technique in various clinical and research scenarios, DTI principles and its various methodological approaches remain unfamiliar to some allied-health professionals. We reviewed some advantages and shortcomings of the most commonly applied analytical methods (ROI, tractography and voxelwise analyses) along with their applications in the investigation of TBI.

Particularly in patients with diffuse axonal injury, natural barriers to free water diffusion, such as the cytoskeleton, axons and myelin sheaths, may be damaged, leading to the ubiquitous finding of reduced FA values in distinct brain areas. Increased radial diffusivity and increased mean diffusivity usually accompany these FA changes. It seems that abnormalities in DTI metrics may correlate with the timing of head injury, severity biomarkers and long-term prognosis. In the future, DWI and DTI may also aid in the selection of TBI patients for targeted therapies and in monitoring the effectiveness of treatments.

Upcoming investigations should attempt to select more homogeneous groups of patients and clearly state inclusion and exclusion criteria. Longitudinal studies with a combined quantification of DTI metrics, instead of transversal studies with putative evaluation of isolated indices, should enhance comprehension of TBI pathophysiology. These studies might foster the goal of alleviating the burden associated with TBI.

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APPENDIX B – Publication 2

https://doi.org/10.1590/0004-282X20180110

LETTER

Reply

Resposta

Daphine Centola Grassi[1], Grasiela Rocha Barros da Silva[1], Celi Santos Andrade[1]

Dear Editor,

We appreciate the effort of Santos and colleagues for writing the letter entitled "Diffuse axonal injury: diffusion tensor imaging and cognitive outcome"[1], about the published article by Grassi et al.[2]. We thank them for all their comments on our paper and we acknowledge the opportunity to reply to their considerations.

Traumatic brain injury remains a major public health concern, directly affecting millions of otherwise healthy individuals, as well as, indirectly, their household members, who usually have to deal with long-term sequelae, including psychiatric symptoms and cognitive deficits. During the last years, advanced magnetic resonance (MR) techniques have played an important role in detecting abnormalities that were once under-recognized when using conventional MR technology. In particular, diffusion tensor imaging represents an important advanced MR tool in the context of traumatic brain injury and diffuse axonal injury[3]. There are already extensive compendiums concerning the physics of diffusion tensor imaging, however, instead, in our work we aimed to briefly review its basic principles and main analytical methods (region-of-interest, tractography and voxelwise analyses), along with the main relevant findings in the context of traumatic brain injury and diffuse axonal injury[2].

Taking into account the advantages of diffusion tensor imaging in the noninvasive exploration of brain microstructure and networks, one should not be surprised by the striking number of recent publications using this technique in the evaluation of patients at different stages after a traumatic episode, ranging from mild to moderate and severe injuries[2,3]. However, there is still an urge to associate diffusion tensor imaging findings with clinical aspects and to correlate the scores with cognitive outcomes, making it valuable and accessible as a prognostic tool in a daily clinical practice.

Fortunately, new scientific studies are evolving steadily and, soon after our recently-published paper[2], new evidences have strengthened the relationship between diffusion tensor imaging abnormalities and diffuse axonal injury outcomes. As pointed out by Santos et al., the work conducted by Hellstrøm and colleagues[4] indicated robust associations between self-reported cognitive, somatic and emotional symptoms, 12 months after mild traumatic brain injury with white matter diffusion tensor imaging parameters, extracted with a voxelwise analysis, dubbed as tract-based spatial statistics. This work also reinforced physiologic effects of aging on brain white matter structures, leaving the older brain more vulnerable to subtle injury-related processes[4]. This also emphasizes the need to control age as a potential confounding variable in case-control diffusion tensor imaging studies.

A work by Leon et al.[5] assessed 217 victims with moderate to severe traumatic brain injury 19 days after the traumatic episode. Twenty-eight white matter fiber bundles were chosen because of their susceptibility to trauma and were evaluated by region-of-interest analysis. Diffusion tensor imaging metrics were highly associated with unfavorable clinical outcomes after six months to one year after the trauma.

Furthermore, a recent meta-analysis of 20 studies investigated correlations between diffusion tensor imaging measures and seven cognitive domains in mild to severe traumatic brain injury victims. All studies pointed to a concordance between diffusion tensor imaging parameters and cognition: increased fractional anisotropy values were associated with higher cognitive performance, especially regarding memory and attention functions[6].

It is expected that diffusion tensor imaging evaluation will potentially have clinical application in head injury survivors in the near future. Nevertheless, most findings heretofore were based on single works and hence upcoming studies are awaited to highlight the prognostic value of diffusion tensor imaging. There is still much work to be done. Larger scale, longitudinal analyses with homogeneous traumatic brain injury groups may play a decisive role in how this technique will prove helpful in predicting a patient's prognosis and also aiding in selection of patients who might benefit from targeted therapies.

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Conflict of interest: There is no conflict of interest to declare.

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APPENDIX C – Publication 3

BRAIN INJURY
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Dynamic changes in white matter following traumatic brain injury and how diffuse axonal injury relates to cognitive domain

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ABSTRACT

Objective: The goal is to evaluate longitudinally with diffusion tensor imaging (DTI) the integrity of cerebral white matter in patients with moderate and severe DAI and to correlate the DTI findings with cognitive deficits.

Methods: Patients with DAI (n = 20) were scanned at three timepoints (2, 6 and 12 months) after trauma. A healthy control group (n = 20) was evaluated once with the same high-field MRI scanner. The corpus callosum (CC) and the bilateral superior longitudinal fascicles (SLFs) were assessed by deterministic tractography with ExploreDTI. A neuropsychological evaluation was also performed.

Results: The CC and both SLFs demonstrated various microstructural abnormalities in between-groups comparisons. All DTI parameters demonstrated changes across time in the body of the CC, while FA (fractional anisotropy) increases were seen on both SLFs. In the splenium of the CC, progressive changes in the mean diffusivity (MD) and axial diffusivity (AD) were also observed. There was an improvement in attention and memory along time. Remarkably, DTI parameters demonstrated several correlations with the cognitive domains.

Conclusions: Our findings suggest that microstructural changes in the white matter are dynamic and may be detectable by DTI throughout the first year after trauma. Likewise, patients also demonstrated improvement in some cognitive skills.

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Introduction

Traumatic brain injury (TBI) is a complex public health issue worldwide because of its high prevalence, morbidity, and mortality (1,2). In the last decades, studies have demonstrated the vulnerability of cerebral parenchyma in the trauma scenario and also the social and economic burden associated with cognitive and psychological impairments in survivors (3,4).

Diffuse axonal injury (DAI) plays an essential role in TBI since it is present in almost half of the patients who need hospitalization and because it is related to brain dysfunctions (5–8). The widespread axonal injury leads to a loss of the brain connectiveness, causing cognitive, motor, and sensory deficits (9–11). Also, DAI is associated with the development of the chronic neurodegenerative traumatic disorder (12,13).

There are different and complex mechanisms involved in the pathophysiology of DAI. Histopathological studies have shown primary and secondary axonal lesions, inflammatory and regeneration processes accompanied by Wallerian degeneration and neuroplasticity that may be the related with the clinical and cognitive outcomes in patients with TBI (7,14). Computed tomography (CT) and conventional magnetic resonance imaging (MRI) are relatively insensitive for these microstructural changes. Nevertheless, diffusion tensor imaging (DTI) is an advanced MRI technique that is able to probe microstructural integrity by exploring the diffusion of water molecules in brain tissues (15–17). Therefore, we hypothesized that these abnormalities would be detectable by DTI metrics along the first year after trauma.

In the last decades, DTI has demonstrated its capability to study brain architecture, geometry and microstructure, and it has been used in the evaluation of several neurological conditions, including brain trauma (18,19). Among different DTI methods of analysis, tractography has been commonly used to parcellate and to assess the white matter tracts in patients with TBI, ranging from mild to severe trauma, and even in those without associated findings in conventional MRI (20–23).

Fibre tracts connect different regions of the brain in order to modulate neuronal impulses. The greatest white matter bundle in the human brain is the corpus callosum (CC), responsible to link homologous regions of both cerebral hemispheres, and also involved in motor, psychological and cognitive activities (24). The genu, body and splenium are the main CC

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Supplemental data for this article can be accessed on the publisher's website.

subdivisions connecting the orbitofrontal regions, the fronto-parietal lobes and the occipital cortices, respectively. Another critical tract involved in different cognitive processes such as language, memory, emotions and attention is the superior longitudinal fasciculus (SLF), which connects Broca´s area (frontal lobe), Geshwind´s area (parietal lobe) and Wernicke´s territory (temporal lobe) in the same brain hemisphere (25). Several studies have demonstrated the vulnerability of these brain tracts in the context of patients with traumatic brain injury (20,22,26,27). However, longitudinal studies assessing the dynamic changes of white matter in DAI are scant in the literature.

This study aims to longitudinally evaluate with DTI the integrity of the CC and the SLFs in patients with moderate and severe DAI at three moments during the first year after the traumatic event, and also in comparison with a matched healthy control group. In addition, correlations between DTI quantitative parameters and neuropsychological data will also be scrutinized.

Materials and methods

Patients

Selection criteria

This study was approved by the Institutional Review Board, and all individuals agreed to be in the study and signed the informed consent form. Patients included in this study were adult outpatients (ages between 18 and 55 years old) admitted to the Emergency Room of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil, due to moderate and severe head trauma according to Glasgow Coma Scale (GCS) scores of 3–12 at initial evaluation, and who met the following criteria: (1) clinical and tomographic diagnosis of DAI, (2) a Marshall score (28) of I, II or III based on CT evaluation, (3) had no focal lesions greater than 10 cm^3, (4) had no midline shift greater than 0.5 cm, (5) had no epidural hematomas that determined compression of the brain parenchyma, (6) had no previous head injury history with hospitalization.

Description of patient's sample

Initially, 225 patients with moderate and severe head trauma were admitted in Emergency Room, but 186 were excluded due to the presence of intra- or extra-axial hematomas or cerebral contusions larger than 10 mm at initial computed tomography examination. Of the 39 remaining individuals, 19 were excluded based on the following reasons: seven losses of follow-up; five individuals had exclusion safety criteria for MRI examination; five DTI artefacts; one patient deceased; and one patient developed epidural compressive hematoma.

For the 20 patients who met the selection criteria, demographic characteristics were as follows: 17 men and 3 women; mean age was 29.6 years (SD±6.8), 14 patients presented with moderate head trauma (GCS of 9–12), and 6 patients presented with severe trauma (GCS of 3–8). The majority of patients suffered motorcycle accidents (n = 10), while the others suffered car accidents (n = 6), running over (n = 3), and physical aggression (n = 1).

Brain imaging

Magnetic resonance imaging

Each patient had a 3.0 Tesla MRI of the brain performed on the same scanner (Intera Achieva, Philips Medical System, Best, The Netherlands) with an eight-channel head coil (Philips Medical System) at three timepoints: (1) 2 months after the trauma, (2) 6 months after the trauma and (3) 12 months after the trauma. A healthy age- and sex-matched control group of 20 individuals was also scanned once.

Anatomical imaging protocol was acquired in the sagittal plane with a 3D T1-weighted Fast Field Echo (3DT1-FFE) sequence covering the entire brain (180 slices), and the following parameters: inversion time (IT) = 700 ms; TR/TE = 6.2 ms/2.7 ms; flip angle = 8°; acquisition matrix = 240 x 240; field of view (FOV) = 240 x 240 × 180 mm; voxel resolution = 1 mm^3 (isotropic); slice thickness = 1.0 mm; completion time = 4 minutes. The susceptibility weighted image protocol consisted of principles of Echo Shifting with a Train of Observations (PRESTO) 3D-T1FFE sequence, axially acquired (a total of 230 slices – 1 mm thick), according to these specifications: TR/TE = 22/29 ms; flip angle = 10°; FOV = 220 x 182 mm; matrix = 224 x 224; voxel size = 0.98 x 0.98 × 1.0 mm; completion time = 3 minutes.

DTI data acquisition

DTI images were collected in the axial plane with gradients applied in 32 non-collinear directions. The entire brain was covered within 70 slices, 2 mm-thick each, with no gaps in between. One image with no diffusion weighting was obtained (b = 0 s/mm^2). Other parameters used were: TR/TE = 8500/61 ms; b value = 1000 s/mm2; matrix = 128 x 128; FOV ("field-of-view") = 256 x 256 mm; $2mm^3$ isotropic voxel; NEX = 1; completion time = 7 minutes.

Pre-processing

All data were pre-processed using the functional MRI brain (FMRIB) software library (FSL), version 5.0 (available at http://www.fmrib.ox.ac.uk/fsl/), following this sequence: brain extraction tool (BET), FMRIB's linear image registration tool (FLIRT) and correction of eddy current induced distortions (29,30). Motion correction was completed using the free toolbox ExploreDTI (A. Leemans, University Medical Center, Utretch, The Netherlands), by rotating the B-matrix in order to keep the orientation input accurate. Investigation for residuals and outliers of the diffusion tensor fit was done with the same software, ending on residual maps similar on all groups. Moreover, the same software was used for tensor calculation and fibre tracking (31,32).

Tractography

A whole-brain tractography was first automatically obtained in the native space using a brute-force approach of every pixel. Deterministic tractography technique was then achieved following a predesigned combination of specific procedures, which included positioning of multiple regions-of-interest (ROIs) on different planes, based on prior anatomical knowledge and previous studies (33,34). The FACT (fibre assignment by continuous tracking) algorithm was calculated with

a fractional anisotropy threshold of 0.25 and maximum angles of 30° for the CC and 60° for the SLF, equally applied to all subjects (35).

The CC was segmented in three parts: genu, body and splenium. According to the Hofer and Frahm's representation (36), the genu was defined as the one-sixth part of the anterior CC, while the splenium the last one-fourth and the body the residual part. The CC was virtually dissected following these steps: first, "SEED" ROIs were marked in the paramedian plane along the CC. To securely track fibres from left and right hemispheres, "AND" ROIs were traced in the midsagittal portion, and, finally, "NOT" ROIs were drawn in the axial and coronal planes in order to eliminate horizontally and vertically oriented fibres (e.g. cingulum and corticospinal tracts, respectively). To dichotomize the SLF fibres, two "SEED" ROIs were delineated in the coronal plane, and two "NOT" ROIS were used to the axial plane to exclude any tracks that had a vertical orientation (toward the corona radiata or the corticospinal fibres) (Figure 1).

The following average quantitative DTI parameters were extracted from each track: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD).

Neuropsychological tests

An experienced neuropsychologist (ALZ) performed specific tests to assess different cognitive domains. The patients were submitted to neuropsychological (NP) assessment only at timepoints 2 and 3 because of comprehension difficulties, mental confusion and agitation typically seen in the early post-trauma stage. The results of each test were converted into a Z-score according to age and years of education. On timepoint 2, three patients were not able to complete the NP tests. On timepoint 3, one patient had missing information concerning the IQ estimation.

The Hopkins verbal learning test (HVLT) evaluates the episodic verbal memory. It consists of immediate, late recall and later recognition of a list containing 12 words (37). The examiner reads the list and the patient is asked to repeat as many words as possible. This procedure is repeated two more times and then, 25 minutes after that, the participant is required to remember the words previously learned (later recognition).

The Victoria Stroop test assesses selective attention and inhibitory control. It consists of three cards. The first card has coloured dots, the second has random coloured nouns words, and the third has the name of the colours with mismatched ink's colour (38). The patient has to say aloud the colour of the ink of the dots, nouns, and coloured names in each card as fast as possible.

Phonologic fluency was assessed by the FAS test – where the patient is asked to say as many words as possible beginning with each letter F, A and S, for 1 min each letter. For the semantic verbal fluency assessment – using animals as a category, the patient has to say as many different animals as possible in one-minute interval (38).

Digit Span (DS) forward and backward (Wechsler Memory Scale 3rd edition, WAIS – III) evaluates the working memory (39). A digit sequence is presented at one digit per second rate. The sequence starts with a two span and increases as the participant repeats it correctly. After the DS forward, the

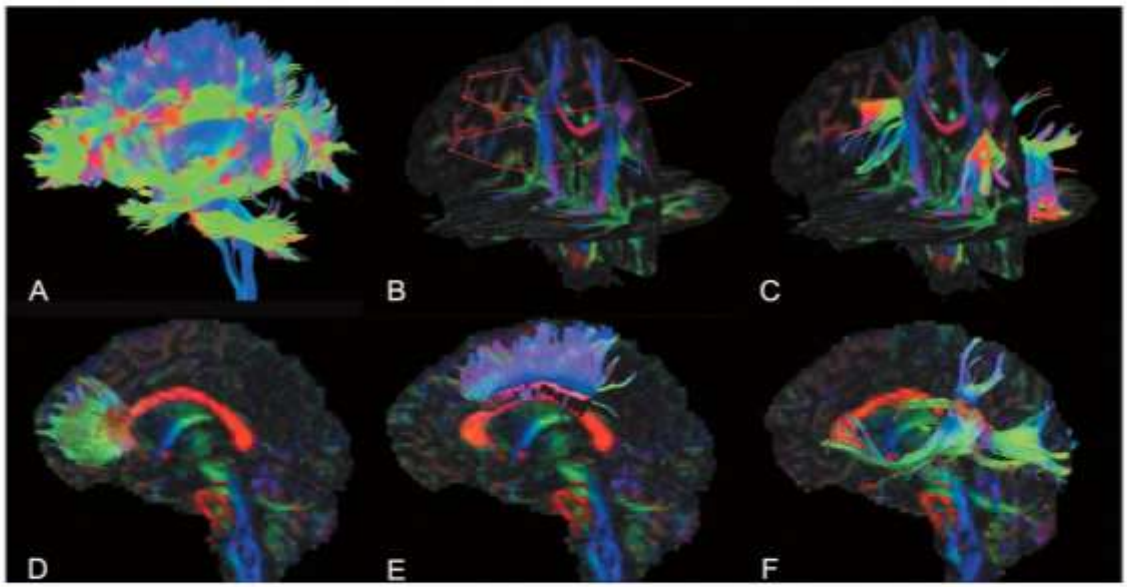

Figure 1. DTI-based tractography post-processing with ExploreDTI. (a), whole-brain tractography is first obtained with a brute-force approach. (b), SEED (in blue) and NOT (in red) ROIs placed in multiple planes of FA maps to virtually dissect the bilateral SLFs. (c). Three-dimensional representation of both SLFs on oblique view. (d, e and f) show the final results of segmentation of the CC in the lateral plane: genu, body, and splenium, respectively.

same procedure is repeated, but the digits are repeated in the backward sequence.

IQ estimation was calculated by combining the performance on both vocabulary and matrix reasoning tests present in the WAIS – III (39,40). The vocabulary test consists of the presentation of words and the patient is asked to define them. In the matrix reasoning test, a matrix of abstract pictures in which there is one picture missing is presented, and the patient has to choose one option that better suits the missing picture.

Statistical analyses

Statistical analysis was performed using IBM – SPSS statistics for Windows, version 25 (International Business Machines Statistical Package for the Social Sciences Inc., Chicago, IL, USA). A professional statistical expert was consulted for all analyses.

Initially, the data were inspected for outliers and distributional characteristics. There were no considerable asymmetries in all DTI quantitative samples or NP tests results.

Comparisons between the patients and the control group were performed with Student´s t-test. A generalized linear function test with robust standard error and unstructured correlation matrix was performed to evaluate changes over time of DTI parameters and neuropsychological tests. After that, the Benjamin-Hochberg procedure for repeated measures was performed. To calculate correlation coefficients, Pearson and Spearman's tests were used. Results were significant with p-value <0.05.

Results

Comparisons of the DTI parameters between patients and healthy controls

The DTI parameters (FA, MD, AD and RD) extracted from the CC and both SLFs in the control group and in the patients' group at all three timepoints can be seen in the supplementary Table S1.

In order to examine the early microstructural abnormalities after moderate and severe TBI, we compared the patients' group at timepoint 1 with the healthy controls (Figure 2). We found significant differences in various DTI parameters at all segments of the CC with lower FA values and higher MD and RD values in the patients' group ($p < .001$). There were no significant differences in AD in any CC segment when comparing both groups. For both SLFs, we found similar results as those found in the corpus callosum, except for AD in the right SLF, which also demonstrated significantly higher values in the patient's group ($p = .04$).

Changes in the DTI parameters along time

Table 1 summarizes the results from the generalized estimating equation (GEE) for the comparison of all quantitative DTI metrics in the patients group considering the three timepoints.

In the body of the CC, FA demonstrated a decrease between timepoints 1 and 2, and then a significant increase at timepoint 3 ($p = .02$). For both MD and AD, we also observed the same pattern of increasing values along time in the body ($p = .003$, p

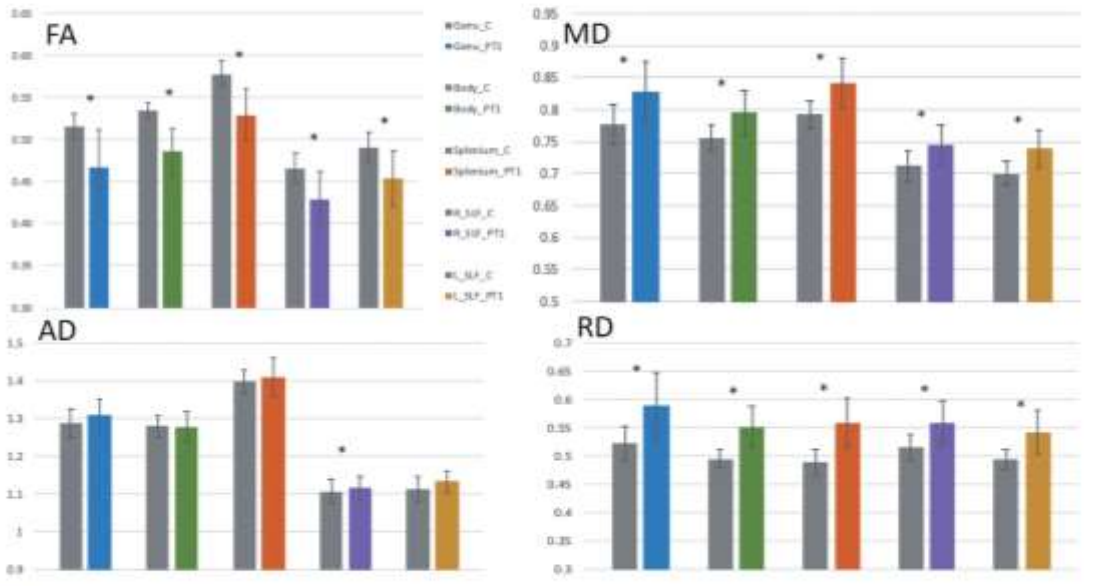

Figure 2. Column bar graphics exhibit the mean values with 95% confidence intervals (error bars) of DTI metrics (FA, MD, AD, RD) for the patients group at timepoint 1 (PT1) in each CC segment (genu in blue, body in green, splenium in orange) and both SLFs (right SLF in purple, left SLF in yellow). The corresponding parameters in the control group are shown in grey colour. MD, AD and RD are expressed in x 10^{-3} mm^2/s. Statistically significant differences ($p < .05$) obtained with Student´s t-test are indicated in the graphics with asterisks (*).

Table 1. Results obtained with generalized estimation equation to evaluate of DTI parameters in the patients group (n = 20) along timepoints 1, 2 and 3.

Tract	DTI metric	*p*-value
Genu	FA	.835
	MD	.063
	AD	.061
	RD	.181
Body	FA	*.020*
	MD	*.003*
	AD	*.025*
	RD	*.016*
Splenium	FA	.992
	MD	*<.001*
	AD	*<.001*
	RD	.070
Right SFL	FA	*.003*
	MD	.764
	AD	.575
	RD	*.035*
Left SLF	FA	*.035*
	MD	.231
	AD	.088
	RD	.180

Significant *p*-values are shown in italics.

Table 2. Z-scores computed for each cognitive domain and the statistical results after generalized estimated equation using time as a model of effect.

Cognitive domain	PT2	PT3	*p*-value
Memory	−2.478 ± 0.171	−1.980 ± 0.205	*.004*
Attention	−1.941 ± 0.249	−1.113 ± 0.251	*.001*
Verbal fluency	−1.348 ± 0.190	−1.239 ± 0.132	.473
Working memory	−0.424 ± 0.158	−0.425 ± 0.155	.993
IQ	−1.006 ± 0.141	−0.885 ± 0.144	.101

The results are shown in mean values ± standard deviations. PT2 and PT3 accounts for the patients groups at timepoints 2 and 3, respectively. Significant *p*-values are shown in italics.

Neuropsychological evaluation

Neuropsychological tests indicated deficits in all cognitive domains in the patients group as indicated by negative Z-scores at both timepoints 2 and 3. Along time, however, patients presented improvement of the performances on memory ($p = .004$) and attention ($p = .001$). Other domains such as verbal fluency, working memory and IQ estimation did not demonstrate significant changes throughout time (Table 2).

= .025), and splenium ($p < .001$, $p < .001$), respectively. For RD, we found increasing values in the body of the CC ($p = .016$). There were no other significant changes in the DTI parameters in the genu of the CC (Figure 3).

We also found significant FA increases along time in both right and left SLFs ($p = .003$, $p = .035$, respectively), accompanied by a significant decrease for RD values in the right SLF ($p = .035$). No other significant changes over time for the other DTI parameters were observed in the SLFs (Figure 3).

Correlations between DTI parameters and neuropsychological tests

There were several significant correlations between DTI parameters and the results of the neuropsychological tests at both timepoints 2 and 3. These results are summarized in the supplementary Table S2.

At timepoint 2, we found positive correlations of FA values in the genu of the corpus callosum with attention (R = 0.508, *p*

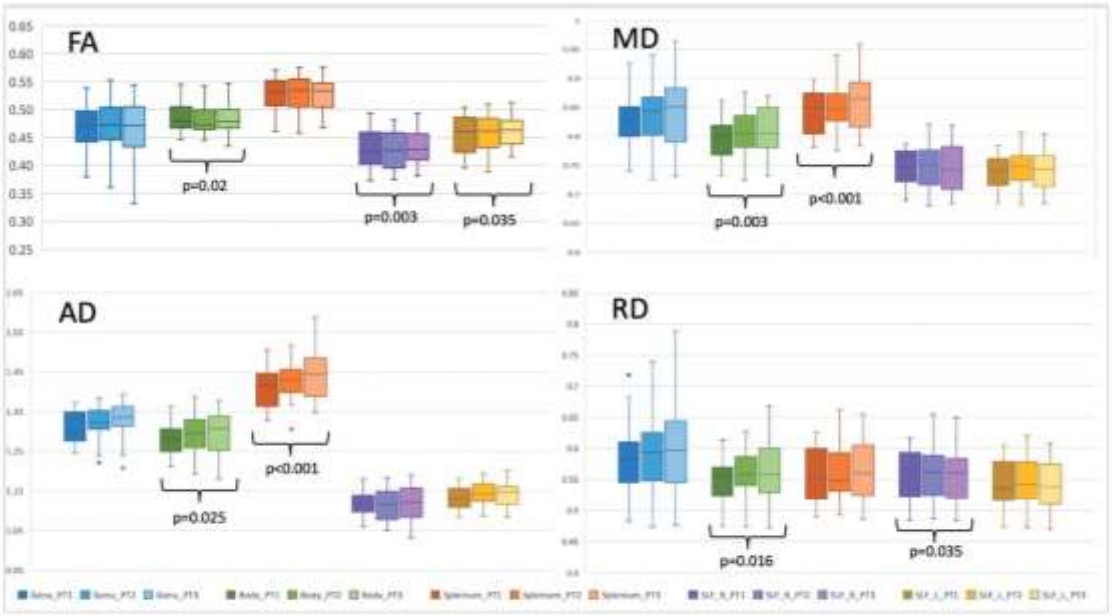

Figure 3. Box-plot graphics exhibit comparisons of DTI metrics (FA, MD, AD, RD) along time for the patients group in each CC segment and in both SLFs (genu in blue shades, body in green shades, splenium in orange shades, right SLF in purple shades, and left SLF in yellow shades). MD, AD and RD are expressed in x 10^{-3} mm^2/s. Statistically significant differences along time found with generalized linear function tests are indicated in the graphics with the corresponding *p*-values.

= .031), and in the splenium with attention (R = 0.496, p = .036) and working memory (R = 0.645, p = .003). There were also positive correlations of FA values in the right (R = 0.489, p = .039) and left (R = 0.6, p = .009) SLFs with IQ. In parallel, MD values in the genu correlated negatively with working memory (R = −0.456, p = .05), as well as in the splenium with attention (R = −0.594, p = .009), verbal fluency (R = −0.529 = p = .020), working memory (R = −0.494, p = .032) and IQ (R = −0.53, p = .024). MD values also correlated negatively with verbal fluency on left SLF (R = −0.481, p = .037). Moreover, RD values in the splenium were correlated negatively with attention (R = −0.549, p = .018), verbal fluency (R = −0.465, p = .045), working memory (R = −0.511, p = .032) and IQ (R = −0.597, p = .009); and in the left SLF with IQ (R = −0.479, p = .044). There was no evidence of correlations between AD at any regions studied and the neuropsychological results at timepoint 2.

At timepoint 3, FA values at the genu also demonstrated a positive correlation with the attention index (R = 0.514, p = .02). Correspondingly, RD values in the same site showed a negative correlation with the same cognitive domain (R = −0.445. p = .049). AD values in the left SLF showed a negative correlation with attention index (R = −0.620, p = .004). There were no other significant correlations between DTI metrics and the cognitive domains at timepoint 3.

Discussion

Our study demonstrates extensive diffusion abnormalities in the white matter, characterized by lower FA and higher MD, in all the evaluated segments in patients with DAI in comparison to healthy controls. This is coherent to the widespread feature of DAI following moderate and severe trauma and is in line with several previous works conducted in animal models and in humans (9,18–20,41–50).

FA is the most commonly used parameter in DTI studies to assess integrity and geometry of axonal fibres. High FA values (close to one) are observed in brain regions containing well-organized parallel axon arrays. On the other hand, brain regions with no internal directional organization are associated with low FA (close to zero). In contrast to FA, MD represents the overall water diffusion, regardless its direction, and is affected by both radial and axial diffusivities (51,52). It has been largely discussed which processes underlie the changes in RD and AD and how they should be interpreted (51–55). Based on mathematical models, observations of in vitro and in vivo experiments (51–53) and also studies with animal models (54,55), we think the more pronounced increments in RD found in our study are possibly associated with demyelination and neuroinflammation, particularly the water accumulation within the myelin sheath (intramyelinic oedema). There were also higher AD values in the patients' group, but this difference was significant only in the right SLF, which most likely reflects abnormalities in cell density and increase in extracellular space. Kinnunen et al. also found the same combination of abnormalities in DTI-derived scalar metrics using a voxelwise approach in patients with TBI in several brain regions, including the CC and SLFs (9). Our work reinforces the usefulness of both FA and MD as sensitive DTI biomarkers of microstructural damage in patients with DAI, even in otherwise normal-appearing parenchyma on conventional MRI.

The longitudinal evaluation of DTI parameters demonstrated that the diffusion abnormalities are not stationary, but also change into some extent along time after trauma. This was particularly evident in the body of the CC, that showed an initial decrease in FA values followed by an increase in the second phase post-injury, accompanied by an increase in MD, AD and RD in the overall study interval. There were also progressive increments in MD and AD values in the splenium. In the SLFs, there were progressive increases in FA mean values, accompanied by significant changes in RD in the right side. Indeed, several works demonstrated that, in addition to the primary axonotmesis directly caused by rotational forces at the moment of the impact, other pathological processes ensue afterwards. There is evidence of a late secondary pro-inflammatory response associated with deposition of myelin debris, overexpression of cytokines, synaptic dysfunction, activation of glial cells, and also deposition of anomalous proteins, such as Tau and beta-amyloid (56–59). On the other hand, in a search for homeostasis and regeneration, a continuous process of debris clearance and anti-inflammatory response is also triggered, with reparative mechanisms that contribute to a neurological recovery (60,61).

One study using a mouse model showed early isolated axonal injury, followed by demyelination, oedema, and persistent axonal damage up to one month after the experiment, which were accompanied by progressive changes in scalar indices, suggesting that DTI may indicate approximate timing of injury (43). Tissue reorganization have been detected to start as early as days after trauma and within 4 weeks, along with an increase in fibre density in affected regions in a rodent model (62). Other investigations conducted in humans that analyzed patients with TBI from mild to severe trauma also demonstrated FA changes from early phases up to several years after trauma (42,45,48). In addition to the continuing process of debris removal and neuronal regeneration, another reasonable explanation for the progressive increment in FA may be related to the vascular injury associated with DAI that causes local bleeding with haemoglobin degradation and iron deposition, which may also determine dynamic changes in FA values (63,64). Noninvasive methods such as DTI might be helpful to foster the understanding of the underlying complex pathophysiologic abnormalities and the microstructural anatomical substrates of the commonly observed cognitive deficits in patients with TBI.

Following recovery from transient loss of consciousness and partial or complete recuperation of acute neurological deficits caused by head trauma, survivors of DAI may present persistent disabilities, loss of productivity and impaired quality of life (1,2). Cognitive impairments depend on multiple variables, such as the trauma severity, rehabilitation and even genetic factors (65–67). A neuropsychological assessment indicated compromise of all cognitive functions in the patients' group in our study up to one year after trauma, but there was significant improvement of episodic verbal memory and attention domains along time. This is in agreement with a meta-analysis review of 39 cross-sectional TBI studies, which indicated that cognitive functions improve after

moderate to severe TBI but remains markedly impaired up to two years post-injury (67).

Moreover, we found significant correlations between DTI metrics and cognitive performances. There were positive correlations between FA values in the genu of the CC with attention at both evaluated phases, as well as negative correlation between MD values and working memory at timepoint 2. The mechanics of head trauma places the ventral and lateral surfaces of the frontal lobes in particular vulnerability for damage (68,69). Given the frontal projections of the genu, it is not surprising that executive functions mediated by these areas could be correlated with microstructural abnormalities as detected by DTI in our study. The splenium is also frequently injured in head trauma due to specific anatomical features such as its close proximity to the fixed falx that determines how the shearing forces propagates in this region. There were significant correlations between DTI indices extracted from the splenium and several cognitive domains, including attention, verbal fluency, working memory and IQ at six months post-trauma. Our results also indicated positive correlations between FA values in both SLFs and IQ at the same timepoint, in addition to negative correlations between the other DTI metrics and verbal fluency and IQ. The correlations were more pronounced in the left SLF, what may be related to the by far more prevalent functional language dominance in the left cerebral hemisphere (70). Furthermore, there were more pronounced correlations in our study at six-moths post-injury, suggesting this interval as the optimal timing of DTI data acquisition for evaluation of cognitive outcomes.

Other authors have also found correlations between DTI parameters and neurocognition. Hashim et al. evaluated 19 subacute (up to one year post-trauma) and chronic (from one up to five years post-trauma) patients with a voxelwise approach and found persistent functional loss in chronic TBI, and also correlations between diffusion indices with memory and visuomotor coordination test scores, but not with executive function (71). Another group conducted a longitudinal study with region-of-interest-based analysis at specific brain sites and demonstrated significant correlations with clinical outcomes up to 15 months after severe trauma (72). There is also evidence of associations between DTI indices and self-reported cognitive and emotional symptoms at 12 months post-injury in mild TBI (4). This study also pointed strongest effects in frontal regions including the forceps minor and the genu of the CC (4).

Lack of correlations between some DTI metrics and cognitive domains at specific sites in our study, especially in the body of the CC, may be related to the relatively low number of participants that are ideally required for correlational studies (73). Indeed, we have applied strict exclusion criteria in order to evaluate a very homogeneous group of patients with moderate and severe TBI with a pure presentation of DAI rather than evaluating patients with a broader spectrum of traumatic injuries, such as large intra-axial and extra-axial hematomas. Furthermore, another possible explanation is that distant rewiring and behavioural compensation may mediate spontaneous improvement of cognitive deficits after TBI. Although these mechanisms are not completely understood and do not represent true pathological recovery, it is supposed that second behaviour in intact circuits overtake the original cognitive function with associated shifts in anatomo-functional maps topography (74,75).

There are several methodological approaches to analyze DTI datasets. Region-of-interest analysis allows straightforward extraction of diffusion parameters from a predetermined area of the brain, but only a limited part of the cerebral structure is evaluated in two-dimensional images. Voxelwise analysis is broadly applied on research scenarios because it is suitable for global analyses of brain parenchyma and allows semi-automated comparison of large groups of patients. Some shortcomings of this approach, however, are the need for alignment and registration of brain volumes to a standard space, with its associated inaccuracies (15,18). Herein, rather than applying an exploratory evaluation prone to multiple error biases, we chose to evaluate with tractography the greatest inter-hemispheric commissure bundle and one long association tract that are known to link critical cortical regions and to modulate several cognitive functions.

Still, some caveats of the deterministic streamline tractography approach should be mentioned. This technique indirectly estimates fibre tract anatomy based on the main direction of water molecules diffusion in each voxel (in the order of millimetres), by far much bigger than the axonal diameter (in the order of microns). This assumption of homogeneous unidirectional vectors is unrealistic and gives erroneous estimations of fibre pathways in areas of crossing fibres. Furthermore, longer acquisition times and motion artefacts limit increases in spatial resolution. Other robust diffusion analysis techniques that soothe some of these limitations are evolving steadfastly, such as global probabilistic tractography, high angular resolution diffusion imaging (HARDI), q-ball imaging and diffusion kurtosis analysis (DKI) (15,18,75–77,78). So far, however, these approaches require more sophisticated processing algorithms and are less feasible for implementation in clinical settings to evaluate individual patients with TBI.

Finally, this study emphasizes the utility of DTI to obtain quantitative information about the white matter microstructure in patients with moderate and severe brain injury. Our results showed extensive and dynamic changes in DTI parameters throughout the first year after trauma. In parallel, patients also demonstrated better performance scores in different neuropsychological domains over time, which could be correlated with DTI metrics at particular brain sites, indicating the potential role of microstructural reorganization and neuroplasticity. DTI is a noninvasive method that could be helpful in monitoring progression of DAI and to select cognitively compromised patients for targeted therapies in the future.

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Declaration of interest

The authors have no conflicts of interest to declare.

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APPENDIX D – Publication 4

Arquivos de Neuro-Psiquiatria

ARQUIVOS DE
NEURO-PSIQUIATRIA

Longitudinal whole-brain analysis of multi-subject diffusion data in diffuse axonal injury

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Keyword:	Craniocerebral Trauma, Diffuse Axonal Injury, Diffusion Tensor Imaging, Glasgow Outcome Scale, Regeneration

Longitudinal whole-brain analysis of multi-subject diffusion data in diffuse axonal injury

Análise longitudinal do encéfalo por tensor de difusão em lesão axonial difusa

Short title: Longitudinal voxelwise analysis of DTI in DAI

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Indication of authors' contributions:

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Contributions in the collection of data: Ana Luiza Zaninotto[3], Fabrício Stewan Feltrin[1,2,5], Fabiola Bezerra de Carvalho Macruz[1,2], Vinicius Monteiro de Paula Guirado[4]

Contributions in the analysis of data: Daphine Centola Grassi[1,2], Celi Santos Andrade[1,2]

Contributions in the writing, critical revision and final approval: Daphine Centola Grassi[1,2], Ana Luiza Zaninotto[3,4], Fabricio Stewan Feltrin[1,2], Fabiola Bezerra de Carvalho Macruz[1,2], Maria Concepción García Otaduy[1,2], Claudia da Costa Leite[1,2], Vinicius Monteiro de Paula Guirado[4], Wellingson Silva Paiva[4], Celi Santos Andrade[1,2]

ABSTRACT

Background: Diffuse axonal injury occurs in high acceleration and deceleration forces in traumatic brain injuries (TBI). This lesion leads to disarrangement of the neural network, which can result in some grade of deficiency. The Extended Glasgow Outcome Scale (GOS-E) is the primary outcome instrument to evaluate TBI victims. Diffusion tensor imaging (DTI) assesses the white matter (WM) microstructure based on the displacement distribution of water molecules.

Objective: Study the WM microstructure along the first year after trauma using DTI, check the patient's clinical outcome, and test for associations.

Methods: We scanned 20 TBI victims of moderate and severe trauma at 2 months and 1 year after the event. Imaging processing was done with the FMRIB software library; we used the tract-based spatial statistics software yielding fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) for statistical analyses. We computed the average difference between the two measures across subjects and performed a one-sample t-test and threshold-free cluster enhancement, p-value < 0.05, corrected. The GOS-E evaluated clinical outcomes. We tested for associations between outcome measures and significant mean FA clusters.

Results: Significant clusters of altered FA were identified anatomically using the JHU WM atlas. We found spotted areas of FA increment along time, in the right brain hemisphere and

the left cerebellum. Extensive regions of increased MD, RD, and AD were observed. Patients presented an excellent overall recuperation. There were no associations between FA and outcome scores, but we cannot exclude a small to moderate association.

Keywords: Craniocerebral Trauma; Diffuse Axonal Injury; Diffusion Tensor Imaging; Glasgow Outcome Scale; Regeneration.

RESUMO

Background: A lesão axonial difusa ocorre em traumas com alta energia de aceleração e desaceleração, determinando desorganização da microestrutura cerebral, levando a algum déficit. A escala de Glasgow Estendida (GOS-E) é indicada na avaliação clínica das vítimas de trauma cranioencefalico. Imagens por tensor de difusão (DTI) estudam a microestrutura cerebral a partir da difusão das moléculas de água.

Objetivo: Analisar a microestrutura cerebral ao longo do primeiro ano após trauma, avaliar clinicamente os pacientes e testar para correlações entre estes resultados.

Métodos: 20 vítimas de TCE moderado e grave foram avaliados 2 meses e 1 ano depois do trauma. O processamento foi feito usando o software FMRIB (FSL) e a análise estatística foi feita com *tract-based spatial statistics software* para extrair os parâmetros de DTI. Calculamos a diferença da média entre as duas observações de cada sujeito e fizemos um teste-t para uma amostra e *threshold-free cluster enhancement*. Realizamos correções para múltiplas comparações e determinamos o valor de $p < 0.05$ como significativo. Um ano após o trauma, a avaliação clínica foi feita usando a GOS-E. Testamos para associações entre os resultados clínicos e os valores médios de FA dos clusters.

Resultados: Os clusters significativos foram identificados usando o atlas JHU WM. Observamos aumento de FA predominantemente no hemisfério cerebral direito e cerebelar à esquerda e também extensas áreas de aumento nos demais parâmetros de DTI. A recuperação dos pacientes foi satisfatória. Não encontramos associações entre os resultados, no entanto alguma associação pequena a moderada não pode ser excluída.

Palavras-chave: Traumatismo Cranioencefálico; Lesão Axonial Difusa; Imagem por Tensor de Difusão; Escala de Coma de Glasgow; Regeneração.

INTRODUCTION

Traumatic brain injury (TBI) causes different complex brain lesions such as hematomas, contusions, vascular injuries, and diffuse axonal injury (DAI). DAI results from high-energy acceleration and deceleration forces, determining shearing strains in the white matter, leading to disconnection or dysfunction of the neural network[1].

Head injuries, particularly DAI, result in distinct functional deficits, such as physical, cognitive, and behavioral impairments, which dramatically affect life quality, return to daily activities, and social reintegration of survivors[2]. In 1975, Jennett and Bond developed the Glasgow Outcome Scale (GOS), and it was used as a primary outcome measure in phase III trials in TBI[3,4]. Afterward, acknowledging some limitations of the GOS, the Glasgow Outcome Scale – Extended (GOS-E) was developed. Since its definition in 1981, it has been primarily used and recommended as the primary outcome measurement in TBI studies[5,6].

DAI is not only restricted to mechanical forces at the moment of the trauma. Many different processes are triggered, such as inflammatory responses, molecular changes, apoptosis, and Wallerian degeneration. Therefore, the pathophysiology of DAI can be divided into primary and secondary lesions. The primary axonal lesion is the complete disconnection related to the kinetic energy in the trauma moment. In contrast, secondary axonal injuries are indirect and progressive lesions to neurons that ensue late after the initial shock[7]. The impact sparks molecular and cellular events that disturb the homeostasis, leading to changes in neurons and the regional microglia that can persist for years[8].

Traditional imaging modalities such as computed tomography and standard magnetic resonance (MR) sequences, such as T1 and T2 weighted sequences, are not sensible to show the white matter (WM) damage related to DAI. Diffusion tensor imaging (DTI) is an advanced MR modality based on water molecules diffusion that measures the preferential displacement along the white matter tracts and has been used to access the brain microstructure in different pathologies, including head injuries[9]. There are diverse methods available to analyze DTI images, such as region-of-interest analysis and tractography. One of the most commonly used is the whole-brain approach to test for group-comparisons, for which tract-based spatial statistics (TBSS) is particularly recommended for voxel wise and cluster-based analyses, constraining statistical analysis to the center of the tracts[9]. It is a semi-automated method, with minimal user dependence, that allows a whole-brain evaluation and is notably suitable for evaluating diffuse lesions in the brain parenchyma such as DAI[10,11].

Other groups have used this approach to assess white matter changes in head injury victims in different stages after trauma[12,13]. Lipton and colleagues conducted a study on patients with mild TBI who presented with persistent cognitive impairment eight months to three years after the trauma. They found decreased fractional anisotropy (FA) and increased mean diffusivity (MD) in the corpus callosum, subcortical white matter, and internal capsules compared to healthy controls[13]. Another group investigated adolescents with mild TBI in the acute phase (from 1 to 6 days after the trauma event) compared to age-matched controls[14]. They found significantly decreased apparent diffusion coefficient (ADC) and radial diffusivity (RD) and increased FA in several white matter regions and the left thalamus, consistent with axonal cytotoxic edema in the acute phase post-injury. However, few published works analyzed the progressive changes in the white matter in DAI, particularly in moderate and severe trauma victims.

This study aims to evaluate longitudinally the white matter of patients with severe and moderate DAI at two moments defined as the subacute (two months) and early chronic phases (one year) following the trauma event. We also assessed patients´ clinical outcome one year after trauma, using the GOS-E scale[6]. Our central hypothesis is that DTI parameters change along time and can have a degree of correlation with functional outcome.

METHODS

Standard protocol approvals

The protocol was reviewed and approved by the institutional review board, the local ethics committee, and all participants gave written informed consent.

Study design and subjects

A prospective study was conducted throughout one year. Adult outpatients admitted at the Emergency Room of Clinics Hospital, Faculdade de Medicina da Universidade de São Paulo, victims of moderate and severe TBI (Glasgow Coma Scale scores between 3 and 12 at initial evaluation), presenting clinical and tomographic findings exclusively of DAI were eligible to be admitted in the study. Exclusion criteria were the presence of contusions greater than 10cm^3, midline shift greater than 0.5 cm, extra-axial collection determining compression of the brain parenchyma, or any indication for surgical intervention. Patients with poor quality

imaging studies that limited analysis, clinical contra-indications that precluded MR scanning, or loss of follow-up were also excluded.

Data acquisition

All data were acquired on a 3T system (Intera Achieva, Philips Healthcare, Best, The Netherlands). Patients were scanned using an 8-channel head proton coil (Philips Healthcare, Best, The Netherlands) at two time-points: two months (subacute phase) and one year (early chronic phase) after the trauma. The routine protocol included fluid-attenuated inversion recovery (FLAIR), diffusion-weighted imaging (DWI), and susceptibility-weighted imaging (SWI) sequences. For the data analysis in this study, we used a volumetric T1-weighted and DTI sequences.

The 3D-T1 fast field echo, acquired in the sagittal plane, was obtained using the following parameters: FOV 240 x 240 x 180 mm^3; matrix 240 x 240mm; isotropic resolution; TR/TE 6,2/2,7ms; and acquisition time 4.13min.

The DTI sequence was acquired in the axial plane, using 32 directions and one b0 using the following parameters: 70 slices; slice thickness 2mm; no gap; field of view 256 x 256 mm; voxel resolution = $2mm^3$ (isotropic); TR/TE 8.500/61ms; b = 1000 s/mm^2; matrix 128 x 128; number of excitations (NEX) = 1; and acquisition time of 7 minutes.

Imaging processing and analysis

Initially, all diffusion images were pre-processed for eddy current corrections and extraction of non-brain voxels, using FMRIB's Diffusion Toolbox (FSL) software, version 5.0.11[9,15]. For motion correction, the free toolbox Explore DTI (A. Leemans, University Medical Center, Utrecht, The Netherlands) was used, which rotates the B-matrix while keeping the exact initial orientation. With this same software, visual quality inspection for residuals and outliers was performed in each data set[16,17].

Thereafter, FA maps were analyzed using TBSS[9]. All individual's FA images were non-linearly registered to the most typical subject of the sample (using -n command), and then the aligned dataset was transformed into the MNI152 standard space ($1mm^3$). The mean aligned FA images were merged into a single four-dimensional (4D) average FA image. A mean FA skeleton was extracted from the generalized 4D image and the tracts were projected into the skeleton, using a 0.2 threshold[18]. To extract mean, axial, and radial diffusivities (MD, AD, and RD, respectively), non-linear warps and skeleton projections were applied to each DTI scalar parameter.

Statistical analysis

To assess differences in FA, MD, RD, and AD along time, we performed one-sample t-tests, using the average difference between the two measures across subjects. Initially, it was first computed the difference between the subacute minus the early chronic phases, and then the early chronic minus the subacute phases. Permutation-based nonparametric inferences were made on unsmoothed statistical maps, using 5000 permutations, and the cluster-like structures were enhanced using the threshold-free cluster enhancement (TFCE) algorithm[19]. This approach was similarly also applied to the MD, AD, and RD maps. Data were corrected for multiple comparisons, using the family-wise error (FWE) rate, setting the significance level at $p < 0.05$.

Thenceforth, the cluster tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Cluster) was applied to extract the exact clusters, followed by the Atlasquery tool to obtain the coordinates (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlasquery) according to the Johns Hopkins University (JHU) white matter tractography atlas.

Outcome measure

We used the GOS-E at 12 months post-injury obtained at the medical appointment follow-up, which has been recommended as the main outcome measurement in TBI studies[7]. It consists in an eight-scale global measure of function, used to estimate physical disability grading[6]. It classifies patients into upper and lower levels of good recovery (GOS-E = 7,8), moderate disability (GOS-E = 5,6), severe disability (GOS-E = 3,4), vegetative state (GOS-E =2) and death (GOS-E =1).

Association analysis

The WM areas with FA differences along time were defined as ROIs and the mean FA values of each one was calculated. Then, to test for association of mean FA values of each ROI with GOS-E grading, we used Cohen's d effect size test. We segmented patients into two different groups: sub-optimal (GOS-E= 5 or 6) and optimal (GOS-E = 7 or 8) performance. We tested for associations of each ROI at two months and one year after trauma.

Taking into account the relatively small patient sample, we also estimated Cohen's d effect size test considering a bigger sample size (4 times our sample, with the same distribution).

RESULTS

In the initial screening, 225 patients with head trauma were evaluated, and the final analysis included twenty patients of those. Demographics of the final sample are described in Table 1. Two hundred and five subjects were excluded for the following reasons:

- 186 had no clinical and/or tomographic criteria for DAI;
- 7 follow-up losses;
- 5 were not eligible for MRI;
- 5 had low-quality DTI studies;
- 1 developed epidural compressive hematoma;
- 1 deceased.

Evaluation of changes along time at two months and one year after trauma (chronic minus subacute volumes) with voxel based TFCE analysis indicated brain regions with FA increment along time, predominantly in the right hemisphere and in the left cerebellum. The significant brain clusters (Table 2) were found in the right superior longitudinal fascicle, the temporal part of the right superior longitudinal fascicle, right inferior front-occipital fascicle, right superior and inferior longitudinal fascicles, the body of corpus callosum, forceps major and left corticospinal tract (Figure 1). Moreover, we found extensive areas of increases in MD, RD, and AD ($p < 0.05$, FWE corrected) (Figure 2).

Of note, the one-sample t-test used to assess the difference between subacute minus the early chronic volumes did not demonstrate significant differences for any DTI parameter.

Correlations between the different FA ROIs and the one-year GOS-E grades were tested with different ROIs at 2-months and 1-year post-trauma (Figures 3 and 4). We did not find any correlations on either moment.

In addition, hypothetically increasing 4 times our sample, we found some associations the one-year GOS-E and the specific ROIs of FA increase at 2 months and 1 year after trauma (Table 3).

DISCUSSION

In our investigation, we did a whole-brain analysis using a semi-automated method to explore white matter changes over time in moderate and severe TBI victims. DTI has mainly

been used to study white matter in the trauma scenario. However, most published articles are related to mild trauma and with different follow-up periods[12,13,20]. It is important to emphasize that our patient sample is very homogeneous, consisting of victims with moderate and severe trauma, explicitly diagnosed and exclusively with DAI, and followed throughout one year after the event.

We found some scattered areas of FA increase, notably in the right brain hemisphere, accompanied by vast regions in the brain and the cerebellum demonstrating an increase in MD, RD, and AD along time. Interestingly, the patients showed relatively good clinical outcomes, according to the GOS-E scale. We also found different associations between each brain region with increased FA and the late clinical outcome (GOS-E) two months and one year after trauma, which were more prominent when tested for a larger sample size. Our results are aligned with previous studies that have described white matter changes on DTI parameters along with time in victims of head trauma[21,22]. These ongoing DTI parameters are related to different pathophysiological processes such as inflammation, degeneration, and regeneration – which have already been described in experimental studies[23,24].

We identified a general area of increase in MD, AD, and RD in brain tracts one year after trauma. We consider that MD increase is mainly a result of high RD values and, in a lower degree to AD increment. MD represents the overall diffusivity of water molecules, which can be related to the increasing content of isotropic tissue with water content (gliosis)[25]. Although the biological basis for the anisotropy and diffusivity changes in tissues revealed by DTI data is still largely debated, studies using animal models have demonstrated that axonal injury itself is represented by AD changes and demyelination is associated with an increase in RD values[26]. Considering that increases in both AD and RD contribute positively to augments in MD values, it is reasonable to assume MD as a more sensitive parameter when compared to FA in our observation.

Moreover, in addition to axonal injury, other important and specific pathophysiological processes are also present in the trauma scenario, such as neuroinflammation, afferent degeneration, and debris clearance, and the magnitude of each one at different stages may imply distinct changes in DTI scalar values. Animal models' studies play an essential role in characterizing these other effects ensued by the trauma event and how they change over time. However, most of the articles published to date describe the changes seen in the early acute time after trauma, and only a limited number of articles evaluate long-term consequences[27]. It is already well established that the overall axonal injury in trauma survivors is a consequence of the secondary axonal injury, which is the indirect damage to neurons related to

neuroinflammation and microglial activation, triggered by the initial impact and that can persist for years[23]. These processes are responsible for biochemical changes leading to local edema and changes in the microvascular circulation, leading to ischemia and demyelination, which can be illustrated by the RD increase over time[28]. Moreover, AD increase has been associated with an increment of the extracellular water content, such as debris clearance, that would ease the water molecule movement in an axis parallel to the axons[29]. Thereby, we suppose that our results can be explained by the Wallerian or Wallerian-like degeneration process due to DAI or related to a secondary pathological process, such as regional ischemia and neuronal death may ultimately lead to brain atrophy[29].

We also found some spotted areas of FA increase in the right brain hemisphere and the left cerebellum over time. Different causes can be associated with FA increase, such as local fibrosis, hemorrhage areas, and neuronal sprouting[30]. FA is related to the microstructural organization, with high values (close to one) related to most anisotropic tissues. Microstructural organization after the trauma has been reported to start as early as days and can persist for years, which is linked to neuroplasticity[31]. The functional recovery accompanied by FA increment may be related somehow to neuroplasticity. Interestingly, we found areas of FA increase in the right brain hemisphere and in the left cerebellum, which may illustrate the involvement of the contralateral cerebellar hemisphere in functional and compensatory changes after trauma, as it has been already reported[32]. An interesting functional study evaluated children with moderate and severe trauma in comparison to controls, and it demonstrated that children with TBI demonstrated changes in functional cerebral activity and demonstrated increased recruitment of neural resources such as the cerebellum[33].

We tested for correlations between mean FA values at the subacute and early chronic phases of the specific regions that presented significant changes in overtime and the GOS-E scores. We couldn't find any significant correlations; however, the lack of significance may be related to our sample size, relatively small when considering the optimal number of individuals required for correlational studies[35]. Still, some specific regions as the right SLF and the body of the corpus callosum demonstrated, at the early chronic phase, promising effect sizes in functional stratification between optimal and sub-optimal GOS-E scores and mean FA values by using a theoretical larger sample size.

Whole-brain voxel-wise analysis has been increasingly used to study DAI since the widespread nature of the disorder, as well as the advantages of minimal intervention for multi-subject group evaluation provided by this analytical method. However, with this

technique, it is mandatory the use strict statistical procedures for correcting for multiple comparison errors, which reduces the sensitivity for detecting subtle changes[12].

One limitation of our study is the relatively small sample size. However, we included a particular and homogeneous group of patients with a minimum one-year survival after the traumatic event, especially considering that victims of moderate and severe head trauma have high mortality rates in the first six months[34,35]. Moreover, we did have a particular group in terms of one-year survival, but these patients also presented an excellent recovery with high one-year GOS-E scores. This may be related to the exclusion of other conditions commonly associated with a head injury, such as contusions and hematomas that can imply a poorer outcome[2].

Concerning the methodology and imaging acquisition, we need to emphasize that more gradient encoding directions and more robust DTI acquisition and analytical methods such as high angular resolution diffusion imaging (HARDI), diffusion kurtosis imaging (DKI), and q-ball imaging are available and could have enhanced the power of the data analysis[11,36]. However, these approaches require longer acquisition times, more sophisticated algorithms, and are still less feasible to implement in clinical and research scenarios.

CONCLUSION

In conclusion, our work indicated changes in all DTI scalar metrics in the brain and cerebellum white matter in a homogeneous group of DAI victims along the first year following moderate and severe head trauma. This study can be important to guide future research in understanding the different pathophysiological processes that ensue at different stages during patient recovery. Upcoming studies are awaited to indicate DTI as a tool in signaling functional outcome, as well as an auspicious method to be used to guide therapies and rehabilitation procedures in trauma survivors.

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LIST OF TABLES

Table 1. Demographics of the 20 patients included in the study.

Gender	Male = 16; Female = 4
Handedness	Right-handed = 16, left-handed = 4
Traumatic event	Motorcycle = 10 Car accident = 6 Run over = 3 Agression = 1
GCS at hospital admission	Moderate (GCS 9-12) = 14 Severe (GCS < 8) = 6
Interval between trauma and hospital admission	39 minutes (15 to 77 minutes)
One-year outcome	GOS-E 5 = 1 GOS-E 6 = 7 GOS-E 7 = 11 GOS-E 8 = 1

Table 1. Demographics.

GCS = Glasgow coma scale, GOS-E = Glasgow outcome scale extended

Table 2. The most significant clusters found in FA analysis.

Cluster Index	Voxels	p	X (mm)	Y (mm)	Z (mm)	Location
7	7	0.007	25	-44	36	**R SLF, R IFOF**
6	4	0.025	3	3	26	**body corpus callosum**
5	4	0.024	32	-41	30	**R SLF, R SLF (temporal part), R ILF**
4	2	0.027	-28	-47	-38	**L CST**
3	1	0.04	29	-68	11	**forceps major, R IFOF, R ILF**
2	1	0.038	30	-43	32	**R SLF and R ILF**
1	1	0.031	33	-35	32	**R SLF, R SLF (temporal part)**

R SLF = right superior longitudinal fascicle, R IFOF = right inferior fronto-occipital fascicle, R ILF = right inferior longitudinal fascicle, L CST = left cortical spinal tract

Table 3. Correlation analysis supposedly increasing by 4 times the sample size.

Cluster index	*p* value (2 months)	*p* value (1 year)
1	0.014	0.009
2	0.066	0.078
3	0.823	0.994
4	0.190	0.398
5	0.020	0.061
6	0.161	0.024
7	0.044	0.685

- *p* value obtained by Cohen's d test.

Figure Legends

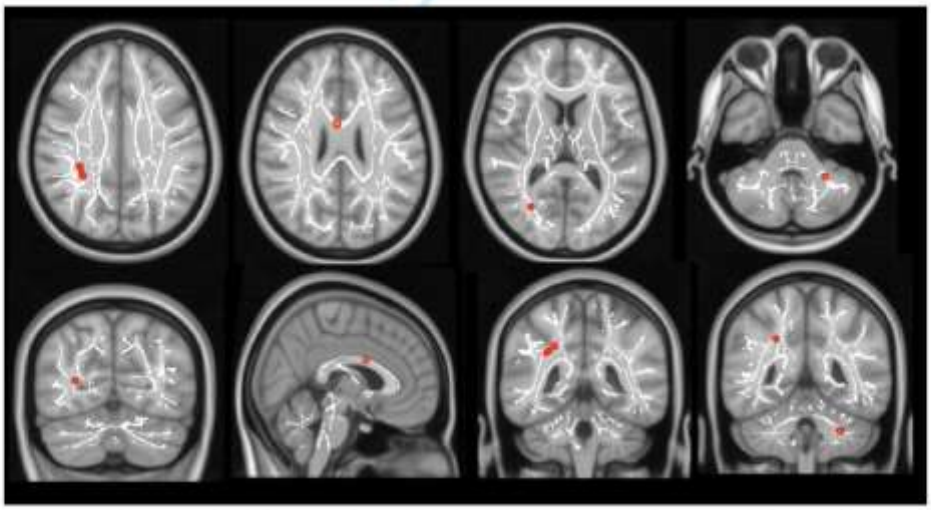


Figure 1. The most significant clusters found with increments in FA (early chronic minus subacute phase) are represented in red, TFCE ($p < 0.05$, FWE corrected). The mean FA skeleton is indicated in white. FA, fractional anisotropy.

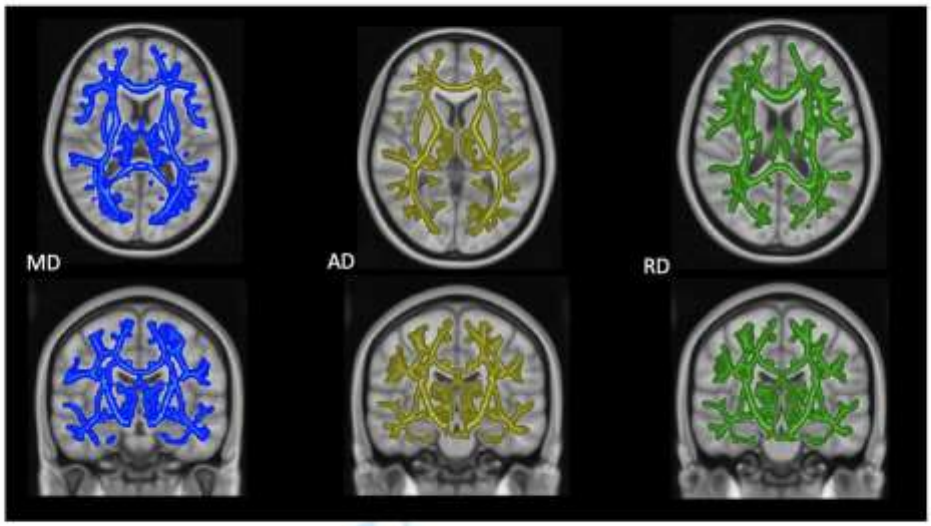
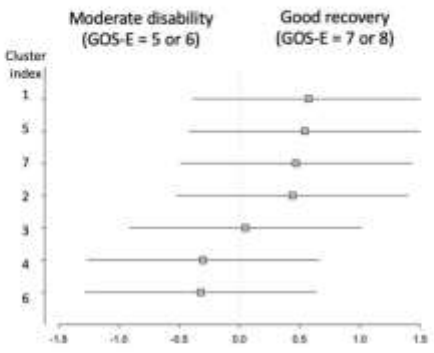


Figure 2. White matter differences between early chronic and subacute phases. Significant clusters ($p < 0.05$, FWE corrected). Blue depicts MD, yellow AD and green RD increases in the chronic phase. FWE, family-wise error; MD, mean diffusivity; AD, axial diffusivity; RD, radial diffusivity.



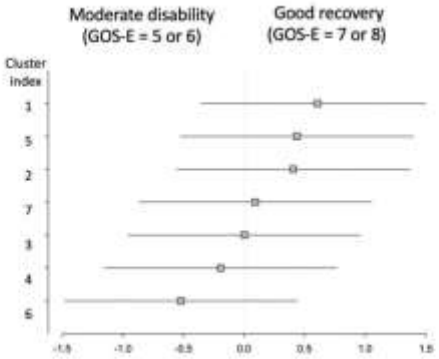
Cluster index	GOS-E Moderate disability	GOS-E Good recovery	d (IC95%)	p value
1	0.52±0.05	0.55 ±0.06	0.57 (-0.39 to 1.54)	0.225
5	0.45±0.06	0.48±0.06	0.54 (-0.42 to 1.51)	0.25
7	0.37±0.06	0.39±0.04	0.47 (-0.49 to 1.43)	0.317
2	0.43±0.04	0.45±0.06	0.44 (-0,52 – o 1.4)	0.347
3	0.53±0.08	0.53±0.06	0.05 (-0.91 to 1.01)	0.912
4	0.68±0.06	0.68±0.05	-0.3 (-1.26 to 0.66)	0.517
6	0.68 ±0.05	0.65±0.07	-0.32 (-1.29 to 0.64)	0.49

Figure 3. Subgroup analysis

The forest plot shows the effect size in the outcome variable across the prespecified subgroup according to GOS-E outcome stratification (moderate disability *vs* good recovery). Association analysis between different ROIs at 2 months after trauma with sub-optimal and

optimal 1-year post-trauma GOS-E scores. Horizontal axis demonstrates differences between the groups of recovery according to each cluster.

Effect sizes values displayed along with respectively confidence interval of 95% and statically significance (*p*) obtained by Cohen's d test (squares).

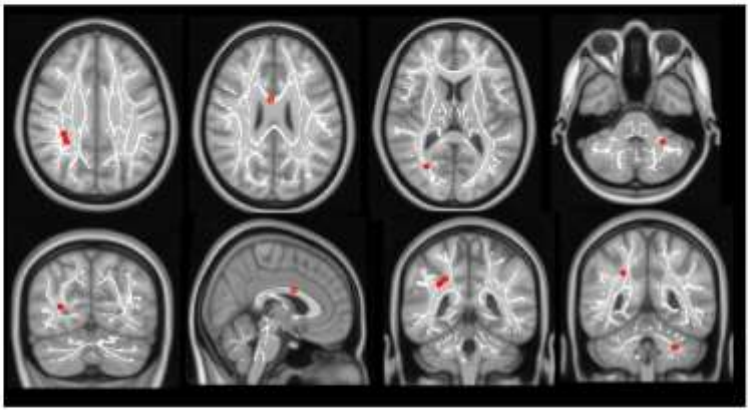


Cluster index	GOS-E Moderate disability	GOS-E Good recovery	d (CI95%)	*p* value
1	0.54±0.04	0.58±0.07	0.41 (-0.36 to 1.57)	0.2
5	0.49±0.07	0.51±0.05	0.44 (-0.53 to 1.4)	0.353
2	0.46±0.4	0.48±0.07	0.41 (-0.56 to 1.37)	0.386
7	0.42±0.05	0.42±0.06	0.09 (-0.87 to 1.06)	0.841
3	0.58±0.09	0.58±0.07	0.00 (-0.96 to 0.96)	0.997
4	0.74±0.04	0739±0.4	-0.19 (-1.16 to 0.77)	0.676
6	0.72±0.03	0.69±0.06	-0.52 (-1.48 to 0.44)	0.268

Figure 4. Subgroup analysis

The forest plot shows the effect size in the outcome variable across the prespecified subgroup according to GOS-E outcome stratification (moderate disability *vs* good recovery). Association analysis between different ROIs at one year after trauma with sub-optimal and optimal 1-year post-trauma GOS-E scores. Horizontal axis demonstrates differences between the groups of recovery according to each cluster.

Effect sizes values displayed along with respectively confidence interval of 95% and statically significance (*p*) obtained by Cohen's d test.



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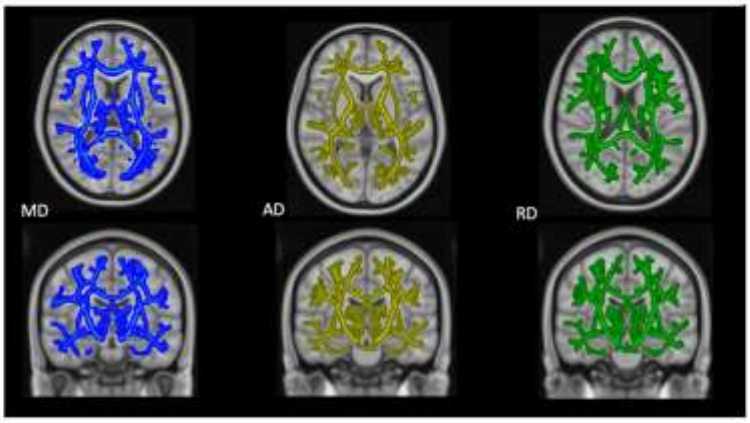
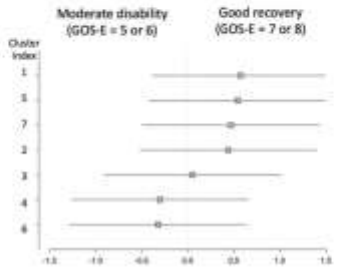


Figure 2. White matter differences between early chronic and subacute phases. Significant clusters ($p < 0.05$, FWE corrected). Blue depicts MD, yellow AD and green RD increases in the chronic phase. FWE, family-wise error; MD, mean diffusivity; AD, axial diffusivity; RD, radial diffusivity.

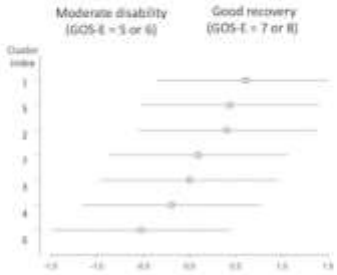
174x97mm (600 x 600 DPI)



Cluster index	GOS-E Moderate disability	GOS-E Good recovery	d (IC95%)	p value
1	0.52±0.03	0.55 ±0.04	0.57 (-0.09 to 1.24)	0.225
5	0.45±0.04	0.48±0.04	0.54 (-0.42 to 1.51)	0.25
7	0.37±0.04	0.39±0.04	0.47 (-0.49 to 1.43)	0.317
2	0.43±0.04	0.45±0.04	0.44 (-0.52 to 1.4)	0.347
3	0.53±0.08	0.53±0.04	0.05 (-0.91 to 1.01)	0.912
4	0.68±0.04	0.68±0.03	-0.3 (-1.26 to 0.66)	0.527
6	0.68 ±0.05	0.65±0.07	-0.32 (-1.29 to 0.64)	0.49

Figure 3. Subgroup analysis
The forest plot shows the effect size in the outcome variable across the prespecified subgroup according to GOS-E outcome stratification (moderate disability vs good recovery). Association analysis between different ROIs at 2 months after trauma with sub-optimal and optimal 1-year post-trauma GOS-E scores. Horizontal axis demonstrates differences between the groups of recovery according to each cluster.
Effect sizes values displayed along with respectively confidence interval of 95% and statically significance (p) obtained by Cohen's d test (squares).

331x146mm (144 x 144 DPI)



Cluster index	GOS-E Moderate disability	GOS-E Good recovery	d (CI95%)	p value
1				
5				
2				
3				
7				
8				
4				
6				

Figure 4. Subgroup analysis
The forest plot shows the effect size in the outcome variable across the prespecified subgroup according to GOS-E outcome stratification (moderate disability vs good recovery). Association analysis between different ROIs at one year after trauma with sub-optimal and optimal 1-year post-trauma GOS-E scores. Horizontal axis demonstrates differences between the groups of recovery according to each cluster.
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329x133mm (144 x 144 DPI)

Gender	Male = 16; Female = 4
Handedness	Right-handed = 16, left-handed = 4
Traumatic event	Motorcycle = 10 Car accident = 6 Run over = 3 Agression = 1
GCS at hospital admission	Moderate (GCS 9-12) = 14 Severe (GCS < 8) = 6
Interval between trauma and hospital admission	39 minutes (15 to 77 minutes)
One-year outcome	GOS-E 5 = 1 GOS-E 6 = 7 GOS-E 7 = 11 GOS-E 8 = 1

Table 1. Demographics of the 20 patients included in the study.
GCS = Glasgow coma scale, GOS-E = Glasgow outcome scale extended

Cluster Index	Voxels	p	X (mm)	Y (mm)	Z (mm)	Location
7	7	0.007	25	-44	36	**R SLF, R IFOF**
6	4	0.025	3	3	26	**body corpus callosum**
5	4	0.024	32	-41	30	**R SLF, R SLF (temporal part), R ILF**
4	2	0.027	-28	-47	-38	**L CST**
3	1	0.04	29	-68	11	**forceps major, R IFOF, R ILF**
2	1	0.038	30	-43	32	**R SLF and R ILF**
1	1	0.031	33	-35	32	**R SLF, R SLF (temporal part)**

Table 2. The most significant clusters found in FA analysis.

R SLF = right superior longitudinal fascicle, R IFOF = right inferior fronto-occipital fascicle, R ILF = right inferior longitudinal fascicle, L CST = left cortical spinal tract

Cluster index	*p* value (2 months)	*p* value (1 year)
1	0.014	0.009
2	0.056	0.078
3	0.823	0.994
4	0.190	0.398
5	0.020	0.061
6	0.161	0.024
7	0.044	0.685

Table 3. Correlation analysis supposedly increasing by 4 times the sample size.

- *p* value obtained by Cohen's d test.