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APPLICATION OF DIFFUSION TENSOR IMAGING IN MULTIPLE SCLEROSIS

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NICOLÁS FRANCISCO LORI, PH.D.
PROF. DOUTORA CATARINA ISABEL NENO RESENDE DE OLIVEIRA

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APPLICATION OF DIFFUSION TENSOR IMAGING IN MULTIPLE SCLEROSIS

Artigo de Revisão

Filipa Costa Sousa[a]

[a] Aluna do 6º ano do Mestrado Integrado em Medicina da Universidade de Coimbra, nº 2009029708

Rua de São Mateus, nº 1450, Cabroelo

4575-200 Capela

filipasousa04@gmail.com

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SUMMARY

RESUMO

A esclerose múltipla é uma doença degenerativa do sistema nervoso central, constituindo uma causa significativa de incapacidade. Durante os últimos anos, a ressonância magnética de difusão tem vindo a ser aplicada no estudo de doentes com esclerose múltipla, na tentativa de melhorar a compreensão do processo patológico subjacente a um nível microestrutural, numa fase precoce da doença. A ressonância magnética de difusão, devido à sua elevada sensibilidade, tem-se mostrado capaz de detetar e quantificar o dano tecidual tanto em áreas de lesão, visíveis em T2, como em áreas aparentemente normais na ressonância magnética convencional.

Este trabalho analisa as aplicações da ressonância magnética de difusão no estudo de doentes com esclerose múltipla, avaliando o interesse da sua possível implementação na prática clínica, como método auxiliar para o diagnóstico, caracterização e seguimento destes doentes.

ABSTRACT

Multiple sclerosis (MS) is a degenerative disease of the central nervous system (CNS), being a significant cause of disability. During the last years, diffusion tensor imaging (DTI) has been applied in the study of MS patients in an attempt to improve the understanding of the pathologic process at a microstructural level, in early stages of the disease. DTI, due to its high sensitivity, has proved to be able to detect and quantify tissue damage within and outside T2-visible MS lesions.

This work analyzes the applications of DTI in the study of MS patients, and evaluates the interest of its implementation in clinical practice for diagnosis, characterization and follow-through of MS patients.

Keywords: Multiple Sclerosis; Diffusion Tensor Imaging; Magnetic Resonance Imaging; White Matter; Gray Matter; Diffuse Axonal Injury; Wallerian Degeneration.

Abbreviations:

9HPT 9-hole peg test;

ADC apparent diffusivity coefficient;

BBB blood-brain barrier;

CC corpus callosum;

CIS clinically isolated syndrome;

cMRI conventional magnetic resonance imaging;

CN caudate nuclei;

CNS central nervous system;

CNV caudate nuclei volume;

LCTs lateral corticospinal tracts;

CUD crossed-uncrossed difference;

DTI diffusion tensor imaging;

EDSS expanded disability status scale;

FA fractional anisotropy;

FLAIR fluid-attenuated inversion recovery;

GM grey matter;

HARDI high angular resolution diffusion imaging;

MD mean diffusivity;

MeSH medical subject headings;

MS multiple sclerosis;

MSFC multiple sclerosis functional composite;

NACN normal appearing caudate nuclei;

NAGM normal appearing grey matter;

NAM normal appearing matter;

NAM normal appearing matter;

NASC normal appearing spinal cord;

NAT normal appearing thalamus;

NAWM normal appearing white matter;

ON optic neuritis;

PASAT paced auditory serial addition test;

PC posterior columns;

PPMS primary-progressive multiple sclerosis;

PRMS progressive-relapsing multiple sclerosis;

RG redundancy gain;

RNFL retinal nerve fiber layer;

ROI regions of interest;

RRMS relapsing-remitting multiple sclerosis;

SPMS secondary-progressive multiple sclerosis;

STIR short tau inversion recovery;

T25FW timed 25-foot walk;

TBSS tract-based spatial statistics;

TMV total macular volume;

VSC voxel-scale connectivity;

WM white matter;

$\lambda_{\parallel}$ parallel diffusivity;

$\lambda_{\perp}$ perpendicular diffusivity.

INTRODUCTION

MS is the most common acquired inflammatory demyelinating disorder of the CNS[1,2] being the major cause of non-traumatic neurological disability in young adults in Europe and North America.[1–3] DTI is a noninvasive imaging technique capable of characterizing the diffusion properties of water molecules *in vivo* and detecting microstructural tissue changes not visible on conventional magnetic resonance imaging (cMRI).[1] The purpose of this work is to analyze the usefulness of DTI in the diagnosis, characterization and follow-through of MS patients.

In 2013, 2.1 million people were estimated suffering of MS in the globe,[4] predominantly affecting young and middle-aged adults,[1] with a female to male ratio of 2:1.[4] The age of onset in typically between 20 and 40 years (slightly later in men that in women) and once diagnosed, MS is present across the life span.[2] Prevalence has increased steadily in several regions around the world over the past half-century.[2] A study from "Direção-Geral da Saúde"[5] indicates that in Portugal, this disease has a prevalence of 54:100.000, ranging from 34 to 74:100.00 with a confidence interval of 95%. Based on these data, it is estimated that MS affects 4287 people in Portugal.

MS is an inflammatory, chronic and degenerative disease that affects the CNS and whose etiopathologic mechanisms are not fully understood. It is thought to have a multifactorial etiology, involving genetic and environmental factors, where the cellular and humoral immunological system play a crucial role in the pathological process.[2]

It is classically characterized by multifocal demyelination plaques affecting mainly the CNS white matter (WM) with a predilection for some areas such as: periventricular WM, optic nerves, brainstem, cerebellum and spinal cord.[2] Though typically thought to be a demyelinating

disease, gliosis and partial or total axonal destruction can also occur, being a major contributor to irreversible neurologic disability.[2,3] Knowledge of the mechanisms responsible for axonal injury is incomplete and, despite the fact that axonal transactions are most conspicuous in acute inflammatory lesions, it is still unclear whether demyelination is a prerequisite for axonal injury in MS. Axons can adapt initially to these injuries but with time distal and retrograde degeneration often occurs.[2]

The presentation of this neurologic disease may be heterogeneous, depending on the affected structures. It may occur with motor disability, cognitive impairment, visual impairment and many other symptoms. Initial symptoms of MS are presented in Table 1. Early in MS, most disease activity is clinically silent and the onset may be abrupt or insidious. Manifestations of MS vary from a benign illness to a rapidly evolving and incapacitating disease requiring profound lifestyle adjustments.[2]

Table 1*: Initial Symptoms of MS

Symptom	*Percent of Cases*	*Symptom*	*Percent of Cases*
Sensory loss	37	Lhermitte's	3
Optic neuritis	36	Pain	3
Weakness	35	Dementia	2
Paresthesias	24	Visual loss	2
Diplopia	15	Facial palsy	1
Ataxia	11	Impotence	1
Vertigo	6	Myokymia	1
Paroxysmal attacks	4	Epilepsy	1
Bladder	4	Falling	1

*Adapted from reference 2.

The clinical course is variable, depending on the number of acute crisis and the clinical manifestations of the patient as the disease progresses. Four clinical types of MS have been described: relapse-remitting MS (RRMS); secondary-progressive MS (SPMS); primary-progressive MS (PPMS); and progressive-relapsing MS (PRMS).[2]

Nowadays the McDonald criteria[6] are widely used to establish the diagnosis (see Table 2). The criteria include clinical and cMRI features and emphasize the need to demonstrate dissemination of lesions in space and time and to exclude alternative diagnoses. The McDonald criteria have resulted in earlier diagnosis of MS with a high degree of both specificity and sensitivity, allowing for better counseling of patients and earlier treatment.[6]

In recent years, cMRI has become an indispensable paraclinical tool in MS for the assessment of clinical diagnosis, natural history, and treatment effects.[3] By cMRI meaning T1-weighted, T2-weighted, T2*-weighted, fluid-attenuated inversion recover (FLAIR), short tau inversion recovery (STIR) and other not diffusion-weighted anatomical magnetic resonance imaging.

Although cMRI provides a direct measurement of the extent of macroscopic pathology in MS, such as lesion plaques, it has little pathological specificity and lacks sensitivity in detecting lesions at the microscopic level.[7] Besides, cMRI does not strongly correlate with clinical evaluation of disease status.[7] It is known that normal appearing matter (NAM) in cMRI is not necessarily organically and functionally normal, since currently there are no cMRI-based parameters that reflect this damage as sensitively as histopathological examination.[8,9]

Table 2*: The 2010 McDonald Criteria for Diagnosis of MS	
Clinical Presentation	*Additional Data Needed for MS Diagnosis*
≥2 attacks; objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack	None
≥2 attacks; objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by: - ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord); or - Await a further clinical attack implicating a different CNS site
1 attack; objective clinical evidence of ≥2 lesions	Dissemination in time, demonstrated by: - Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time; or - A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or - Await a second clinical attack
1 attack; objective clinical evidence of 1 lesion (CIS)	Dissemination in space and time, demonstrated by: For DIS: - ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord); or - Await a second clinical attack implicating a different CNS site; and For DIT: - Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time; or - A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or - Await a second clinical attack
Insidious neurological progression suggestive of MS (PPMS)	1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria: - Evidence for DIS in the brain based on ≥1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions; - Evidence for DIS in the spinal cord based on ≥2 T2 lesions in the cord; - Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)
MS = multiple sclerosis; CNS = central nervous system; MRI = magnetic resonance imaging; DIS = dissemination in space; DIT = dissemination in time; PPMS = primary progressive multiple sclerosis; CSF = cerebrospinal fluid; IgG = immunoglobulin G.	

*Adapted from reference 6.

In the study of patients with MS there are many advantages of having a sensitive and reliable *in vivo* method for investigating the specific pathological changes of the CNS. For this purpose DTI is one of the most promising techniques.

DTI is a noninvasive imaging technique capable of characterizing the diffusion properties of water molecules *in vivo*. This advanced MRI technique provides unique quantitative information regarding the structural and orientational features of the CNS enabling the detection of microstructural tissue changes that are not visible in cMRI. This technique has been applied increasingly in MS research over the last few years and has helped to a better understanding of the mechanisms underlying tissue injury thought to be responsible for neurological dysfunction.[10]

In this review DTI will be schematically described, and an analysis of the use of DTI in MS will be done. Thus describing the strengths and limitations of the use of DTI in the diagnosis, characterization, and treatment of MS.

METHODS

The research carried out for this review came from a first search on Pubmed database, on October 2013, with the support of a differentiated professional of the library of the University of Coimbra's Hospital. The used search terms were "MS" and "diffusion magnetic resonance imaging" (*Medical Subject Headings*, from the *National Library of Medicine*), for articles published in the last ten years. In order to obtain recent studies in this area, still not indexed with *Medical Subject Headings* search terms, an additional research was made using the keywords: "*MS AND (mri OR mr imaging OR magnetic) AND diffusion*". From the total research more than 200 articles were obtained, and the most relevant were selected based on the following criteria: journal of publication and its current impact factor, main author's professional class and number of citations, and adequacy to the aim of this work. Additional search was performed during the elaboration of this work whenever necessary.

Selected articles were obtained using both the Faculty of Medicine, University of Coimbra's, and University of Coimbra's Hospital databases.

Diffusion magnetic resonance imaging

Molecular diffusion

DTI is a quantitative, non-invasing *in vivo* imaging technique that measures a single phenomenon: the dephasing of proton spins in the presence of a spatially-varying magnetic field linear gradient. In other words: it is a technique that quantifies at different places the orientational variability of the amount of random water diffusion, thus obtaining information about the tissues surrounding that water.[3,11–15]

The characteristics of diffusion are influenced by several tissue components, including cell membranes and organelles,[12,16] providing unique *in vivo* information about the pathological processes of brain microstructural damage.[3,12]

Using mathematical models of the underlying tissue, it is possible to determine parameters describing the tissue microstructure. Although being able to be applied in any body part, this imaging technique is most frequently used in brain and spinal cord.[12]

Any particle at a temperature above absolute zero possesses thermal energy that manifests as random movement, the molecular diffusion. By the application of two radiofrequency pulses, a 90 degree pulse followed by a 180 degree pulse, it is induced a spatially dependent phase shift that makes this sequence sensitive to the effects of diffusion. The diffusion of the spins, after the application of these two gradients, causes a phase dispersal which leads to signal attenuation.

Diffusion weighted imaging

The echo signal in a typical spin echo sequence combines T2 and diffusion-weighting. By measuring the signal at two different b-values the effects of T2 decay can be removed

leaving just the diffusion-weighted attenuation and it is possible to obtain information about the water's diffusion by the corresponding MRI signal intensity reduction. The apparent diffusion coefficient (ADC)[17] is a scalar measure that reflects the amount of apparent diffusivity in a particular direction.[7] Although the ADC is largely independent of the direction of the diffusion gradients in grey matter (GM), the same is not true in WM. The ADC is higher when the diffusion gradients are aligned with the predominant fiber direction, reflecting that water diffuses more freely parallel (||) to the length of an axon than perpendicular (⊥) to it.[3,12]

Diffusion tensor imaging

If b is the b-value of the diffusion MRI data defined by the data acquisition parameters, and $\hat{q}$ is a unit-vector with the orientation and proportional to the diffusion-sensitizing magnetic field gradient, then the magnetic field intensity reduction caused by the diffusion is equal to $e^{-b\,ADC}$ and the ADC is equal to $\hat{q}^T \cdot D \cdot \hat{q}$ where D is the diffusion tensor. The diffusion tensor is a 3x3 matrix that quantifies diffusion, with for example, D_{xy} quantifying the correlated diffusion along the x and y axes. Naturally then, D_{xy} is equal to D_{yx}, so the diffusion tensor has 6 independent elements[12,16] (Figure 1).

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix} \qquad \mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix}$$

Figure 1: The diffusion tensor is a matrix which is symmetric, and so it has six independent parameters.

The diffusion tensor is calculated from images acquired with diffusion-weighting gradients applied in at least 6 noncollinear directions and provides information on both the

magnitude and direction of water diffusion.[11,12] The axes of the diffusion correspond to the eigenvectors of the tensor (e_1, e_2, and e_3), and the degree of diffusion along these principle axes is given by the three eigenvalues (λ_1, λ_2, and λ_3).[7,12]

Tensor representation

Diffusion tensors are often visualized as ellipsoids with the size and shape reflecting the degree of diffusion along each principal axis. The diffusion ellipsoid representation has axes aligned with the eigenvectors with a magnitude proportional to the square roots of the corresponding eigenvalues, the principal axis being the eigenvector with the highest eigenvalue[7,12] (Figure 2).

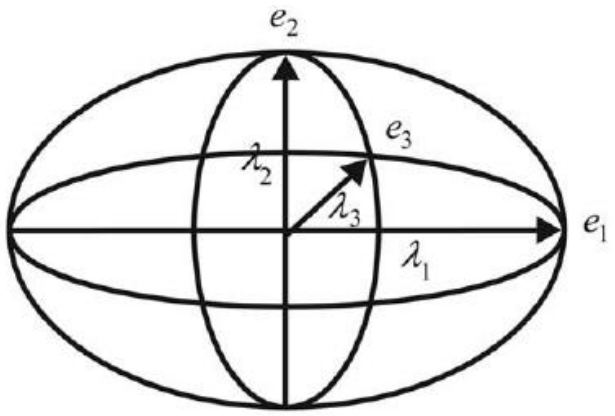

Figure 2: Elliptical representation of a tensor, reflecting the degree of diffusion along each axis. The axes of diffusion correspond to the eigenvectors of the tensor (e_1, e_2, and e_3), and the relative size of each axis is determined by the square root of the eigenvalues of the tensor (λ_1, λ_2, and λ_3). Principal axis in this representation corresponds to the eigenvector e1. Figure extracted from reference 7.

Scalar measurements

The mean diffusivity (MD) provides a measure of the average diffusion of water, representing the mean of the three eigenvalues.[11,12,18] This index parameter is independent of the spatial orientation of the coordinate system used.[16] Higher values of MD indicate greater levels of diffusivity, thus indicating more space for diffusion to occur.[11,19]

$$MD = \frac{\lambda_1+\lambda_2+\lambda_3}{3}$$

The values of MD are remarkably similar across GM and WM, between different subjects and across mammalian species.[12] Furthermore, MD is not influenced by patient positioning or fiber orientation,[11] being affected only by cellular size and integrity.[20]

The ADC can be decomposed into two components, the longitudinal (parallel, axial) diffusivity ($\lambda_\parallel$), and the perpendicular (radial, transverse) diffusivity ($\lambda_\perp$).

$$\lambda_\parallel=\lambda_1 \qquad \lambda_\perp = \frac{\lambda_2 + \lambda_3}{2}$$

The eigenvalue $\lambda_\parallel$ measures the diffusivity parallel to the main fiber direction. Research in this area has shown that $\lambda_\parallel$ can been used as a marker for axonal integrity.[8,12] Reduced $\lambda_\parallel$ values have been associated with decreased axonal density within the brain and spinal cord.[8] Normal $\lambda_\parallel$ can be found despite the presence of demyelination and/or axon loss due to loss of small caliber axons and relative preservation of large caliber axons.[8]

The $\lambda_\perp$ measures the diffusivity perpendicular to the main direction of fibers, and has essentially been associated with myelin integrity.[1,8,12,21] However, it has been demonstrated that it also reflects changes of axonal membrane and extracellular space since loss of axonal structures may lead to the less restricted diffusion perpendicular to the main direction of fibers

leading to an increase in this parameter.[1,21] However, these parameters may not portray an accurate reflection of demyelination especially in areas of low anisotropy due to complex tissue architecture such as crossing fibers.[12,22]

Fractional anisotropy (FA) quantifies the degree of anisotropy of the diffusion tensor (A), in other words, FA is a measure of the directionality of diffusion.[11,16,19,23] FA ranges from 0 to 1, with 0 reflecting completely isotropic diffusion and 1 reflecting diffusion constrained to occur in one direction only.[11,19] Although being an oversimplification due to not taking into account the possible existence of crossing WM fibers, FA has been used as a sensitive index of the WM structural integrity.[12,19,24]

$$FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

FA is related to many factors including axonal count, axonal density, degree of myelination, and fiber organization.[12] While changes are frequently attributed to one or more of these factors, DTI alone cannot distinguish them and is thus non-specific.

Following the observation that diffusion is predominantly isotropic in GM but anisotropic in WM, it is relevant to try to understand whether it relates to a specific microstructural component (e.g. cytoskeleton's neurofilaments and microtubules, the axonal membranes, and/or the myelin sheath). Studies using animal models have demonstrated that the cytoskeleton does not appear to be a significant contributor do diffusion anisotropy.[12] In contrast, axonal membranes and thus axonal density are likely to play a central role whereas, although modestly, myelin is likely to contribute to anisotropy.[12] Anisotropy is higher in regions with more axons per cross-sectional area and thus an increased higher packing density and number of axonal membranes.[12]

Image processing

In clinical studies, involving DTI, a suitable parameter must be extracted and then compared between groups to be properly interpreted, or information about correlations of these parameters with clinical scores or *ex-vivo* analysis may be extracted. To obtain these results, appropriate data processing with specific software must be done.

Two common techniques are employed to extract the DTI parameters:

1. Voxel-based analysis/ Tract-based spatial statistics

The voxel-based analysis is based on the mapping of diffusion indices, such as FA, that are then spatially normalized to a stereotaxic space. Statistical tests are then applied to examine the significance of the group-wise differences.[25,26]

A variant of this technique, known as tract-based spatial statistics (TBSS),[25] determines a WM skeleton representing the 'core' of the tracts from the group and projects each subject's FA map onto this skeleton[12] (Figure 3).

2. Region-of-interest based analysis.

The structures of interest are delineated either manually by an operator or automatically from an atlas, then, the mean of a diffusion parameter (such as FA) is determined in each structure for each subject.[12] Having extracted a parameter and performed a group comparison, any differences must be interpreted.

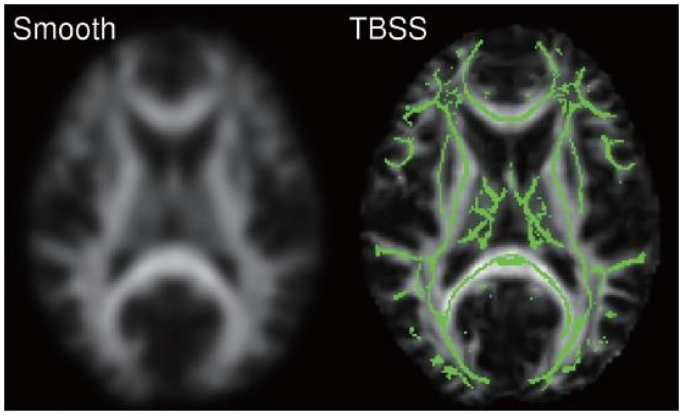

Figure 3: Voxel-based techniques for analyzing DTI parameters. In conventional voxel-based analysis the FA map is non-linearly registered to a template and smoothed (left). In TBSS following normalization, the FA map is projected onto a WM skeleton (right, in green). Figure extracted from reference 12.

DTI tractography

The diffusion tensor model assumes that there are no crossing WM fibers, and so the direction of the principal eigenvector (the eigenvector with highest eigenvalue) in each voxel is aligned with the predominant direction of WM fibers locally.[7,12] This principle allows the representation and visualization of WM connections using the information contained in DTI data, giving rise to DTI tractography.[27,28]

DTI tractography is a highly sensitive imaging technique used to delineate WM fiber tracts and thus construct 3D tract traces of the pathways, representing WM fiber tracts.[7] The principal eigenvectors' orientation can be displayed either as a vector field or through color coding.[7,12] The directionally encoded color scheme represents the x, y and z components in red, green and blue, respectively, so typically the fibers running left-right are red, antero-posterior are green, and supero-inferior are blue[12] (Figure 4).

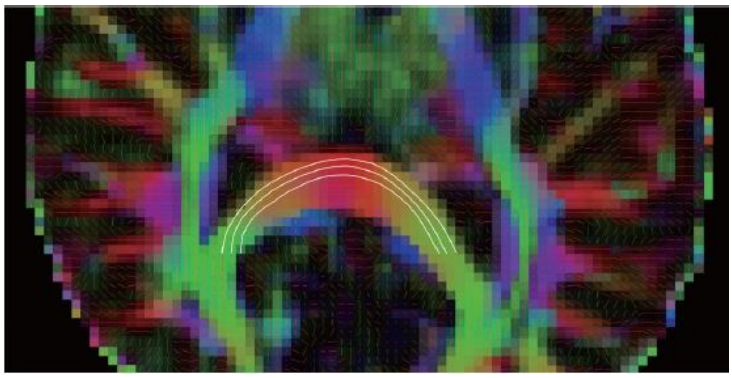

Figure 4: Close up of the corpus callosum with the vector field from the principal eigenvectors and white lines indicating how DTI tractography algorithms follow the vector field. Figure extracted from reference 12.

Additionally, quantitative parameters may be extracted, including the volume of connection and fiber volume.[12] This information may be used qualitatively for surgical planning or applied in patients suffering from degenerative disease of the CNS, namely MS.

Thus, DTI tractography is a valuable tool for a better understanding of the pathophysiology of microstructural damage and its association with clinical measures. Such type of information will ultimately lead to improved monitoring of patients, better prediction of the course of the disease, and more rapid assessment of new treatments or therapies.[7]

Clinical assessment

A vast number of studies using DTI embrace a clinical assessment to establish correlations between specific DTI parameters obtained and the clinical condition of the patient.

There are a number of instruments that describe severity and progression of MS that are currently used in clinical trials.

Expanded disability status scale

The expanded disability status scale (EDSS) of Kurtzke[29] is a clinician-administered assessment scale evaluating the functional systems of the CNS. It is the most popular and widely used instrument for evaluating MS in clinical trials. It remains a useful tool for classifying MS patients according to disease severity and describes disease progression.[30,31]

MS functional composite

MS functional composite (MSFC),[32] which was developed by the MS Society's Clinical Assessment Task Force, is as an additional clinical measurement of MS disability progression. In recent years, the MSFC has been increasingly used in clinical trials.[30]

The MSFC is a three-part performance scale for evaluating the degree of impairment in MS patients. It includes the assessment of leg function by moving a short walking distance: timed 25-foot walk (T25FW), the assessment of arm function using breadboard test: 9-hole peg test (9HPT) and an attention/concentration test to assess cognitive functions: paced auditory serial addition test (PASAT).

Crossed-uncrossed difference

Crossed-uncrossed difference (CUD) is a measure of inter-hemispheric transfer time. It is based on visual reaction times. In MS patients, CUD has special interest in the study of corpus callosum (CC), the largest white matter fiber bundle of brain that connects both cerebral hemispheres.[33]

Redundancy gain task

Redundancy gain (RG) refers to the common finding that the presentation of multiple, redundant stimuli tends to evoke responses more quickly and accurately when compared to presentation of a single stimulus.[33,34] Simple reaction time is measured in divided attention tasks with visual stimuli presented to the left or right of fixation, or redundantly, to both sides.[33,34] The redundancy gain paradigm has been used extensively together with reliable information about inter-hemispheric processing, thus being a useful tool in the evaluation of CC function in MS patient.[33,34]

APPLICATIONS OF DTI IN MS

From cMRI to DTI

In recent years, cMRI has become an indispensable paraclinical tool in MS for the assessment of clinical diagnosis, natural history, and treatment effects.[3]

Nowadays cMRI remains an essential tool in the study of MS patients both in clinical practice and research areas. By scanning MS patients with cMRI and defining a map of lesions and normal appearing WM (NAWM) using regions of interest (ROI), and then doing a DTI scan in order to more accurately study the tissue characteristics; we can use histophatologic studies as a gold standard of tissue damage, in order to evaluate the extent of the disease and to analyze the sensitivity and specificity of imaging techniques. Moreover, clinical assessment of these patients for subsequent correlation with the parameters of DTI may be of great value in understanding the implications of these microstructural abnormalities in clinical manifestation and patient disability.

For all areas that appear to be normal to conventional MRI, and in the context of following DTI study, they are named normal appearing matter (NAM), subdivided in normal appearing WM (NAWM) and normal appearing GM (NAGM). DTI derived metrics have found tissue damage not only in the area of the T1- and T2-weighted lesion but also in the lesion's surrounding area and in remote NAM.[3,7,14,35]

T1- and T2-weighted lesion

MS lesions are pathologically heterogeneous and appear as different patterns on cMRI with variable sizes and appearance. They may represent areas of acute inflammatory changes, while others may show extensive and irreversible tissue destruction.[3]

MS acute lesions generally produce a hyperintense signal in both proton density and T2-weighted images, while the hypointense T1-weighted lesions are considered to be chronic.[7]

An increase in vascular permeability from a major breakdown of the blood-brain-barrier (BBB) is detected by leakage of intravenous gadolinium into the parenchyma. Such leakage-contrast-enhancing lesions occur early in the development of an MS lesion and are a useful marker of inflammation. Contrast-enhancing lesions usually disappear within 6 weeks or less.[2,3]

DTI studies have shown that diffusion abnormalities in lesions are always more pronounced than those found in the corresponding NAM, regardless of the type of injury. Higher degrees of diffusivity and lower FA are DTI findings shared by different types of lesions[7,36] suggesting more extensive microstructural damage, as expected (Figure 5). Despite all the injuries presenting abnormalities detected by DTI, their degree of severity varies with the type of injury,[37,38] which might be expected, taking into account their different pathological substrates.

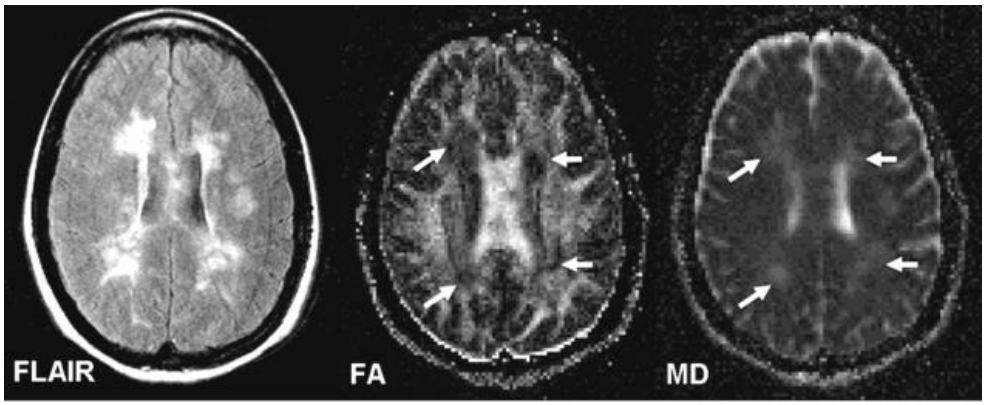

Figure 5: FLAIR and DTI-derived FA and MD maps in a 29-year-old patient with RRMS showing decreased FA and increased MD in MS lesions. Figure extracted from reference 3.

In MS lesions, the highest diffusion values appear to be found in non-enhancing T1-hypointense lesions (also called T1 "black holes,") as compared with enhancing lesions and non-enhancing T1-isointense lesions, suggesting a more extensive damage.[37,39–41] This finding may be due to the long-standing destructive damage in those hypointense lesions,[36,42] in which water diffusion is less restricted.

Some studies have shown that enhancing lesions can be differentiated from non-enhancing lesions by measuring their MD values[40,41] but other studies have failed to show this utility.[39] Thus DTI cannot yet differentiate enhancing and non-enhancing lesions by measuring their MD. This discrepancy may be due to the variable degree of tissue damage during the lesion active period as reflected by their variable appearance on MRI. However, DTI studies have shown that FA is always lower in enhancing than in non-enhancing lesions,[36,43] suggesting more pronounced tissue destruction of the WM microstructure at the site of enhancement. These data also indicate that FA is more sensitive in differentiating pathological substrates of MS lesions.

Table 3: DTI findings in the study of T1- and T2-weighted lesions in MS
1. Increased MD
2. Decreased FA
3. DTI is more sensitive than cMRI for the detection of tissue damage.
4. Highest degrees of abnormalities in non-enhancing T1-hypointense as compared with enhancing lesions and non-enhancing T1- isointense lesions
5. More extensive tissue destruction in enhancement lesions as compared with non-enhancing.
6. FA is more sensitive than MD in differentiation of enhancing versus non-enhancing lesions

NAWM

NAWM is defined as WM with normal signal intensity on T2-weighted images.[3,24] However histological studies have shown that abnormalities in NAWM occur early in the disease process, and may be present before clinical manifestation.[9]

The application of DTI in the study of NAWM of patients with MS has identified microstructural abnormalities when compared with matched control subjects, suggesting the presence of microscopic pathology beyond the resolution of cMRI. A decrease in FA has been consistently reported,[3,38,44–47] probably secondary to myelin sheath and axonal destruction. These abnormalities tend to expand the extracellular space, resulting also in increased MD, also reported in several studies.[3,24,38]

The pathological mechanism underlying NAWM microstructural abnormalities is not fully understood. Two mechanisms have been presented as possible explanations: Wallerian degeneration and a primary neurodegenerative hypothesis. According to the first one, NAWM abnormalities are secondary to axonal transection within WM lesions, occurring distal to damaged axons, that remain below the threshold detectable by cMRI.[8,48,49] The former ascribe NAWM microstructural damage to primary microscopic lesions to which cMRI are more sensitive.[48] There are evidences that Wallerian degeneration starts immediately after the occurrence of a primary lesion.[50] Moreover, it is reasonable to think that degenerative processes start early in the disease, after evidence of lesions. Hence some authors consider that both mechanisms may contribute to the microstructural abnormalities in NAWM.[48,50] A positive correlation between the lesion load and the detected abnormalities in DTI findings in NAWM would be in favor of Wallerian degeneration. Some studies have reported positive correlations,[19,50,51] while others report no significant correlations.[19,31,33,52–54] To solve this

controversy, further studies with clear histopathology are needed. The results for specific anatomical areas are shown forward in this work.

Although the DTI abnormalities seem to be quite widespread in NAWM, they tend to be more severe as we get closer to the lesion, with more abnormal values in lesions and their periphery than in more distant regions.[3,45] Significantly reduced anisotropy both inside the T2-weighted lesions and in the immediately adjacent NAWM regions was found.[46] These results indicate that the real size of the lesions is often substantially greater in DTI than what is seen in cMRI, showing its higher sensitivity for the detection of tissue microstructural damage.

The sequence of events in lesion evolution is not fully understood and it would be of major interest to establish whether BBB leakage is the initiating event in new lesion formation or a consequence of earlier subtle pathological changes in NAWM. Werring and colleagues[55] performed a longitudinal study in an attempt to address this question. They have demonstrated a steady and moderate increase of MD in pre-lesional NAWM areas followed by a rapid and marked increase at the time of contrast enhancement of the lesion. Although this new pathological activity may develop for many months prior to focal lesion formation, a preexisting pathological process must occur in the NAWM, which can be detected by DTI.

Therefore, the degree of diffusion changes in NAWM measured by DTI may have predictive value about the subsequent lesion activity and evolution.[3] This knowledge may have important implications for understanding lesion pathogenesis, for early treatment intervention and more sensitive treatment monitoring.[8,55]

Table 4: DTI findings in the study of NAWM in MS
1. DTI has higher sensitivity for the detection of tissue microstructural damage
2. Decreased FA
3. Increased MD
4. Both, Wallerian degeneration and primary microscopic lesions, may be responsible for microstructural damage in NAWM
5. DTI study of NAWM may be useful in predicting areas of future lesion

Corpus callosum

Several post mortem studies have demonstrated that the CC is a commonly affected brain structure in MS patients[24] with a lesion frequency of 93%, in this population.[33] In MS, CC is commonly affected by direct inflammatory processes and secondary Wallerian degeneration, resulting in callosal atrophy.[18,19]

The CC is the largest fiber bundle of the brain [3,18,24] and a paradigmatic example of highly organized WM brain structure[24] (Figure 6). Callosal tracts connect cortical and subcortical brain regions,[3,24] being also the connection of both cerebral hemispheres and promoting functional integration of sensory and motor functions.[3,24,56] Callosal structural integrity has also been linked to high-level tasks in various domain, including cognition.[31]

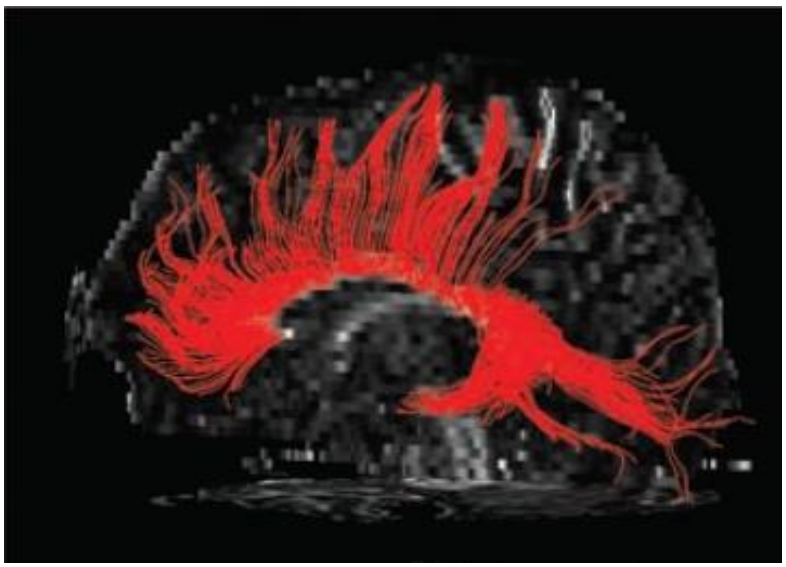

Figure 6: DTI tractography showing 3D projections of CC fibers in a right-handed, 34-year-old healthy man. Figure extracted from reference 37.

Identification and evaluation of occult injury of the CC *in vivo* can provide a better understanding of underlying pathological changes and allow therapy implementation, early in the disease process.

Although CC is a good example of NAWM in normal humans, with high concentration of WM tracts, it was poorly studied in MS patients.[24] Moreover, the reasonably defined borders of the CC limits inadvertent tissue class mixing, which could facilitate the analysis of the NAWM. As a result, the CC is an ideal anatomical structure to evaluate the NAWM in MS patients.[9,24]

DTI in the study of CC

Using DTI, significant differences in CC of MS patients, when compared with selected healthy controls has been demonstrated. One of most common findings is a reduced volume of CC, reflecting callosal atrophy, even in the absence of lesions on conventional MRI.[19,31,33] Significant DTI abnormalities, such as significantly lower FA, higher $\lambda\perp$, $\lambda\parallel$ and MD have been consistently reported in several studies, not only in lesions but also in normal appearing CC (NACC).[7,18,19,24,31,33] However, these abnormalities are more pronounced in callosal lesions, as expected, taking into account the greatest tissue destruction.[19,31]

Regional variation

The human CC has been divided into five anatomical regions, which include from front to back: genu; rostrum; body, often subdivided into anterior, middle and posterior body; isthmus; and splenium (Figure 7).[57] The different callosal regions have different fiber compositions and connect different brain areas, being therefore involved in distinct brain functions. Thus, it is not surprising that when different regions of the CC are considered individually, significant differences in DTI parameters are found among them. Although several

studies have reported conflicting results for some CC regions, a general trend is to find more significant abnormalities in the body of the CC, while genu seems to be relatively spared.[7,24,31]

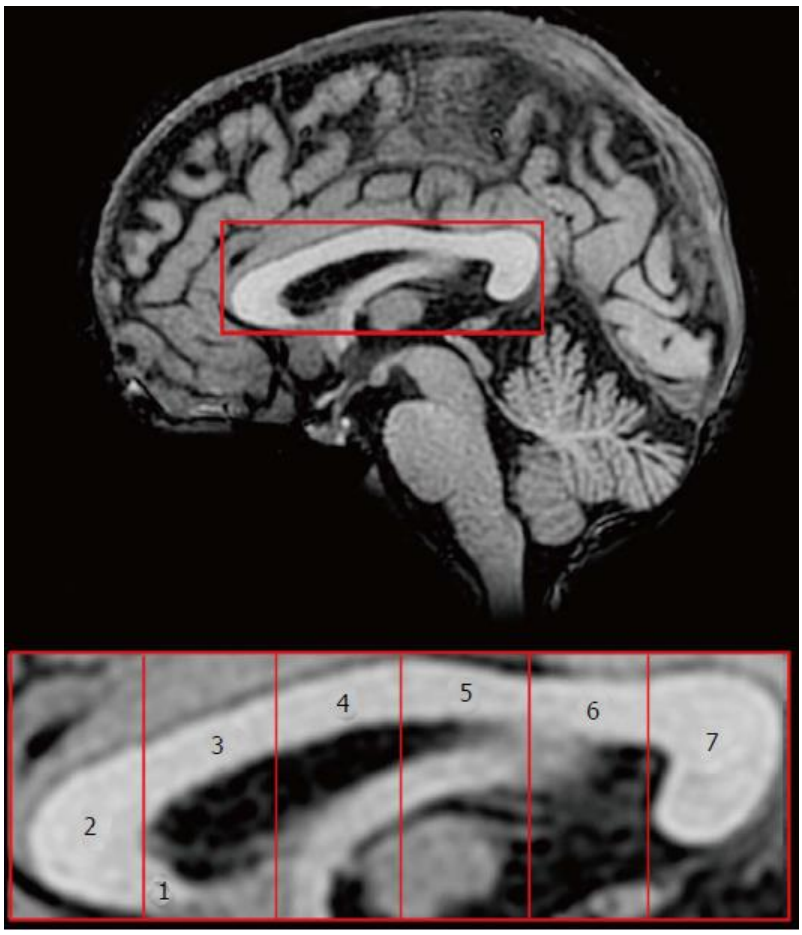

Figure 7: Subdivisions of the human CC. Midsagittal cMRI of the CC (above) and its seven anatomical regions. Region 1: rostrum; 2: genu; 3: anterior midbody; 4: central midbody; 5: posterior midbody; 6: isthmus; 7: splenium. Figure extracted from reference 57.

Cognitive (dys)function

Taking into consideration the central role of CC in brain function, it is expected that callosal abnormalities have a significant clinical impact. There are several methods for evaluating clinical impairment in MS patients, some of them more specific for CC function, as demonstrated in the two studies that are reviewed in detail:

Study 1

Ozturk et al[31] have studied 69 MS patients and 29 matched controls to assess DTI parameters (FA, MD, $\lambda\perp$, and $\lambda\parallel$) in several CC regions, using ROI and DTI tractography. The results obtained were consistent with these previously published for CC. Posteriorly, in order to clarify the clinical meaning of DTI abnormalities for each region, they have applied EDSS and MSFC scores in their MS patients.

The main results of this study show that the anterior body and splenium of the CC contributed most strongly to the association between FA and PASAT-3. On the other hand, the isthmus and splenium contributed most strongly for the association with 9HPT. Further, there was no specific segment of the CC in which FA was associated with T25FW nor EDSS.

From these findings the authors concluded that the projections of the anterior body to some of the frontal areas and subcortical nuclei, that are involved in task performance,[31,57] explain the strongest correlations found in this callosal region, since PASAT-3 requires attention and verbal working memory.[10] The involvement of the splenium, which primarily carries visual information, is more difficult to explain; one possibility is that individuals use an implicit visual representation to solve this task.[31]

Compared to other components of the MSFC, the 9-hole peg test more specifically involves pathways that connect the supplementary motor areas, requiring somatosensory,

motor, and visual circuits, which traverse the corpus callosum.[31] The multimodality nature of this task explains the strongest contribution from isthmus and splenium.[31,57]

EDSS poorly correlated with the DTI parameters analyzed. Since cognitive and noncognitive impairment do not always develop in parallel in MS, and the EDSS is heavily weighted toward simple motor disability, these findings are not particularly surprising since walking is a highly automatic function that may not require the corpus callosum.

Study 2

Another study[33] has also evaluated DTI parameters as well as interhemispheric communication tasks in 16 female MS patients and 16 age and education matched female controls allowing the establishment of clinical correlations. For behavioral evidence of abnormal inter-hemispheric processing, RG and CUD were used.

The main results of this study show that an increased RG for the MS group, outside the normal range, were found. CUD was on the normal range and did not differ between groups.

The authors conclude that these diverging results for RG and CUD suggest that they rely on different callosal mechanisms, or that RG paradigm measure is more sensitive than the CUD measure to pick up small callosal dysfunction in MS.

When looking for significant associations between DTI parameters and these behavioral data, it was found a significant correlation between the increase of RG and the decrease of FA. This correlation was due to transverse diffusivity that explained 27% of RG variance. On the other hand, RG did neither correlate significantly with callosal or total brain lesion load, nor with $\lambda_{\parallel}$.

In resume

From the studies previously analyzed we can conclude that DTI is a more sensitive tool than cMRI in the detection of WM abnormalities in MS patients. A subtle multifocal and diffuse abnormality in the CC, was proven to occur earlier in the disease process and potentially with more severity than in other NAWM areas. This finding may be explained by the fact that CC is the brain's largest fiber tract and connects a wider cortical area.[19]

The understanding of regional variation of CC microstructural abnormalities can be valuable for clinical correlations. However, more studies are needed for a better characterization of regional variation since results are controversial. DTI findings have demonstrated to correlate moderately with clinical scores.

Table 5: DTI findings in the study of CC in MS
1. CC is an ideal anatomical structure to evaluate the NAWM
2. Reduced volume due to callosal atrophy
3. Decreased FA
4. Increased MD
5. Increased $\lambda_{\perp}$
6. Increased $\lambda_{\parallel}$
7. CC is preferential for microscopic injury preceding other NAWM changes
8. Regional variations of damage
9. DTI parameters correlated with clinical scores

Optic nerve and optic pathways

Visual impairment is common in MS patients, affecting up to 80% of patients and being the most common visual syndrome in MS.[58] Approximately 50% of people with MS have an episode of optic neuritis (ON) in their lifetime,[31] being a common clinical presentation of the disease (in 21%),[9] referred as clinically isolated syndrome (CIS) as long as MS is suspected.[9] Although visual dysfunction in MS is commonly related to ON, it can be observed even without prior ON since any path of visual pathway can be affected[31,58,59] (Figure 8).

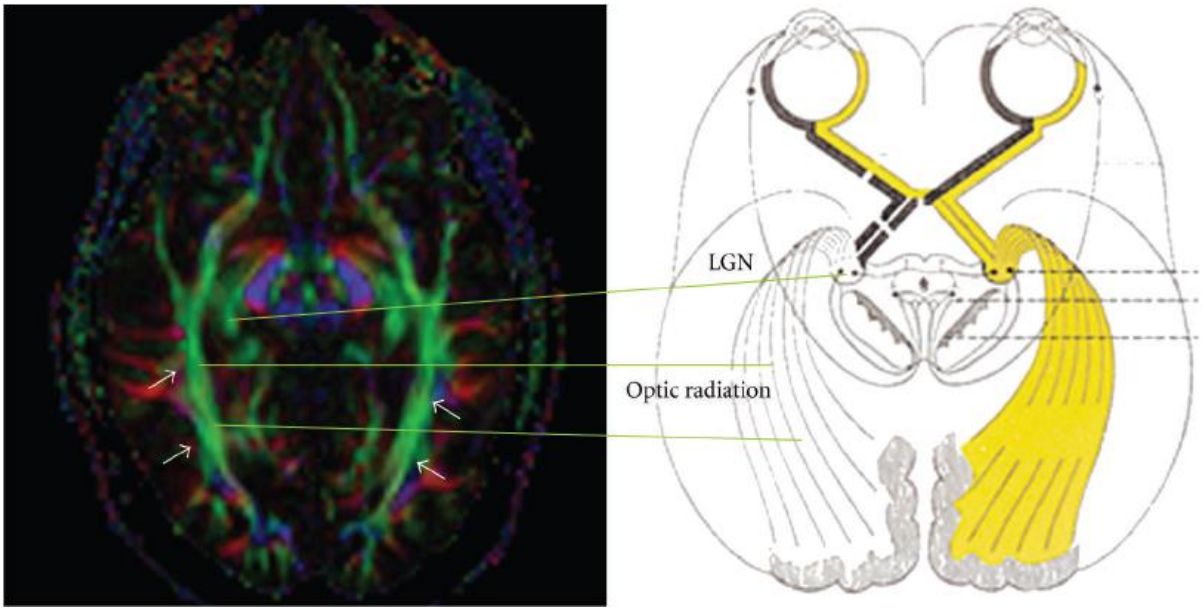

Figure 8: On the left, DTI FA-weighted principal axis orientation map of the optic system in a healthy subject revealing the optic radiation in the occipital lobe (arrows). On the right, the schematic drawing shows the anatomy of the visual pathway, particularly of the optic radiation and lateral geniculate nucleus (LGN). Figure extracted from reference 61.

DTI in the study of optic pathway

Some studies using DTI have been conducted in MS patients in order to evaluate microstructural damage along the optic pathway, relating it with visual impairment. DTI evaluation reveals subclinical abnormalities in many MS patients.[31,58]

Thinning of the retinal nerve fiber layer (RNFL) has been reported[31] and microstructural abnormalities have been found in optic tract: significantly increased diffusivities (MD and $\lambda_{\parallel}$) and mildly reduced FA.[31] Optic radiation has been studied as well, and significant microstructural abnormalities were detected, similar to that found in optic tract: increased diffusivities (MD, $\lambda_{\parallel}$ and $\lambda_{\perp}$) and reduced FA.[58] These DTI parameters are in conformity with those obtained in NAWM of other structures, reflecting chronic tissue injury.

Correlations of DTI parameters within optic pathway

The relationship among DTI parameters in different structures of optic pathway have been studied and interesting results have been found. The RNFL thinning and the loss of total macular volume (TMV) that have been correlated with several abnormalities in the entire optic pathway: lower optic-tract FA was correlated with RNFL thinning and TMV reduction;[31] and the average RNFL thickness was significantly correlated with FA and $\lambda_{\perp}$ in NAWM within optic radiations.[58] Additionally, FA and higher diffusivities in optic radiations were associated with RNFL thinning.[58]

These finding highlight the links between abnormalities in WM and the associated tissue of origin of the axons contained within that WM (in this case, the macula) supporting the hypothesis that Wallerian degeneration plays an important role in NAWM abnormalities. Thus, abnormalities in both RNFL and optic tract reflect damage of the optic nerve, but other factors may play an important role.

Voxel-scale connectivity maps

In an attempt to better understand the underlying phatologic mechanism in patients with ON, Ciccarelli et al used DTI tractography to generate voxel-scale connectivity maps in the optic radiation in 7 patients, 1 year after isolated unilateral ON and in 10 controls.[52] These maps provide, for each voxel in the brain, a scalar value that ranks the degree of connection to a particular seed point. Regions of voxels with the highest voxel-scale connectivity (VSC) are interpreted as defining the WM pathways connected to the seed point.[52]

The main results of this study show that patients had reduced VSC values in both optic radiations when compared with controls. The lesions also showed a reduced VSC value as compared to that of voxels in the adjacent WM. No relationship was found between optic radiation VSC and lesion load, and the VSC of the tract downstream of lesion was not affected by the presence of the lesion itself.

From these findings the authors concluded that the lack of correlation between patients VSC and lesions suggests that optic radiation abnormalities are secondary to the optic nerve damage rather than to local pathology, enhancing the role of optical neuritis and subsequent microstructural changes.

The authors suggest that reduced VSC may reflect a reduction in axonal density and volume of the optic radiation fibers, which originate in the LGN, which may be explained by the mechanism of trans-synaptic dystrophy secondary to optic nerve damage and loss of afferent axons in the LGN.

ON in optic pathway damage

As expected, optic-neuritis history has been associated with thinning of the RNFL in the ipsilateral eye.[31]

In order to access the role of ON in optic pathway damage it is important to correlate microstructural data with previous history of ON. A study on optic nerve[59] reported the lowest FA and the highest diffusivities in optic nerves with previous ON, suggesting diffuse neurodegeneration in addition to prior inflammation. The authors also found that ON plays a major role in the alterations of retinal structure and loss of total macular volume (TMV).[59] In contrast, another study failed to establish a correlations between lower optic-tract FA and ON history.[31]

For a better understanding of this conflicting results it is necessary to take into account that changes in optic nerve can occur even in the absence of overt attacks of ON and yet, secondary damage in the remaining optic pathway may still occur.

Visual acuity

It is of major interest in this patients, the assessment of visual acuity. For this purpose low contrast letters are used.

Low-contrast visual acuity have been related with previous ON (decreased visual acuity at 2.5% and 1.25%contrast)[59] and an impaired visual acuity has also been shown to be related with lower FA and higher diffusivities on optic nerve[58,59] and optic radiation.[58] Optic radiation lesion fraction (but not DTI indices within lesions) was strongly associated with visual acuity scores. Correlations between visual scores and DTI indices (optic radiation specific FA and $\lambda_{\perp}$) demonstrated to be primarily within the NAWM.[58]

In contrast, when visual acuity of patients is accessed and correlated with diffusion indices along the visual pathway, no special correlation was found within the optic tract.[31] The authors that failed to stablish this latter correlation advanced the "clinical-radiological paradox" as a possible reason, suggesting that this is partly because cMRI is more sensitive to inflammation and demyelination than to axon damage, the presumed cause of disability.

In resume

FA and diffusivities are potentially useful quantitative imaging biomarkers of entire optic pathway damage in MS. Such damage is associated with retinal injury and visual disability. ON seems to play a role in visual disability, as well as in microstructural damage to optic pathway structures. However evidences are sparse and more studies are needed.

Table 6: DTI findings in the study of optic nerve and optic pathways in MS
1. Vision impairment is common in MS patients
2. ON is the most common visual syndrome
3. Thinning of the retinal nerve-fiber-layer
4. TMV loss
5. Lower FA and increased diffusivities in optic tract and optic radiation
6. ON is associated with damage along other optic pathway structures
7. There is a correlation between microstructural abnormalities and visual acuity

Spinal cord

Spinal cord injury caused by MS may be associated with a high morbidity and functional incapacity, being its study of utmost importance.

MS commonly involves the spinal cord, as shown by postmortem studies and cMRI studies reporting spinal cord lesions in about 90% of patients with established disease, regardless the clinical evidence of spinal cord involvement.[53]

cMRI has an important role in the evaluation of MS patients with spinal cord involvement,[61] being of great value to detect lesions in patients who do not have clinical spinal cord involvement.[54] Although the degree of such a damage is expected to be associated with the severity of the neurologic deficits, previous cMRI studies have failed to show such correlation.[54,61,62] This is likely to be the consequence of the inability of cMRI to accurately quantify the overall extent of spinal cord damage.[62]

Also, in clinical practice, patients often present with clinically suspected MS, with cMRI findings that fail to meet the criteria for diagnosis.[63] cMRI still lacks of sensitivity in the detection of tissue damage, since MS also affects normal appearing spinal cord (NASC) that was proven to be damaged on histological examination.[54,61,63]

In contrast, DTI is able to provide information about tissue microstructural properties, making this technology potentially more sensitive to detect spinal cord involvement in MS patients than cMRI is.[20,54]

The mechanism by which MS affects NASC is unknown and may be related with Wallerian degeneration, with a primary ischemic/vasculitic process, or with early local demyelination.[63]

DTI in the study of spinal cord

Several studies have applied this technology in the study of spinal cord in MS patients, having achieved promising results. In same way as for CC, spinal cord lesions and NASC are first identified by cMRI, and then several DTI metrics are obtained and compared with matched healthy controls. Under this scope, clinical correlations are also possible to be established.

Applying large ROI and calculating mean DTI parameters of spinal cord, some relevant information has been obtained (Figure 9). The FA average has been demonstrated to be reduced in both lesions and NACS in MS patients as compared with healthy controls.[53,54,63] When studied in more detail, by the ROI method, FA values are shown to be significantly lower in the lesions as compared with the perilesional region, in both NASC and controls. The FA mean values in the NASC were also lower than the values obtained in the control group.[54] This finding is not surprising as tissue destruction, which has been related to lower values of FA, is progressively more pronounced when going from NACS to plaques. Higher MD average values have been reported in MS patients.[20,53] Evidences exists showing that the models that used FA were better than those that used MD.[63]

No significant correlations between DTI parameters and lesion load on T2-weighted imaging have been reported.[53,54] In addition no diffusion differences were found between the NASC of patients with and without spinal cord lesions.[54] However, as expected, diffusion measurements are significantly different in the spinal cord plaques as compared with the measurements of the control subjects,[1,54] and cMRI also consistently reports a reduced cross sectional area of spinal cord.[20,53]

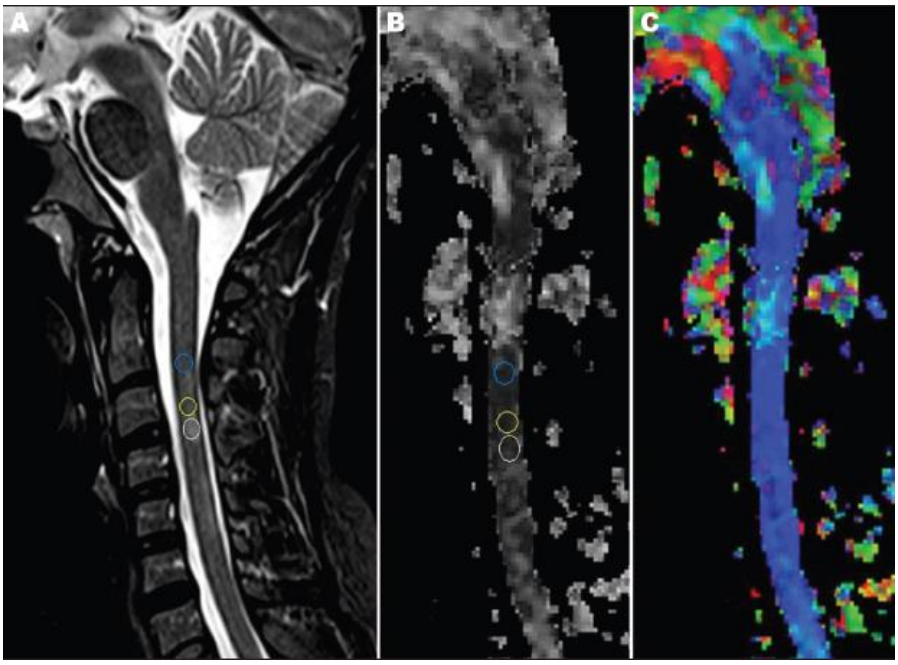

Figure 9: 32-years-old female patient with MS. Cervical spine cMRI showing sagittal STIR (A), FA map (B) and FA color map (C). In A and B, the placement of the ROI is demonstrated in the plaque (white), NASC around the plaque (yellow) and NASC more than 1cm from the plaque (blue). Figure extracted from reference 64.

Regional variation

Spinal cord contains several tracts carrying relevant information of distinct systems, such as motor, sensory, and autonomic systems. DTI imaging of spinal cord, able to study tissue microstructure, has particular advantages, allowing to study the extent of lesion in different anatomical regions and to elucidate structure-function relationships.[1]

This kind of study in MS patients may be of great value once demyelination may have a propensity for specific spinal tracts, depending on clinical state.[1,63] For example, lesions in primary demyelination have a predilection for the posterior columns (PCs) of the spinal cord. For this purpose it is significantly more accurate to determine DTI parameters in ROIs

representing specific spinal cord anatomical areas, rather than representing the entire spinal cord transverse area.[63]

Nevertheless, some studies have failed to demonstrate significant FA differences among different anatomical regions.[63,64]

Clinical correlations:

Spinal cord injury often leads to substantial MS-caused disability.[1] Weakness and loss of proprioception can impair ambulation and diminish functional independence.[1]

Several studies have tested DTI use in predicting clinical disability and the results are promising. In a study involving 10 RRMS patients,[20] cervical spinal cord average FA and MD were measured and the correlation with EDSS was assessed. Although no correlation between EDSS and average FA was found, MD showed a moderate correlation.

Results of DTI analysis of discrete spinal cord tracts, as opposed to the study of average parameters, were shown to correlate with specific clinical functions carried by these tracts.[1]

Herein, Naismith et al[1] have studied specific tracts of the spinal cord - PCs and lateral corticospinal tracts (LCTs) - by measuring DTI parameters and relating them with clinical scores, in MS patients. Their main hypothesis was that DTI, as an imaging biomarker of tissue integrity, could differentiate the level of residual function in patients with remote inflammatory spinal cord disease.

Vibratory sense was reduced in MS patients and was specifically related to abnormal $\lambda_{\perp}$ and FA in PCs, and no correlation was observed with DTI parameters within LCTs. Additionally, more integrative neurologic functions tested by 9HPT, 25FTW, and EDSS showed a consistent relationship with $\lambda_{\perp}$ and FA within both PCs and LCTs. These results are consistent with what is expected, taking into account that vibratory sense is conveyed by PCs

of spinal cord, while complex tasks require both PCs and LCTs. Moreover, for 9HPT and T25FW, combined PC and LCT injury was more frequent in individuals with more disability, as compared with when only one tract was affected.[1] Results of DTI analysis of discrete spinal cord tracts were shown to correlate with specific clinical functions carried by these tracts.

In resume

Taken together these findings suggest that DTI can be used as an imaging biomarker of spinal cord tissue injury at the tract level and that lesion extent is related with disability. Thus DTI is a promising tool in predicting clinical course and monitoring disease progression. In order to support its application in clinical practice, further investigation is still necessary.

Table 7: DTI findings in the study of spinal cord pathways in MS
1. Reduced cervical cord cross-sectional area
2. Reduced FA
3. Increased MD
4. DTI metrics do not correlate with number of T2-visible lesions in the cord
5. No significant regional variations
6. DTI metrics correlate with clinical disability
7. DTI as an imaging biomarker of spinal cord tissue injury at the tract level

NAGM

The study of WM, although being of great value, still does not entirely correlate with clinical manifestations and patient disability.[65] This may be due to the fact that MS is a diffuse disease, affecting not only WM but also GM. Indeed, postmortem and *in vivo* studies have demonstrated that the deep GM is also affected in MS.[65]

Also, diffuse microscopic damage in NAGM can be present in the thalamus and basal ganglia,[65] namely decreased FA and increased MD, indicating the lack of sensitivity of cMRI to the study of GM.

Though still in a lesser extent than for WM, some research on application of DTI in GM has been carried out and promising results are emerging.

Thalamus

Thalamus can be seen as a convergence zone of the brain, densely interconnected with most cortical regions, that conveys a variety of brain functions including motor, sensory, and cognitive abilities.[65] It consists of a mixed-composition of GM and WM (the former representing approximately 5% of its content).[66] Despite this minor WM content, it is usually included in the study of GM.

DTI in the study of Thalamus

Several pathologic and DTI studies have demonstrated consistently that thalamic involvement is, from the earliest stages of the disease, a prominent feature of MS.[66]

DTI-derived metrics were sensitive to detect thalamic damage: an increased FA, as well as increased MD were reported.[65,66] These results may suggest thalamic atrophy possibly related

to demyelination and/or axonal damage. However, histopathological studies are needed for a better understanding of these findings.

Correlations were found between DTI-derived metrics in the thalamus and remote WM damage reflected by T1 and T2 lesion volume or brain-parenchymal fraction.[65,66] One plausible hypothesis is that structural damage to WM networks could induce trans-synaptic axonal degeneration within thalamic nuclei (Wallerian degeneration).

Clinical correlations

Because of its abundant neuronal connections, both direct and indirect microstructural damage to thalamic nuclei could be intimately related with functional disability in MS.[66] Therefore, DTI implementation of thalamic study could potentially explain part of patients' disabilities and be a valuable tool in predicting disease progression.

This was the hypothesis tested by Tovar-Moll et al[66] by the application of cMRI and DTI in normal appearing thalamus (NAT) in a group of 24 MS patients with 24 healthy volunteers age- and sex-matched.

By the application of EDSS and PASAT scores, physical and cognitive ability of patients were assessed and correlated with DTI parameters. In the overall, patients with RRMS had relatively mild disability, suggesting that subtle thalamic damage can be present in patients with mild disease.

In patients with RRMS, thalamic involvement, mainly MD increase, explained patient cognitive and motor disability in a large extent. On the contrary, no significant correlations between clinical scores and DTI-derived metrics were observed in the SPMS group.

S. Mesaros et al[65] have also brought up the question of the applicability of assessing damage to the thalamus by DTI to convey prognostic information. For that purpose, they have

studied 54 PPMS patients by cMRI and DTI at baseline and after a mean follow-up of 15 months.

At baseline, patients experience a significant atrophy, as well as, DTI microstructural abnormalities of thalamus, when compared with healthy controls. When evaluated for diffusional changes, an increase of thalamic MD and a progressive decrease of thalamic FA were observed, but only this latter was significantly different between patients and controls.

In resume

There are still few studies focused on DTI application in thalamus characterization in MS patients. These preliminary findings suggest that microstructural damage is present early in the disease progression. Furthermore, DTI may correlate with disability and may have prognostic value. It should be noted that more studies about this issue are needed.

Table 8: DTI findings in the study of thalamus in MS
1. Thalamic atrophy
2. Increased FA
3. Increased MD
4. DTI abnormalities at early stages of disease
5. Mild clinical correlations
6. DTI may have a prognostic value

Caudate nuclei

The caudate nuclei (CN), one of the components of the basal ganglia, is involved in fine motor and cognitive functions. Thus, DTI study of caudate nuclei seems promising regarding the possibility of establishing clinical correlations.

Hasan et al[67] have investigated the value of CN macro and microstructural metrics as markers of GM degeneration in healthy adults and MS patients. They have focused their attention on NACN. This study demonstrated that both the caudate nuclei volume (CNV) and their volume ratio relative to the intracranial volume, decrease with age, in both men and women, in the healthy and MS groups, but to a bigger extent in this last group, reflecting a general trend of atrophy of deep and cortical GM in healthy controls. Also, consistently with what happens in other GM studies,[65] strong and significant correlations between whole brain lesion load and caudate volume ratio, relative to the total intracranial volume, were noted.

A high caudate FA, along with increased MD, compared to the age-matched adult controls was also observed. These preliminary findings indicate that caudate DTI-derived metrics can serve as potential quantitative radiological markers of MS pathology. However more studies are needed to support this hypothesis.

Table 9: DTI findings in the study of caudate nuclei in MS
1. Caudate atrophy
2. Correlations between CNV and whole brain lesion load and whole brain CSF fraction
3. Increased FA
4. Increased MD

DISCUSSION

DTI has been increasingly applied to the study of MS patients due to its ability to detect and quantify disease-related changes of the tissue microstructure within NAM and T2-visible lesions of both grey and white matter. Throughout this work, various applications of DTI in MS patients were reviewed, and results have shown great potential for the application of this imaging technology in future clinical practice.

DTI has been capable of detecting tissue microstructural abnormalities in NAM, even in early stages of disease,[9] showing greater sensitivity than cMRI in the assessment of the disease extent.[3,38,44–47] Therefore, DTI may contribute for an early diagnosis of MS-suspected patients, namely those presenting CIS, allowing an earlier treatment implementation.

In addition, tissue microstructural damage, as shown by DTI abnormalities, significantly correlated with clinical cognitive and motor scores,[1,20,31,33,66] having also a prognostic value.[65] These findings highlight the role of microstructural damage in disability, which supports the DTI implementation as a sensitive tool for patients monitoring.

Despite the promising results, DTI still is a developing technology, and there are some limitations in the applications of DTI in MS patients that must be addressed.

MS may present different clinical phenotypes and distinct disease courses, being also expected different patterns of tissue injury. If different disease phenotypes are not taken into account, this heterogeneity among patients may hamper results comparison.

Some studies have explored DTI parameters characteristics of MS disease phenotypes and significant differences have been reported, reflecting variable severity of pathological changes among them.[68] Yet, few studies show strong evidence with respect to this issue. Whereby, fundamental differences among disease phenotypes are not presently stablished.[69] In

the matter of correlation between DTI parameters and disability scores, there were no clear distinctions among the different MS subtypes, as well.[69] More studies in an attempt to address this issue are needed.

DTI studies still are affected by several technical constraints, including acquisition schemes, image analysis, and post-processing techniques. With regard to acquisition, it has been shown that pulse sequences and the use of different scanners influence the measurements of quantities derived from MR imaging.[70] Echo-planar imaging (EPI) is a pulse sequence that acquires image data in a very short time, thereby freezing any patient motion.[70] Because patients motion is a particular problem in MS patients, EPI is the most widely used acquisition method for DTI studies. Nevertheless, this pulse sequence because of the rapid acquisition, suffers from lower resolution than cMRI. This problem is easily overcome by the complementation of DTI study with higher resolution cMRI scans.[70]

The use of differing methods may hamper results comparison and knowledge integration[71] Standardization of these technical approaches to DTI would allow for a substantial improvement of the ability to compare results between studies, and obtain a better knowledge integration. However, the best methods for DTI studies of MS patients remains a matter of debate.[37,70]

Once acquisition is performed and the diffusion tensor is estimated, indexes can be calculated and further analyzed by using several approaches, such ROI-based analyses, TBSS and DTI tractography.[12,70,72]

Several of the studies analyzed in this work used a ROI based analyses. This post-processing technique has special interest when the study is aimed to a specific normal appearing brain structure, such as specific columns of the spinal cord and CC regions, or lesions identified by cMRI.[70] ROI may be outlined manually or semi-automatically by expert observers and then

superimposed on the DTI parameter maps in order to calculate the average properties within each ROI.[70] However, this technique has some limitations, including a very poor reproducibility of ROI positioning between different study subjects,[26,70] which means that slight changes in ROI position can result in large changes of DTI metrics. Additionally, using ROI-based analysis, only a limited number of specific regions can be examined.[54,73,74]

TBSS enables DTI data analysis in a voxel-wise fashion minimizing multi-subject registration errors by carrying out the analysis in a common skeleton of major WM structures.[10] This high reproducible post-processing technique allows for a voxel-wise assessments of changes in diffusion metrics, without identifying a specific anatomical target.[54,73] TBSS however, cannot reliably estimate and interpret the voxel-wise statistics at the crossing regions of fibers due to the inherent limitations associated with DTI.[70]

The DTI tractography is a highly sensitive imaging technique for the representation and visualization of WM.[7] DTI tractography has been shown to be a robust tool to analyze WM structures, such as spinal cord and CC, being more reproducible and reliable compared with an ROI-based analysis to evaluate the diffusion measures.[54] Moreover, some limitations of DTI tractography still need to be overcome by further technical developments. DTI can only resolve a single fiber orientation within each imaging voxel.[75] WM fiber crossing, bending, or twisting, as well as, focal diffuse alteration of tissue organization, result in a decreased FA and a consequent increase in uncertainty of the primary eigenvector of DTI.[37,76] Consequently, the low FA can erroneously terminate the tracking algorithm or cause a deviation of the bundles at those sites, thus limiting the application of DTI tractography.[26,70,76]

To overcome the limitations of DTI-based fiber tractography and its derived metrics, more complex models have been used leading to the development of methods beyond DTI, such as high angular resolution diffusion imaging (HARDI)[76] HARDI is a sampling strategy that acquires diffusion along different directions with a high angular resolution.[26,70] The resulting

3D representation of diffusivity can be decomposed into a set of orthogonal 3D functions, such as spherical harmonics, providing information about orientations of multiple axonal fiber populations at each voxel.[26,70,75] After acquisition, HARDI signal can be reconstructed using approaches such as Q-ball or spherical harmonics decomposition, in order to obtain the orientation distribution function at each voxel and so allowing fiber tracking.[26,70]

These and other innovative techniques for the study of CNS are under development, showing great promise for the study of patients with neurodegenerative diseases such as MS.

CONCLUSION

DTI evidences of microstructural abnormalities in NAM demonstrate its higher sensitivity relatively to cMRI, showing possible benefit in its use as a complementary technique in the study of MS patients. Being DTI more sensitive to MS-related damage than cMRI, a more accurate differential diagnosis and earlier diagnosis may be possible when using DTI, allowing earlier and more targeted therapeutic intervention.

The relationship between DTI metrics and clinical manifestations will potentially allow clinicians to better correlate fiber tract disruption and MS symptoms, such as cognitive impairment. Furthermore, it would ultimately lead to improved monitoring of patients, better prediction of the course of the disease, and more rapid assessment of new treatments.

However, in order to better understand the possible applications of DTI in the study of MS patients and to overcome some of its limitations, more studies are needed.

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