Directional diffusivity changes in the optic nerve and optic radiation in optic neuritis

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Objective: Optic neuritis (ON) is defined as an inflammation of the optic nerve and provides a useful model for studying the effects of inflammatory demyelination of white matter. The aim of this study was to assess the diffusion changes in both the optic nerve and optic radiation in patients with acute and chronic ON using diffusion tensor (DT) MRI.

Methods: 33 patients with idiopathic demyelinating optic neuritis (IDON) and 33 gender- and age-matched healthy controls were examined with DT-MRI and with T_1 and T_2 weighted MRI.

Results: Compared with controls, both first-episode and recurrent patients with IDON in the acute stage showed significantly increased radial diffusivity ($\lambda_{\perp}$) and decreased mean fractional anisotropy (FA) in the affected nerves. Reduced FA, increased $\lambda_{\perp}$, mean diffusivity (MD) and axial diffusivity ($\lambda_{\parallel}$) were determined in patients with subacute IDON. We found no significant difference in the directional diffusivity of optic radiation in patients whose disease had lasted less than 1 year compared with healthy controls. However, significant changes in the FA and $\lambda_{\perp}$ of the optic radiation were detected in patients with disease duration of more than 1 year.

Conclusion: These results show the great potential and capacity of DT-MRI measures as useful biomarkers and indicators for the evaluation of myelin injury in the visual pathway.

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Optic neuritis (ON) is an inflammatory disease of the optic nerve [1]. Acute idiopathic demyelinating optic neuritis (IDON) is an early and frequent manifestation of multiple sclerosis (MS), an illness whose pathology is characterised by the development of multifocal demyelinating white matter lesions. The optic nerve, as a part of the central nervous system (CNS), transmits visual information from the retina to the brain. Its function can be assessed quantitatively in a very reliable manner by clinical and electrophysiological measurements. Hence, studying IDON offers a unique opportunity to explore the pathophysiology of inflammatory demyelination in the CNS.

Optic nerve sheath dilation can be detected using conventional T_2 weighted MRI, as has been reported by Hickman et al [2, 3]. These two studies assessed the effects of a single inflammatory process and its accompanying demyelination in a cohort of patients during their first episode of acute unilateral ON. The authors reported a consistent pattern of changes associated with demyelination lesions caused by inflammation in the optic nerve. It is of great clinical importance to determine prodromal changes and the underlying pathological

mechanisms in patients with ON. However, as the hyperintensity can be a result of inflammation, gliosis or axonal degeneration, T_2 weighted images fail to identify the cause underlying the pathology. Diffusion tensor (DT)-MRI, a widely recognised imaging technique that identifies the dominant direction of water diffusion and the magnitude of anisotropy *in vivo* [4], has recently gained more prominence for the investigation of white matter structure, integrity and connectivity. The demyelination damage in the optic nerve and optic radiation can be located with the help of DT-MRI parameters, such as mean diffusivity (MD), fractional anisotropy (FA), axial diffusivity ($\lambda_{\parallel}$) and radial diffusivity ($\lambda_{\perp}$) [5, 6].

Post-mortem examination of MS patients suggests that the pathological mechanisms of ON may include a combination of inflammation, demyelination, astrogliosis and axonal destruction [7]. Studies of mouse optic nerves after retinal ischaemia have revealed subtle changes of axons and myelin in the white matter, and found $\lambda_{\parallel}$ and $\lambda_{\perp}$ values to be associated with axonal pathological changes [8, 9]. These results suggest DT-MRI to be superior to other conventional imaging techniques for exploring the pathological mechanisms of ON. Particular challenges associated with DT-MRI of the optic nerve are the small diameter of the nerve and the mobile structures that are surrounded by cerebrospinal fluid (CSF) and orbital fat [10, 11]. In light of this, and despite using different sequences and protocols, it is remarkable that several different groups have

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reported similar values in healthy controls (MD $1.0\text{--}1.3 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ and FA 0.4–0.6) and altered diffusion parameters in chronic ON patients [12, 13].

The different developmental stages of ON seem to be associated with different pathological mechanisms. The acute stage is characterised by inflammation and possibly demyelination of the optic nerve. The chronic stage, on the other hand, typically shows axonal damage, possibly even axonal death leading to atrophy of the optic nerve [14–16]. Increased MD and decreased FA were observed in a heterogeneous cohort of patients with chronic ON [17]; increased apparent diffusion coefficient (ADC) values were found especially in chronic patients [18, 19]. A study closely related to our work showed axial diffusivity $\lambda_{\parallel}$ in the acute stage to provide important prognostic information and the radial diffusivity $\lambda_{\perp}$ in the subacute stage to represent the best measure correlated with the visus [20]. A recent study proved tractography to be a method sensitive enough to detect pathological abnormalities in the optic radiations after ON [21].

Understanding the connection between altered diffusion parameters in the optic nerve, optic radiation and visual performance will provide insight into the underlying pathological mechanisms and may be valuable in predicting visual development after ON. The previous studies mentioned above have shown different pathological mechanisms during the different stages of ON. We were interested in confirming these findings by DT-MRI, a novel and sensitive methodological approach. We hypothesised that the pathological changes occurring during the illness might impact the diffusion indices differently, and therefore that we might find differing diffusion values in the acute and subacute stages of ON based on the final clinical diagnosis.

Methods and materials

Patients

33 patients who fulfilled the clinical criteria set by the Optic Neuritis Study Group [22, 23] were recruited from May 2008 to December 2008 at the Beijing Tongren Hospital. The patient group consisted of 12 males and 21 females from 10 to 58 years (mean age, 31.1 ± 12.8 years).

Table 1. Demographic and clinical characteristics of patients with idiopathic demyelinating optic neuritis

Characteristic	
Gender (number)	
Male	12
Female	21
Age (years)	
Median	31.1
Range	10–58
Stage of disease (cases)	
Acute	33
First	26
Relapse	7
Subacute	18
First	6
Relapse	12

The demographic data assessed during the MRI study are included in Table 1. 33 gender- and age-matched healthy controls (12 male and 21 female, mean ages 29.21 ± 12.09 years; range, 10–60 years) with normal neurological examinations and no history of neurological disorders served as controls.

In this paper, we will further refer to the individual eyes of the subjects as cases; altogether the study included 51 cases in the patient group. In 33 cases with acute IDON, we managed to obtain the MRI data within 30 days of the onset of symptoms. 26 of these cases were first manifestations of the acute form of ON, the other 7 were recurrent. We defined the ON as acute if a patient experienced an episode of ON within 30 days from the onset of visual symptoms as defined in previous studies [20, 22, 24]. In 18 cases with subacute IDON, the MRI data were acquired more than 30 days after the onset of symptoms. 6 cases were first episodes and 12 recurrent episodes. At the same time, we selected 9 subjects whose disease had lasted longer than 1 year and 14 subjects who had had symptoms for less than 1 year to investigate the secondary effects of ON.

The study was approved by the ethics committee of the Beijing Tongren Hospital and a written informed consent was obtained from each subject according to the Helsinki Declaration.

Data acquisition

All measurements were performed on a 1.5T Signa MRI system (General Electric, Milwaukee, WI). Head motion was minimised by the restraining foam pads provided by the manufacturer. Subjects were asked to close their eyes in order to minimise any effects of deliberate eye movement during the acquisition time.

Each subject was scanned using a high-resolution T_2 weighted fluid-attenuated inversion recovery (FLAIR) sequence (repetition time (TR)=9000 ms, echo time (TE)=120 ms, time interval (TI)=2125 ms, field of view (FOV)= $24 \times 21 \text{ cm}^2$, matrix size 256×222 , 32 slices, 4.0 mm slice thickness with 0.8 mm interslice gap) in order to detect any brain abnormalities. At the time of the optic neuritis, the patients had no significant image deterioration or other signs of neurologic lesions in the optic radiation.

Figure 1. Position of the slices viewed on an axial localiser view of the optic nerve. There are eight slices from the anterior part (adjacent to the optic papilla) to the posterior part (near the orbital apex) of the optic nerve.

The images of the optic nerves were obtained with an eight-channel head coil using a coronal-oblique spin-echo echo planar imaging (SE-EPI) sequence with parallel acquisition. The coronal-oblique slices were set orthogonal to the nerves (Figure 1). The covering range was from the optic papilla to the orbital apex of the optic nerve. We used the following acquisition parameters for the optic nerve: one b_0 and six non-collinear gradient directions with $b=600 \text{ s mm}^{-2}$, $\text{FOV}=22 \times 22 \text{ cm}^2$, matrix size 128×128 , number of excitations (NEX)=16 and eight contiguous 5.0 mm slices. By focusing solely on the optic nerve, the signal:noise ratio (SNR) of images was set at 35–40. The diffusion acquisition parameters of the optic radiation were as follows: 1 b_0 and 15 non-collinear directions with $b=1000 \text{ s mm}^{-2}$, $\text{TR}=6000 \text{ ms}$, $\text{TI}=71 \text{ ms}$, $\text{FOV}=24 \times 24 \text{ cm}^2$, matrix size 128×128 , NEX=6 and 22 contiguous 4.0 mm slices. In addition, a whole-brain three-dimensional (3D) T_1 spoiled gradient-echo sequence (SPGR) ($\text{TR}=10 \text{ ms}$, $\text{TE}=4.4 \text{ ms}$, $\text{TI}=600 \text{ ms}$, $\text{FOV}=26 \times 26 \text{ cm}^2$, matrix size= 256×256 , NEX=1 and 152 contiguous 1.0 mm slices) was used as a source image for the subsequent co-registration of the optic radiation.

Data processing

The first step was to validate the quality of the raw images. The images with insufficient quality were re-scanned until they met the SNR criteria set for the analysis. Then, eddy current distortions and motion artefacts in the DT-MRI data were corrected by applying affine alignment, using FMRIBs diffusion toolbox (FSL, Oxford, UK.) [25, 26]. In order to be able to compare diffusion properties in patients and controls, a method to identify corresponding anatomical regions was required.

The first necessary step was to find a consistent spatial normalisation for the two separate groups. Because of the different protocols for optic nerves and optic radiations, we present two different methods for the processing of the respective MRI data, and describe these methods in the following sections.

Optic nerve fibre

Image registration

The maps of MD, FA and eigenvector were calculated on a voxel-by-voxel basis. The reconstructed tensor matrix was then diagonalised in order to obtain eigenvalues (λ_1 , λ_2 and λ_3) and eigenvectors using DTIStudio (MRI Studio Software, Johns Hopkins University, Baltimore, MD). To be able to correct for global morphological differences, a grand average b_0 data set was created from all subjects. This average image was then used as a reference relative to which each subject was positioned (individual b_0 to template b_0) with a 12-parameter affine model. The same transformation parameters were then used to co-register the MD and FA images to the template b_0 .

Regions of interest selection

The intraorbital fourth layer of the nerve (about 2.0 cm after the eyeball) was used for the following analysis (Figure 2). The region of interest (ROI) were defined manually on the b_0 -template (average non-diffusion weighted) consisting of two square 2×2 voxels in the same way as Trip et al [17] (Figure 2a). To avoid bias caused by the partial volume effect, the ROIs were placed in the centre of the nerve. After averaging the images across the population, the b_0 images contained significantly less

Figure 2. Region of interest (ROI) selection in the fourth slice of the optic neuritis (ON). (a) Non-diffusion-weighted b_0 image, (b) colour map, (c) fractional anisotropy (FA) map and (d) mean diffusivity (MD) map. The arrows point to the optic nerve. The ROIs were placed on the b_0 -averaged images and then transferred onto the FA and MD maps.

noise than the individual images. The associated colour-coded maps were used for optimal ROI placement (Figure 2b). To guarantee objectiveness, ROI selection was performed by an experienced radiologist blinded to the identity of the subject. The ROIs of individual cases were mapped from the template b_0 using an inverse transformation. These ROIs were then overlaid to the MD, FA and eigenvalue maps, where mean values from the four voxels were obtained (Figure 2c,d).

Optic radiation fibre

All tracts in the data were reconstructed using a fibre assignment-continuous tracking algorithm [6]. In order to minimise the anatomical brain variability between

subjects, a group-based atlas framework was introduced constructing a population-specific template. We applied the joint analysis framework for group-based co-registration combining structural and diffusion tensor MRI, as did Tao et al [27], but with the diffeomorphic anatomical registration using exponentiated lie (DARTEL) algebra registration method [28]. As a high-dimensional diffeomorphic registration method, this novel technique utilises the large deformation framework parameterised by velocity vector fields to preserve topology. The sum of square differences between the source and target images are minimised simultaneously to the registration, and the linear elastic energy of the deformations are used to warp the target image, which can improve the realignment performance of small inner structures [29, 30].

Figure 3. Optic radiation analysis pipeline jointly using structural and diffusion images. DWI, diffusion-weighted imaging; DARTEL (diffeomorphic anatomical registration using exponentiated lie algebra); GM, grey matter; WM, white matter.

Combined DT-MRI and structural analysis pipeline

Group template maps were created using statistical parametric mapping (SPM8, Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK) to determine the normal intersubject variability of white matter pathways. We built a structural atlas from all subjects' T_1 images with the DARTEL toolbox. After this step, the b_0 -volume of each DT-MRI data set was registered to the associated T_1 image using a 12-parameter affine transformation. The corrected diffusion images for each subject were algebraically transformed to compose a structural atlas space, enabling anatomical identification and comparison of the optic radiation in the aforementioned atlas space. An overview of the procedure for detecting differences between the diffusion properties of fibre tracts is shown in Figure 3.

Regions of interest selection

After the atlas construction procedure, we used a multiple ROI approach to exploit the fibre tract. The starting ROI was manually placed in the lateral geniculate body on a reconstructed axial image. It was designed to include the optic radiation of each side and the entire surrounding white matter. For each tract, a second spherical ROI with a 4 mm radius was placed in the occipital lobe near the midline. Reconstructed fibres penetrating both ROIs were considered representative of the optic radiation (Figure 4). These two ROIs were then overlaid on the FA maps and transferred to other directional diffusivity maps. Diffusivity in all spatial directions was obtained from every voxel along the route of the optic radiation.

Statistical analysis

All statistical analyses were performed using SPSS v13.0 (SPSS Inc., Chicago, IL). In a first step, ipsilateral differences between patients and controls were explored using a paired t -test. To avoid the bias arising from the classification of the bilateral involved nerves of one patient being at the same ON stage and in the same group, we applied the generalised estimating

equation (GEE). This method, introduced by Zeger et al [31], extends generalised linear models to accommodate correlated data from subjects with similar characteristics.

Results

Directional diffusivity of the optic nerve

Acute optic neuritis

The study consisted of 33 acute-stage cases: 26 first manifestations and 7 recurrent cases. Significant differences within the ROIs were detected in all DT-MRI measurements (except $\lambda_{\perp}$ in recurrent ON) when the two subgroups of patients and their controls were compared (paired t -test, Table 2 and Figure 5). The mean FA was significantly reduced and the mean MD and $\lambda_{\perp}$ were increased in the acute-stage IDON cases compared with healthy controls. In patients with first manifestation, significantly decreased $\lambda_{\parallel}$ values were detected ($t=2.10$, $p=0.046$). Increased $\lambda_{\parallel}$ were found in recurrent patients compared with controls, but this difference was not significant ($t=0.84$, $p=0.434$). As there were only seven cases with a recurrent disease history, we decided to evaluate only the first manifestation group and the matched healthy group using GEE (Table 3). This study proved the mean FA from ON patients to be significantly lower than that in healthy controls ($z=61.053$, $p < 0.001$). Compared with healthy controls, we found drastically elevated $\lambda_{\perp}$ ($z = 19.181$, $p < 0.001$) in the patients and slightly decreased $\lambda_{\parallel}$, but the latter did not reach statistical significance ($z=3.414$, $p=0.065$).

Subacute optic neuritis

The DT-MRI data from 18 remitting cases are illustrated in Table 4. Cases with both first manifestation of IDON (paired t -test, $n=6$) and recurrent IDON (paired t -test, $n=12$) showed a similar and significant trend, with reduced FA values and increased MD, $\lambda_{\parallel}$ and $\lambda_{\perp}$ when compared with controls (Table 4). However, there was

Figure 4. Diffusion tensor (DT)-MRI fibre tracking and extraction of optic radiation. DT-MRI fibre tracks (green) were launched from a starting region of interest (white box) in a plane posterior to the lateral geniculate nucleus. Fibre tracks were filtered with a second region of interest (two balls) in a plane adjacent to the visual cortex.

Table 2. Diffusion parameters during the acute phase of IDON (first and recurrent onset cases)

Indices		Acute	Controls	t-value	p-value
FA	First	0.39 ± 0.08	0.59 ± 0.09	8.40	0.000
	Recurrent	0.33 ± 0.05	0.64 ± 0.11	7.46	0.000
MD	First	1.50 ± 0.20	1.40 ± 0.30	2.22	0.036
	Recurrent	1.80 ± 0.28	1.20 ± 0.36	3.54	0.012
$\lambda_{\parallel}$	First	2.18 ± 0.31	2.39 ± 0.45	2.10	0.046
	Recurrent	2.50 ± 0.31	2.27 ± 0.64	0.84	0.434
$\lambda_{\perp}$	First	1.10 ± 0.20	0.80 ± 0.27	5.40	0.000
	Recurrent	1.50 ± 0.28	0.70 ± 0.28	5.45	0.002

33 IDON cases were in the acute stage (disease duration from examination to last onset day less than 1 month), of which 26 cases in 19 subjects were first affected and 7 cases in 7 subjects were suffering recurrent episodes. Axial ($\lambda_{\parallel}$), radial ($\lambda_{\perp}$) and mean diffusivities (MD) are given in $\mu\text{m}^2 \text{ms}^{-1}$. Fractional anisotropy is without units. All values of DTI indices are given as the mean $\pm$ standard deviation. IDON, idiopathic optic neuritis; FA, fractional anisotropy.

no significant difference in $\lambda_{\parallel}$ ($t=2.46$, $p=0.057$) between subacute first-episode IDON patients and controls. We suspect that this may be owing to the small sample size ($n=6$).

Directional diffusivity of the optic radiation

The DT-MRI examination of the optic radiation was performed on 23 patients. 9 patients whose disease had lasted from 1 year to 13 years and 14 patients whose

Figure 5. Quantitative analysis of diffusion tensor (DT)-MRI indices in optic neuritis. (a) fractional anisotropy (FA), (b) mean diffusivity (MD), (c) axial diffusivity ($\lambda_{\parallel}$) and (d) radial diffusivity ($\lambda_{\perp}$) in each optic nerve from controls and cases of first onset during the acute stage (expressed as range (red box), mean (horizontal line) $\pm$ standard deviation (T-bars)). Paired t -tests demonstrated that MD and $\lambda_{\perp}$ were significantly elevated and FA was notably reduced in affected nerves.

Table 3. The GEE-determined diffusion indices from the 26 cases of first-onset acute-stage optic neuritis in 19 patients compared with controls

Indices	Parameter estimation	Standard deviation	z-value	p-value
FA	-0.201	0.026	61.053	0.000
MD	0.137	0.000	3.253	0.071
$\lambda_{\parallel}$	-0.208	0.000	3.414	0.065
$\lambda_{\perp}$	0.309	0.000	19.181	0.000

The values of axial ($\lambda_{\parallel}$), radial ($\lambda_{\perp}$) and mean (MD) diffusivities are given in $\mu\text{m}^2 \text{ms}^{-1}$. Fractional anisotropy (FA) is without units. All values are expressed as the natural logarithm of the ratio between controls and patients with IDON in the acute stage. GEE, generalised estimating equation; IDON, idiopathic optic neuritis.

Table 4. Diffusion indices from cases during the subacute phase of IDON (both first and recurrent onset)

Indices		Acute	Controls	t-value	p-value
FA	First	0.39 ± 0.08	0.56 ± 0.03	4.42	0.007
	Recurrent	0.35 ± 0.10	0.56 ± 0.05	8.01	0.000
MD	First	1.80 ± 0.28	1.40 ± 0.08	3.87	0.012
	Recurrent	2.10 ± 0.44	1.50 ± 0.140	4.73	0.001
$\lambda_{\parallel}$	First	2.64 ± 0.36	2.34 ± 0.10	2.46	0.057
	Recurrent	2.96 ± 0.49	2.52 ± 0.29	2.35	0.038
$\lambda_{\perp}$	First	1.40 ± 0.27	0.90 ± 0.09	4.25	0.008
	Recurrent	1.70 ± 0.45	1.00 ± 0.10	5.88	0.000

Eighteen cases with IDON were in the subacute stage (the duration of the disease from the examination day to the last onset had been more than 1 month). Of these, 6 cases in 5 subjects had been affected for the first time and 12 in 8 subjects had been affected previously. Patients were defined as "recurrent" if they had had more than two onsets of symptoms at the time of the MRI assessment. Axial ($\lambda_{\parallel}$), radial ($\lambda_{\perp}$) and mean (MD) diffusivities are given in $\mu\text{m}^2 \text{ms}^{-1}$. Fractional anisotropy is without units. All values of diffusion tensor-MRI indices are given as the mean $\pm$ standard deviation. IDON, idiopathic optic neuritis.

diseases had lasted from 8 days to 4 months were included in this analysis. The route of the reconstructed fibres and the tract-specific quantification was consistent with the known anatomy of the human visual pathway [21, 32]. These findings showed connections from the posterior part of the optic radiation to visual areas, and connections between the medial part and the nucleus of the lateral geniculate body. Table 5 illustrates the mean FA, MD, $\lambda_{\perp}$ and $\lambda_{\parallel}$ within the reconstructed optic radiation of 9 patients whose disease had lasted more than 1 year (paired *t* test, *n*=9). Compared with the control group, the FA values from these patients show a statistically significant decrease (*t*=3.45, *p*=0.009) and the $\lambda_{\perp}$ value shows a dramatic increase (*t*=3.92, *p*=0.004; Figure 6). Compared with the controls, there is no statistical change in the mean FA, MD, $\lambda_{\perp}$ and $\lambda_{\parallel}$ within the reconstructed optic radiation of the 14 patients whose disease had lasted less than 1 year (paired *t*-test, *n*=14; Table 6).

Table 5. Diffusion parameters in IDON patients whose disease had lasted more than 1 year (*n*=9) in comparison with controls

Parameter	ON	Controls	t-value	p-value
FA	0.46 ± 0.04	0.50 ± 0.03	3.45	0.009
MD	0.89 ± 0.05	0.84 ± 0.02	2.14	0.065
$\lambda_{\parallel}$	1.38 ± 0.07	1.37 ± 0.07	0.41	0.691
$\lambda_{\perp}$	0.64 ± 0.06	0.58 ± 0.02	3.92	0.004

The values of axial ($\lambda_{\parallel}$), radial ($\lambda_{\perp}$) and mean (MD) diffusivities are given in $\mu\text{m}^2 \text{ms}^{-1}$. Fractional anisotropy (FA) is without units. All values of diffusion tensor-MRI indices are given as the mean $\pm$ standard deviation. IDON, idiopathic optic neuritis; ON, optic neuritis.

Discussion

The most common cause for IDON is believed to be an autoimmune reaction against the myelin surrounding the fibres in the optic nerve, which induces an inflammatory response that can result in nerve damage. In some cases, early symptoms of ON may indicate an outbreak of MS, a disease also caused by inflammation and axon damage in the brain and spinal cord. Thus, a surrogate biomarker is needed to reveal the underlying pathological processes of ON. In the current study, we used the directional diffusivities from DT-MRI to investigate the abnormalities in the optic nerves and optic radiation after ON.

The diameter of the human optic nerve is about 3–4 mm. The nerve is surrounded by several membrane layers, including the nerve sheath and orbital fat. Artefacts caused by eye movement and the susceptibility effects caused by nearby sinuses make it difficult to acquire reliable diffusion image data and to maintain an adequate SNR. Methods such as SE-EPI [33], inner volume imaging (IVI) or the reduced FOV technique [34–36] were introduced to improve image quality. In this study, the SE-EPI protocol, a relatively low maximum *b*-value with 600 s mm^{-2} , six independent directions and a high number of acquisitions were used to ensure a sufficiently high SNR. This method has previously been validated by several authors [12, 17, 34, 36, 37]. In addition, we scanned the optic nerve bilaterally in the coronal plane because the image distortion was greater when separate unilateral images were acquired.

Kolbe et al [12] scanned the optic nerves individually in ten coronal oblique slices set orthogonal to the nerve and analysed the first six slices. They found that the

Figure 6. Fractional anisotropy (FA) and radial diffusivity ($\lambda_{\perp}$) in the optic nerves of controls and patients who had suffered more than 1 year of visual impairment (expressed as range (red box), mean (horizontal line) $\pm$ standard deviation (T-bars)).

diffusivity values changed drastically along the length of the optic nerve. The FA values were considerably lower and the MD values considerably higher in the first and second slices than in other parts. No significant differences in FA or MD were found in the three last slices. In our study, we divided the optic nerve into eight orthogonal coronal oblique slices. The layered pattern of DT-MRI diffusivity was confirmed in a pre-test study: the optic nerve on slices 6–8 was indistinguishable in most cases, and the diffusion indices were susceptible to vitreous body in slices 1 and 2. By contrast, robust directional diffusivity was observed in slices 3–5. FA and MD values showed no significant differences between the right and the left optic nerves in healthy controls (Table 7). Randomised variance block-analysis indicated significant differences in FA but not in MD among the slices (Table 8; FA: $F=17.54$, $p<0.001$; MD: $F=0.500$, $p=0.613$). In addition, the FA values in the fourth and fifth slices were higher than the one in the third slice ($p<0.000$ (third *vs* fourth); $p<0.000$ (third *vs* fifth), but did not differ statistically from each other ($p=0.757$ (fourth *vs* fifth)). We suggest that the effect of eye movements is smaller in the posterior part of the optic nerve.

We assume that two factors may affect the diffusivity values: first, the possible mobility of the optic nerve may be reduced in the mid-posterior part; second, there may be more directional movement of water molecules in

the well-organised and compact fibres. The fifth layer of the optic nerve (about 2.5 cm distal from the eyeball) could be located clearly in most subjects, but measurements failed in five teenagers and in one 60-year-old patient because of reconstruction problems. For that reason, we had to use the fourth layer (about 2 cm after the eyeball) in this study.

DT-MRI uses water diffusion characteristics to reconstruct white matter structure through diffusion direction and amplitude. Altered diffusion parameters were found in patients with chronic ON compared with healthy controls: MD was increased and FA decreased [13]. According to Smith and McDonald [38], the pathophysiological mechanisms underlying the clinical symptoms of acute ON include inflammation, oedema, demyelination and loss of axons in the optic nerve. A spontaneous visus recovery a few weeks or even months after the injury has been reported in some cases. Many factors, such as a decreasing inflammatory response, remyelination, restoration of conduction in demyelinated axons [38] or cortical or subcortical plasticity [14, 15, 39, 40] may lead to the visual recovery. Since the demyelination is presumably a dynamic process, we hypothesised that different DTI indices may change at different stages of ON.

Naismith et al [20] discovered the FA and $\lambda_{\perp}$ to be the first parameters to change in acute IDON. They found significantly decreased $\lambda_{\parallel}$ in acute IDON, and suggested it may be correlated with the visual outcome. In our study, we found significantly increased mean $\lambda_{\perp}$ and decreased FA in both first-episode and recurrent acute cases when compared with controls. We also detected a decrease in the $\lambda_{\parallel}$ of patients with first-episode acute-stage IDON using a paired *t*-test ($t=2.10$, $p=0.046$), although that difference did not reach statistical significance ($z=3.414$, $p=0.065$) after GEE model analysis was performed. Because the pathological changes in recurrent cases are more complex than those in cases with first-time manifestation, and as the sample size of recurrent cases was small ($n=6$), we will only discuss the first-episode subgroup as we assume that this model probably reflects the pathological changes in acute period more closely. In cases with white matter injury

Table 6. Diffusion parameters in IDON patients whose disease had lasted less than 1 year ($n=14$) in comparison with controls

Indices	ON	Controls	t-value	p-value
FA	0.49 ± 0.04	0.48 ± 0.03	0.62	0.547
MD	0.88 ± 0.04	0.87 ± 0.04	1.06	0.308
$\lambda_{\parallel}$	1.41 ± 0.03	1.38 ± 0.06	2.06	0.062
$\lambda_{\perp}$	0.62 ± 0.05	0.61 ± 0.04	0.34	0.738

The values of axial ($\lambda_{\parallel}$), radial ($\lambda_{\perp}$) and mean (MD) diffusivities are given in $\mu\text{m}^2 \text{ms}^{-1}$. Fractional anisotropy (FA) was without units. All values of diffusion tensor-MRI indices are given as the mean $\pm$ standard deviation. IDON, idiopathic optic neuritis; ON, optic neuritis.

Table 7. Lateral differences in FA and MD values in slices 3–5 from 10 healthy controls in the pre-test study

	FA				MD			
	Right side	Left side	t-value	p-value	Right side	Left side	t-value	p-value
Third	0.57 ± 0.04	0.56 ± 0.06	0.297	0.774	1.57 ± 0.14	1.60 ± 0.19	−0.795	0.452
Fourth	0.67 ± 0.05	0.67 ± 0.05	−0.291	0.779	1.61 ± 0.23	1.58 ± 0.18	0.853	0.418
Fifth	0.67 ± 0.05	0.68 ± 0.05	−0.472	0.65	1.50 ± 0.20	1.52 ± 0.20	−0.628	0.548

Fractional anisotropy (FA) is without units. Mean diffusivities (MD) are given in $\mu\text{m}^2 \text{ms}^{-1}$.

involving only myelin degeneration, we hypothesise that $\lambda_{\perp}$ is likely to increase, reflecting the increased freedom of water molecules to undergo Brownian motion perpendicular to the axons as myelin integrity is lost. The results of our study confirm consistent pathological changes and support our assumption.

Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model, which can simulate many features of human MS. ON is one of the phenotypes of EAE mice. Xu et al [13] report that $\lambda_{\parallel}$ and $\lambda_{\perp}$ appear to be both sensitive and specific for axonal injury and demyelination. Wu et al [41] studied an EAE murine model in the acute stage using *in vivo* diffusion-weighted imaging with diffusion sensitising gradients parallel and perpendicular to the axonal tracts. They detected that progressive acute axonal damage resulted in a 23% decrease in $\lambda_{\parallel}$ at 20 days after immunisation. Using a paired *t*-test, we found that $\lambda_{\parallel}$ decreases in patients with first-episode acute IDON, a finding we ascribe to axonal injury during the acute stage. However, this conclusion needs to be reconfirmed by more research.

Trip et al [17] and Kolbe et al [12] found increased MD and reduced FA values in patients with unilateral IDON who had suffered from visual symptoms for a minimum of 1 year. These authors considered these changes to be caused mainly by axonal loss, with demyelination and gliosis playing a partial role. In our study, 18 cases (both first manifestations and recurrent cases) with IDON in the subacute stage showed significantly decreased FA and increased $\lambda_{\perp}$, $\lambda_{\parallel}$ and MD when compared with controls, supporting the findings of the aforementioned authors.

The DT-MRI fibre tracks and segmentation of the optic radiation from the lateral geniculate nucleus to the visual cortex have already been studied by Yamamoto et al [42] and by Berman et al [43]. Bajraszewski et al [44] also found significantly increased MD and reduced FA in the optic radiation in patients with ON (4.0 ± 0.4 years after symptom onset) compared with controls. They suggested

that the changes are caused by the anterograde effects of the nerve damage.

Our study found no significant changes in diffusion parameters in patients with ON of less than 1 year in duration, but a significantly decreased FA and higher $\lambda_{\perp}$ if the disease duration exceeded 1 year. This difference indicates more serious atrophy of the optic radiation after the recurrence of symptoms. The most likely pathogenesis of abnormal diffusion in the optic radiation would appear to be secondary lesions induced by axonal degeneration after ON. We also observed an increased MD value in the optic radiation in chronic ON patients when compared with control subjects. However, this change was not significant ($t=2.14$, $p=0.065$), possibly because of the small number of patients. These findings support our hypothesis that abnormal diffusion in the optic radiation is an important characteristic of ON. Further research is needed to strengthen the role of DT-MRI measurements in ON evaluation and degree assessment.

Conclusions

In the current study, we applied DT-MRI methodology to investigate changes in the optic nerve and optic radiation. The changes in diffusion parameters measured during both acute and remitting ON support and extend previously reported findings. In addition, we found significantly decreased FA and increased $\lambda_{\perp}$ in the optic radiation of chronic ON patients. We were able to detect dynamic changes in the diffusion parameters during the development of chronic ON, possibly indicating ongoing myelin damage. On the basis of our novel findings, we suggest directional diffusivity to possess great potential as a specific biomarker and evaluation measure for myelin injury. Further investigations are needed to determine whether these indices can make practical contributions to the diagnosis and determination of prognosis for patients with ON.

Table 8. Comparison of fractional anisotropy (FA) and mean diffusivities (MD) values in slices 3–5 from 10 healthy controls in the pre-test study

Indices	Slice			Statistic	
	Third (mean ± std)	Fourth (mean ± std)	Fifth (mean ± std)	F-value	p-value
FA	0.56 ± 0.04	0.67 ± 0.05	0.68 ± 0.05	17.54	<0.001
MD	1.58 ± 0.15	1.60 ± 0.21	1.51 ± 0.21	0.500	0.613

Randomised variance block-analysis indicated significant differences in FA but not in MD among third to fifth slices. In addition, after multiple comparisons by the least significant difference (LSD) test, we found that the FA values in the fourth and fifth slices were higher than those in the third slice ($F=17.54$, $p<0.001$; $p<0.000$ [third vs fourth], $p<0.000$ [third vs fifth]), but did not differ statistically from each other ($p=0.757$ [4th vs 5th]). FA is without units. MD are given in $\mu\text{m}^2 \text{ms}^{-1}$.

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