A potential tool for the diagnosis of ALS based on diffusion tensor imaging

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Abstract
Our objective was to quantify and better understand white matter (WM) impairment in patients with amyotrophic lateral sclerosis (ALS) and to propose a model based on diffusion tensor imaging (DTI) for diagnosing patients with suspected ALS with upper motor neuron (UMN) signs. Twenty-six ALS patients (24 with prominent UMN signs and two with an isolated lower-motor neuron (LMN) syndrome) and 22 healthy volunteers were examined using DTI. Data analysis included voxel-based WM tract-based spatial statistics (TBSS), volume-of-interest analysis of the TBSS results and streamline tractography analysis. Converging evidence revealed WM impairment along the corticospinal tracts and in the mid-body of the corpus callosum. This was demonstrated by reduced fractional anisotropy values caused by increased radial diffusivity, without significant changes in axial diffusivity. There were no significant correlations between diffusivity indices and patients’ disability or disease duration. A discriminant analysis model based on the tractography results was designed to distinguish between patients with UMN signs and controls, yielding 87.5% sensitivity and 85% specificity. In conclusion, DTI can detect WM impairment in patients with ALS in several brain regions, and might be a sensitive tool for the diagnosis of ALS in the early stages of the disease with UMN involvement.

Key words: Amyotrophic lateral sclerosis, diffusion tensor imaging, white matter impairment, corticospinal tracts, tract-based spatial statistics

Introduction
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with an incidence of 2–2.4/100,000 persons per year (1,2). The basic pathologic process is a progressive degeneration predominantly of motor neurons, both in the motor cortex (upper motor neurons (UMN)), and the anterior horn cells and neurons in the motor ganglia of the brainstem (lower motor neurons (LMN)).

It is sometimes difficult to separate ALS from other motor neuron syndromes (3) and a reliable diagnosis can only be made in relatively advanced stages of the disease based on the El Escorial criteria (4). Conventional magnetic resonance imaging (MRI) is used in the diagnostic process mainly to rule out other diseases with similar clinical features. Unspecific high signal intensity of the corticospinal tracts (CST) may be seen occasionally in T2-weighted and fluid-attenuated inversion recovery (FLAIR) images (5,6). However, currently there are no definitive imaging or other biological markers for UMN involvement in ALS.

Diffusion tensor imaging (DTI) is currently the gold standard for studying white matter (WM) organization, maturation and pathology (7,8). Direct information on the axonal microstructure can be obtained from the three diffusion tensor eigenvalues \( \lambda_1, \lambda_2, \lambda_3 \) that represent diffusion along \( \lambda_1 = \text{axial diffusivity, (Da)} \) and perpendicular \( (\lambda_2 + \lambda_3)/2 = \text{radial diffusivity, (Dr)} \) to the principal axes of the fibres (9,10). Two
other commonly calculated indices are the apparent diffusion coefficient/mean diffusivity:

\[
MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}
\]

and fractional anisotropy (FA):

\[
FA(\lambda_1, \lambda_2, \lambda_3) = \frac{1}{\sqrt{2}} \left( \frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{\lambda_1 + \lambda_2 + \lambda_3} \right).
\]

Reduced FA values were demonstrated in several demyelination processes (10).

DTI appears to be a promising diagnostic tool for patients with ALS (11). Reduced WM integrity in patients with ALS was detected via various DTI approaches. Common findings in DTI studies using region-of-interest (ROI) and tractography analyses are increased MD values and reduced FA values at multiple levels along the CST (12–21). In other studies using whole-brain voxel-based analysis and tract-based spatial statistics (TBSS) (22,23), changes were mostly confined to the CST, but were also reported in the corpus callosum (CC) (24–27). Most of these studies refer only to changes in the FA and MD, while a few further investigated changes in the axial and radial indices to better understand the pathogenic mechanism underlying the changes in FA values (28). Yet, these studies did not provide a clinical diagnostic tool based on imaging results that would enable a preclinical diagnosis of UMN involvement in patients with suspected ALS.

The current study aims to further evaluate the extent of damage to WM fibres in patients with ALS by applying different analytical approaches to the same data set. To this end, various diffusivity indices – FA, MD, Da and Dr – were studied. Imaging results were correlated with clinical assessment and a model was created by the diffusion indices that showed high sensitivity and specificity in distinguishing between UMN-ALS patients and healthy controls and promising results for its predictive value in two patients with subclinical UMN involvement.

**Patients and methods**

**Subjects**

Subjects’ characteristics are presented in Table I.

**Study group**

Twenty-six patients were included in this prospective study. In order to exclude unrelated ischaemic changes in WM that are common in the elderly and could interfere with the present analysis, only patients less than 60 years of age with no more than two cardiovascular risk factors were included. Additionally, FLAIR images were reviewed and patients with periventricular WM disease were excluded. Patients underwent a thorough clinical examination, assessing muscle strength, and checking for the presence of UMN and LMN signs, non-motor features (sensory loss, extrapyramidal symptoms, cognitive impairment), and bulbar signs. Disability was evaluated according to the revised ALS functional rating scale (ALSFRS-R) (29).

Twenty-four patients fulfilled criteria for definite/probable ALS, according to the revised El Escorial criteria (4) and exhibited prominent UMN signs (brisk reflexes, increased tone) along with weakness in at least one limb at the time of examination. The remaining patients were a 57-year-old male with pure LMN involvement and a 58-year-old female with clear signs of LMN and very subtle signs of UMN involvement. These two patients were included in order to test the proposed model for the diagnosis of subclinical UMN involvement.

**Control group**

Twenty-two subjects volunteered to participate in this study. Only individuals with no history of neurological disease and without any abnormalities detected on conventional MR images (T1 and T2 weighted images) were included.

The study was approved by the Institutional Review Board of the Tel-Aviv Sourasky Medical Centre and the Israeli Ministry of Health, and written informed consent was obtained from all participants.

**Data acquisition and processing**

MRI scans were performed on a 3.0T MRI scanner (GE Signa-EXCITE, Milwaukee, WI, USA). The protocol included anatomical sequences (T1/T2 weighted images and FLAIR), and DTI, acquired along 15 diffusion gradient directions (b = 0, 1000 s/mm²). Axial slices were

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(3 mm thickness/0 gap), covering the whole brain (TR/TE = 11,000/91 ms, field of view 220 mm, acquired matrix of 128 × 128; reconstructed to 256 × 256). Preprocessing included eddy currents and motion corrections performed using FSL software (www.fmrib.ox.ac.uk/fsl). DTI analysis included: voxel-based TBSS, volume-of-interest analysis of the TBSS results and stream-line tractography analysis, based on the TBSS results.

**TBSS analysis**

FA, MD, Da and Dr maps were calculated using the FSL FDT tool and were aligned into a 1 × 1 × 1 mm MNI152 standard space (22). A mean FA skeleton \( n = 46 \) was created with threshold of FA > 0.2. Diffusivity maps were compared using the FSL randomized tool (patients > controls and controls > patients). The results included maps corrected at cluster level (threshold-free cluster enhancement (TFCE)) threshold at a level of \( p < 0.05 \), fully corrected for multiple comparisons across space (TFCE corrected).

**TBSS-VOI analysis**

In order to correlate the TBSS results with clinical assessment, VOI analysis was performed. VOIs were defined based on results obtained from the TBSS analysis and using anatomical marks obtained from atlases (the JHU-ICBM-DTI-81 WM atlas for VOIs in the CC, and the Juvelich-Histological atlas for the longitudinal tracts). Longitudinal tracts were further divided into fibres at the level of the ventricles and above, and fibres at the level of the internal capsule. Mean values of the diffusion indices in those areas were calculated for each subject.

Groups of the mean values of the DTI indices were compared using the two-tailed group \( t \)-test for independent samples (Bonferroni correction, \( p < 0.0025 \)). Pearson's correlation coefficients were calculated to evaluate the relationship between clinical variables (ALSFRS-R and disease duration) and the diffusivity values (Bonferroni correction, \( p < 0.001 \)).

**Tractography analyses**

Stream-line tractography analysis was performed using DTISTUDIO software (Johns Hopkins University cmrm.med.jhmi.edu). Two major fibre bundles, the longitudinal fibres and the CC, were studied. The longitudinal fibres were reconstructed separately for the motor and sensory fibres and for each hemisphere (Figure 1). Fibres were calculated as they passed through three ROIs, manually defined on colour-coded maps in the: 1) anterior (motor: yellow and blue) or posterior (sensory: green and orange) to the central sulcus (Figure 1a); 2) posterior limb of the internal capsule (Figure 1b); and 3) mid-brain. The entire CC was defined on a mid-sagittal plane using a single ROI approach. Further segmentation of the CC into five segments was performed according to Witelson (30) (Figure 1d, e). The CC was subdivided into five regions (on the mid-sagittal plane) comprising the anterior third (CC-w1: yellow), the anterior and posterior midbody (CC-w2: orange and CC-w3: blue), the posterior third (CC-w4: green), and the posterior one-fifth (CC-w5: pink). Each segment was used as a seed region for the tractography analysis. The mean FA, MD, Da and Dr values were calculated for each of the reconstructed fibres/segments.

Between-group comparisons of mean values were conducted using two-tailed group \( t \)-tests for independent samples (Bonferroni correction, \( p < 0.0018 \)). Pearson's correlation coefficients were calculated to evaluate the relationship between clinical assessment and diffusivity values (Bonferroni correction, \( p < 0.001 \)).

**Discriminant analysis**

In order to obtain a model that could distinguish between patients with UMN-ALS and healthy controls, a discriminant analysis with minimal Wilks' lambda as a criterion for conversion was performed, using the diffusivity indices obtained from the tractography analysis as predictors. The sensitivity and specificity of the model were tested using the 'leave one out' method based on 24 UMN patients and 22 healthy subjects.

**Results**

**Study population**

Twenty-six patients and 22 healthy controls participated in this study. The between-group comparisons...
were performed for the 24 patients with UMN involvement and the control group. There were no significant age ($p = 0.48$) or gender ($p = 0.32$) differences between groups.

**Tract-based spatial statistics – TBSS**

Significantly reduced FA values were detected in the longitudinal fibres at the level of the ventricles and above in both hemispheres as well as in the mid-body of the CC of patients compared to controls (Figure 2a). A significant increase in MD was detected in the longitudinal fibres at the level of the ventricles and above and at the level of the internal capsule (IC), in the right hemisphere of the patients compared to the controls (Figure 2b). A significant increase in Dr was detected in the longitudinal fibres at the level of the ventricles and above and at the level of the IC in the right hemisphere and in the mid-body of the CC (Figure 2c) in patients compared to controls. No significant group differences were detected in Da in any brain region.

**TBSS – VOIs**

Three VOIs were defined based on the TBSS results in areas in which significant reduction in FA values in the TFCE-corrected map was obtained: the right and left longitudinal fibres at the level of the ventricles and above (defined as superior corona radiata – SCR) and the mid-body of the CC. Two additional VOIs were defined based on our TBSS results in areas where reduction in FA values was obtained (TFCE-uncorrected) – the right and left longitudinal fibres at the level of the IC. Findings in these areas were also previously reported in patients with ALS (16,17,19). Mean diffusivity values were calculated separately for each VOI and are presented in Table II. As expected, significantly reduced FA and an increased Dr were detected in all VOIs ($p < 0.0025$) in patients compared to controls. A significantly increased MD was detected in the right longitudinal fibres at the level of the IC and SCR in patients compared to controls ($p < 0.0025$). No significant group differences were detected for the Da in any of the VOIs. There were no significant correlations between diffusivity values and ALSFRS-R or disease duration.

**Tractography results**

Mean diffusivity values in the five CC segments and in the motor and sensory fibres in both groups are summarized in Table III. No significant differences were detected for any of the diffusivity values between the right and left hemispheres for either motor or sensory fibres, and therefore a mean value between hemispheres was used for each diffusivity value, separately for the motor and sensory fibres. Significantly reduced FA values and increased MD and Dr values were found in patients with ALS compared to controls in the motor fibres and in Witelson segment 3. There were no significant differences in the Da values, nor were there any significant correlations between diffusivity values and ALSFRS-R or disease duration.

**Discriminant analysis**

A stepwise discriminant analysis was performed on the diffusivity indices obtained from the tractography analysis in order to differentiate between patients with UMN involvement and healthy controls. Three measures entered the formula reaching a Wilks’ lambda of 0.357 ($\chi^2(3) = 33.5, p < 0.001$). The three variables accepted for the function and their standardized canonical discriminant coefficients were: Dr-CST = 0.463, FA-CCw2 = 0.933 and FA-CCw3 = −1.24. The functions at group centroids were 1.17 for patients and −1.46 for controls. This model yielded a sensitivity of 87.5% and a specificity of 85.0% ($n = 46, 24$ patients and $22$ healthy subjects). The discriminant function was additionally applied to two patients with a predominant LMN syndrome. The first patient, who had only LMN signs, was correctly classified as not UMN-ALS, while the second patient, who had clear-cut LMN signs and a mild UMN sign (an extensor plantar response in one leg), was diagnosed by this model as having UMN involvement, compatible with a diagnosis of ALS. At clinical follow-up 27 months later, the first patient still had a pure LMN syndrome, with little progression, while the second patient died 23 months after the MRI examination with a clinical picture of definite ALS with both UMN and LMN involvement.
Table II. Tract-based spatial statistics (TBSS) volumes-of-interest analysis results.

<table>
<thead>
<tr>
<th>Area</th>
<th>FA (a.u.)</th>
<th>MD ($\times 10^{-3}$ mm$^2$/s)</th>
<th>Da ($\times 10^{-3}$ mm$^2$/s)</th>
<th>Dr ($\times 10^{-3}$ mm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>ALS</td>
<td>F</td>
<td>CON</td>
</tr>
<tr>
<td>Midbody - CC</td>
<td>0.53 ± 0.05</td>
<td>0.45 ± 0.05</td>
<td>12.8*</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>Lt IC</td>
<td>0.67 ± 0.03</td>
<td>0.62 ± 0.03</td>
<td>19.8*</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>Rt IC</td>
<td>0.68 ± 0.03</td>
<td>0.62 ± 0.04</td>
<td>15.1*</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>Lt SCR</td>
<td>0.43 ± 0.03</td>
<td>0.39 ± 0.02</td>
<td>16.4*</td>
<td>0.64 ± 0.02</td>
</tr>
<tr>
<td>Rt SCR</td>
<td>0.43 ± 0.02</td>
<td>0.39 ± 0.03</td>
<td>17.9*</td>
<td>0.63 ± 0.02</td>
</tr>
</tbody>
</table>

FA (a.u.): functional anisotropy (arbitrary units); MD: mean diffusivity; Da: axial diffusivity; Dr: radial diffusivity; CON: control; ALS: amyotrophic lateral sclerosis; CC: corpus callosum; Lt: left; Rt: right; IC: internal capsule; SCR: superior corona radiata.

*p < 0.0025.

Table III. Tractography analysis results.

<table>
<thead>
<tr>
<th>Area</th>
<th>FA (a.u.)</th>
<th>MD ($\times 10^{-3}$ mm$^2$/s)</th>
<th>Da ($\times 10^{-3}$ mm$^2$/s)</th>
<th>Dr ($\times 10^{-3}$ mm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>ALS</td>
<td>F</td>
<td>CON</td>
</tr>
<tr>
<td>Motor</td>
<td>0.54 ± 0.03</td>
<td>0.51 ± 0.03</td>
<td>16.7*</td>
<td>0.65 ± 0.01</td>
</tr>
<tr>
<td>Sensory</td>
<td>0.54 ± 0.03</td>
<td>0.52 ± 0.02</td>
<td>5.3</td>
<td>0.67 ± 0.02</td>
</tr>
<tr>
<td>CC-All</td>
<td>0.56 ± 0.02</td>
<td>0.55 ± 0.02</td>
<td>0.2</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>CC-w1</td>
<td>0.51 ± 0.03</td>
<td>0.51 ± 0.03</td>
<td>0.0</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>CC-w2</td>
<td>0.52 ± 0.03</td>
<td>0.51 ± 0.04</td>
<td>1.2</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>CC-w3</td>
<td>0.54 ± 0.02</td>
<td>0.49 ± 0.04</td>
<td>27.7*</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>CC-w4</td>
<td>0.52 ± 0.03</td>
<td>0.49 ± 0.03</td>
<td>6.0</td>
<td>0.74 ± 0.03</td>
</tr>
<tr>
<td>CC-w5</td>
<td>0.61 ± 0.02</td>
<td>0.62 ± 0.01</td>
<td>1.0</td>
<td>0.74 ± 0.03</td>
</tr>
</tbody>
</table>

FA (a.u.): functional anisotropy (arbitrary units); MD: mean diffusivity; Da: axial diffusivity; Dr: radial diffusivity; CON: control; ALS: amyotrophic lateral sclerosis; CC-w(n): Witelson segment n’ within the corpus callosum.

*p < 0.0025.
Discussion

In this study we used TBSS and tractography analysis methods, which demonstrated converging evidence of reduced WM integrity in patients with ALS compared to controls. WM impairments were detected in the longitudinal fibres as well as in the mid-body of the CC. Several diffusivity indices were studied in order to better understand the changes in FA and to provide additional information on the mechanisms underlying the WM impairment. These diffusivity indices yielded a model that enabled the differentiation between ALS patients with UMN involvement and healthy controls with a sensitivity of 87.5% and a specificity of 85.0%.

Our TBSS results of the FA values were significant only for the longitudinal fibres at the level of the ventricles and above, while results at the level of the IC were not significant after correction for multiple comparisons. Previous studies reported inconsistent results regarding the location of WM impairment along the longitudinal fibres (17,21,23,24,31,32), which may be due to the use of different methodologies for data analysis. Nevertheless, all these studies indicated white matter impairment in the longitudinal fibres.

In this study, significant results in the TBSS analysis were largely confined to the right hemisphere. Similar trends were also detected in the left hemisphere; however, they did not reach significance when controlling for multiple comparisons. These asymmetries did not correlate with patient presentations. Similar results, with higher statistical significance in the right hemisphere, were previously reported in other studies (24,32). Further studies with a larger number of patients are required in order to expand on and interpret these findings.

Tractography analysis detected WM impairment in the motor fibres, while results in the sensory fibres were not significant after correction for multiple comparisons. These results support ALS as a degenerative disease of the motor system.

In this study, we referred to results obtained from the TBSS analysis as reduced WM integrity in the longitudinal fibres and not specifically in the CST. Although the location was defined by various atlases as being the CST, the exact separation between the afferent and efferent fibres in this analysis is less reliable. Both probabilistic and stream-line tractography can track fibres according to their directionality and their anatomical locations, specifically when defined by using a multi-ROI approach. Therefore, these methods may be preferable in separating motor from sensory fibres.

Reduced FA values were detected in the mid-body of the CC (in the TBSS results) and in Witelson segment 3 (in the tractography analysis) of our patients, which are referred to as the callosal motor fibres (33). These findings are in line with previous reports of abnormalities in the CC in patients with ALS compared to healthy controls, using both conventional imaging and DTI studies (24–27,34–36).

Reduced FA values, both in the longitudinal fibres and in the CC, were shown to be a result of increased Dr without concomitant significant changes in the Da. Changes in Dr have been suggested as being correlated with demyelinating processes (10). This might indicate WM damage in parallel or secondary to the neuronal injury in ALS patients. These results may provide additional information regarding the pathophysiological process underlying ALS, although correlation with pathological data is needed.

Converging results were obtained with the TBSS and tractography analysis, two distinct methods each with its own advantages. TBSS is a fully automated voxel-based comparison of the entire WM skeleton, does not require a priori hypothesis and can detect localized changes. Tractography analysis examines a specific fibre tract based on a priori hypotheses (26), and since it yields an average value across the entire tract it may miss localized differences in a specific location that can be better detected using voxel-based methods. In favour of tractography analysis, anatomical location (i.e. the specific tract) can be defined within the subject’s space and the separation between sensory and motor fibres becomes more reliable when using a multi-ROI approach for the tractography algorithm. In whole brain analysis methods, the anatomical localization requires the alignment of each individual to a given standard space; this may increase error and can present problems in some populations.

There were no significant correlations between diffusivity indices and ALSFRS-R or disease duration. Although some correlations were detected, even reaching significance (p < 0.01), none of the results was significant after correction for multiple comparisons. Other studies reported inconsistent results between DTI indices and clinical assessment, with absence or different levels of significance (16,17,20,31,34,37). The lack of correlation between MRI findings and ALSFRS-R is probably due to the variance in LMN involvement that contributes significantly, in addition to the UMN involvement, to the ALSFRS-R score. The rate of progression in ALS varies between patients, therefore disease duration was not expected to be related to the MRI findings. A variable that might be correlated to the MRI findings is the level of functional disability related to upper motor neuron dysfunction; however, this variable is currently not accurately quantifiable.

The discriminant analysis was performed only on the tractography results, a simple and practical method that can easily be integrated in the clinical setting. Although the final analysis was based on VOI selection, these results were shown to be statistically significant in the voxel based analysis (TBSS), demonstrating converging results. Interestingly, two of the variables accepted for the discriminant function...
were the FA-CCw2 and FA-CCw3. Currently, most studies focus on WM impairment along the CST; yet the results presented here highlight the importance of the integrity of the CC in the diagnosis of ALS. The third variable was the Dr in the CST, emphasizing the significance of the various diffusivity indices in studying patients with ALS. Preliminary results in two patients demonstrate the model’s sensitivity and its potential for early identification of UMN involvement that is not yet clinically detectable, in addition to enabling earlier diagnosis of ALS.

In summary, WM impairment was demonstrated along the longitudinal and within the callosal fibres of ALS patients. Of the various studied diffusivity indices, Dr was the main cause of reduced WM anisotropy and was thus important in establishing a diagnosis of ALS. Two different analytical methodologies were applied to the same data set and presented converging results. High sensitivity and specificity were obtained using the discriminant analysis model based on 46 subjects. From the two cases with LMN syndromes described, it seems that this method is able to predict subclinical UMN involvement in patients with LMN syndromes and therefore to differentiate between ALS and non-ALS LMN syndromes (e.g. paraneoplastic, spinal muscular atrophy, Kennedy syndrome, monomelic atrophy). However, further studies are needed to evaluate the usefulness of the technique as a predictive model in these patients, as well as in patients with ALS with different clinical phenotypes, such as progressive bulbar palsy, pure LMN syndromes, and older individuals with coexistent cerebrovascular disease.

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