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Structural and diffusion imaging versus clinical assessment to monitor amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis is a progressive neurodegenerative disease that affects upper and lower motor neurons. Observational and intervention studies can be tracked using clinical measures such as the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) but for a complete understanding of disease progression, objective in vivo biomarkers of both central and peripheral motor pathway pathology are highly desirable. The aim of this study was to determine the utility of structural and diffusion imaging as central nervous system biomarkers compared to the standard clinical measure, ALSFRS-R, to track longitudinal evolution using three time-point measurements. $N = 34$ patients with ALS were scanned and clinically assessed three times at a mean of three month time intervals. The MRI biomarkers were structural T1-weighted volumes for cortical thickness measurement as well as deep grey matter volumetry, voxel-based morphometry and diffusion tensor imaging (DTI). Cortical thickness focused specifically on the precentral gyrus while quantitative DTI biomarkers focused on the corticospinal tracts. The evolution of imaging biomarkers and ALSFRS-R scores over time were analysed using a mixed effects model that accounted for the scanning interval as a fixed effect variable, and, the initial measurements and time from onset as random variables. The mixed effects model showed a significant decrease in the ALSFRS-R score, ($p < 0.0001$, and an annual rate of change (AROC) of -7.3 points). Similarly, fractional anisotropy of the corticospinal tract showed a significant decrease ($p = 0.009$, AROC $= -0.0066$) that, in turn, was driven by a significant increase in radial diffusivity combined with a trend to decrease in axial diffusivity. No significant change in cortical thickness of the precentral gyrus was found ($p > 0.5$). In addition, deep grey matter volumetry and voxel-based morphometry also identified no significant changes. Furthermore, the availability of three time points was able to indicate that there was a linear progression in both clinical and fractional anisotropy measures adding to the validity of these results. The results indicate that DTI is clearly a superior imaging marker compared to atrophy for tracking the evolution of the disease and can act as a central nervous biomarker in longitudinal studies. It remains, however, less sensitive than the ALSFRS-R score for monitoring decline over time.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of unknown aetiology that causes degeneration of upper and lower motor neurons. Establishing biomarkers to help in the development of therapeutic agents, has become a key goal of clinical research. In recent years, 14 longitudinal MRI studies focused on tracking disease progression in ALS. Eight studies used diffusion tensor imaging (DTI) (Agosta et al., 2009a; Agosta et al., 2010; Blain et al., 2007; Keil et al., 2012; Menke et al., 2012; Muller et al., 2012; van der Graaff et al., 2011; Zhang et al., 2011). Four studies focused on structural measures using

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various approaches: cortical thickness (Schuster et al., 2014; Verstraete et al., 2012); tensor based morphometry (TBM) (Agosta et al., 2009b); and volumetry of deep grey matter structures (Westeneng et al., 2015). Only two studies compared structural and diffusion imaging in the same cohort (Kwan et al., 2012; Menke et al., 2014). Results are quite inconsistent across studies at present. A particular problem, potentially, is that all published studies used only two time-points making it difficult to judge if decline is linear or otherwise, and moreover, if some conflicting results may have been spurious—with greater than two time-points, in contrast, one can assess whether a coherent pattern of change is emerging over time as opposed to apparently 'significant' changes that may represent random error. Furthermore, longitudinal studies to date have typically contained small numbers—only five (Menke et al., 2014; Schuster et al., 2014; Verstraete et al., 2012; Verstraete et al., 2014; Westeneng et al., 2015) of the above studies listed included 20 or more patients. Finally, with most studies investigating only a single biomarker in isolation, the question arises of whether an apparently significant change in such instances adds true value—i.e. a change may be statistically significant, but, if the effect is order(s) of magnitude below other outcome measures, of little value. For further discussion of the challenges facing imaging studies in ALS, see Verstraete et al. (2015).

With all of these considerations in mind, the present study investigated $n = 34$ patients with ALS who were scanned on three occasions at an average of approximately three month intervals. The performance of DTI metrics and structural imaging—analysed using both the cortical thickness approach, VBM and automated deep grey matter volumetry—was assessed and also contrasted to the standard clinical outcome measure, the revised ALS Functional Rating Scale (ALSFRS-R) (Cedarbaum et al., 1999).

2. Materials and methods

2.1. Participants

Patients were recruited from specialist ALS clinics as part of a prospective study that has recruited $N = 125$ cases to date. Clinical diagnosis of ALS was made according to the revised El Escorial criteria (Brooks et al., 2000) with all patients fulfilling criteria for clinically definite or probable ALS. Patients suffering from flail limb or upper motor neuron only and/or showing symptoms of any of the frontotemporal lobar degeneration syndromes were excluded; this was established on clinical grounds, including caregiver interview. The Montreal cognitive assessment (MoCA) (Nasreddine et al., 2005) score was used to assess cognitive performance. Patients needed to have had three MRI examinations using the research scan protocol. Of those meeting these criteria, $N = 38$, four were excluded because they lacked at least one image modality at one time point, leaving $N = 34$ patients with complete data sets at each time-point, ALS-TP1, ALS-TP2, ALS-TP3. $N = 31$ patients had a classic (Charcot's) phenotype and $N = 3$ had a pyramidal phenotype (Chiò et al., 2011). ALSFRS-R (Cedarbaum et al., 1999) severity score was assessed by the same experienced neurologist on all occasions (SV) and to derive ALS Milano-Torino Staging (ALS-MITOS) scores (Chiò et al., 2015). Demographics are summarised in Table 1.

For some imaging comparisons, 29 healthy control subjects were recruited and screened to exclude neurological illness and cognitive impairment (MoCA ≥ 26). All subjects gave written informed consent; the ethics committee of Otto-von-Guericke University approved the study.

2.2. Image acquisition

All MRI scans were performed on the same Siemens Verio 3T system (Siemens Medical Systems, Erlangen, Germany) equipped with a gradient coil capable of 45 mT/m and 200 T/m/s slew rate. A standard 32-channel phased array imaging coil was used in receive mode. The field of view was aligned in all cases to the anterior commissure–posterior commissure line.

The DTI acquisition had a resolution of $2 \times 2 \times 2$ mm^3 and consisted of diffusion weighted data along 30 non-collinear diffusion directions with b = 1000 s/mm^2, and one scan without diffusion weighting (b = 0 s/mm^2). Full details of the acquisition scheme have been previously published (Cardenas-Blanco et al., 2014). T1-weighted, high-resolution structural MRI images were obtained using a three dimensional magnetization prepared rapid acquisition gradient-echo (MPRAGE) sequence with the following parameters: echo time/repetition time = 4.82/2500 ms, inversion time = 1100 ms, flip angle = 7°, receiver bandwidth = 140 Hz/pixel, distance factor 50% and a matrix size of $256 \times 256 \times 192$, yielding an isotropic resolution of 1 mm^3. A T2-weighted FLASH sequence acquired during the same session was used to exclude vascular pathology (no vascular lesions were identified in the dataset).

2.3. Image analysis

2.3.1. Diffusion tensor imaging

Diffusion tensor images were processed using The Oxford Centre for Functional MRI of the Brain (FMRIB) software library (Smith et al., 2004). Each diffusion weighted volume was affined-aligned to its corresponding b0 image using FMRIB's linear image co-registration tool (FLIRT v5.4.2) (Jenkinson and Smith, 2001) to correct for motion artefacts and eddy-current distortions. A binary brain mask of each b0 image was created, using the brain-extraction tool (BET v2.1) (Smith, 2002) with fractional threshold f = 0.1 and vertical gradient g = 0. FMRIB's diffusion toolbox (FDT v2.0) was used to fit the tensor and compute the eigenvalues L1 (axial diffusivity), L2 and L3 at each brain voxel and generate mean diffusivity (MD), fractional anisotropy (FA) and radial diffusivity (RD). Whole-brain analyses were performed using tract-based spatial statistics (TBSS). Spatial normalisation was achieved by warping all FA images to the $1 \times 1 \times 1$ mm^3 FMRIB58_FA standard template (FMRIB, University of Oxford, UK) in MNI152 space (Montreal Neurological Institute, McGill University, Canada) using FMRIB's nonlinear registration tool (FNIRT v1.0).

A cross-sectional analysis comparing ALS-TP3 and controls was completed to map the distribution of DTI changes. All warped ALS-TP3 and control FA maps were averaged to create a mean FA template, from which the mean FA skeleton was derived, using FA > 0.2. Finally, all spatially normalised FA, axial diffusivity (L1), RD and MD data were projected onto the skeleton and non-parametric statistics applied, where 10,000 permutations were run using randomize v2.1 with threshold free cluster

Table 1
Study participant demographics.

	Controls ($N = 29$)	ALS-TP1 ($N = 34$)	ALS-TP2 ($N = 34$)	ALS-TP3 ($N = 34$)
M/F	23/6	22/12	22/12	22/12
Age (years)	61.8 (10)	57.3 (9.9)	57.6 (9.9)	58.0 (9.9)
Symptom duration (mo)	–	23.6 (21.0)	27.0 (20.8)	31.3 (21.3)
ALSFRS-R score (/48)	–	40.2 (4.4)	37.9 (5.3)	35.1 (6.4)
ALS-MITOS stage 1[a]	–	2	3	7
MOCA (/30)	27.0 (0.8)	25.5 (2.1)	25.9 (3.0)	26.9 (2.7)

[a] Number of patients with an ALS-MITOS score of 1, all other patients scored zero; no patients progressed beyond a score of 1 over the course of the study.