Region-specific impairment of the cervical spinal cord (SC) in amyotrophic lateral sclerosis: a preliminary study using SC templates and quantitative MRI (DTI/ihMT).

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Region-specific impairment of the cervical spinal cord (SC) in amyotrophic lateral sclerosis: a preliminary study using SC templates and quantitative MRI (DTI/ihMT).

In this preliminary study, our objective was to investigate the potentiality of high-resolution anatomical imaging, Diffusion Tensor Imaging and conventional/inhomogeneous Magnetization Transfer imaging (MT/ihMT) at 3T analyzed with template-extracted regions of interest to measure the atrophy and the structural changes of white and gray matter spinal cord (SC) occurring in ALS patients.

Ten ALS patients and 20 age-matched healthy controls were recruited. SC gray matter (GM) and white matter (WM) areas were automatically segmented using dedicated templates. Atrophy indices were evaluated from T2-weighted images at each vertebral level from cervical C1 to C6. DTI and ihMT metrics were quantified within the corticospinal tracts (CST), the posterior sensory tracts (PST) and the anterior GM horns (aGM) at C2 and C5 levels. Clinical disabilities of ALS patients were evaluated using Revised ALS Functional Rating Scale, Upper Motor Neuron (UMN) and Medical Research Council scorings and correlated to MR metrics.*

Compared to healthy controls, GM and WM atrophies were observed in ALS patients, especially at lower cervical levels, where a strong correlation was additionally observed between GM atrophy and the UMN score ($R=-0.75$, $p=0.05$ at C6). Interestingly, significant decrease of ihMT ratios was found in all the regions of interest ($p\text{-values}<0.0008$), FA and MT ratios significantly decreased in CST especially at C5 ($p\text{-values}<0.005$) and $\lambda_{//}$ significantly decreased in CST ($p=0.0004$) and PST ($p=0.003$) at C2. Strong correlations between MRI metrics and clinical scores were also found ($0.47<|R|<0.87$, $p\text{-values}<0.05$).

Altogether, these preliminary results suggest that high-resolution anatomical imaging and ihMT imaging, in addition to DTI, are valuable to characterize SC tissue impairment in ALS. In this study, in addition to an important SC WM demyelination, we also observed for the first time in ALS impairments of cervical anterior GM.

Keywords:

ALS, spinal cord, diffusion tensor imaging, inhomogeneous magnetization transfer, motor neuron, GM atrophy, spinal cord templates

Abbreviations

(a)GM	(Anterior) Gray Matter
ADC	Apparent Diffusion Coefficient
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Revised Amyotrophic Lateral Sclerosis Functional Rating Scale
ANOVA	Analysis of Variance
CSA	Cross-sectional area
CSF	Cerebrospinal Fluid
CST	Corticospinal tract
DPR	Disease Progression Rate
DTI	Diffusion Tensor Imaging
ECG	Electrocardiogram
FA	Fractional anisotropy
FOV	Field of view
HASTE	Half-Fourier acquired single-shot turbo spin echo
ihMT(R)	Inhomogeneous Magnetization Transfer (Ratio)
LMN	Lower Motor Neuron

<i>Mp-</i>	<i>Multi-parametric</i>
<i>MR(I)</i>	<i>Magnetic Resonance (Imaging)</i>
<i>MRC</i>	<i>Medical Research Council</i>
<i>MT(R)</i>	<i>Magnetization Transfer (Ratio)</i>
<i>PST</i>	<i>Posterior Sensitive tract</i>
<i>RF</i>	<i>Radiofrequency</i>
<i>ROI</i>	<i>Region of Interest</i>
<i>SC</i>	<i>Spinal Cord</i>
<i>SE-EPI</i>	<i>Spin-echo Echo-Planar Imaging</i>
<i>STD</i>	<i>Standard deviation</i>
<i>UMN</i>	<i>Upper Motor Neuron</i>
<i>WM</i>	<i>White Matter</i>
$\lambda_{//}$	<i>Axial diffusivity</i>
$\lambda_{\perp}$	<i>Radial diffusivity</i>

107

108 **Introduction**

109 Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease affecting both upper and
110 lower motor neurons (UMN and LMN, respectively), which leads to progressive muscle weakness,
111 physical disability, and eventual death within 1 to 10 years from symptom onset with a mean
112 survival of 3 years^{1,2}.11/20/2019 10:31:00 AM Given this marked heterogeneity of the disease,
113 there is an unmet need for diagnostic, prognostic and monitoring biomarkers that could improve our
114 understanding of ALS physiopathology. Such indicators have already been the subject of important
115 investigations in both animal and human studies using a wide variety of techniques including
116 electrophysiology, serology, as well as *postmortem* and *in vivo* Magnetic Resonance Imaging
117 (MRI), and evidence of progressive damages of the corticospinal tracts (CST), involvement of
118 sensory pathways³⁻⁵, and presence of gray matter (GM) atrophy and degeneration^{6,7} have thereby
119 been reported. *In vivo* MRI bears particularly great potential to investigate the pathology. Numerous
120 studies have hence been conducted on the brain⁸, however only a few focused on the SC so far,
121 mostly due to technical challenges related to small diameter, physiological motions and
122 susceptibility artifacts^{9,10}.

123 Thanks to recent improvements including data sampling strategies or triggering method^{11,12}, cord
124 MRI can now indirectly help probing abnormalities in spinal GM motor neurons. Correlations
125 between cord atrophy and hand muscle deficit have for instance been reported⁵. Cord MRI also
126 helps characterizing impairment of white matter (WM) pathways. Degeneration of CST but also
127 posterior sensory tracts (PST) have notably been detected using Diffusion Tensor Imaging (DTI)
128 and Magnetization Transfer (MT)^{4,5,13,14}.

129 To go further, we now propose to specifically target GM atrophy and WM demyelination and see
130 whether this could impact our knowledge on ALS disease. More precisely, given the relatively
131 insufficient specificity of both DTI and conventional MT techniques to identify different
132 pathophysiological mechanisms¹⁵⁻¹⁷, our aim here is to combine them with high-resolution
133 anatomical imaging and the emerging inhomogeneous MT technique (ihMT)^{18,19}.

134 Indeed, DTI has largely been used to quantitatively assess structural changes in both brain and SC
135 WM. DTI metrics, especially axial and radial diffusivities ($\lambda_{//}$ and $\lambda_{\perp}$ respectively) are now
136 extensively used to evaluate axonal degeneration and demyelination in several pathologies
137 involving the SC¹². However, changes in DTI metrics could reflect various pathological
138 mechanisms such as inflammation, edema, demyelination or axonal degeneration, hence limiting
139 the specificity of the technique¹¹. In the same way, MT has also largely been used and the derived
140 MT metric (Magnetization transfer ratio or MTR) has proven to be highly sensitive to

141 microstructural tissue changes, especially to myelin alterations^{5,20,21}. MT has however been reported
142 having specificity limitation towards myelin, being sensitive to all the macromolecular pool and not
143 only to myelinated structures. On the other hand, the recently proposed inhomogeneous MT
144 technique bears great potential for myelin specific imaging. This new contrast mechanism has been
145 interpreted as a dipolar order effect within the macromolecular pool which is characterized by a
146 relaxation time constant T_{ID} ²². The ihMT technique provides a new mean to enhance the selectivity
147 of MRI by properly weighting the signal to specific T_{ID} range. Hence, thanks to its long T_{ID} ^{23,24} the
148 dipolar order contribution of the myelinated structures can be isolated from the broad MT signal,
149 giving rise to an enhanced myelinated tissue contrast as compared to conventional MT^{19,25}.
150 Although extensive work is still on-going for the full characterization of this technique^{24,26}, it has
151 already been used to assess brain and SC tissue demyelination in normal aging and multiple
152 sclerosis^{27,28}. Never applied in ALS so far, it could bring new insights into the pathophysiology of
153 ALS.

154 As for the resolution, most SC studies have so far focused on the characterization of the whole cord
155 itself rather than its substructures, mostly due to the low spatial resolution of conventional clinical
156 images with regards to the small SC cross-sectional dimensions. Over the last few years, high-
157 resolution anatomical imaging has been used to derive morphometric analyses in both WM and GM
158 separately, allowing for instance studying GM atrophy occurring with age^{28,29}. High-resolution
159 anatomical imaging hence also offers great potential to distinctively assess GM and WM
160 impairments in ALS.

161 In this context, by using a multi-parametric (mp-) MR protocol combining DTI, ihMT and high-
162 resolution anatomical imaging along with SC templates and clinical evaluation, this preliminary and
163 pilot study aimed to separately evaluate GM and WM atrophy at the cervical spine level, to assess
164 structural changes occurring within specific SC regions of interest (ROIs) and ultimately, to provide
165 new biomarkers and insights useful for the study of ALS disease mechanisms.

166

167 **Material and methods**

168 ***Subjects***

169 Ten patients (8 men, 2 women, age: 55.6±9.0 years old, ranging from 40 to 70) diagnosed with
170 probable or definite ALS with spinal-onset according to the Awaji criteria³⁰ and 20 age-matched
171 healthy control (HC) subjects (10 men, 10 women, age: 53.8±10.0 years old, ranging from 39 to 74)
172 were included. The local institutional ethics review board approved the study and written informed
173 consent was obtained from all subjects before participation. Exclusion criteria were history of
174 cervical trauma and acute medical condition.

175 ***Clinical Assessment***

176 All patients underwent clinical examination before MR scanning (*Table 1*). Motor disability was
177 evaluated using the revised ALS Functional Rating Scale (ALSFRS-R) (normal value, 48;
178 minimum, 0)³¹, from which the disease progression rate (DPR) was derived such that DPR (*in*
179 *months*⁻¹) = $(48 - \text{ALSFRS-R}) / \text{disease duration (in months)}$. By removing bulbar-related sub-score
180 (normal value: 12; minimum 0) from total ALSFRS-R, Spinal ALSFRS-R sub-score (normal value:
181 36; minimum 0) was extracted. An adapted Upper Motor Neuron score (UMN)³², grading limb
182 tendon and jaw jerk reflexes, as well as Hoffman and Babinski signs, was also evaluated (normal
183 value, 24 to 34; maximum: 55).

184 Muscle strength in the upper limbs was measured in 14 muscles (7 on each side) using the Medical
185 Research Council (MRC)^{32,33} rating scale (normal value per muscle, 5; range, [0 - 5]). Hand Grip
186 strength was measured to evaluate upper limb muscle deficits using the Martin Vigorimeter (Martin
187 Medizintechnik, Tuttlingen, Germany)^{34,35}.

188 ***MR Imaging and preprocessing***

MR acquisitions were performed on a 3T-MRI (MAGNETOM Verio; Siemens Healthcare, Erlangen, Germany) using the body coil for transmission, and the standard 12-channel head, 4-channel neck and 24-channel spine matrix array coils for reception. The MR protocol, including anatomical, DTI and ihMT acquisitions (cf. *Table 2* for general sequence parameters), was optimized to last about one hour to minimize patient discomfort.

Anatomical imaging included one sagittal 3D T_2 -weighted turbo spin-echo (TSE) sequence covering the entire cervical spinal cord from cervical level C1 to C7 and two axial 2D T_2^* -weighted multi-echo gradient echo sequences (Multi-Echo Data Image Combination, MEDIC) providing good WM and GM contrast. One slab (9 slices) was centered and optimized to be strictly perpendicular to the SC axis at mid-C2 (covering from C1 to C3) and the other one centered at mid-C5 (covering from C4 to C6-C7).

Diffusion Tensor Imaging (DTI) consisted in two axial slabs acquired using a monopolar single-shot spin-echo echo-planar imaging (SE-EPI) prototype sequence (ECG-gating³⁶, trigger delay 300ms, TR ~ 2500ms (3 heart beats), fat saturation, 4 averages), placed perpendicularly to the SC axis: one was positioned mid-vertebrae at C2 level and the other one at C5 level. Local B_0 shimming was performed prior to the acquisition. Diffusion parameters were 30 independent diffusion-encoding directions, 2 b-values (0 and 800s/mm²), and δ/Δ 15.42/34.24 ms. For each acquisition, in-line correction of eddy-current-induced distortions was performed using dynamic field correction³⁷. The DTI metrics (*Fractional Anisotropy (FA)*; *Apparent Diffusion Coefficient (ADC)*; *Radial diffusivity ($\lambda_{\perp}$)*; *Axial diffusivity ($\lambda_{\parallel}$)*) were calculated online using Siemens Neuro 3D software.

Classic and inhomogeneous Magnetization transfer (MT/ihMT) imaging was similarly performed at C2 and C5 levels with a customized pulsed saturation preparation optimized from¹⁹, (Hann-shaped pulses lasting 0.5ms each, repeated every 1ms for a total saturation duration of 500ms, alternating single- and dual-frequency with a $f=7$ kHz saturation offset, for a 4.9 μ T root-mean-square B_1 calculated over the whole saturation duration, hence delivering a total energy of 12.1 μ T².s) using a single-slice half-Fourier acquired single-shot turbo spin echo (HASTE) readout. At each level, 4 unsaturated images (M_0) along with 4 repetitions of 30 interleaved images per saturation scheme were acquired (124 images per time series in total). ECG data were recorded for retrospective correction of cerebrospinal fluid pulsatility-induced artifact as described in¹⁹. MT/ihMT ratios (*MTR/ihMTR*) were subsequently reconstructed using Matlab scripts (MATLAB R2012a, Mathworks, Natick, MA, USA), according to:

$$MTR = 1 - MT_{(+f)}/M_0 \quad (\text{Eq. 1})$$

$$ihMTR = [MT_{(+f)} + MT_{(-f)} - MT_{(+/-f)} - MT_{(-/+f)}]/M_0 \quad (\text{Eq. 2})$$

Where $MT_{(+f)}$ and $MT_{(-f)}$ correspond to a single offset saturation performed at positive and negative offset respectively; $MT_{(+/-f)}$ and $MT_{(-/+f)}$ correspond to dual offset saturations.

Post-processing

For each subject, individual anatomical T_2^* -weighted and multi-parametric MR data were first co-registered. The sagittal 3D T_2 -w volume was then automatically segmented using PropSeg³⁸ and non-linearly registered to the MNI-Poly-AMU template³⁹ so as to provide an automated vertebral labeling of each subject scan. Then, for each level, automatic spinal cord segmentation and ROI delimitation based on the T_2^* -w images were performed using the probabilistic WM/GM AMU₄₀⁴⁰ and WM tracts⁴¹ atlases, as summarized in *Figure 1* and previously described in²⁸. The whole processing was performed using a customized MATLAB (MATLAB R2012a, Mathworks, Natick, MA, USA) script based on functionalities included in the SC Toolbox⁴².

After post-processing, the dataset for each subject consisted of 4 DTI metrics and 2 MT metrics extracted from the bilateral CST, PST, and anterior GM (aGM) horns at C2 and C5 levels. Total GM was not considered for mp-MRI quantification due to potential partial volume effect. AMU₄₀-

based GM/WM segmentations were systematically visually checked, and when needed, manually adjusted (19 % of the data). Then, Cross-Sectional Areas (CSA), in mm^2 , were extracted from the segmentations. Mean values for each vertebral level (identified by registration to the MNI-Poly-AMU template) were obtained by averaging values from all corresponding slices. Finally, SC CSA was obtained by computing the sum of GM and WM CSAs at each level (C7, which was not systematically included within the slab, was not considered).

Statistical Analysis

Univariate statistical analyses were performed using JMP9 (SAS institute, Cary, NC, USA). CSA from ALS patients and HC were compared in both GM and WM using two-way analyses of variance (ANOVA) with age as covariate. Similarly, differences of DTI and MT/ihMT metrics between the two groups (ALS and HC) were also assessed at each cervical level, within CST, PST and aGM, as well as in the whole WM. P-values are reported without correction for multiple comparisons such that $*p<0.05$, $**p<0.005$ and $***p<0.0005$. Comparisons that survive Bonferroni correction for multiple comparisons with a threshold of $p<0.05$ are marked with (■) in the figures and tables.

Correlations between MR metrics and clinical disability scores (total ALSFRS-R, spinal ALSFRS-R, DPR, disease duration, UMN score, MRC scale, Hand Grip Strength) were assessed using Spearman rank test.

Results

Cross-sectional areas.

Figure 2 shows representative WM/GM segmentations obtained at C2 and C5 for a HC and an age-matched ALS patient. Figure 3 shows WM/GM CSA group analyses between controls and patients at each cervical level from C1 to C6. No age-related variations were found when performing the two-way ANOVA. As compared to HC, patients presented significantly lower GM CSA values ($\text{CSA} \leq 28 \text{ mm}^2$, dropout rate up to 37%, $***$) in lower cervical levels (C4-C6). Significantly decreased WM CSA values ($***$) were also observed along the whole cervical SC, with higher decrease percentage (-36% to -41%, $\text{CSA} < 53 \text{ mm}^2$) at lower levels. As a consequence, whole SC CSA values were also significantly decreased in ALS patients (data not shown), the highest decrease being found at C4-C6 levels (-35% to -39%, $\text{CSA} < 63 \text{ mm}^2$).

Regional multi-parametric MRI

Characteristic mp-MRI maps collected in a HC subject and two age-matched ALS patients, one with moderate disease progression (ALS1, age=55, total ALSFRS-R=41, spinal ALSFRS-R=29, disease duration 41 months, $\text{DPR}=0.17 \text{ months}^{-1}$) and one with severe disease progression (ALS2, age=56, total ALSFRS-R=29, spinal ALSFRS-R=18, disease duration 17 months, $\text{DPR}=2.71 \text{ months}^{-1}$) are shown in Figure 4.

Metrics obtained for both populations in the different ROIs are summarized in Figure 5 (see also Supplementary Table 1 for a complete report of the results) and detailed below. According to the ANOVA, age did not influence any of the metric variations.

a) Bilateral CST (Figure 5.A): in ALS patients, FA and ihMTR were found significantly lower as compared to controls (more than 12% and 17% of decrease, respectively). $\lambda_{\perp}$ was higher (up to +23%) although non-significantly different after correction for multiple correction. A moderate but significant decrease was also observed for MTR (-10% at C5) and $\lambda_{\parallel}$ (-9% at C2).

b) Bilateral PST (Figure 5.B): Similar variations although less pronounced and less significant than in the CST were observed for all metrics. A significant ihMTR decrease down to -15% was notably observed at C2 and down to -10% at C5 level.

c) Whole WM (Figure 5.C): FA showed a significant decrease (down to -18% at C5), $\lambda_{\perp}$ a large and significant increase (more than +25%) whilst ADC increase was moderate but significant

at C5 after Bonferroni correction. More importantly, MTR and ihMTR were significantly decreased at both C2 and C5 levels (down to -25% and -15%, respectively).
d) Anterior GM horns (Figure 5.D): In average, slightly but non-significantly increased ADC values (between +9% and +12%) in association with increase of both $\lambda_{||}$ and $\lambda_{\perp}$ were observed for the patients as compared to HC. A moderate decrease tendency of MTR values along with a significant decrease of ihMTR values (down to -12%) were also found at both C2 and C5 levels.

Correlations with clinical scores

Whole SC CSA was found significantly correlated to both total ALSFRS-R score ($\rho=0.63$, $p=0.05$) and spinal ALSFRS-R sub-score ($\rho=0.64$, $p=0.05$) (cf. *Figure 6A*) at C5, and a correlation tendency was observed at C6. Altogether, DTI and MT/ihMT metrics impairments were largely correlated to disease duration and MRC, especially at the C2 level in all regions. Complete correlation results are summarized in *Supplementary Table 2*. CST metrics at C2 ($0.46 \leq |\rho| \leq 0.80$) were more correlated to clinical scores than at C5 ($0.49 \leq |\rho| \leq 0.61$). MTR/ihMTR in aGM at both levels were more correlated to clinical scores than DTI metrics. Representative significant correlations are illustrated in *Figure 6B, C, and D*.

Discussion

The main purpose of this study was to probe regional and microstructural changes of SC tissue occurring in ALS using a template-based analysis of multi-parametric MRI including high-resolution anatomical imaging, DTI and conventional/inhomogeneous MT. Results showed evidences for: 1) WM and SC atrophy throughout the whole cervical segment and GM atrophy mainly at lower levels; 2) significant DTI and ihMT metrics variations compared to HC in the lateral CST, the PST and the aGM at both C2 and C5 cervical levels; 3) Correlations between MR metrics and clinical scores within the investigated ROIs.

Spinal cord, Gray Matter and White Matter atrophies

Most neuroimaging studies in ALS were conducted in the brain and showed evidence of GM and WM degeneration^{6,8,43–49}. The few studies conducted so far in the cervical SC reported a reduction of cord CSA^{5,21,43,50,51}, but they did not investigate GM/WM separately, probably due to insufficient GM/WM image contrast and low spatial resolution. In this study, thanks to the high GM/WM contrast and the higher resolution of our T₂*-weighted images, we were able, not only to confirm the atrophy of cervical SC but also to quantify atrophy occurring in both GM and WM.

GM atrophy has already been widely investigated in ALS at the brain level^{6,7,52,53} and reported to reflect degeneration of UMN. Similarly, the GM atrophy we found here probably reflects degeneration/destruction of LMN as expected in ALS pathophysiology. Although no MR studies comparing the different forms of ALS (bulbar and spinal onset³) has yet been performed, the GM atrophy observed here is nonetheless highly consistent with the spinal-onset ALS form of our patients. As for WM atrophy, although WM loss have previously been reported by several studies conducted in the brain^{54,55}, studies conducted in the spinal cord only reported WM tracts degeneration, particularly the CST^{5,21,56,57}, but did not directly evaluate WM atrophy. In this study, based on automatic GM/WM segmentations of high-resolution anatomical T₂*-weighted images, we were able to probe WM atrophy in ALS patients which could be explained by WM tracts degeneration as will be discussed in the next section. Further longitudinal assessment will provide more insights to these GM and WM atrophies and their temporal evolution patterns.

ALS, a multi-system degenerative process

CST degeneration in ALS has already been shown by numerous MR studies using various techniques including DTI and MT in both the brain^{47,58,59} and the SC^{4,5,14,21,43}. Focusing on the spinal level, these studies reported reduced FA and increased $\lambda_{\perp}$ in the CST along the cervical SC^{4,5,14}, as well as reduced MTR^{5,21} and non-significant ADC increase and $\lambda_{||}$ decrease. Our findings confirm these results and add further evidence of CST impairment from the ihMTR

334 significant decrease observed at both C2 and C5 cervical levels. FA, $\lambda_{\parallel}$ and ADC changes may
335 suggest axonal degeneration or destructuration, whereas the increase of $\lambda_{\perp}$ combined with
336 decreased MTR and, more importantly with decreased ihMTR, the presumably myelin-specific
337 contrast, suggest demyelination of the CST. Interestingly, ihMTR demonstrated the most significant
338 variations as compared to the more conventional MTR and $\lambda_{\perp}$.

339 Although a moderate number of patients was included in this preliminary study, regression analyses
340 showed significant correlations between FA, $\lambda_{\perp}$ and total ALSFRS-R, spinal ALSFRS-R, MRC and
341 Hand Grip strength, at both C2 and C5 levels. Previous studies have reported correlations between
342 disease severity and FA in the lateral CST^{5,21}, but did not find any other significant correlations.

343 ALS neurodegeneration is however not restricted to the primary motor system. Although less
344 important and slower, it also extends to the sensory system as reported, not only in the brain^{4,44,60},
345 but also in the SC by Cohen-Adad et al.⁵ and Iglesias et al.⁴. Both SC studies reported significant
346 decrease of FA and increase of $\lambda_{\perp}$ while $\lambda_{\parallel}$ showed no change in the dorsal columns. Moreover,
347 Cohen-Adad et al. observed a decrease tendency of MTR values. In the present study, similar
348 patterns of metric variations were observed in the PST with a trend for $\lambda_{\perp}$ increase and MTR
349 decrease. Additionally, a significant ihMTR decrease was observed at both C2 and C5 levels, hence
350 suggesting similar, although less pronounced, alterations in PST than in CST, in agreement with the
351 two aforementioned studies reporting a less important impairment of sensory pathways than that of
352 motor systems. Again, interestingly, ihMT variations were found more important and more
353 significant than that of MTR and $\lambda_{\perp}$. Results of preliminary correlation analyses between MR
354 metrics and clinical score suggested degeneration/demyelination of PST as functional and muscular
355 deficits get worse, also in line with the hypothesis of progressive sensory pathways degeneration
356 observed by Iglesias et al.⁴.

357 Considering the whole cross-sectional WM, FA, MTR and ihMTR significantly decreased, $\lambda_{\perp}$
358 significantly increased, and ADC tended to increase. To our knowledge, the present study is the
359 first to investigate DTI and MT/ihMT metrics variations in the whole SC WM. The metric
360 variations observed along with the tendency of correlation to clinical scores are in line with those
361 measured in CST and PST taken separately, although the variation amplitude for all metrics in
362 whole WM tended to be higher. One hypothesis for this tendency would be the alteration of other
363 WM tracts such as anterior CST, rubrospinal or reticulospinal tracts. However, investigation of
364 these latter was beyond the scope of this pilot study due to their small size (less than 10 pixels).
365 Further studies with higher spatial resolution involving all WM tracts must thus be conducted to
366 identify all the altered tracts.

367 Even though ALS is primarily characterized by involvement of both UMN and LMN, assessing
368 reliably with MRI the spinal anterior GM that contains the motor neurons of interest remains a
369 challenge. Thanks to the highly contrasted and resolved T₂*-w images and the automatic AMU₄₀
370 WM/GM segmentation and ROI delimitation used in this study, we successfully investigated *in vivo*
371 spinal aGM degeneration with mp-MRI. Changes in ALS patients' metrics were found: FA tended
372 to decrease and ADC and $\lambda_{\parallel}$ to increase, which could be explained by neuronal loss. MTR tended to
373 decrease whilst ihMTR significantly did. GM oligodendroglial involvement in the SC has already
374 been reported in *in vitro* and *postmortem* ALS studies^{61,62}, and the ihMTR decrease observed here
375 might thus suggest GM demyelination. As for now, the ihMTR values obtained here in GM should
376 cautiously be interpreted as spatial blurring associated to the HASTE imaging readout might have
377 slightly contaminated GM voxel with WM signals. Results obtained in aGM are nonetheless of
378 particular interest for future explorations, and further studies using an alternative readout module
379 will be conducted to minimize the potential WM signal contamination effect. Finally, when
380 performing regression analyses, significant correlations between ihMTR and MTR and disease
381 duration at both C2 and C5 levels, between ihMTR and MTR and spinal ALSFRS-R, MRC and
382 Hand Grip strength at C2, and between $\lambda_{\parallel}$ and MRC at C5 were found. Taken together with atrophy,
383 these results suggest a more important neurodegeneration in lower cervical levels. This process

could be explained by the anatomical topography of motor neurons in the cervical SC described by Routal and Pal⁶³, which suggest that spinal aGM horns at lower cervical levels contain numerous and thicker motor neuron groups as compared to upper cervical levels. These motor neurons could be involved in early stage of ALS neurodegeneration according to the four-stage model of ALS proposed by Braak et al.⁶⁴. Further longitudinal follow-ups and multi-level investigations will be conducted to answer this specific question.

Limitations and perspectives

As aforementioned, we chose to limit the protocol to a one-hour acquisition time for the sake of patient comfort, hence precluding mp-MRI investigation of all cervical levels. To address this issue, further methodological developments are under consideration to allow for multi-slice and multi-angle capability for both DTI and ihMT sequences, hence providing multiple pure cross-sectional SC slices within a single acquisition.

Although challenging due to SNR loss, further developments towards a reduction of DTI and ihMT in-plane resolution ($0.9 \times 0.9 \text{ mm}^2$) and slice thickness (10 mm) are also to be considered. Increasing the spatial in-plane resolution would allow exploration of the posterior GM horns, so far unexplored for mp-MRI due to GM-WM partial volume effect (PVE). Reducing the slice thickness may also help limiting PVE, even though through-slice morphological variation in the SC is expected to be small when the slice is positioned strictly perpendicular to the cord axis. It is worth noting that this choice of slice thickness (10 mm) was driven by the important need of SNR of the ihMT acquisition and was applied to DTI to ensure the same PVE-induced contamination. Current strategies developed to enhance the ihMT signal²⁶ will greatly help to handle these issues in future studies.

These sequence improvements along with longitudinal patients' follow-ups will allow assessing both spatial and chronological progression of ALS. Additionally, further investigations on a larger cohort using a multivariate approach could lead to a better stratification of patients and finer characterization of SC degeneration. Furthermore, since some patients in this study showed asymmetric deficits when considering clinical scores (MRC score, Hand Grip strength) from left and right upper limbs, a careful investigation and correlation with mp-MRI metrics in lateralized ROIs, currently unavailable given the small cohort of patients, has to be performed in order to assess whether such asymmetry might also be observed in mp-MRI metrics variation.

Finally, when considering DTI and MT/ihMT metrics, ihMTR decreases were found systematically more important and more statistically significant than DTI and conventional MT metrics variations within all ROIs, suggesting a marked sensitivity of the ihMT technique to tissue microstructural changes. This technique will therefore be of great interest when performing longitudinal follow-ups on ALS patients to monitor spatial and temporal tissue demyelination. Moreover, it would also offer great perspectives for investigating other demyelinating pathologies such as Multiple Sclerosis. In this context, it is worth noting that the ihMT contrast, which relies on dipolar mechanisms, is magnetic-field independent and has been previously applied at both 1.5T and 3T. Translation to higher field strengths (e.g. 7T) should be feasible in principle as long as the RF energy deposition is maintained within regulatory constraints. In this perspective, recent strategies aiming at reducing the RF demand to optimize the ihMT ratio are of great utility²⁶.

Despite some limitations, this pilot study combining high-resolution morphological and quantitative MRI showed great potential to investigate WM/GM tissue degeneration and demyelination, two major processes occurring in ALS. It more particularly suggests that: 1) spinal GM atrophy as measured using T_2^* -weighted-derived CSA may be a sensitive biomarker of anterior GM horn cell degeneration in ALS; and 2) DTI and MT metrics, especially ihMT ratio, may be sensitive and potentially early biomarkers of demyelination occurring in spinal cord WM; 3) multi-parametric MRI using different but complementary MR techniques may be a great tool for a finer and more exhaustive characterization of SC-involving pathologies.

Conclusion

433 Thanks to dedicated template-based analysis, an efficient and reproducible way of extracting
434 information from anatomical structures, and a mp-MRI protocol combining high-resolution
435 anatomical imaging, DTI and the emerging myelin-specific inhomogeneous MT, we were able to
436 probe evidences, for the first time, for both SC GM and WM atrophy in ALS. We also brought new
437 elements clarifying the cervical GM horns degeneration, as well as corticospinal and posterior
438 sensory tracts impairments.

439 In addition to CST and PST destructure/degeneration shown by DTI metrics and preliminary
440 correlations with clinical scores, important demyelination has particularly been highlighted by
441 ihMT ratio decrease. Moreover, direct evidence of anterior GM horns involvement has also been
442 observed mostly through atrophy but also through ADC, $\lambda_{\perp}$ and ihMTR variations.

443 Further longitudinal studies in a larger population and with refined MR sequences are to be
444 conducted to investigate the prognostic role of GM atrophy and WM demyelination as markers of
445 disease progression and to provide more insights to the pattern of degeneration and underlying
446 processes involved in ALS pathophysiology.

447

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455

456 **Declaration of interest:**

457 The authors report no conflicts of interest to disclose.

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684

685 **Tables**

	Median	Interquartile range	Min - Max
Disease duration (months)	15.5	[11-22]	[7-53]
Total ALSFRS-R (0-48)	41	[31-42]	[29-44]
Spinal ALSFRS-R sub-score	26.2	[20.2-31.9]	[18-32]
Disease progression rate	0.41	[0.28-1.06]	[0.17-2.71]
Total MRC Score (0-70)	58.5	[42-67]	[19-70]
Upper motor neuron score	40	[33-42]	[30-47]
Hand Grip strength (kPa)	27	[4-80]	[0-100];

686 **Table 1:** Clinical features of the 10 ALS patients included in this study.

687

688

	DTI	ihMT	2D T2*-w	3D T2-w
Orientation / Readout ⁽¹⁾	Axial / Single Shot SE-EPI	Axial / HASTE	Axial / MGE	Sagittal / TSE
TR (s) / TE (ms)	~2.5/79	~4/TE _{eff} =48	0.523/5 echoes, TE _{eff} =27	1.5/124
Field of View	117x128mm ²	172x172mm ²	135x180mm ²	256x256mm ²
Voxel size	0.9x0.9x10mm ³	0.9x0.9x10mm ³	0.47x0.47x5mm ³	1x1x1mm ³
Location (spatial coverage)	C2 (5 slices) C5 (5 slices)	C2 (1 slice) C5 (1 slice)	C1-C3 (9 slices) C4-C7 (9 slices)	C1-C7
Flip angle	90/180°	90/120°	28°	110°
Parallel acquisition	GRAPPA reconstruction (R=2)	-	GRAPPA reconstruction (R=2)	-
Phase-Encoding direction	Right-Left	Right-Left	Anterior-Posterior	Head-Feet
Acquisition time	2x(~7min)	2x(~8min)	2x(3:44min)	5:53min

Table 2: Main MR parameters used in this study.

⁽¹⁾ SE-EPI: Spin-Echo Echo-Planar Imaging; HASTE: Half-Fourier Acquired Single-shot Turbo spin-Echo; MGE: Multiecho Gradient Echo, TSE: Turbo Spin-Echo; GRAPPA: Generalized auto calibrating partially parallel acquisitions; CSF: Cerebrospinal Fluid.

		FA (no units)			ADC ($\times 10^{-3} \text{ mm}^2/\text{s}$)			$\lambda_{//}$ ($\times 10^{-3} \text{ mm}^2/\text{s}$)			$\lambda_{\perp}$ ($\times 10^{-3} \text{ mm}^2/\text{s}$)			ihMTR (%)			MTR (%)		
		HC	ALS		HC	ALS		HC	ALS		HC	ALS		HC	ALS		HC	ALS	
C2	CST	6.96 ± 0.57	5.91 ± 0.92	** †	1.02 ± 0.10	1.05 ± 0.15		1.98 ± 0.14	1.81 ± 0.11	*** †	0.54 ± 0.12	0.67 ± 0.20	*	6.25 ± 0.70	5.20 ± 0.54	*** †	32.03 ± 2.06	30.34 ± 2.03	*
	PST	7.12 ± 0.63	6.67 ± 0.68		1.06 ± 0.09	1.04 ± 0.11		2.10 ± 0.14	1.96 ± 0.08	** †	0.54 ± 0.12	0.59 ± 0.15		6.09 ± 0.55	5.20 ± 0.64	*** †	32.15 ± 2.02	30.23 ± 2.61	*
	Whole WM	6.99 ± 0.68	6.01 ± 0.74	** †	1.02 ± 0.11	1.07 ± 0.12		1.99 ± 0.12	1.87 ± 0.05	** †	0.53 ± 0.14	0.67 ± 0.16	* †	6.29 ± 0.42	5.13 ± 0.52	*** †	33.01 ± 1.77	30.42 ± 1.94	** †
	aGM	5.26 ± 0.61	4.94 ± 0.63		0.95 ± 0.09	1.04 ± 0.15	*	1.57 ± 0.11	1.65 ± 0.13	*	0.64 ± 0.10	0.73 ± 0.16		6.24 ± 0.39	5.48 ± 0.35	*** †	34.66 ± 2.14	33.46 ± 2.10	
C5	CST	6.25 ± 0.58	5.51 ± 0.79	** †	1.06 ± 0.13	1.12 ± 0.16		1.88 ± 0.14	1.83 ± 0.12		0.64 ± 0.15	0.76 ± 0.19		5.60 ± 0.47	4.45 ± 0.56	*** †	30.03 ± 2.08	26.90 ± 2.10	*** †
	PST	6.50 ± 0.66	6.25 ± 0.84		1.08 ± 0.13	1.08 ± 0.15		1.97 ± 0.12	1.91 ± 0.09		0.64 ± 0.16	0.67 ± 0.20		5.23 ± 0.40	4.72 ± 0.49	* †	29.15 ± 1.93	27.40 ± 1.53	*
	Whole WM	6.57 ± 0.60	5.41 ± 0.71	** †	1.05 ± 0.13	1.19 ± 0.17	* †	1.94 ± 0.11	1.92 ± 0.10		0.60 ± 0.16	0.82 ± 0.20	** †	5.53 ± 0.45	4.14 ± 0.34	*** †	30.01 ± 2.08	25.52 ± 1.85	*** †
	aGM	4.57 ± 0.80	4.59 ± 0.61		0.93 ± 0.15	1.04 ± 0.19		1.44 ± 0.23	1.59 ± 0.20		0.68 ± 0.14	0.77 ± 0.19		5.47 ± 0.36	4.76 ± 0.39	*** †	31.01 ± 1.48	29.34 ± 1.45	*

Supplementary Table 1: DTI and MT/ihMT metrics obtained for ALS and HC subjects in CST, PST, whole WM and anterior GM at both C2 and C5 levels. Values are mean ± standard deviation. P-values resulting from two-way analyses of variance are reported such as *p<0.05, **p<0.005, ***p<0.0005, uncorrected for multiple comparisons. (†) indicate statistical differences that survive Bonferroni correction for multiple comparisons.

			Total ALSFRS-R	Spinal ALSFRS-R	Disease duration	MRC	Hand Grip
C2	CST	FA	0.50 *	0.56 *	-0.47 *	0.79 ***	0.52 *
		ADC	-0.55 *	-0.60 **	0.56 *	-0.80 ***	-0.59 *
		$\lambda//$	-0.50 *	-0.54 *	0.53 *	-0.49 *	-0.45 *
		$\lambda_{\perp}$	-0.54 *	-0.58 *	0.54 *	-0.80 ***	-0.54 *
		ihMTR	0.46 *	0.47 *	-0.56 *	0.26	-0.00
		MTR	0.29	0.26	-0.34	0.24	0.15
	PST	FA	0.02	0.03	-0.36	0.61 **	0.17
		ADC	-0.17	-0.19	0.46 *	-0.62 **	-0.10
		$\lambda//$	-0.23	-0.27	0.35	-0.48 *	0.01
		$\lambda_{\perp}$	-0.05	0.06	0.43	-0.60 **	-0.05
		ihMTR	0.29	0.31	-0.73 ***	0.36	0.06
		MTR	0.37	0.35	-0.61 **	0.34	-0.05
	WM	FA	0.21	0.19	-0.50	0.66 *	0.37
		ADC	-0.34	-0.33	0.69 *	-0.69 *	-0.53
		$\lambda//$	-0.64 *	-0.70 *	0.87 **	-0.64 *	-0.90 **
		$\lambda_{\perp}$	-0.34	0.33	0.69 *	-0.69 *	-0.53
		ihMTR	0.51	0.48	-0.85 **	0.47	0.52
		MTR	0.37	0.33	-0.66 *	0.16	0.23
	aGM	FA	0.33	0.36	-0.58 *	0.64 **	0.51 *
		ADC	-0.03	0.00	0.46 *	-0.29	-0.19
		$\lambda//$	0.13	0.21	0.10	-0.05	-0.09
		$\lambda_{\perp}$	-0.16	-0.16	0.52 *	-0.44 *	-0.34
		ihMTR	0.50 *	0.55 *	-0.70 ***	0.70 **	0.50 *
		MTR	0.45 *	0.48 *	-0.57 *	0.52 *	0.35
C5	CST	FA	0.53 *	0.49 *	-0.14	0.57 *	0.61 *
		ADC	-0.44	-0.38	0.04	-0.48 *	-0.47
		$\lambda//$	-0.06	-0.04	-0.15	-0.20	-0.06
		$\lambda_{\perp}$	-0.49 *	-0.44	0.10	-0.54 *	-0.52 *
		ihMTR	0.01	0.02	-0.18	0.19	0.03
		MTR	0.01	0.00	-0.03	0.11	-0.20
	PST	FA	0.58 *	0.49 *	-0.35	0.46 *	0.46
		ADC	-0.63 **	-0.54 *	0.49 *	-0.49 *	-0.45
		$\lambda//$	-0.10	-0.03	0.49 *	-0.38	-0.06
		$\lambda_{\perp}$	-0.65 **	-0.56 *	0.42	-0.48 *	-0.50 *
		ihMTR	0.02	0.05	-0.36	0.39	0.21
		MTR	0.10	0.12	-0.35	-0.05	-0.26
	WM	FA	0.39	0.32	-0.02	0.26	0.08
		ADC	-0.46	-0.36	0.06	-0.27	-0.10
		$\lambda//$	-0.26	-0.17	0.05	-0.19	-0.2
		$\lambda_{\perp}$	-0.47	-0.40	0.06	-0.29	-0.12
		ihMTR	-0.24	-0.28	-0.35	-0.11	-0.15
		MTR	-0.03	-0.07	-0.21	-0.40	-0.58
	aGM	FA	0.44	0.47	-0.33	0.37	0.66 **
		ADC	-0.44	-0.38	0.38	-0.43	-0.28
		$\lambda//$	-0.24	-0.15	0.35	-0.51 *	-0.07
		$\lambda_{\perp}$	-0.50 *	-0.46 *	0.38	-0.35	-0.38
		ihMTR	-0.05	0.00	-0.53 *	0.12	0.33
		MTR	0.16	0.19	-0.53 *	0.23	0.20

Supplementary Table 2: Spearman's coefficient correlations (ρ) between MRI metrics and clinical scores in ALS patients. Significant correlations are highlighted in bold and marked with * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

Figures

Figure 1: Post-processing pipeline for MRI data, shown for C2 cervical level, and including spinal cord segmentation on T_2^* -w images, registration to the AMU₄₀ template, GM/WM segmentations in the subject space followed by cross-sectional area extraction, as well as multi-parametric data intra-subject normalization allowing metrics extraction in whole WM, corticospinal tracts, posterior sensitive tracts and anterior GM horns.

Figure 2: Gray and white matter atrophy in ALS patients.

T_2^* -w images and representative GM/WM automatic segmentations (blue and yellow masks, respectively) extracted from a healthy control subject (top) and an age-matched ALS patient (bottom, ALSFRS-R=30, disease duration 16 months) at both C2 and C5 levels.

Figure 3: Cross-sectional areas in mm² in healthy controls and ALS patients.

Mean values extracted from GM/WM segmentations in the subject spaces and averaged for each cervical level from C1 to C6. The boxplot bars represent from bottom to top: the intra-group outermost value that falls within the distance computed as $1^{\text{st}} \text{ quartile} - 1.5 \cdot (3^{\text{rd}} \text{ quartile} - 1^{\text{st}} \text{ quartile})$, the first quartile (25%), the median value, the third quartile (75%) and the outermost value computed as $3^{\text{rd}} \text{ quartile} + 1.5 \cdot (3^{\text{rd}} \text{ quartile} - 1^{\text{st}} \text{ quartile})$, respectively for each cervical level; isolated markers are outliers.

CSA variation rate (%) between controls and patients for each level is calculated using the following formula: $\% = (\text{CSA}_{\text{patients}} - \text{CSA}_{\text{controls}}) / \text{CSA}_{\text{controls}}$, negative percentage indicating a decrease in patients' values. P-values are reported as: * $p < 0.05$ and *** $p < 0.0005$, uncorrected for multiple comparisons. (■) indicate statistical differences that survive Bonferroni correction for multiple comparisons (adjusted Bonferroni $p < 0.0083$).

Figure 4: Representative multi-parametric maps at C2-level extracted from an age-matched healthy control subject (top) and two representative ALS patients: ALS1 (middle, ALSFRS-R=41, disease duration 41 months) with moderately altered metrics and ALS2 (bottom, ALSFRS-R=29, disease duration 7 months) with severely altered metrics.

Figure 5: DTI and MT/ihMT metrics obtained for ALS and HC subjects in (A) CST, (B) PST, (C) whole WM and (D) anterior GM at both C2 and C5 levels. Metrics variation rates (%) between ALS (in blue) and HC (in black) are indicated along with p-values (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, uncorrected for multiple comparisons) resulting from two-way analyses of variance. (■) indicate statistical differences that survive Bonferroni correction for multiple comparisons.

Figure 6: Representative correlations between MRI metrics and clinical scores in ALS patients.

Correlation coefficients and p-values are derived from Spearman's correlation. When existing, statistical level of significance is indicated as: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.