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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 (diffusion tensor imaging/inhomogeneous magnetization transfer)**

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

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

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

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

90 *Compared to healthy controls, GM and WM atrophies were observed in ALS patients, especially at*  
91 *lower cervical levels, where a strong correlation was additionally observed between GM atrophy*  
92 *and the UMN score ( $R=-0.75$ ,  $p=0.05$  at C6). Interestingly, significant decrease of ihMT ratios was*  
93 *found in all the regions of interest ( $p$ -values $<0.0008$ ), FA and MT ratios significantly decreased in*  
94 *CST especially at C5 ( $p$ -values $<0.005$ ) and  $\lambda_{//}$  significantly decreased in CST ( $p=0.0004$ ) and PST*  
95 *( $p=0.003$ ) at C2. Strong correlations between MRI metrics and clinical scores were also found*  
96 *( $0.47<|R|<0.87$ ,  $p$ -values $<0.05$ ).*

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

101

102 Keywords:

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

105

106 **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<sup>1,2</sup>.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 pathophysiology. 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 pathways<sup>3-5</sup>, and presence of gray matter (GM) atrophy and degeneration<sup>6,7</sup> 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 brain<sup>8</sup>, 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<sup>9,10</sup>.

123 Thanks to recent improvements including data sampling strategies or triggering method<sup>11,12</sup>, 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 reported<sup>5</sup>. 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)<sup>4,5,13,14</sup>.

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 mechanisms<sup>15-17</sup>, our aim here is to combine them with high-resolution  
 133 anatomical imaging and the emerging inhomogeneous MT technique (ihMT)<sup>18,19</sup>.

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 SC<sup>12</sup>. 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 technique<sup>11</sup>. 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<sup>5,20,21</sup>. 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  $T_{ID}$ <sup>22</sup>. 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  $T_{ID}$ <sup>23,24</sup> 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<sup>19,25</sup>.  
150 Although extensive work is still on-going for the full characterization of this technique<sup>24,26</sup>, it has  
151 already been used to assess brain and SC tissue demyelination in normal aging and multiple  
152 sclerosis<sup>27,28</sup>. 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<sup>28,29</sup>. 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 criteria<sup>30</sup> 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)<sup>31</sup>, from which the disease progression rate (DPR) was derived such that  $DPR$  (*in*  
179 *months*<sup>-1</sup>) =  $(48 - \text{ALSFRS-R}) / \text{disease duration (in months)}$ . 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)<sup>32</sup>, 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)<sup>32,33</sup> 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)<sup>34,35</sup>.

### 188 ***MR Imaging and preprocessing***

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

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

200  
 201 **Diffusion Tensor Imaging (DTI)** consisted in two axial slabs acquired using a monopolar single-  
 202 shot spin-echo echo-planar imaging (SE-EPI) prototype sequence (ECG-gating<sup>36</sup>, trigger delay  
 203 300ms, TR ~ 2500ms (3 heart beats), fat saturation, 4 averages), placed perpendicularly to the SC  
 204 axis: one was positioned mid-vertebrae at C2 level and the other one at C5 level. Local B<sub>0</sub>  
 205 shimming was performed prior to the acquisition. Diffusion parameters were 30 independent  
 206 diffusion-encoding directions, 2 b-values (0 and 800s/mm<sup>2</sup>), and δ/Δ 15.42/34.24 ms.  
 207 For each acquisition, in-line correction of eddy-current-induced distortions was performed using  
 208 dynamic field correction<sup>37</sup>. The DTI metrics (*Fractional Anisotropy (FA)*; *Apparent Diffusion*  
 209 *Coefficient (ADC)*; *Radial diffusivity (λ<sub>⊥</sub>)*; *Axial diffusivity (λ<sub>∥</sub>)*) were calculated online using  
 210 Siemens Neuro 3D software.

211  
 212 **Classic and inhomogeneous Magnetization transfer (MT/ihMT)** imaging was similarly performed  
 213 at C2 and C5 levels with a customized pulsed saturation preparation optimized from<sup>19</sup>, (Hann-  
 214 shaped pulses lasting 0.5ms each, repeated every 1ms for a total saturation duration of 500ms,  
 215 alternating single- and dual-frequency with a f=7kHz saturation offset, for a 4.9μT root-mean-  
 216 square B<sub>1</sub> calculated over the whole saturation duration, hence delivering a total energy of  
 217 12.1μT<sup>2</sup>.s) using a single-slice half-Fourier acquired single-shot turbo spin echo (HASTE) readout.  
 218 At each level, 4 unsaturated images (M<sub>0</sub>) along with 4 repetitions of 30 interleaved images per  
 219 saturation scheme were acquired (124 images per time series in total). ECG data were recorded for  
 220 retrospective correction of cerebrospinal fluid pulsatility-induced artifact as described in<sup>19</sup>.  
 221 MT/ihMT ratios (*MTR/ihMTR*) were subsequently reconstructed using Matlab scripts (MATLAB  
 222 R2012a, Mathworks, Natick, MA, USA), according to:

$$223 \quad \mathbf{MTR} = \mathbf{1} - \mathbf{MT}_{(+f)}/\mathbf{M}_0 \quad (\text{Eq. 1})$$

$$224 \quad \mathbf{ihMTR} = [\mathbf{MT}_{(+f)} + \mathbf{MT}_{(-f)} - \mathbf{MT}_{(+/-f)} - \mathbf{MT}_{(-/+f)}]/\mathbf{M}_0 \quad (\text{Eq. 2})$$

225 Where  $\mathbf{MT}_{(+f)}$  and  $\mathbf{MT}_{(-f)}$  correspond to a single offset saturation performed at positive and  
 226 negative offset respectively;  $\mathbf{MT}_{(+/-f)}$  and  $\mathbf{MT}_{(-/+f)}$  correspond to dual offset saturations.

## 227 **Post-processing**

228 For each subject, individual anatomical T<sub>2</sub>\*-weighted and multi-parametric MR data were first co-  
 229 registered. The sagittal 3D T<sub>2</sub>-w volume was then automatically segmented using PropSeg<sup>38</sup> and  
 230 non-linearly registered to the MNI-Poly-AMU template<sup>39</sup> so as to provide an automated vertebral  
 231 labeling of each subject scan. Then, for each level, automatic spinal cord segmentation and ROI  
 232 delimitation based on the T<sub>2</sub>\*-w images were performed using the probabilistic WM/GM AMU<sub>40</sub><sup>40</sup>  
 233 and WM tracts<sup>41</sup> atlases, as summarized in *Figure 1* and previously described in<sup>28</sup>. The whole  
 234 processing was performed using a customized MATLAB (MATLAB R2012a, Mathworks, Natick,  
 235 MA, USA) script based on functionalities included in the SC Toolbox<sup>42</sup>.

236 After post-processing, the dataset for each subject consisted of 4 DTI metrics and 2 MT metrics  
 237 extracted from the bilateral CST, PST, and anterior GM (aGM) horns at C2 and C5 levels. Total  
 238 GM was not considered for mp-MRI quantification due to potential partial volume effect. AMU<sub>40</sub>-

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

## 245 *Statistical Analysis*

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

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

## 257 **Results**

### 258 *Cross-sectional areas.*

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

### 268 *Regional multi-parametric MRI*

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

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

277 *a) Bilateral CST (Figure 5.A):* in ALS patients, FA and ihMTR were found significantly lower  
278 as compared to controls (more than 12% and 17% of decrease, respectively).  $\lambda_{\perp}$  was higher (up to  
279 +23%) although non-significantly different after correction for multiple correction. A moderate but  
280 significant decrease was also observed for MTR (-10% at C5) and  $\lambda_{\parallel}$  (-9% at C2).

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

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



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

### 292 *Correlations with clinical scores*

293 Whole SC CSA was found significantly correlated to both total ALSFRS-R score ( $\rho=0.63$ ,  $p=0.05$ )  
294 and spinal ALSFRS-R sub-score ( $\rho=0.64$ ,  $p=0.05$ ) (cf. *Figure 6A*) at C5, and a correlation tendency  
295 was observed at C6. Altogether, DTI and MT/ihMT metrics impairments were largely correlated to  
296 disease duration and MRC, especially at the C2 level in all regions. Complete correlation results are  
297 summarized in *Supplementary Table 2*. CST metrics at C2 ( $0.46 \leq |\rho| \leq 0.80$ ) were more correlated to  
298 clinical scores than at C5 ( $0.49 \leq |\rho| \leq 0.61$ ). MTR/ihMTR in aGM at both levels were more correlated  
299 to clinical scores than DTI metrics. Representative significant correlations are illustrated in *Figure*  
300 *6B, C, and D*.

### 301 **Discussion**

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

### 309 *Spinal cord, Gray Matter and White Matter atrophies*

310 Most neuroimaging studies in ALS were conducted in the brain and showed evidence of GM and  
311 WM degeneration<sup>6,8,43-49</sup>. The few studies conducted so far in the cervical SC reported a reduction  
312 of cord CSA<sup>5,21,43,50,51</sup>, but they did not investigate GM/WM separately, probably due to insufficient  
313 GM/WM image contrast and low spatial resolution. In this study, thanks to the high GM/WM  
314 contrast and the higher resolution of our  $T_2^*$ -weighted images, we were able, not only to confirm  
315 the atrophy of cervical SC but also to quantify atrophy occurring in both GM and WM.

316 GM atrophy has already been widely investigated in ALS at the brain level<sup>6,7,52,53</sup> and reported to  
317 reflect degeneration of UMN. Similarly, the GM atrophy we found here probably reflects  
318 degeneration/destruction of LMN as expected in ALS pathophysiology. Although no MR  
319 studies comparing the different forms of ALS (bulbar and spinal onset<sup>3</sup>) has yet been performed, the  
320 GM atrophy observed here is nonetheless highly consistent with the spinal-onset ALS form of our  
321 patients. As for WM atrophy, although WM loss have previously been reported by several studies  
322 conducted in the brain<sup>54,55</sup>, studies conducted in the spinal cord only reported WM tracts  
323 degeneration, particularly the CST<sup>5,21,56,57</sup>, but did not directly evaluate WM atrophy. In this study,  
324 based on automatic GM/WM segmentations of high-resolution anatomical  $T_2^*$ -weighted images,  
325 we were able to probe WM atrophy in ALS patients which could be explained by WM tracts  
326 degeneration as will be discussed in the next section. Further longitudinal assessment will provide  
327 more insights to these GM and WM atrophies and their temporal evolution patterns.

### 328 *ALS, a multi-system degenerative process*

329 CST degeneration in ALS has already been shown by numerous MR studies using various  
330 techniques including DTI and MT in both the brain<sup>47,58,59</sup> and the SC<sup>4,5,14,21,43</sup>. Focusing on the  
331 spinal level, these studies reported reduced FA and increased  $\lambda_{\perp}$  in the CST along the cervical  
332 SC<sup>4,5,14</sup>, as well as reduced MTR<sup>5,21</sup> and non-significant ADC increase and  $\lambda_{\parallel}$  decrease. Our  
333 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<sup>5,21</sup>, 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<sup>4,44,60</sup>,  
345 but also in the SC by Cohen-Adad et al.<sup>5</sup> and Iglesias et al.<sup>4</sup>. 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.<sup>4</sup>.

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 T<sub>2</sub>\*-w images and the automatic AMU<sub>40</sub>  
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<sup>61,62</sup>, 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

384 could be explained by the anatomical topography of motor neurons in the cervical SC described by  
385 Routal and Pal<sup>63</sup>, which suggest that spinal aGM horns at lower cervical levels contain numerous  
386 and thicker motor neuron groups as compared to upper cervical levels. These motor neurons could  
387 be involved in early stage of ALS neurodegeneration according to the four-stage model of ALS  
388 proposed by Braak et al.<sup>64</sup>. Further longitudinal follow-ups and multi-level investigations will be  
389 conducted to answer this specific question.

### 390 *Limitations and perspectives*

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

396 Although challenging due to SNR loss, further developments towards a reduction of DTI and ihMT  
397 in-plane resolution ( $0.9 \times 0.9 \text{ mm}^2$ ) and slice thickness (10 mm) are also to be considered. Increasing  
398 the spatial in-plane resolution would allow exploration of the posterior GM horns, so far unexplored  
399 for mp-MRI due to GM-WM partial volume effect (PVE). Reducing the slice thickness may also  
400 help limiting PVE, even though through-slice morphological variation in the SC is expected to be  
401 small when the slice is positioned strictly perpendicular to the cord axis. It is worth noting that this  
402 choice of slice thickness (10 mm) was driven by the important need of SNR of the ihMT acquisition  
403 and was applied to DTI to ensure the same PVE-induced contamination. Current strategies  
404 developed to enhance the ihMT signal<sup>26</sup> will greatly help to handle these issues in future studies.

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

413 Finally, when considering DTI and MT/ihMT metrics, ihMTR decreases were found systematically  
414 more important and more statistically significant than DTI and conventional MT metrics variations  
415 within all ROIs, suggesting a marked sensitivity of the ihMT technique to tissue microstructural  
416 changes. This technique will therefore be of great interest when performing longitudinal follow-ups  
417 on ALS patients to monitor spatial and temporal tissue demyelination. Moreover, it would also offer  
418 great perspectives for investigating other demyelinating pathologies such as Multiple Sclerosis. In  
419 this context, it is worth noting that the ihMT contrast, which relies on dipolar mechanisms, is  
420 magnetic-field independent and has been previously applied at both 1.5T and 3T. Translation to  
421 higher field strengths (e.g. 7T) should be feasible in principle as long as the RF energy deposition is  
422 maintained within regulatory constraints. In this perspective, recent strategies aiming at reducing  
423 the RF demand to optimize the ihMT ratio are of great utility<sup>26</sup>.

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

### 432 **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 destructuration/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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685 **Tables**

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

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

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

**Table 2: Main MR parameters used in this study.**

<sup>(1)</sup> 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.

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

**Supplementary Table 2:** Spearman's coefficient correlations ( $\rho$ ) 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<sub>2</sub>\*-w images, registration to the AMU<sub>40</sub> 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<sub>2</sub>\*-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 mm<sup>2</sup> 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<sup>st</sup> quartile – 1.5\*(3<sup>rd</sup> quartile – 1<sup>st</sup> quartile), the first quartile (25%), the median value, the third quartile (75%) and the outermost value computed as 3<sup>rd</sup> quartile + 1.5\*(3<sup>rd</sup> quartile – 1<sup>st</sup> 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:  $\% = (CSA_{patients} - CSA_{controls}) / CSA_{controls}$ , 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.