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SCIENTIFIC ARTICLE

A pilot study assessing T1-weighted muscle MRI in amyotrophic lateral sclerosis (ALS)

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Abstract

The authors set out to study the role of T1-weighted muscle MRI in the diagnostic phase of ALS, comparing images from ten patients and nine age-matched healthy controls (HCs). All subjects underwent MRI of 68 muscles in the hands, paraspinal regions and lower limbs; the images were semi-quantitatively scored. Atrophy was more frequent in muscles of ALS patients than HCs ($p < 0.0001$); fatty infiltration was particularly marked in iliopsoas ($p = 0.046$), anterior ($p = 0.020$) and posterior ($p = 0.047$) calf muscles in patients. A trend towards agreement was found between MRI and clinic-EMG data for the first dorsal interosseous, paraspinal, and tibial anterior muscles. Muscle T1-weighted MRI can distinguish ALS patients from HCs for specific regions (i.e., legs). MRI abnormalities could be found in pauci-symptomatic spinal muscles in bulbar-onset patients. Muscle MRI may be a useful diagnostic tool in ALS, in particular for muscles difficult to investigate using clinical-EMG methods.

Keywords Amyotrophic lateral sclerosis · Muscle magnetic resonance imaging · Biomarkers · Electromyography · Muscle damage

Introduction

Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative disease usually assumed to target motor neurons (MNs). However, evidence of involvement of other cells types [1, 2], also outside the central nervous system, has challenged this "neurocentric" view of ALS, which may thus be defined as a "multi-systemic" disease [3]. Although muscle abnormalities in ALS are habitually considered secondary to MN damage [4], muscle, too, can be a primary target [5, 6], with "dying back" degeneration of MNs occurring subsequently. In recent years, efforts to clarify the pathogenesis of ALS have focused on muscle tissue [7]. Muscle magnetic resonance imaging (MRI), shown to be useful to define prognostic biomarkers of ALS in the G93A-SOD1 mouse model [8], is an established diagnostic tool in inherited and acquired neuromuscular disorders [9, 10]. In particular, T2-weighted (T2-w) images show acute pathological processes (e.g., edema)

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[11], and T1-weighted (T1-w) sequences disclose chronic alterations (atrophy, fatty substitution) [11]. However, literature data on the role of muscle MRI in ALS are scarce and heterogeneous. Longitudinal studies in small cohorts [12, 13] have evaluated, respectively, qualitative T1-w/T2-w images of the legs and volumetric T1-w images of the tongue, hands, and legs. Other authors [14] used T1-w and T2-w MRI to study nerve and muscle abnormalities in the upper and lower limbs of multifocal motor neuropathy (MMN) and ALS patients. Furthermore, brachial plexus and limb-girdle muscle MRI has been shown to play a pivotal role in the differential diagnosis between ALS and inflammatory conditions [15]. Recently, Jenkins et al. investigated the role of T2-w whole-body muscle MRI as a biomarker of denervation in MN disease [16].

To study the role of muscle MRI in the diagnosis of ALS, we compared T1-w images of hand, paraspinal and lower limb muscles in newly diagnosed ALS patients and in age-matched healthy controls (HCs) to look for evidence of muscular atrophy and remodeling (i.e., fatty substitution) and to relate the radiological findings to clinical and electromyographic (EMG) data.

Materials and methods

Patients

In this cross-sectional pilot study, we enrolled ten right-handed patients newly diagnosed with probable or definite ALS, using the El Escorial criteria [17], between January 1, 2015 and December 31, 2015 at the C. Mondino National Neurological Institute. Exclusion criteria were inability to give informed consent, a contraindication to MRI, respiratory failure impairing ability to lie flat in the scanner. We scored clinical severity using the ALS Functional Rating Scale revised (ALS-FRSr) [18], and upper MN (UMN) involvement using the UMN burden score [19]. Nine age-matched healthy controls (HCs) were also recruited for MRI analysis. The institute's ethics committee approved the study and all subjects gave their written informed consent.

MRI data analysis

With subjects supine, a 1.5-T MRI scanner (Philips Gyroscan, Koninklijke, The Netherlands) was used to obtain sequential axial T1-w images (slice thickness, 10 mm; interslice gap, 1 mm; repetition time (TR), 500 ms; echo time (TE), 15 ms; signal averages, 1; voxel size 1.19 × 0.95 × 10) from the femoral head to the ankle, in order to study of the thigh and calf muscles. Axial T1-w (slice thickness, 10 mm; interslice gap, 0.3 mm; TR, 500 ms; TE, 8 ms; signal averages, 4; voxel size, 0.8 × 1.01 × 10 mm) and coronal T1-w images (slice thickness, 5 mm; interslice gap, 1 mm; TR, 500 ms; TE, 15 ms; signal averages, 3; voxel size, 0.9 × 1.2 × 5 mm) were also obtained to study the paravertebral muscles from the intermediate dorsal to the sacral region. Finally, with subjects prone, hand muscles were studied with axial (slice thickness, 3 mm; interslice gap, 5 mm; TR, 300 ms; TE, 10 ms; signal averages, 4; voxel size, 1.19 × 1 × 3 mm) and coronal T1-w sequences (slice thickness, 3 mm; interslice gap, 3 mm; TR, 300 ms; TE, 10 ms, signal averages, 4; voxel size, 1.19 × 1 × 3 mm). Sequence parameters varied between individuals to ensure full anatomical coverage. Total scanning time was usually under 90 min.

Scans were examined by expert neuroradiologists (A.P., S.B.), blinded to clinical data. They looked for normal and abnormal muscle bulk (atrophy – yes/no) and normal and abnormal signal intensity. Intrarater and inter-rater reproducibility was first confirmed by the two independent raters, who achieved coefficients of variation < 5% for all regions of interest. Each muscle was assessed on T1-w sequences and graded for degree of fatty substitution using the Mercuri scale [20], i.e.:

- Normal: no traces of increased signal intensity in the muscle, graded 0;
- Mild: only traces of increased signal intensity in otherwise well-preserved muscle, graded 1;
- Moderate: increased signal intensity in less than 50% of the muscle, graded 2;
- Severe: increased signal intensity in at least 50% of the muscle, graded 3.

EMG data analysis

We performed a semiautomatic quantitative analysis of the motor unit action potentials (MUAPs) in the first dorsal interosseous, thoracic paravertebral, and anterior tibial muscles, using Medelec Synergy SYN5-C (©Viasys Healthcare, Manor Way, Old Woking, Surrey, UK). We recorded at least ten MUAPs per muscle and considered their mean duration (ms). Mean MUAP duration was considered normal (< 10 ms), mild altered (10–12.5 ms), moderate altered (12.5–15 ms), severe altered (> 15 ms) for the tibial anterior muscle, and normal (< 9 ms), mild altered (9–11.25 ms), moderate altered (11.25–13.5 ms), severe altered (> 13.5 ms) for first dorsal interosseous, and thoracic paravertebral muscles.

Statistical analysis

Data are reported as median and IQR range for quantitative variables and percentages for categorical ones. A UMN burden score of 13 was used to stratify patients by UMN involvement, and clinically considered suitable for distinguish

patients with mild and severe UMN involvement; this cut-off was determined by the range and distribution of values, and following a clinical decision. To compare differences in MRI data among groups, Chi-square tests and non-parametric Mann–Whitney tests were performed. Spearman correlation coefficient was used to evaluate the association between MRI and ALSFRS-r items. *P* values ≤ 0.05 were considered significant (two-sided). The statistical software STATA V.14 was used for the analysis.

Results

Ten patients (six males, four females; median age, 67.7 years, IQR 63.1–71.8) with definite ($n = 3$), probable ($n = 4$) or probable laboratory-supported ($n = 3$) ALS, and nine age-matched HCs (six males, three females; median age, 69.6 years, IQR 64.4–73.4) were recruited. Clinical and demographic data are summarized in Table 1.

Lower limb MRI was performed in all patients and HCs, paraspinal MRI in all patients and 8/9 HCs, right-hand MRI in 7/10 patients and 7/9 HCs, and left-hand MRI in 6/10 patients and 6/9 HCs (Table 1 - supplementary data). Muscle atrophy was more frequent in patients than HCs (22.46 vs. 0.72%, $p < 0.0001$). Median rates of fatty substitution analyzing muscles by region and singly are reported in Table 2. No difference was found between right and left side. There are no statistically significant differences between patients and HCs for hand ($p = 0.142$), thoracic paraspinal ($p = 0.100$), gluteus maximus ($p = 0.482$), anterior thigh ($p = 0.793$), posterior thigh ($p = 0.664$). We found statistically significant differences for iliopsoas ($p = 0.046$), anterior calf ($p = 0.020$), and posterior calf ($p = 0.047$). Figures 1 and 2 show sample MRI scans.

Median rates of fatty substitution between spinal ALS patients and HCs were not significant for hand ($p = 0.081$), thoracic paraspinal ($p = 0.079$), iliopsoas ($p = 0.117$), gluteus maximus ($p = 0.307$), anterior thigh ($p = 0.462$), and posterior thigh ($p = 0.609$). Hence, significant differences were found for anterior calf ($p = 0.009$) and posterior calf ($p = 0.031$). All the comparisons of median rates of fatty substitution between bulbar ALS patients and HCs were not statistically significant except for iliopsoas ($p < 0.05$). Median rates of fatty substitution between spinal and bulbar ALS patients were all not statistically significant. The same results were obtained after stratifying patients for UMN involvement ($p > 0.05$, data not shown).

In all patients except one, mean duration of MUAPs (EMG analysis) was calculated for the first dorsal interosseous, thoracic paravertebral, anterior tibial muscles, and in all patients

Table 1 Clinical and demographic data of ALS patients (A, summary) and (B) for each patient

A)

Data	
Gender, M/F	6/4
Age at onset, median in years (IQR)	66.5 (62–70)
Time from onset to diagnosis, median in months (IQR)	7 (5–11)
UMN burden score, median (IQR)	13 (8–14)

B)

Patient ID	Gender	Age at onset (years)	Site of onset	Type of involvement	UMN score	ALSFRSr
1	M	58	Spinal	UMN + LMN right leg	16	40
2	M	86	Bulbar	UMN + LMN Tongue	13	43
3	M	78	Bulbar	UMN + LMN Tongue	2	44
4	F	63	Spinal	LMN Right leg	13	34
5	M	69	Spinal	LMN Arms	14	48
6	M	67	Spinal	LMN Left arm	13	44
7	F	68	Spinal	UMN Right leg	16	33
8	F	71	Spinal	LMN Left leg	1	38
9	M	66	Spinal	LMN Arms	8	39
10	F	57	Spinal	LMN Right arm	10	40

M male, *F* female, *ALS-FRSr* Amyotrophic Lateral Sclerosis Functional Rating Scale revised, *UMN* upper motor neuron, *LMN* lower motor neuron

Table 2 MRI data (degree of fatty substitution using the Mercuri scale) for patients and HCs

Muscle *Median (IQR)*	Patients ($n = 10$)	HCs ($n = 9$)	*P* value
Right hand	0 (0–0.5)	0 (0–0)	0.142
Right paraspinals	1 (1–2)	1 (0.5–0)	0.100
Right iliopsoas	1 (0–1)	0 (0–0)	0.046
Right gluteus maximus	1 (1–2)	1 (1–2)	0.482
Right anterior thigh	0.88 (0.5–1)	1 (0–1)	0.793
Right posterior thigh	1 (0.86–1)	1 (0–1)	0.664
Right anterior calf	0.875 (0.5–1)	0 (0–0.5)	0.020
Right posterior calf	1 (1–1.3)	0.67 (0.33–1)	0.047
Left hand	0 (0–0.5)	0 (0–0)	0.142
Left paraspinals	1 (1–2)	1 (0.5–1)	0.100
Left iliopsoas	1 (0–1)	0 (0–0)	0.046
Left gluteus maximus	1 (1–2)	1 (1–2)	0.482
Left anterior thigh	0.88 (0.5–1)	1 (0–1)	0.793
Left posterior thigh	1 (0.86–1)	1 (0–1)	0.664
Left anterior calf	0.875 (0.5–1)	0 (0.5–1)	0.020
Left posterior calf	1 (1–1.3)	0.67 (0.33–1)	0.047

MRC scale (clinical muscles strength) was registered for the first dorsal interosseous and tibial anterior muscles (table 2–supplementary data). ALS-FRSr items related to hand function, in particular writing and handling a knife were summarized and an inverse correlation with dominant hand MRI data was obtained ($r = -0.7217$) (Fig. 3).

Discussion and conclusions

Literature on muscle MRI in ALS is scarce and heterogeneous in terms of study type (longitudinal [12, 13, 16], cross-sectional [14, 15]), region scanned (upper limbs [13–15], lower limbs [12–15], tongue [13], whole body [16]), type of images obtained (volumetric [12, 13, 15], qualitative T1-w/T2-w [12, 15], quantitative T1-w/T2-w [12, 14, 15]), and use of controls (age-matched HCs [15], no age-matched HCs [12, 13], other neurological controls [14]). To date, authors have mainly performed lower limb MRI with T1-w sequences. Bryan et al. [12] used T1-w and T2-w sequences to assess leg muscles over a longitudinal 4-month follow-up in 11 ALS patients and eight HCs. Visual inspection revealed abnormalities ("moth-eaten" appearance) on T1-w images in six patients, but mean muscle T1 time and volume were not different between patients and HCs. Instead, the ALS patients showed increased muscle T2 time, evaluated using quantitative analysis. In a longitudinal study, Jenkins et al. [13] evaluated possible atrophy of the thenar eminence, first dorsal interosseous, tibialis anterior and tongue using volumetric muscle 3-T MRI in four ALS patients and 11 HCs. Progressive atrophy was found only in the thenar eminence and tibial anterior, and only in patients showing clinical progression. No data on subclinical atrophy were given. Clinical-radiological discordance in other muscles was suggested to reflect a contribution of UMN pathology. More recently, Staff et al. [14] studied the role of nerve and muscle 3-T/1.5-T MRI with T1-w and T2-w sequences in differential diagnosis between ALS and MMN. They showed upper and lower limb muscle MRI alterations in 57% of ALS patients ($n = 60$), specifically atrophy in 35%, while MMN patients had no atrophy. Gerevini et al. [15] suggested that muscle and nerve root/plexus MRI might aid differential diagnosis between ALS and inflammatory neuropathies. They showed T1-w and T2-w abnormalities in the subscapular, supra- and infraspinatus muscles of all ALS patients ($n = 23$). In particular, the supraspinatus was the most affected by T2-w alterations, while no difference in fatty substitution or atrophy was found. Very recently, Jenkins et al. [16] proposed a very fast protocol for T2-w whole-body muscle MRI in order to longitudinally study the denervation in motor neuron disease. They did not focus on chronic muscle alterations that are pivotal in neurodegenerative disorders.

In our explorative study, we qualitatively assessed hand, paraspinal, and lower limb muscles with T1-w MRI in ten newly diagnosed ALS patients and nine age-matched HCs. We selected those muscles because they belong to different regions (cervical, thoracic, lumbosacral) that are habitually evaluated in ALS patients in order to stage the disease using the El Escorial classification [17]. We did not perform MRI for all subjects because the requested position was extremely uncomfortable during the exam (i.e., prone position for hands). We detected statistically significant differences between the two groups for iliopsoas ($p = 0.046$), anterior calf ($p = 0.020$), and posterior calf ($p = 0.047$). The small range of values is due to the selection of patients with new diagnosis of ALS. No specific pattern of muscle involvement was found, as well as no asymmetry was detected. However, single muscle difference between left and right side was evident but it not emerged from the statistical analysis, probably due to a low sample size. We demonstrated that UMN damage is independent of muscle abnormalities, as no difference emerged on stratifying patients for UMN burden score.

Muscle analysis considering spinal ALS patients and HCs confirmed differences for anterior calf ($p = 0.009$), and posterior calf ($p = 0.031$). Furthermore, there is significant result for iliopsoas ($p < 0.05$) between bulbar ALS patients and HCs. No difference emerged between bulbar and spinal ALS patients. In this subgroup analysis, the disequilibrium of bulbar ($n = 2$) and spinal ($n = 8$) patients is pivotal to consider for the results, and it is necessary to increase the power size and improve the balance of recruitment to confirm these data. At the moment, it is possible to affirm that our spinal ALS

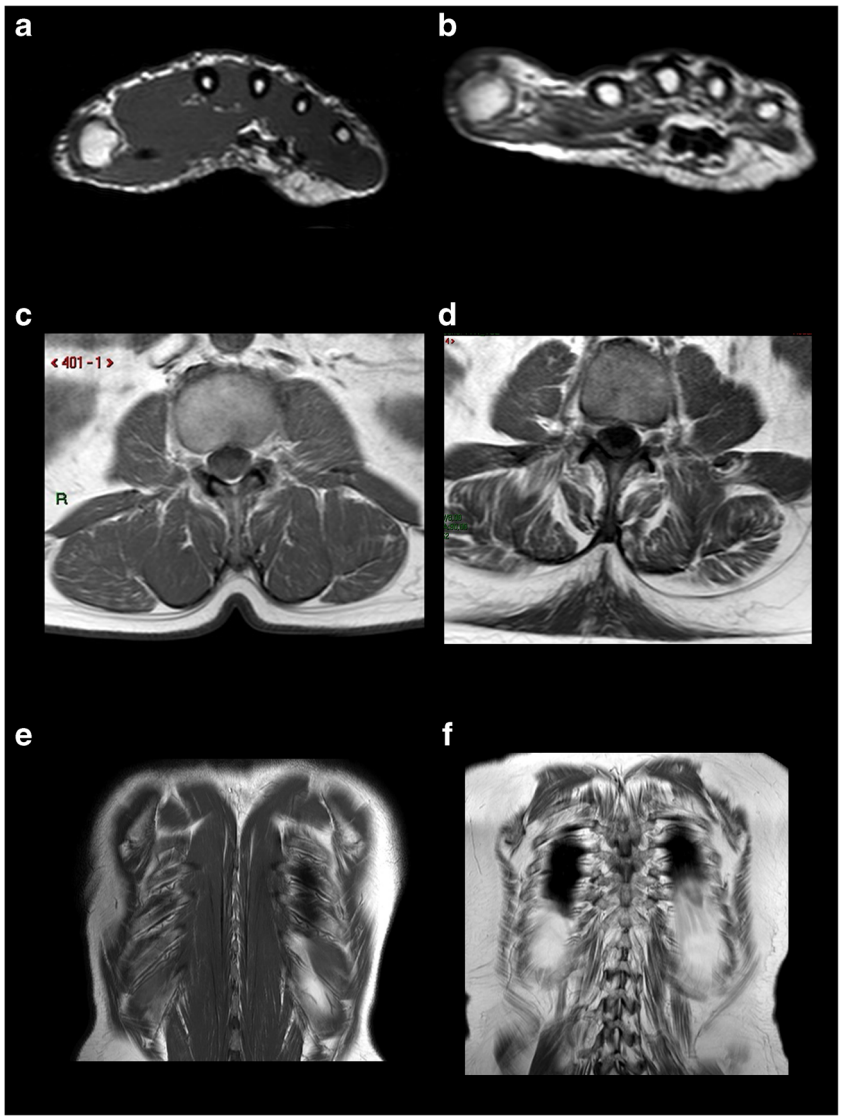

Fig. 1 MRI scans of healthy controls (**a**, **c**, **e**) and patients (**b**, patient #6, **d**, patient #9, **f**, patient #8): left-hand axial T1-w (**a**, **b**, patient #6), paraspinal axial T1-w (**c**, **d**, patient #9), paraspinal coronal T1-w (**e**, **f**, patient #8)

patients often present distal lower limbs clinical onset (i.e., Patrikios form). Moreover, iliopsoas, a spinal muscle, could be studied to support the diagnosis in bulbar patients.

Concerning the comparison of results between EMG and MRI, a trend towards agreement was appreciable for first dorsal interosseous, paraspinal, and tibial anterior muscles. The level of muscle alterations is different between EMG and MRI, and the discrepancy was likely due to the greater sensitivity of EMG for the quantification of muscle damage. Indeed, many moderate and severe alterations were identified using the EMG grading in muscles characterized by low grade of fatty substitution in MRI. MRI could, instead, be more useful for studying deeper muscles that are difficult to assess using EMG. Further data should be obtained increasing the sample size.

Finally, a trend towards agreement can be also appreciated for clinical aspects (MRC for first dorsal interosseous and tibial anterior muscles) and MRI. A concordance emerged between clinical and MRI data for the dominant hand. Indeed, low scores for specific ALSFRS-r items correspond to a high level of MRI alterations. This region was tested because muscles assessed by MRI are selectively involved in writing and using a knife (both ALS-FRSr items).

Limitations of our explorative study included the low number of patients recruited, which reflects the rarity of ALS. To be able to collect significant data, and also take into account the clinical heterogeneity of ALS onset, we decided to assess numerous muscles in each patient. The full protocol included the evaluation of 68 muscles belonging to the cervical, thoracic, and lumbar segments. We excluded bulbar muscles because the relevant literature data are not homogeneous [21], mainly for technical reasons. Finally, we could not perform quantitative

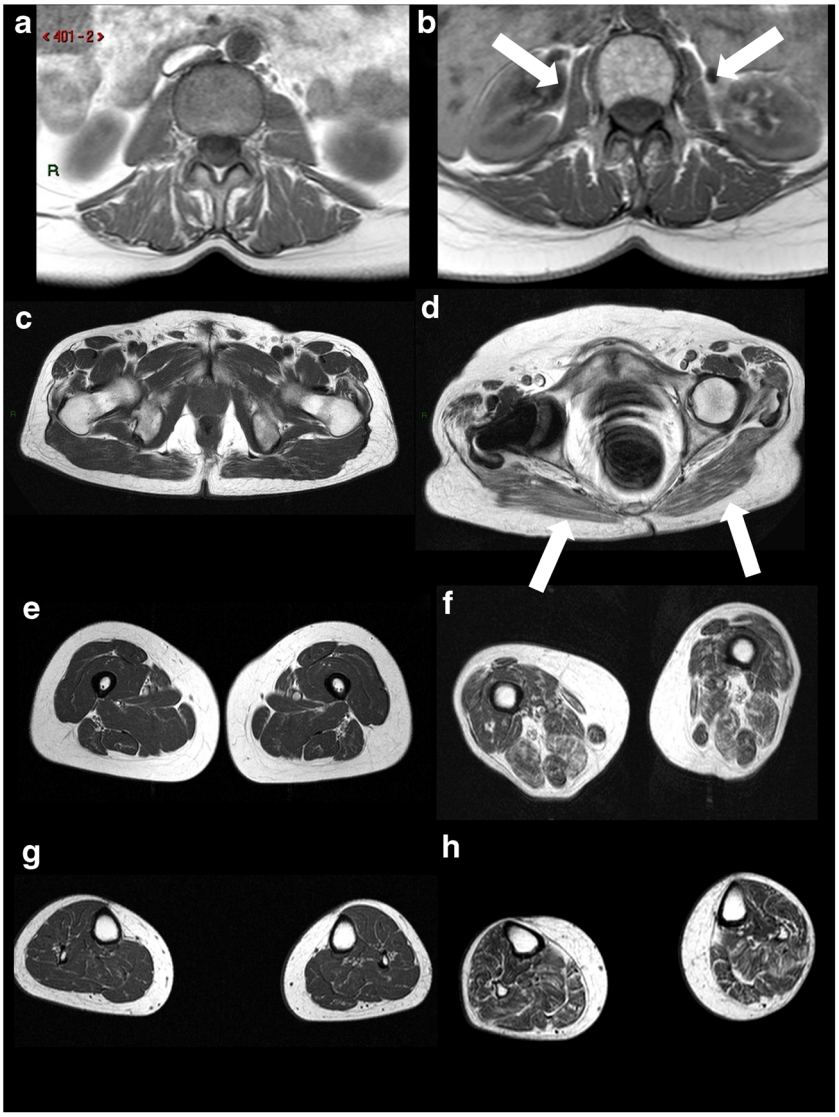

Fig. 2 MRI scans of healthy controls (**a**, **c**, **e**, **g**) and patients (**b**, patient #10, **d**, patient #8, **f**, patient #8, **h** patient #8): iliopsoas axial T1-w (**a**, **b**, patient #10), gluteus maximus axial T1-w (**c**, **d**, patient #8), thigh axial T1-w (**e**, **f**, patient #8), calf axial T1-w (**g**, **h**, patient #8)

assessment of muscle T1 time or volume because quantitative sequences require greater technical resources, and time-consuming post-acquisition processing [22]. The main technical problems we encountered were related to the coil at our disposal and to hand MRI: the prone position was poorly tolerated both by patients and controls. For this reason, and also because since EMG data are sufficient to highlight abnormalities, we decided not to further analyze this region. In future studies, we will consider to acquire more MRI data on specific regions, where it is possible to provide significant data but poorly amenable to clinical and EMG assessment (e.g., the paraspinal muscles).

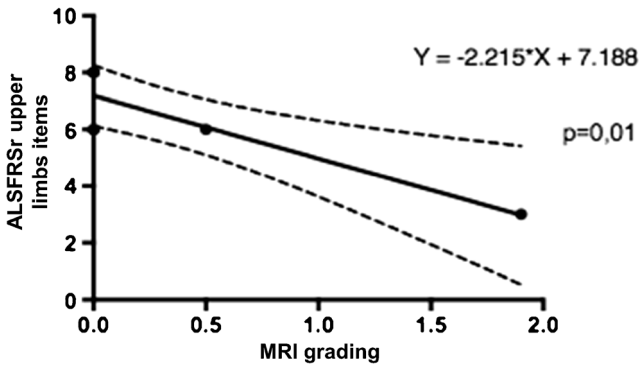

Fig. 3 Correlation between ALSFRSr items for upper limbs and MRI scans of hands

In conclusion, muscle T1-w MRI can distinguish ALS patients from HCs for specific regions (i.e., legs). MRI abnormalities could be found in pauci-symptomatic spinal muscles in bulbar-onset patients (i.e., iliopsoas). Paraspinal and leg muscle MRI may be a useful diagnostic tool in early ALS. Form these preliminary results, we plan to conduct a longitudinal study in order to investigate more in depth the role of muscle through MRI measurements as a prognostic or predictive biomarker in ALS patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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