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ORIGINAL ARTICLE

Amyotrophic lateral sclerosis regional progression intervals change according to time of involvement of different body regions

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Abstract

Background and purpose: The prediction of disease course is one of the main targets of amyotrophic lateral sclerosis (ALS) research, particularly considering its wide phenotypic heterogeneity. Despite many attempts to classify patients into prognostic categories according to the different spreading patterns at diagnosis, a precise regional progression rate and the time of involvement of each region has yet to be clarified. The aim of our study was to evaluate the functional decline in different body regions according to their time of involvement during disease course.

Methods: In a population-based dataset of ALS patients, we analysed the functional decline in different body regions according to time and order of regional involvement. We calculated the regional progression intervals (RPIs) between initial involvement and severe functional impairment using the ALS Functional Rating Scale revised (ALSFRS-r) subscores for the bulbar, upper limb, lower limb and respiratory/thoracic regions. Time-to-event analyses, adjusted for age, sex, ALSFRS-r pre-slope (ΔALSFRS-R), cognitive status, and mutational status were performed.

Results: The duration of RPI differed significantly among ALS phenotypes, with the RPI of the first region involved being significantly longer than the RPIs of regions involved later. Cox proportional hazard models showed that in fact a longer time between disease onset and initial regional involvement was related to a reduced duration of the RPI duration in each different body region (bulbar region: hazard ratio [HR] 1.11, 95% confidence interval [CI] 1.06–1.16, $p < 0.001$; upper limb region: HR 1.16, 95% CI 1.06–1.28, $p = 0.002$; lower limb region: HR 1.11, 95% CI 1.03–1.19, $p = 0.009$; respiratory/thoracic region: HR 1.10, 95% CI 1.06–1.14, $p = 0.005$).

Conclusions: We found that the progression of functional decline accelerates in regions involved later during disease course. Our findings can be useful in patient management and prognosis prediction.

KEYWORDS
ALSFRS-R, amyotrophic lateral sclerosis, clinical trial, disease spreading, prion-like mechanism

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a widely variable progressive course [1]. Much clinical [2], neuroimaging [3] and neuropathological [4] evidence supports the idea of a focal beginning of motor neurodegeneration that progresses following functional and anatomic pathways in a region-oriented manner, combining over time with variable progression rates.

Prediction of disease course is one of the main targets of ALS research. The ALS functional rating scale revised (ALSFRS-r) is the most widely used scale in clinical practice and research [5], although it lacks unidimensionality when considered as a single total score [6]. In a recent study [7], we pointed out that spatial spreading of functional motor and cognitive impairment is one of the major determinants of patient survival, together with age of onset, progression rate, and respiratory dysfunction. Early single- or multiple-district symptom progression rate has been related to overall survival [8], but progression rate and time of involvement of each region has yet to be clarified.

The aim of our study was to evaluate the duration of functional decline in the four body regions according to their time of involvement during the course of the disease.

METHODS

Data collection

All patients ($N = 1105$) meeting the revised El Escorial criteria for defined, probable, and probable laboratory-supported ALS diagnosed in the period 2007–2014 in the Piemonte and Valle d'Aosta regions, Italy, were included.

For each patient we collected sex, date of onset, site of onset, date of diagnosis, and date of death/tracheostomy from January 1, 2007 until December 31, 2018 (censoring date). Neuropsychological evaluation was performed within 3 months from diagnosis in 633 patients (58.6%). The neuropsychological battery used for classification has been reported in a previous paper [9]. Patients' cognitive status was classified according to the revised ALS-frontotemporal spectrum disorder (FTD) consensus criteria [10]. To evaluate the effect of cognitive impairment, we subdivided patients into two categories: patients with impaired cognition (corresponding to the ALS-FTD, ALS with behavioral impairment [ALSbi], ALS with cognitive impairment [ALSci] and ALS with combined cognitive and behavioral impairment [ALScbi] categories in the revised ALS-FTD consensus criteria), and patients with normal cognition (corresponding to the ALS-CN category). Genetic analysis for *C9orf72*, *SOD1*, *TARDBP* and *FUS* was considered when available (*c9orf72* $N = 62$, 5.6%; other mutations $N = 37$, 3.4%; genetic information not assessed $N = 179$, 16.2%) [11].

The ALSFRS slope at time of diagnosis (ΔALSFRS-R) was calculated for each patient using the following formula: (48 − ALSFRS-R score at diagnosis)/(time from onset to diagnosis) [12].

Regional progression interval calculation

The regional progression intervals (RPIs) for each patient were calculated as the time interval between initial involvement and severe functional impairment (Inv – SevI interval) of each body region (bulbar region, upper limbs, lower limbs, and respiratory/thoracic region). The date of initial involvement was considered when at least 1 point was lost from the maximum total ALSFRS-r subscore of that region: <12 for the bulbar region for items 1, 2 and 3; <8 for the upper limbs for items 4 and 5; <8 for the lower limbs for items 8 and 9; and <12 for the respiratory region for items 10, 11 and 12. ALSFRS-r data were completed using the patient's medical history for the initial involvement before diagnosis: we considered specifically dysphagia/dysarthria/excessive drooling for the bulbar region, muscle strength reduction/functional impairment/muscle hypotrophy for the upper and lower limb regions, and dyspnea/orthopnea for the respiratory/thoracic region. This method was based on the King's staging system calculation using ALSFRS-R and used in our previous work [7, 13]. The date of severe functional impairment was obtained according to both medical history and ALSFRS-r subscores, considering regional-specific milestones: (i) need of enteral/parenteral nutrition (score <2 for ALSFRS item 3) for the bulbar region; (ii) inability to perform any manipulation with hands (score <1 for ALSFRS item 5a/5b) for upper limbs; (iii) non-ambulatory functional movement only (score <2 for ALSFRS item 8) for lower limbs; (iv) need for continuous use of non-invasive mechanical ventilation (NIMV) throughout the night (score <3 for ALSFRS item 12) for the respiratory/thoracic region. The date of initial involvement or severe functional impairment corresponded to the date of the visit when the region was observed to be involved for the first time or reached the regional milestone described. For severe impairment of the bulbar and the respiratory/thoracic regions we also interrogated the clinical dataset (the Piemonte and Valle d'Aosta Registry for ALS–PARALS) for the dates of percutaneous gastrostomy positioning, parenteral nutrition, and overnight NIMV start. RPI was calculated as the fraction of years between date of Initial involvement and date of Severe impairment for each body region.

Statistical analysis

Differences in discrete and continuous variables of interest were analyzed using the χ^2 test and Student's *t*-test or Kruskal–Wallis test by ranks, the Mann–Whitney *U*-test with Bonferroni correction and

Dunn's multiple comparison test, as appropriate. A p value <0.05 was taken to indicate statistical significance.

Time-to-event analysis was modeled performing Cox proportional hazard models, first treating each body region alone and then considering all body regions together. In these models severe functional impairment was considered as the event. In patients reaching the event, the observation time corresponded to the RPI; in patients not reaching the event, it corresponded to the interval between initial involvement and date of death/tracheostomy/censoring. We considered as covariates the time between disease and initial involvement (Onset – Inv interval, covariate of interest), sex, age at onset and ΔALSFRS-R. In the subgroup of patients with cognitive evaluation, the same analysis was performed stratifying for cognitive status (normal vs. cognitively impaired) and genetic status.

We also calculated the King's staging system score from the ALSFRS-R score [13] and the Milano-Torino staging system (MiToS) [14] at each visit. We calculated the time between King's Stage 1 and Stage 2 (corresponding to the interval from disease onset/involvement of the first body region to the second body region involvement) and between King's Stage 2 and Stage 3 (corresponding to the interval from the second to the third body region involvement). For MiToS score, we performed the same analysis from Stage 1 to Stage 2 and from Stage 2 to Stage 3. Through this analysis, we wanted to understand if the longitudinal regional spreading of functional involvement and impairment across different body regions also differed according to disease stage.

Finally, we computed a Cox proportional hazard model considering all body regions together and their order of involvement (first, second, third and fourth region involved) as the covariate of interest. Collinearity tests were performed to avoid information duplication and multicollinearity by examining the tolerance and the variance inflation factor for each covariate. A Kaplan–Meier curve with log-rank test was performed to analyze the RPIs according to the order of involvement.

Anonymous data were collected in a dataset developed in Excel (Version 14.0.4760.1000, 64 bit). Data were analysed using Stata V.13.1 (StataCorp) and IBM SPSS Statistics for Windows, version 25.0. (IBM Corp.).

Ethical approval

The study design was approved by the Ethical Committee of the Azienda Ospedaliero-Universitaria Città della Salute (Prot. N. 0036344).

RESULTS

Patients' characteristics

A total of 9658 ALSFRS-R scores for 1105 ALS patients were included in the analysis, with the scores performed during their follow-up: median (interquartile range [IQR]) visit-to-visit interval 2.50 (1.77–3.67) months; median (IQR) number of visits/patient: 5 (2–10). A total of 907 patients (82.1%) died or underwent tracheostomy before the end of follow-up. The main descriptive statistics are summarized in Table 1.

In Table 2 we summarize the RPIs for all body regions in our study population. In 119 patients (10.8%) we obtained all four region-specific progression intervals, in 776 patients (70.2%) we were able to calculate more than one RPI, while in 329 (29.8%) patients no RPI was obtained.

In the overall cohort, the Onset – Inv interval and RPI (Inv – SevI interval) of the bulbar, upper and lower limb regions did not differ significantly (Mann–Whitney test with Bonferroni correction for multiple comparison $p > 0.05$), while the respiratory/thoracic region was involved significantly later (longer Onset – Inv interval) during the disease course (respiratory median [IQR] Onset – Inv interval 1.40 [0.81–2.51] years; $p < 0.001$), showing also a shorter RPI (0.31 [0.00–0.77] years; $p < 0.001$).

When stratifying patients according to the site of onset, the Onset – Inv interval differed significantly across diverse regions, reflecting the different spreading pathways in spinal and bulbar phenotypes (Table 2 and Figure S1). In addition, RPIs differed significantly

TABLE 1 Descriptive statistics

	Median (IQR)
Age at onset, years	67.7 (60.0–74.2)
Onset-diagnosis interval, years	0.7 (0.4–1.1)
ΔALSFRS-R at diagnosis (point loss per month)	0.6 (0.3–1.3)
Survival, years	2.5 (1.6–3.9)
	***n* (%)**
Sex	
Male	589 (53.3)
Female	516 (46.7)
Cognitive status classification	
Normal cognition	335 (30.3)
Impaired cognition	298 (27.0)
Not assessed	472 (42.7)
Site of onset	
Bulbar region	385 (34.8)
Upper limbs	313 (28.3)
Lower limbs	386 (34.9)
Respiratory/thoracic region	21 (1.9)
Genetic mutations	
Wild-type	827 (74.8)
c9orf72	62 (5.6)
SOD1, *TARDBP*, *FUS*	37 (3.4)
Not assessed	179 (16.2)
Total	1105 (100.0)

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale revised; IQR, interquartile range; RPI, regional progression interval.

TABLE 2 Regional progression interval determination in different body regions

Body region	Bulbar, *n* (%)	Upper limbs, *n* (%)	Lower limbs, *n* (%)	Respiratory, *n* (%)	
Initial involvement determination	Dysarthria, dysphagia, drooling	Strength reduction, impairment, muscle hypotrophy	Strength reduction, impairment, muscle hypotrophy	Dyspnea, orthopnea	
Initial involvement determination by ALSFRS-R score	Item 1, 2, 3 < 12	Item 4, 5 < 8	Item 8, 9 < 8	Item 10, 11, 12 < 12	
Initial involvement (%)	927/1105 (83.9)	1001/1105 (90.6)	1003/1105 (90.8)	791/1105 (71.6)	
Severe impairment determination	Need of enteral/parenteral nutrition	Inability to perform any manipulation with hands	Non-ambulatory functional movement only	Continuous use of NIMV throughout the night	
Severe impairment determination by ALSFRS-R score	Item 3 < 2	Item 5 < 1	Item 8 < 2	Item 12 < 3	
Severe impairment (%)	450/927 (48.5)	341/1001 (34.1)	470/1003 (46.9)	394/791 (49.8)	
	Onset – Inv interval, years				
	Bulbar	**Upper limbs**	**Lower limbs**	**Respiratory**	***p****
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	
Overall cohort	0.46 (0.00–1.48)	0.50 (0.00–1.40)	0.42 (0.00–1.23)	1.40 (0.81–2.51)	**<0.001**
Bulbar region onset	–	0.94 (0.49–1.60)	1.00 (0.50–1.64)	1.14 (0.71–1.91)	**0.002**
Upper limb onset	1.15 (0.58–2.03)	–	0.88 (0.42–1.79)	1.62 (0.92–2.59)	**<0.001**
Lower limb onset	1.41 (0.85–2.75)	1.08 (0.50–2.23)	–	1.71 (0.94–3.17)	**<0.001**
Respiratory/thoracic region onset	0.74 (0.17–1.25)	0.42 (0.17–0.80)	0.50 (0.17–1.36)	–	0.252
	RPI, years				
	Bulbar	**Upper limbs**	**Lower limbs**	**Respiratory**	***p***[§]
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	
Overall cohort	1.27 (0.69–2.00)	1.27 (0.69–2.26)	1.37 (0.68–2.41)	0.31 (0.00–0.77)	**<0.001**
Bulbar onset	1.52 (1.08–2.17)	0.90 (0.42–1.16)	0.67 (0.37–1.00)	0.26 (0.00–0.67)	**<0.001**
Upper limbs onset	0.91 (0.52–1.44)	1.87 (1.10–3.02)	1.32 (0.62–2.09)	0.31 (0.00–0.72)	**<0.001**
Lower limbs onset	0.75 (0.37–1.62)	1.08 (0.51–2.03)	1.97 (1.24–3.06)	0.31 (0.00–0.84)	**<0.001**
Respiratory/thoracic onset	0.09 (0.04–0.57)	–	0.42 (0.19–1.08)	0.86 (0.45–1.17)	0.066

Note: Summary of clinical features and ALSFRS-R items scores used for the determination of initial regional involvement and severe impairment and the number of initial involvement and severe functional impairment values obtained. In each body region, the interval from disease onset to initial regional involvement was calculated as a fraction of years, while the RPI was calculated as the time interval between the date of initial regional involvement and date of severe functional impairment.

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale revised; IQR, interquartile range; NIMV, non-invasive mechanical ventilation; Onset – Inv, disease onset to initial regional involvement interval; RPI, regional progression interval.

Bold indicates statistical significant value ($p < 0.05$).

*Kruskal–Wallis test.

[§]Kruskal–Wallis test; the significant difference found was related only to the respiratory/thoracic region, which was found to be significantly different from all other body regions, considering both time of involvement and RPI duration (Mann–Whitney test with Bonferroni correction for multiple comparison: respiratory/thoracic region vs. other body regions, $p < 0.001$; other body region comparisons, p = nonsignificant). Median and IQR were unobtainable due to number of RPIs being < 2 per group.

among the bulbar, upper and lower limb onset groups, as shown in the boxplots in Figure 1: using Kruskal–Wallis test and Dunn's multiple comparison test, we observed that the RPI of the region of onset was significantly longer than RPIs of regions involved later ($p < 0.001$ for all comparisons). The same trend, although not significant, was observed for respiratory onset patients ($p = 0.066$).

Time-to-event analyses are shown in Table 3. All collinearity tests resulted in nonsignificant multicollinearity among covariates (Table S1).

In the whole cohort, a longer Onset – Inv interval was related to a reduced RPI duration, considering both each region separately (Table 3a) and all regions together (Table 3b). To confirm the trend

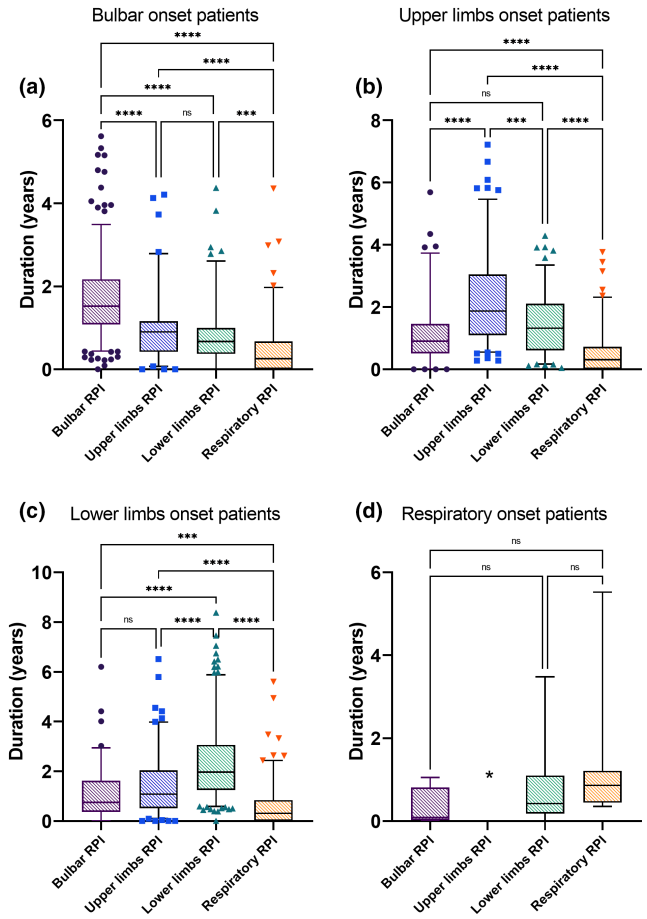

FIGURE 1 Regional progression intervals (RPIs) according to the site of onset. (a) Bulbar onset patients. (b) Upper limbs onset patients. (c) Lower limbs onset patients. (d) Respiratory/thoracic onset patients. Kruskall–Wallis comparison for nonparametric distribution results are shown in Table 1 in the main text. Dunn's multiple comparison test results are shown (***$p < 0.001$; ****$p < 0.0001$; ns, $p > 0.05$).

of reduction of RPI, we performed the same analysis stratifying Onset – Inv into five interval lengths (≤6 month, 6 months–1 year, 1 year–2 years, 2–3 years, and >3 years) and observed a trend towards a progressive hazard ratio increase (test for trend $p < 0.001$; Table 3c).

We further analyzed RPIs according to the order of involvement of the body regions. Cox proportional hazard adjusted models demonstrate that the RPI duration decreases according to the order of involvement (Table 3d).The Kaplan–Meier curve (Figure 2) confirmed this trend ($p < 0.001$, log-rank for pairwise comparison; Table S2).

Using King's and MiToS staging, we observed that the median (IQR) duration of the interval between King's Stage 1 and Stage 2 was significantly longer than the interval between King's Stage 2 and Stage 3 (median [IQR] Stage 1–2 interval 0.75 [0.42–1.56] years vs. Stage 2–3 interval 0.47 [0.25–0.95] years; Mann–Whitney *U* test $p < 0.001$). Also using MiToS, the time interval between stages decreased during progression (median [IQR] Stage 1–2 interval 0.59 [0.26–0.98] vs. Stage 2–3 interval 0.36 [0.20–0.66] years; Mann–Whitney *U* test $p < 0.001$).

Stratification for mutational status was not possible because of the limited number of patients, except for *C9orf72* carriers. When considering genetic status, both wild-type ($N = 827$) and *C9orf72* patients ($N = 62$, Tables S3 and S4) showed the same progressive reduction of RPI duration according to time of involvement.

Finally, using a smaller cohort of patients ($N = 633$) with neuropsychological evaluations at diagnosis, we observed that cognitive status did not significantly change the distribution of RPI; the RPI in the onset region was significantly longer than other RPIs in patients with normal cognition and in those with impaired cognition (Table S5).

DISCUSSION

In this study, in a population-based cohort of ALS patients, we found that there was a progressive shortening of the Inv – Sevl intervals in body regions during disease progression. This phenomenon was present in all four examined regions (bulbar, upper limbs, lower limbs and respiratory/thoracic), regardless of sex, age of onset, progression rate, cognitive impairment status, and C9orf72 mutation status.

Progression of ALS has been considered for a long time to be a linear phenomenon, but more recent models have depicted the overall progression as curvilinear [15] or sigmoidal [16]. The nonlinearity of functional impairment was also evidenced in a recent study that analyzed the occurrence of plateaus during ALS progression [17].

Despite the widespread interest in disease prediction modeling, the current literature about the progression rates in different body regions and the reaching of clinical milestones is still relatively sparse. One of the first attempts to distinguish between overall and regional progression was a study by van der Kleij et al. [18]. Using the ALSFRS-r score, they produced a model to measure the regional burden of the disease and thus predict overall survival. Clinical staging systems, such as the MiToS [14], the King's college staging system [19], and the "fine-till-9" (FT9) system [20], have been developed to measure the regional burden of functional impairment in different ways. The transition through clinical stages is influenced by the overall progression rate; its duration (or transit time) has recently been proposed as a surrogate outcome measure in clinical trials [21].

In this study, we used ALSFRS-r subscores as a proxy to detect symptom progression in the four body regions, and we found that RPIs were significantly reduced when regional involvement occurs later on in the disease course; therefore, in the different ALS phenotypes, the regional progression rates change over time.

Our observations could have several possible interpretations. First, they could be explained as a consequence of the patient's biological system undergoing degeneration rather than the disease itself, especially considering the high impact of the disease on nutrition, metabolic expenditure, global motility, and respiratory function.

TABLE 3 Cox proportional hazard models

	Adjustments	Time	Covariate of interest	HR	CI	*p*
(a)	Sex, Age at onset ΔALSFRS-R score at diagnosis	Bulbar RPI	Onset – bulbar involvement interval	1.11	1.06–1.16	**<0.001**
		Upper limb RPI	Onset – upper limb involvement interval	1.16	1.06–1.28	**0.002**
		Lower limb RPI	Onset – lower limb involvement interval	1.11	1.03–1.19	**0.009**
		Respiratory/thoracic RPI	Onset – respiratory/ thoracic involvement interval	1.10	1.03–1.18	**0.005**
(b)	Sex Age at onset ΔALSFRS-R score at diagnosis	Every RPI	Onset – Inv	1.10	1.06–1.14	**<0.001**
(c)	Sex Age at onset ΔALSFRS-R score at diagnosis	Every RPI	Onset – Inv ≤6 months	1		
			Onset – Inv 6 months – 1 year	1.34	1.15–1.55	**<0.001**
			Onset – Inv 1 year – 2 years	1.43	1.25–1.64	**<0.001**
			Onset – Inv 2 years– 3 years	1.50	1.24–1.81	**<0.001**
			Onset – Inv >3 years	1.53	1.26–1.86	**<0.001**
(d)	Sex Age at onset ΔALSFRS-R score at diagnosis	Every RPI	First region involved	1		
			Second region involved	1.25	1.10–1.42	**0.001**
			Third region involved	1.51	1.31–1.74	**<0.001**
			Fourth region involved	1.84	1.55–2.19	**<0.001**

Note: Severe functional impairment was considered as the event. The observation time corresponded to the RPI in patients reaching the event or to the interval between initial involvement and date of death/tracheostomy/censoring (patients without event). The covariate of interest is reported in the appropriate column. All models considered as covariates sex, age at onset and progression rate (ΔALSFRS-r score) at diagnosis. (a) Cox proportional hazard model performed considering each region separately, adjusted for sex, age at onset, ΔALSFRS-R score at diagnosis. (b) Cox proportional hazard model performed considering all body regions together, adjusted for sex, age at onset, ΔALSFRS-R score at diagnosis. (c) Cox proportional hazard model performed considering all body regions together, stratified by Onset – Inv intervals (≤6 month, 6 months – 1 year, 1 year–2 years, 2–3 years, and >3 years), adjusted for sex, age at onset, ΔALSFRS-R score at diagnosis. (d) Cox proportional hazard model performed considering the order of involvement of the body region (first, second, third and fourth body region involved), adjusted for sex, age at onset, ΔALSFRS-R at diagnosis.

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale revised; CI, confidence interval; HR, hazard ratio; Onset – Inv, disease onset to initial regional involvement interval; RPI, regional progression interval.

Bold indicates statistical significant value ($p < 0.05$).

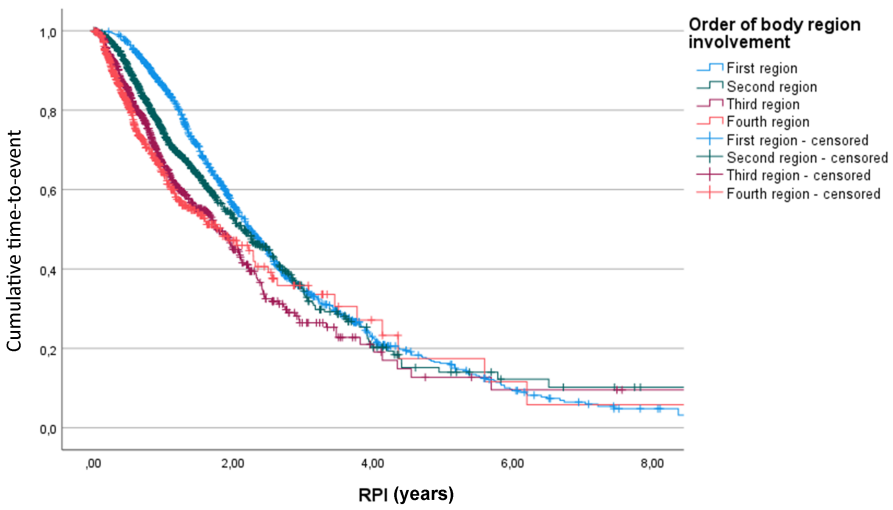

FIGURE 2 Regional progression interval (RPI) duration according to the order of involvement, independently from the specific region involved. Kaplan–Meier analysis was performed using RPI as time in patients that reach the severe impairment and time from initial involvement to death/tracheostomy/censoring date in patients that did not reach severe impairment in that region (censored). The four groups correspond to the order of involvement (first, second, third and fourth region involved). Log-rank test $p < 0.001$.

Second, they could be related to the different timing of the involvement of the upper motor neurons (UMNs) and lower motor neurons (LMNs), which varies throughout the disease course [2]. LMN signs (muscle atrophy and weakness) are the major determinants of functional impairment, measured by ALSFRS-R [22] and, in the majority of ALS patients, they are more prominent in more

advanced stages of the disease. Our study is also in agreement with recent studies on plateaus in ALS: using ALSFRS-R and the Medical Research Council scale [15, 23] these studies showed that pauses in functional decline are more frequent in patients with UMN phenotypes and at the beginning of the disease, becoming rarer and rarer during progression.

Third, some neuropathological features highlighted in ALS disease models the prion-like behaviour of TDP43 and SOD1 protein aggregates [24] that could spread from UMNs to other functionally connected cells, such as LMNs and frontal neurons [25]. These models could explain not only the initial focality of disease, but also the exponential dissemination to other body regions, with progressive involvement of a growing number of motor cells. In light of this, our data on regional longitudinal spreading, using RPI or a clinical staging system, agreed with prion-like models by showing a progressive reduction in the time to impairment.

In addition, the modification of neuroinflammatory cell profiles from tolerant to aggressive [26, 27] could determine the progressive deterioration of a growing number of motor neurons, which also fits well with our clinical observations. However, despite the fact that in many forms of neurodegeneration the kinetics of neuronal loss appears to be exponential [28], all these possible interpretations remain speculative.

Our study has some limitations. First, our observations were derived from the ALSFRS-r scale and did not strictly represent the underlying biological process; we were also unable to distinguish between UMN and LMN involvement. Moreover, the intrinsic limitation of this scale, namely, its multidimensionality and floor effect [29], did not allow comparison of the severity among the different regions during disease progression. Nevertheless, the choice of this scale also lends importance to our results because ALSFRS-r progression is routinely used in clinical trials as a primary or secondary endpoint. Considering the availability of both clinical and trial-based retrospective datasets, the ALSFRS-r scale offers the possibility to easily validate our data in different patients cohorts. Second, the lack of longitudinal evaluation of cognitive impairment could limit our findings regarding cognitive status stratification. Cross-sectional neuropsychological assessment stratified by motor stages [30–32] and evidence from different neurodegenerative conditions [33, 34] support the hypothesis of exponential cortical damage, but no study using "cognitive milestones" has been performed to date. Third, the observation of a shorter respiratory RPI could be biased by many different factors that could have led to an underestimation of this interval. The ALSFRS-r respiratory items poorly reflect pulmonary function [35], indeed a high number of ALS patients showed both forced vital capacity (FVC) reduction and arterial blood gases alteration without any respiratory symptoms detected by ALSFRS-r [36]. Also, the definition of severe respiratory impairment based on non-invasive ventilation usage could have reduced the number of respiratory RPIs evaluated, excluding patients who did not tolerate it. To overcome these limitations, further studies considering repeated respiratory measurements, such as FVC, should be performed.

Fourth, we could not derive from the ALSFRS-R the time of initial involvement for body regions involved before the first clinical evaluation, therefore, we used information reported by the patients. While this could affect the accuracy of the RPI calculation, a different solution could not be found. Fifth, we could not compare severe impairment, and consequently the RPIs, for different regional functions (e.g., bulbar RPIs vs. lower limb RPIs).

In conclusion, our results provide novel insights into the dynamics of ALS functional worsening over time and offer relevant clues to the understanding of the underlying pathological mechanisms. The evidence of "snowball-like" behavior observed in our study could improve the prediction of disease progression trajectories and milestones in the clinical setting.

AUTHOR CONTRIBUTIONS

Manera Umberto: conceptualization, data curation, formal analysis, methodology, software, validation, visualization, writing – original draft; **D'Ovidio Fabrizio**: conceptualization, data curation, formal analysis, methodology, validation, writing – review and editing; **Cabras Sara**: data curation, validation, visualization, writing – review and editing; **Torrieri Maria Claudia**: data curation, validation, visualization, writing – review and editing; **Vasta Rosario**: data curation, validation, visualization, writing – review and editing; **Palumbo Francesca**: data curation, visualization, writing – review and editing; **Grassano Maurizio**: data curation, visualization, writing – review and editing; **De Marchi Fabiola**: data curation, visualization, writing – review and editing; **Mazzini Letizia**: data curation, supervision, validation, visualization, writing – review and editing; **Mora Gabriele**: supervision, validation, visualization, writing – review and editing; **Moglia Cristina**: data curation, resources, validation, visualization, writing – review and editing; **Calvo Andrea**: data curation, funding acquisition, project administration, resources, supervision, validation, visualization, writing – review and editing; **Chiò Adriano**: conceptualization, data curation, funding acquisition, methodology, project administration, resources, supervision, validation, visualization, writing – review and editing.

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CONFLICT OF INTEREST

Umberto Manera, Fabrizio D'Ovidio, Sara Cabras, Maria Claudia Torrieri, Antonio Canosa, Rosario Vasta, Francesca Palumbo, Maurizio Grassano, Fabiola De Marchi, Letizia Mazzini, Gabriele Mora, Cristina Moglia report no conflicts of interest. Andrea Calvo has received a research grant from Cytokinetics. Adriano Chiò serves on scientific advisory boards for Mitsubishi Tanabe, Roche, Biogen, Denali Pharma and Cytokinetics, and has received a research grant from Italfarmaco. The sponsor organizations had no role in data collection and analysis and did not participate in writing or approving the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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