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Regional spreading of symptoms at diagnosis as a prognostic marker in amyotrophic lateral sclerosis: a population-based study

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Abstract

Objective

The lack of prognostic biomarkers in ALS patients induced researcher to develop clinical evaluation tools for stratification and survival prediction. We assessed the correlation between patterns of functional involvement, considered as a cumulative number of body region involved, and overall survival, in a population-based series of ALS patients (PARALS).

Methods

We derived the functional involvement of four body regions at diagnosis using ALSFRS-R subscores for bulbar, upper limbs, lower limbs and respiratory/thoracic regions. We analysed the effect of NBRI at diagnosis on overall survival, adjusting for age at onset, sex, site of onset, onset-diagnosis interval, forced vital capacity, body-mass index, *c9orf72* mutational status, and comparing it to King's staging system.

Results

The number of body region involved (NBRI) was strongly related to survival, with a progressive increase of death/tracheostomy risk among groups (2 body regions HR=1.24, 95% CI=1.06-1.45, p=0,007; 3 body regions HR=1.65, 95% CI= 1.38-1.98, p<0.001; 4 body regions HR=2.68, 95% CI 2.11-3.39, p<0.001). Using ALSFRS-R score, the consistency between the number of regions involved and King's clinical stage at diagnosis was very high (81%). The inclusion of the functional involvement of respiratory/thoracic region, which is frequently underestimated, and the evaluation of cognitive impairment, allowed to subdivide patients into different prognostic categories. Regional spreading of the disease is associated with overall survival, independently from the initial region involved.

Conclusions

The evaluation of NBRI, with the inclusion of initial respiratory/thoracic involvement and cognition, can be useful in many research fields, leading to a better stratification of patients. Our findings highlight the importance of the spatial spreading of functional impairment in the prediction of ALS outcome.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the involvement of upper and lower motor neurons in different body regions. Disease onset occurs in bulbar or spinal regions, but spreading patterns remains largely unpredictable in the single patient. ALS phenotypic heterogeneity is widely recognized [1] and its impact on disease progression and overall survival has been confirmed also in population-based series [2]. The underlying mechanisms of this variability are still poorly understood different genetic or environmental factors have been analysed, but genotype-phenotype correlations and environmental risk factor-phenotype correlations remain limited and inconclusive [3]

Older age, bulbar onset and diagnostic delay are generally considered as negative prognostic factors for overall survival, while gender and El Escorial criteria have shown inconsistent results [4]. Also nutritional status [5], cognition [6] and respiratory function [7] at diagnosis have been considered as prognostic markers. Among genetic mutations, *c9orf72* expansion leads to a shorter survival compared to non-mutated ALS patients [8].

Recently, innovative prognostic biomarker, such as neurofilaments [9] showed consistent association in disease progression prediction. Considering phenotypic heterogeneity, a multimodal approach was used to develop the ENCALS Survival Prediction Model [10], a validated model able to stratify patients in prognostic categories according to different candidate predictors.

In clinical practice, ALSFRS-R is the most widely used surrogate marker of disease progression [11]. Total ALSFRS-R score at diagnosis is a strong survival predictor, even when adjusted for forced vital capacity (FVC), age, sex and symptom duration [12] and its progression rate provides an additional predictive index beyond ALSFRS-R alone [13]. Two main ALS clinical staging systems, the King's College staging system and the Milano-Torino staging (MITOS) system, can be calculated from ALSFRS-R score [14, 15]. Recently the metric quality of ALSFRS-R has been questioned, demonstrating that the total score lacks unidimensionality and does not fulfil fundamental measurement requirements [16, 17].

The aim of the present study was to assess the patterns of functional involvement in four body regions (upper limbs, lower limbs, bulbar and respiratory/thoracic regions) at diagnosis and their correlation to overall survival, using ALSFRS-R score as a multidimensional scale in a population-based series of ALS patients.

Methods

Data collection

All patients (N=1,105) meeting the revised El Escorial Criteria for defined, probable and probable-laboratory supported ALS [18] diagnosed in the period 2007-2014 in Piemonte and Valle d'Aosta regions, Italy, were included. Thirteen patients were excluded from the analyses because of missing information on clinical

evolution and further 30 cases were excluded for their long disease course (survival from diagnosis >10 years, see supplementary material).

For each patient we collected age at onset, sex, site of onset, date of diagnosis, diagnostic delay, death/tracheostomy. Survival was assessed from January 1st, 2007, until December 31st, 2017, or until death/tracheostomy. In a subgroup of patients (58.5%) we obtained also forced vital capacity (FVC) and body-mass index (BMI). Mutational status for *c9orf72*, *SOD1, TARDBP,* and *FUS* was available in 84.1% of patients. Neuropsychological evaluation was performed at diagnosis (diagnosis-evaluation interval ≤ 3 months) in 622 patients (58.6%). Patients' cognitive status was classified according to the revised ALS-FTD Consensus Criteria [19]: the neuropsychological batteries used for classification were described in an our previous work [20]. To evaluate the effect of cognitive impairment, we subdivided patients into two categories: cognitively impaired patients (corresponding to ALS-FTD, ALSbi, ALSci, ALScbi categories in the revised ALS-FTD Consensus Criteria) and cognitively normal patients (corresponding to ALS-CN category).

Functional involvement assessment using ALSFRS-R score

We derived the number of body region involved (NBRI) at diagnosis using ALSFRS-R subscores for bulbar, upper limbs, lower limbs and respiratory/thoracic regions. We considered ALSFRS-R items 1, 2 and 3 for the bulbar region, items 4 and 5 for the upper limb region, items 8 and 9 for the lower limb region, and items 10, 11 and 12 for the thoracic/respiratory region. A region was considered to be affected when at least 1 point was lost from the maximum total subscore. Item 6 (dressing/hygiene) and item 7 (turning over in bed) were excluded from the analysis, being not specific for upper or lower limbs involvement. In our series, as in a previous paper [21], these items never occurred alone in patients with upper and/or lower limbs involvement.

We determined also King's stage at diagnosis using ALSFRS-R [14] in order to compare our results with a validated clinical staging system [22], and to assess their different prognostic yield.

Functional involvement assessment using ALSFRS-R score and neuropsychological evaluation

In the subset of 622 patients with neuropsychological evaluation at diagnosis, we considered the presence of cognitive impairment as another body region involved, stratifying patients in a further classification based on the number of motor body regions plus cognitive (NBRI-C). Using this classification, for example, a patient with bulbar and upper limbs motor involvement and cognitive impairment has been considered as having three body region involved (NBRI-C = 3).

Statistical analysis

Differences of discrete and continuous variables of interest between numbers of regions involved were analysed using the $\chi 2$ test, Student's t test or Kruskal–Wallis test by ranks and Mann-Whitney U test, as appropriate. A p value <0.05 was considered significant.

The association between numbers of regions involved and survival was assessed using Cox proportional hazards models, adjusted for sex, age groups (15-44, 45-59, 60-74 and over 75 years), diagnostic delay, ALSFRS-R total score, site of onset. Kaplan-Meier curves and log rank test were calculated. We also performed sensitivity analysis considering the effect of FVC (below and above 80%) and BMI categories (BMI $\leq$ 18.5 "underweight", BMI 18.5-25 "normal weight", BMI 25 -30 "overweight or preobesity", BMI $\geq$ 30 "obesity") according to the WHO classification [23], and excluding genetic mutation (*c9orf72*, *SOD-1*, *TARBDP*, *FUS*) carriers.

Data were analysed using Stata V.13.1 (StataCorp, College Station, Texas, USA).

Standard Protocol Approvals, Registrations, and Patient Consents. The study design was approved by the Ethical Committee of the Azienda Ospedaliero-Universitaria Città della Salute. Patients signed a written informed consent.

Results

Patients' characteristics

Of the 1,062 patients included, 937 (88.2%) deceased or underwent tracheostomy before the end of follow-up. Five-hundred and sixty-five were males (53.2%), and the mean age at onset was 66.3 years (Standard Deviation, SD=11.0). At diagnosis, one region was functionally affected in 492 patients (46.3%), two regions in 288 patients (27.1%), three in 196 patients (18.5%), and four in 86 patients (8.1%). The number of affected regions at diagnosis progressively increased with the increase of the age at onset (p=0.0001). Patients' characteristics are shown in Table 1.

Table 1 Descriptive statistics of the main variables of interest, stratified by the NBRI at diagnosis. . Valid raw (%r) and column (%c) percentages by brackets.

NBRI	**1**	**2**	**3**	**4**	**Total**	
	n (%^{r})	***n (%^{r})***	***n (%^{r})***	***n (%^{r})***	***n (%^{c})***	***P-value***
Sex						
Male	262 (46.4)	154 (27.3)	107 (18.9)	42 (7.4)	565 (53.2)	0.854
Female	230 (46.3)	134 (27.0)	89 (17.9)	44 (8.8)	497 (46.8)	
Age at onset						
15-44	27 (71.1)	8 (21.0)	3 (7.9)	0 (0.0)	38 (3.6)	< 0.001
45-59	113 (56.8)	65 (32.7)	13 (6.5)	8 (4.0)	199 (18.7)	
60-74	250 (45.3)	159 (28.8)	101 (18.3)	42 (7.6)	552 (52.0)	
Over 75	102 (37.4)	56 (20.5)	79 (28.9)	36 (13.2)	273 (25.7)	
El Escorial revised						
Defined ALS	119 (23.5)	162 (32.0)	139 (27.5)	86 (17.0)	506 (47.6)	< 0.001
Probable ALS	159 (48.8)	110 (33.7)	57 (17.5)	0 (0)	326 (30.7)	
Probable-laboratory supported ALS	214 (93.0)	16 (7.0)	0 (0)	0 (0)	230 (21.7)	
Site of onset						
Bulbar	185 (49.2)	77 (20.5)	77 (20.5)	37 (9.8)	376 (35.4)	< 0.001
Upper limbs	132 (43.7)	100 (33.1)	51 (16.9)	19 (6.3)	302 (28.4)	
Lower limbs	171 (47.1)	109 (30.0)	60 (16.5)	23 (6.4)	363 (34.2)	
Respiratory/thoracic	4 (19.1)	2 (9.5)	8 (38.1)	7 (33.3)	21 (2.0)	
Mutational status						
Wild-type	372 (46.5)	227 (28.4)	140 (17.5)	61 (7.6)	800 (75.3)	0.337
c9orf72	32 (51.6)	17 (27.4)	11 (17.7)	2 (3.3)	62 (5.8)	
SOD-1	9 (56.3)	4 (25.0)	3 (18.8)	0 (0)	16 (1.5)	
TARDBP	8 (61.5)	3 (23.1)	0 (0)	2 (15.4)	13 (1.2)	
FUS	3 (100)	0 (0)	0 (0)	0 (0)	3 (0.3)	
Not assessed	68 (40.0)	37 (22.0)	42 (25.0)	21 (12.5)	168 (15.8)	
BMI						
Underweight (≤ 18.5)	13 (52.0)	4 (16.0)	5 (20.0)	3 (12.0)	25 (2.3)	0.543
Normal (18.5-25)	177 (51.6)	93 (27.1)	58 (16.9)	15 (4.4)	343 (32.3)	
Overweight (25-30)	99 (53.2)	50 (26.9)	28 (15.1)	9 (4.8)	186 (17.5)	
Obesity (≥ 30)	30 (41.1)	26 (35.6)	13 (17.8)	4 (5.5)	73 (6.9)	
Not assessed	173 (39.8)	115 (26.4)	92 (21.2)	55 (12.6)	435 (41.0)	
FVC						
< 79%	116 (42.2)	69 (25.0)	67 (24.4)	23 (8.4)	275 (25.9)	< 0.001
> 80%	205 (56.8)	109 (30.2)	38 (10.5)	9 (2.5)	361 (34.0)	
Not assessed	171 (40.1)	110 (25.8)	91 (21.4)	54 (12.7)	426 (40.1)	
Cognitive classification						
Normal cognition	184 (56.1)	88 (26.8)	45 (13.7)	11 (3.4)	328 (30.9)	0.003
Impaired cognition	136 (46.3)	74 (25.2)	58 (19.7)	26 (8.4)	294 (27.7)	
Not assessed	172 (39.1)	126 (28.6)	93 (21.1)	49 (11.1)	440 (41.4)	
Total	**492 (46.3)**	**288 (27.1)**	**196 (18.5)**	**86 (8.1)**	**1062 (100)**	

NBRI	1	2	3	4	Total	
						P-value
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	
ΔALSFRS-R (point/month)	0.33 (0.21-0.67)	0.70 (0.40-1.22)	1.27 (0.75-2.00)	1.69 (1.08-3.00)	0.64 (0.29-1.31)	<0.001
Onset-diagnosis interval = diagnostic delay	0.67 (0.41-1.03)	0.75 (0.50-1.08)	0.75 (0.50-1.08)	0.81 (0.50-1.16)	0.75 (0.42-1.08)	0.040
Total	**492 (46.3)**	**288 (27.1)**	**196 (18.5)**	**86 (8.1)**	**1062 (100)**	

NBRI was related to site of onset ($p<0.001$) with a more widespread disease at diagnosis in patients with respiratory onset compared to other groups. Mutational status did not affect the spreading of the disease at diagnosis ($p=0.337$).

The overall mean diagnostic delay was 0.98 years. The intervals between onset and diagnosis were marginally different in relation to the NBRI (1 region: 0.67 years, IQR=0.41-1.03; 2 regions: 0.75 years, IQR=0.50-1.08; 3 regions: 0.75 years, IQR=0.50-1.08; 4 regions: 0.81 years, IQR=0.50-1.16; $p=0.04$), not showing a significant increase trend among groups (see Mann-Whitney U test in supplementary table). The distribution of ALSFRS-R total scores at diagnosis was significantly different (1 region: median score 45, IQR=44-46; 2 regions: 42, IQR=39-44; 3 regions: 37, IQR=33-40; 4 regions: 29, IQR=24-36; $p<0.001$), as well as the rate of disease progression (ΔALSFRS-R = 48 – ALSFRS-R at diagnosis/diagnostic delay in months, $p < 0.001$) and cognitive impairment ($p=0.003$).

A more widespread functional involvement at diagnosis in the elderly could be explained postulating a greater diagnostic delay due to the existence of several clinical conditions that could mimic an initial motor neurons impairment (such as sarcopenia, brain vascular aging, etc.): in our series we do not confirm this hypothesis, finding no difference in the diagnostic delay among different age classes (Kruskall-Wallis test $p=0.376$). Another possible explanation is the presence of a more aggressive disease in the elderly, which is confirmed considering the overall mean survival reduction across different age classes (see supplementary material)

Different combinations of NBRI are shown in Table 2

Table 2 Different combination of body regions involved (bulbar, upper limbs, lower limbs, respiratory/thoracic) subdivided by total number of regions involved (NBRI) at diagnosis. In the last column, *n* represents the total number of patients with a specific combination of regions involved, followed by its relative percentage *(%)* referred to the single NBRI category.

Body region involved		Bulbar	Upper limbs	Lower limbs	Respiratory/ Thoracic	*n*	*(%)*
NBRI	1	X				185	(37.6)
			X			132	(26.8)
				X		171	(34.8)
					X	4	(0.8)
	2	X	X			67	(23.3)
		X		X		50	(17.4)
		X			X	14	(4.8)
			X	X		142	(49.3)
			X		X	7	(2.4)
				X	X	8	(2.8)
	3	X	X	X		126	(64.3)
		X	X		X	18	(9.2)
		X		X	X	17	(8.7)
			X	X	X	35	(17.8)
	4	X	X	X	X	86	(100.0)
	Total					1062	

Survival analysis according to the number of regions involved at diagnosis

Median survival time was inversely related to NBRI at diagnosis (1 region: 2.90 years, IQR=2.1-5.6; 2 regions: 2.6 years, IQR=1.7-4.2; 3 regions: 1.8 years, IQR=1.2-3.1; 4 regions: 1.5 years, IQR=0.8-2.4; p=0.0001)

Table 3 shows the Cox proportional Hazard models for different adjustments. Adjusting for age and sex, the NBRI was strongly related to survival, with a progressive increase of death/tracheostomy risk among groups (2 body regions HR=1.24, 95% CI=1.06-1.45, p=0,007; 3 body regions HR=1.65, 95% CI= 1.38-1.98, p<0.001; 4 body regions HR=2.68, 95% CI 2.11-3.39, p<0.001) (Table 3).

The association resulted to be even more significant adjusting also for diagnostic delay and site of onset (2 body regions HR=1.53, 95% CI=1.30-1.79, p<0,001; 3 body regions HR=2.09, 95% CI= 1.75-2.50, p<0.001; 4 body regions HR=4.63, 95% CI 3.61-5.93, p<0.001).

To assess the effect of global functional impairment we adjusted the analysis also for ALSFRS-R total score at diagnosis. Also in this case the association to the number of regions involved remained significant (Table 3).

Kaplan-Meier curves for each group of NBRI are reported in Figure 1. The number of involved regions proportionally increased the risk of death/tracheostomy (log-rank test, $p \leq 0.001$).

Insert Figure 1 about here

Figure 1. Kaplan-Meier curve of ALS patients by NBRI at diagnosis.

Sensitivity analysis: wild-type patients, nutritional status and respiratory function

We further performed a sensitivity analysis considering only wild-type patients (800 patients) and the association remained significant.

In a second sensitivity analysis in 621 patients (58.5%), which considered also measures of both FVC and BMI at diagnosis, the NBRI remained significantly associated with a cumulative increased risk of death/tracheostomy

Sensitivity analysis: cognitive impairment spreading

In this sensitivity analysis we considered cognition as a further body region involved. As motor impairment derived by ALSFRS-r reflects neuronal damage, in the same way cognitive impairment assessed by neuropsychological evaluation could show disease spreading through non-motor areas.

We performed Kaplan-Meier curves (log-rank test, $p \leq 0.001$, see Supplementary material) and Cox proportional Hazard models adjusted for age, sex and diagnostic delay, showing that also NBRI-C classification is a strong survival determinant (Table 3)

Table 3 Hazard Ratios of NBRI and King's staging at diagnosis. Cox proportional hazard models adjusted for age classes, sex, onset-diagnosis interval, site of onset, total ALSFRS-R score. Sensitivity analysis in wild-type patients, according FVC and BMI status. Sensitivity analysis considering cognition as a further body region (NBRI-C).

		NBRI			King's stage at diagnosis			
Adjustments		HR	CI	P		HR	CI	p
Age classes, sex	1	1			1	1		
	2	1.24	1.06-1.45	0.007	2	1.35	1.16-1.57	<0.001
	3	1.65	1.38-1.98	<0.001	3	1.72	1.43-2.06	<0.001
	4	2.68	2.11-3.39	<0.001	4*	1.94	1.48-2.55	<0.001
Age classes, sex, diagnostic delay, site of onset	1	1			1	1		
	2	1.53	1.30-1.79	<0.001	2	1.64	1.40-1.91	<0.001
	3	2.09	1.75-2.50	<0.001	3	2.51	2.08-3.03	<0.001
	4	4.63	3.61-5.93	<0.001	4*	2.08	1.53-2.82	<0.001
Age classes, sex, diagnostic delay, site of onset, total ALSFRS-R score	1	1			1	1		
	2	1.30	1.09-1.54	0.003	2	1.24	1.05-1.48	0.012
	3	1.40	1.11-1.79	0.005	3	1.26	0.96-1.63	0.090
	4	2.48	1.74-3.55	<0.001	4*	0.85	0.59-1.24	0.411

		HR	CI	P	N
Wild-type patients[#] (N=800) - Age classes, sex, diagnostic delay, site of onset	1	1			372
	2	1.40	1.15-1.70	0.001	227
	3	1.50	1.13-1.97	0.005	140
	4	2.91	1.89-4.48	<0.001	61
Age classes, sex, site of onset, FVC > 80%, BMI status (N=609)	1	1			312
	2	1.44	1.19-1.75	<0.001	169
	3	1.52	1.20-1.93	<0.001	98
	4	3.79	2.54-5.67	<0.001	30

NBRI-C[∞]		HR	CI	P	N
Age classes, sex, diagnostic delay (N=622)	1	1			184
	2	1.27	1.02-1.58	0.036	224
	3	1.58	1.22-2.05	<0.001	119
	4	2.45	1.80-3.35	<0.001	69
	5	5.86	3.73-9.19	<0.001	26

**** King's stages 4a and 4b were considered in a single category (stage 4)***

[#] Patients included in this sensitivity analysis resulted wild-type for SOD-1, TARDBP and FUS mutations and c9orf72 repeated expansion

∞In this sensitivity analysis, we classified patients considering cognitive impairment as another region involved

Number of regions and King's stage comparison at diagnosis

The study population was also stratified according to King's stage at diagnosis (Table 4).

Table 4 King's stages according to NBRI at diagnosis

N. of body regions / King's stages	1	2	3	4	Total
1	483	39	6	0	528
%col	98.2%	13.5%	3.1%	0.0%	49.7%
%row	91.5%	7.4%	1.1%	0.0%	100.0%
2	5	240	52	3	300
%col	1.0%	83.3%	26.5%	3.5%	28.2%
%row	1.7%	80.0%	17.3%	1.0%	100.0%
3	0	4	110	57	171
%col	0.0%	1.4%	56.1%	66.3%	16.1%
%row	0.0%	2.3%	64.3%	33.3%	100.0%
4	4	5	28	26	63
%col	0.8%	1.7%	14.3%	30.2%	5.9%
%row	6.3%	7.9%	44.4%	41.3%	100.0%
Total	492	288	196	86	1,062
%col	100.0%	100.0%	100.0%	100.0%	100.0%
%row	46.3%	27.1%	18.5%	8.1%	100.0%

Using ALSFRS-R score, the consistency between the NBRI and King's clinical stage at diagnosis was 81%. The inconsistency between the two staging system was mainly related to an under-representation of initial respiratory/thoracic involvement in King's system [14].

Survival models using the King's staging system were also assessed and their HRs were reported and compared with NBRI. King's staging, adjusted only for age and sex, showed statistically cumulative and significant association to overall survival (Table 3). Nevertheless, adjusting for diagnostic delay and site of onset the trend of increasing risk of death/tracheostomy resulted to be inverted in stages 3 and 4.Adjusting also for ALSFRS-R total score both stage 3 (HR 1.26, 95% CI=0.96-1.63) and stage 4 (HR 0.85, 95% CI=0.59-0.24) lose their significance.

Discussion

In this population-based study, which included 96% of ALS patients incident in Piemonte and Valle d'Aosta Italian regions during the 2007-2014 period, we evaluated the importance of assessing the diffusion of motor involvement at diagnosis as prognostic biomarker. First, we observed that the spatial spreading at diagnosis, considered as NBRI, was strongly related to overall survival, independently from the specific body region involved. Second, we found that the inclusion of the involvement of respiratory/thoracic region provides useful information for the classification in a more or less widespread disease. Third, we pointed out that evaluation of cognitive dysfunction lead to a better stratification of patients' outcome.

The phenotypical heterogeneity in ALS is determined by a various combination of clinical traits, such as site of onset, the predominant involvement of upper or lower motor neurons, and the rate of disease progression [24]. Such heterogeneity has profound implications in determining clinical outcomes and in designing clinical trials [25, 26]

Recent neuropathological studies have proposed an explanation to the focality of onset and to upper and lower motor neurons involvement as a corticofugal spreading of TDP43 pathology [27,28], while the determinants of progression rate are far to be elucidated. They can reflect individual differences in disease-modifying genes, environmental modifiers exposure or both [29].

The pattern of disease spreading has been related to survival [30]; for example, in lower limb-onset, the time to involvement of the following limb has been shown to be a prognostic factor regardless of initial direction of spread [31]. Besides, the time to bulbar involvement in spinal onset patients [32], the time to generalisation (i.e. the time of spreading of the clinical signs from spinal or bulbar localization to both) [33] and the interval from onset to involvement of the second region have been reported to correlate with survival [21].

In the present study, we have shown that the regional spreading of ALS symptoms can be easily derived from the ALSFRS-R scale, which is characterised by high internal consistency, reliability and responsiveness to change [34]. The use of a validated scale minimises the risk of misclassifications of patients due to variability in neurological evaluation, allowing its administration in different clinical settings. Moreover, considering the worldwide use of ALSFRS-R score, our findings can be easily replicated using data from other population-based or clinical trial datasets. The use of ALSFRS-R regional subscores considered the multidimensionality of the scale, allowing to derive information on spatial spreading of lesions in a way which is otherwise impossible to obtain with the total raw score. The NBRI at diagnosis resulted to have a remarkable prognostic value, independent from age, sex, site of onset, diagnostic delay, ALSFRS-R total score, FVC and nutritional status.

C9orf72 gene expansion is generally considered a negative prognostic factor, leading to early age at onset, increased risk of dementia [35] and shorter survival [36]. Considerable phenotypic heterogeneity occurs

across the other ALS-related genetic mutations [37]. We found that the regional spreading of functional impairment at diagnosis is not significantly different in patients with *c9orf72* expansion and other genetic mutations compared to wild-type patients, suggesting that the negative effect of *c9orf72* mutations on overall survival is probably related to other factors, such as cognitive impairment and to its negative influence on mechanical ventilation and enteral nutrition adherence [38].

We compared the prognostic yield of NBRI to King's staging system, a validated clinical score that summarises the anatomical spread of disease [39]. The King's staging system considers as milestones, the progressive involvement of bulbar, upper and lower limbs regions to classify stages 1, 2 and 3, but it does not consider the initial respiratory/thoracic involvement [22]. In our population-based series, this difference resulted to be the major determinant of the lack of consistency between NBRI and King's stage.

The involvement of the thoracic region is frequently underestimated, especially in the early phases of the disease: this is can be due to many factors, such as the difficulty of assessing UMN and LMN signs at thoracic level, the limited number of neurophysiological studies of paravertebral muscles performed, and the need of pulmonary function tests to detect early respiratory impairment, which can be less accurate in patients with bulbar symptoms. Despite these limitations, we have found that the evaluation of functional respiratory involvement at diagnosis derived from ALSFRS-R scale can provide reliable information about the involvement of thoracic region. According to our findings, as indicated by the sensitivity analysis adjusted for FVC, thoracic region involvement attains a prognostic value as a sign of a more widespread disease.

Our findings are in keeping with other studies focused on the symptoms burden in early disease stages [12, 13, 21, 30, 33, 40]. However, we have provided novel evidences on the prognostic value of number of body regions functionally involved.

Cognitive impairment has been widely recognised as one of the major determinants of disease outcome [6]. Recent findings suggested that ALS motor and cognitive components may worsen in parallel during disease progression [20]. In our sensitivity analysis with NBRI-C, we found an exponential increase in risk across categories, confirming that the spread of the disease through cognitive areas is a cause of worse prognosis, independently from specific motor impairment.

Our study is not without limitations. First, we could not discriminate whether the functional involvement of a body region is mainly related to UMN or LMN dysfunction. We assume that functional involvement that leads to the loss of point in ALSFRS-R regional subscore is related to the presence of either UMN or LMN signs or both, considering that the body region with the highest UMN involvement at onset in general also had the highest frequency of LMN signs and vice versa [39]. Second, we could not include in our analysis any specific biomarkers (such as neurofilaments – pNfH and NfL): recent findings showed a correlation between pNfH and NfL levels and the number of regions with both UMN and LMN involvement, demonstrating their higher correlation to disease spreading than to rate of progression [9]. Third, our study

has not been designed to provide a personalised prediction model, like the ENCALS Survival Prediction Model, but to specifically untangle the role of the spatial spread of functional impairment and the underlying pathology, from the mere progression rate. For this reason, the NBRI is not recommended to be used as prognostic marker in a single patient. Fourth, due to their retrospective nature, our findings should be confirmed in prospective cohorts from different populations, to assess their validity and generalizability.

For this reason, we suggest that the evaluation of NBRI can be useful in many research fields (i.e. neuropathology, prognostic biomarkers and neuroimaging), shedding new light on the importance of evaluating the spreading of functional impairment in the prediction of ALS outcome.

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