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ORIGINAL COMMUNICATION

Riluzole and other prognostic factors in ALS: a population-based registry study in Italy

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Abstract

Objective In this prospective population-based registry study on ALS survival, we investigated the role of riluzole treatment, together with other clinical factors, on the prognosis in incident ALS cases in Emilia Romagna Region (ERR), Italy.

Methods A registry for ALS has been collecting all incident cases in ERR since 2009. Detailed clinical data from all patients diagnosed with ALS between 1.1.2009 and 31.12.2014 have been analyzed for this study, with last follow up date set at 31.12.2015.

Results During the 6 years of the study, there were 681 incident cases with a median tracheostomy-free survival of 40 months (95% CI 36–44) from onset and of 26 months (95% CI 24–30) from diagnosis; 573 patients (84.14%) were treated with riluzole, 207 (30.39%) patients underwent gastrostomy, 246 (36.12%) non invasive ventilation, and 103 (15.15%) invasive ventilation. Patients who took treatment for ≥ 75% of disease duration from diagnosis had a median survival of 29 months compared to 18 months in patients with < 75% treatment duration. In multivariable analysis, factors independently influencing survival were age at onset (HR 1.04, 95% CI 1.02–1.05, $p < 0.001$), dementia (HR 1.56, 95% CI 1.05–2.32, $p = 0.027$), degree of diagnostic certainty (HR 0.88, 95% CI 0.78–0.98, $p = 0.021$), gastrostomy (HR 1.46, 95% CI 1.14–1.88, $p = 0.003$), NIV (HR 1.43, 95% CI 1.12–1.82, $p = 0.004$), and weight loss at diagnosis (HR 1.05, 95% CI 1.03–1.07, $p < 0.001$), diagnostic delay (HR 0.98, 95% CI 0.97–0.99, $p = 0.004$), and % treatment duration (HR 0.98, 95% CI 0.98–0.99, $p < 0.001$).

Conclusions Independently from other prognostic factors, patients who received riluzole for a longer period of time survived longer, but further population based studies are needed to verify if long-tem use of riluzole prolongs survival.

Keywords Amyotrophic lateral sclerosis · Survival · Prognostic factors · Therapeutic intervention · Riluzole

Introduction

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease characterized by progressive motor deficits over the course of weeks to months leading to severe disability and death. The disease is highly heterogeneous in clinical presentation, age at onset, involvement of other than motor systems (cognitive impairment in up to 50%, variable sensory, autonomic, extrapyramidal signs), family history and genetic background, disease course and survival [1, 2]. Although survival of ALS patients from symptom onset is reported to be 3–5 years, published studies report a wide range of outcomes influenced by age and site of onset, severity and rate of disease progression, diagnostic delay, cognitive impairment, nutritional and respiratory status, functional/disability scores, Revised El Escorial diagnostic Criteria (EEC-R), multidisciplinary approach, and therapeutic interventions [3–5].

Despite significant efforts and a large number of well-designed clinical trials, published studies on ALS treatments gave negative results, probably because of ALS phenotypic, genetic and pathophysiological heterogeneity [6]. So far,

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riluzole is the only drug that has been shown to increase survival in ALS patients [7], although with a poorly understood mechanism of action [8] and a way of drug delivery still investigational [9, 10].

Riluzole has been tested in four randomized clinical trials (RCTs) involving 1477 patients, showing a significant effect on survival which was quantified as a gain of 3 months in 50% of patients or a 9% gain in the probability of surviving 1 year [11].

Conversely, subsequent cohort studies have suggested that treatment with riluzole may be associated with a median survival increase of 6 months to as many as 12 months, with diverse impact on different subpopulations of ALS patients [5, 12–15]. Two recent studies (one retrospective [16] and one prospective [17]) considered the cumulative defined daily dose to measure treatment duration, and obtained contrasting results.

In this prospective population-based registry study on ALS survival, our aim was to study the role of riluzole treatment and its duration on prognosis in incident ALS cases in Emilia Romagna Region (ERR), Italy, between 2009 and 2014.

Methods

Patient data collection

The study was performed in Emilia Romagna Region (ERR), northern Italy, considering patients diagnosed with ALS from 1/1/2009 to 31/12/2014. The region of Emilia-Romagna covers an area of 22,453 km^2, ranking 6th in Italy, and during the evaluated time period, the local population increased from 4,395,569 (2009) to 4,450,508 (2014).

A prospective registry (Emilia Romagna Registry for ALS–ERRALS) has been active in our region since 2009 [18], collecting all incident ALS cases among residents of ERR, diagnosed according to EEC-R [19]. Caring physicians collected a detailed phenotypic profile of each ALS patient, including the following parameters: age at onset and diagnosis, gender, type of onset, site and time of onset, affected regions, upper and lower motor neuron signs, EEC-R classification, clinical phenotype [1], presence of dementia (diagnosed by neuropsychological testing) and/or extrapyramidal signs, family history, and drugs (including riluzole).

These data have been included at diagnosis into an electronic database available through a dedicated web-site, accessible only to the investigators of ERR Neurological Departments. In each department, one or more investigators were identified as study referents, and had to upload data on new ALS cases as soon as possible after diagnosis. Clinical follow up has been performed in the 17 neurological departments of ERR until death, collecting and uploading information on ALS course, including forced vital capacity (FVC), ALS Functional Rating Scale-Revised (ALSFRS-R) [20, 21], riluzole intake and discontinuation, gastrostomy [22], non-invasive (NIV) or invasive (IV) ventilation support, and cause, place and time of death. A regular supervision on the data has been performed by the coordinating center, checking for accuracy and completeness.

Conforming to the EFNS Guidelines [23], patients underwent a regular multidisciplinary follow-up at least every 3–4 months. When patients were no longer able to reach the centers, they were monitored at their home or in a nursing home by the Integrated Home Care of the Italian National Health System (INHS) [24].

For this study we focused on clinical variables and riluzole intake, considering date of riluzole administration and discontinuation (if applicable) with respect to disease duration.

The study was approved by the ethical committees of the coordinating center and of ERR provinces.

Statistical methods

Continuous variables are reported as mean and standard deviation (SD) from the mean. Discrete variables are reported as number and proportion of subjects with the characteristic of interest. Descriptive statistics were performed using Student's *t* test and Chi square test where appropriate. Survival was calculated from onset and from diagnosis to death/tracheostomy or the censoring date (last day of follow-up, 31/12/2015) using the Kaplan–Meier method. The curves were compared with the Log-Rank test. Multivariable analysis was performed with Cox's proportional hazards model, to evaluate the association between demographic and clinical factors, riluzole use/treatment duration and survival.

Riluzole use was defined as a categorical variable (0 = "no drug", 1 = "drug user" if treatment was taken at a dosage of 100 mg for at least 1 month), but we also classified individuals in different groups based on the duration of riluzole treatment (in days from treatment initiation to treatment end or death/tracheostomy) with respect to ALS duration (in days from diagnosis to death/tracheostomy) (proportion of days covered -PDC) [16]. PDC definition was adopted because an evaluation of survival times exclusively in dependence of the days of riluzole treatment would have biased the results since patients with longer therapy durations will have lived necessarily longer [16]. Mean PDC was 75%, median PDC was 90%; we considered these values as cut off values for the individuation of three treatment groups.

We also calculated the delay from onset of symptoms to start of riluzole (months). Regarding the analysis of riluzole's effect on survival we accounted for a possible bias due to a delay in the start of riluzole treatment after diagnosis

by adjusting for the immortal time bias, which refers, in observational studies, to the time during which the outcome could not have occurred [25]. To correct for this bias we used a time-varying covariate for time from diagnosis to Riluzole exposure in order to avoid misclassification of exposed patients' survival time before the first prescription as the exposed follow-up time [25].

We measured ALSFRS-R at diagnosis and ALSFRS-R monthly decline, subtracting ALSFRS-R total score at diagnosis from the score of 48 presumed at onset and dividing the results for the number of months of diagnostic delay [26].

For all Cox proportional hazard regression analyses, we considered age at onset, phenotypes, type of onset, EEC-R classification, presence of dementia and/or extrapyramidal signs, comorbidities using the Charlson Comorbidity Index (CCI) [27], family history of ALS, monthly ALSFRS-R decline at diagnosis and riluzole use, to assess the independent contribution of the variables of interest to death or tracheostomy.

A p value < 0.05 was considered significant. All calculations were performed with STATA statistical package,V.12 (2013).

Results

Clinical characteristics of ALS patients

During the 6 years of study, there were 681, 371 men and 310 women (men:women = 1.20), incident cases in ERR. Table 1 shows the clinical features of ALS patients. Median survival time was 40 months (95% CI 36–44) from onset and 26 (95% CI 24–30) from diagnosis. The overall 1-, 2-, 3- and

Table 1 Patients' characteristics ($N = 681$)

Explanatory variables	Total $N = 681$ *n* (%) *m* [SD]	Men $N = 371$ *n* (%) *m* [SD]	Women $N = 310$ *n* (%) *m* [SD]	*p* value
ALS Onset (Bulbar)	238 (34.95)	107 (28.84)	131 (42.26)	**< 0.001**
Age at onset	66.50 [± 11.09]	65.87 [± 10.66]	67.26 [± 11.57]	0.102
Diagnostic delay	13.16 [± 13.09]	13.13 [± 13.85]	13.20 [± 12.14]	0.947
Phenotype				**< 0.001**
Bulbar	211 (30.98)	92 (24.80)	119 (38.39)	
Classic	286 (42.00)	177 (47.71)	109 (35.16)	
Flail arm and leg	119 (17.47)	68 (18.33)	51 (16.45)	
UMNp	38 (5.58)	16 (4.31)	22 (7.10)	
Respiratory	21 (3.96)	15 (4.04)	6 (1.94)	
Unknown	6 (0.88)	3 (0.81)	3 (0.97)	
Revised El Escorial criteria				0.250
Definite	189 (27.75)	94 (25.34)	95(30.65)	
Clinically probable	216 (31.72)	122 (32.88)	94 (30.32)	
Probable lab-supported	92 (13.51)	48 (12.94)	44 (14.19)	
Possible	136 (19.97)	82 (22.10)	54 (17.42)	
Dementia	64 (9.39)	31 (8.36)	33 (10.65)	0.308
BMI at diagnosis (kg/m^2)	24.39 [± 3.98]	24.94 [± 3.72]	23.73 [± 4.19]	**< 0.001**
Riluzole (yes)	573 (84.14)	319 (85.98)	254 (81.94)	0.150
Treatment duration (days)	869.24 [± 645.00]	867.84 [± 639.67]	870.99 [± 652.84]	0.954
Delay from onset to riluzole intake (days)	421.13 [± 372.12]	408.82 [± 367.45]	436.48 [± 378.02]	0.378
Delay from diagnosis to riluzole intake (days)	41.53 [± 97.98]	39.74 [± 80.31]	43.76 [± 116.46]	0.626
Gastrostomy (yes)	207 (30.39)	96 (25.88)	111 (35.81)	**0.005**
Non invasive ventilation (yes)	246 (36.12)	147 (39.62)	99 (31.94)	**0.038**
Invasive ventilation (yes)	103 (15.15)	65 (17.52)	38 (12.26)	0.059
ALSFRS-R at diagnosis	38.58 [± 7.95]	39.28 [± 7.26]	37.75 [± 8.63]	**0.013**
ALSFRS-R monthly decline (points/month) measured at diagnosis	1.14 [± 1.66]	1.08 [± 1.41]	1.21 [± 1.92]	0.300
Charlson Comorbidity Index	2.73 [± 1.56]	2.69 [± 1.50]	2.77 [± 1.62]	0.221

Bold indicates statistically significant results ($p < 0.05$)

SD standard deviation, *NA* not appropriate, *UMN-P* upper motor neuron predominant, *BMI* body mass index, *ALSFRS-R* ALS functional rating scale-revised

5-year survival rates from onset were 89.6, 68.8, 53.8 and 36.6%, respectively. The overall 1-, 2-, 3- and 5-year survival rates from diagnosis were 73.3, 52.8, 39.9 and 32.7%, respectively. Five hundred and 73 patients (84.14%) were treated with riluzole, 207 (30.39%) underwent gastrostomy, 246 (36.12%) NIV, and 103 (15.15%) IV.

Univariate analysis of factors associated with ALS survival

According to univariate analysis, factors related to tracheostomy-free survival from diagnosis were (Table 2): age at onset, diagnostic delay, site of onset, phenotype, presence of dementia, degree of diagnostic certainty according to EEC-R, weight change between healthy status and diagnosis, ALSFRS-R score at diagnosis, and ALSFRS-R monthly decline, NIV and gastrostomy, comorbidities (CCI). The same factors influenced survival from disease onset (Table 2).

Overall riluzole treatment did not influence ALS survival (Fig. 1a). In contrast, the duration of treatment in relation to disease duration, expressed as percent of days of riluzole use (until discontinuation, tracheostomy or death)(PDC), influenced survival (Table 2). Standard daily dose of riluzole was 50 mg twice a day. Median PDC was 90%. Mean PDC was 75%. Patients that took riluzole for a number of days corresponding to at least 75% of disease duration from diagnosis (≥ 75% PDC) had a median survival of 29 months as compared to 18 months in patients with a shorter percentage of riluzole treatment (Fig. 1b). Patients that took riluzole for a number of days corresponding to at least 90% of disease duration from diagnosis (≥ 90% PDC) had a median survival of 46 months as compared to 15 months in patients with a shorter percentage of riluzole treatment (Fig. 1c). The delay from disease onset to start of treatment significantly influenced survival (Table 2) as the earlier the patients took riluzole, the worse the prognosis. Half of treated patients took riluzole treatment after 317 days (~ 10 months) from onset.

Patients who took riluzole for ≥ 90% PDC were younger, had a shorter diagnostic delay, a lower CCI and ALSFRS-R decline, had more frequently a spinal onset, were less frequently affected by dementia and underwent more often NIV and IV (Table 3). There was a mean difference of 2 months in the delay from diagnosis to treatment initiation between the two groups.

Multivariable analysis of factors associated with ALS survival

We then did a multivariable analysis including PDC, the delay from onset to start of treatment, and all variables possibly influencing survival available at time of diagnosis. In the initial Cox multivariable model, we included the following variables (retention criterion of < 0.10): diagnostic delay, age at onset, site of onset, phenotypes, presence of dementia, EEC-R classification, PDC, delay from onset to start of treatment, ALSFRS-R score at diagnosis, ALSFRS-R monthly decline at diagnosis, weight loss at diagnosis, comorbidities (CCI), gastrostomy and NIV. We also used a time-varying covariate for time from diagnosis to riluzole exposure.

After dropping non-significant terms (stepwise backward), the final model included age at onset, diagnostic delay, dementia, EEC-R classification, gastrostomy, NIV, weight loss at diagnosis, PDC (Table 4). As for PDC (%), after correcting for immortal time bias, we obtained a HR of 0.98, i.e. a decrease of the risk of death/tracheostomy of 2% for each % increase in treatment duration.

Subgroups analysis of survival

Hypothesizing that early treatment discontinuation was most frequent in more severely affected patients, we excluded from our population those patients who died before 6 months from diagnosis (79 cases). In the remaining 602 patients the results were unchanged and factors independently influencing survival (multivariable analysis) were age at onset (HR 1.03, 95% CI 1.02–1.04, $p < 0.001$), diagnostic delay (HR 0.97, 95% CI 0.96–0.99, $p = 0.001$), EEC-R classification (HR 0.86, 95% CI 0.76–0.98, $p = 0.020$), NIV (HR 1.71, 95% CI 1.31–2.24, $p < 0.001$), gastrostomy (HR 1.61, 95% CI 1.23–2.11, $p = 0.001$), weight loss at diagnosis (HR 1.04, 95% CI 1.01–1.06, $p = 0.002$), and PDC (HR 0.98, 95% CI 0.98–0.99, $p = 0.001$). As compared to the remaining study population, the 79 patients dying early were older (mean age at onset 73.78 ± 7.89 vs 65.53 ± 11.10 years), with bulbar onset (55.70 vs. 44.30%), more frequently demented (78.48 vs. 21.52%), and with a greater monthly decline of the ALSFRS-R (2.15 ± 2.87 vs. 1.00 ± 1.36 points/months). The factors influencing survival in these patients were PDC (HR 0.95, 95% CI 0.94–0.97, $p = 0.001$), and CCI (HR 1.71, 95% CI 1.08–2.69, $p = 0.021$).

The same results were obtained removing from the initial population patients who survived less than 4 months (46 patients) and less than 5 months (64 patients) (data not shown).

We investigated if treatment with riluzole and its duration were ineffective in the patients with the lowest ALSFRS-R score at diagnosis i.e. the lowest quartile according to this variable (ALSFRS-R global score < 35). In this group, multivariable analysis showed that factors predicting survival were PDC (HR 0.98, 95% CI 0.97–0.99, $p < 0.001$), weight loss at diagnosis (HR 1.07, 95% CI 1.03–1.11, $p < 0.001$), age at onset I (HR 1.05, 95% CI 1.02–1.08, $p = 0.002$), and diagnostic delay (HR 0.97, 95% CI 0.94–0.99, $p = 0.009$). When we limited the analysis to 25% of patients having the

Table 2 Demographic and clinical factors and survival (from onset/diagnosis to death or tracheostomy) in incident patients of Emilia Romagna, Italy (univariate analysis) ($N = 681$)

Variable	Categories	Survival from onset			Survival from diagnosis		
		HR	95% CI	*p* value	HR	95% CI	*p* value
Sex	Woman	1	(Reference)	0.239	1	(Reference)	0.308
	Man	1.12	0.93–1.37		1.11	0.91–1.35	
Onset	Spinal	1	(Reference)	**< 0.001**	1	(Reference)	**< 0.001**
	Bulbar	1.77	1.45–2.16		1.69	1.39–2.06	
Diagnostic delay	Months	0.41	0.33–0.50	**< 0.001**	0.98	0.97–0.99	**< 0.001**
Age at onset	Years	1.04	1.03–1.05	**< 0.001**	1.04	1.03–1.05	**< 0.001**
Phenotype	Bulbar	1	(Reference)	**< 0.001**	1	(Reference)	**0.015**
	Classic	0.63	0.50–0.78		0.80	0.64–1.00	
	Flail arm/leg	0.47	0.35–0.64		0.70	0.52–0.94	
	Upper MN-predominant	0.32	0.19–0.56		0.52	0.31–0.86	
	Respiratory	1.29	0.77–2.16		1.15	0.65–2.09	
Dementia	No	1	(Reference)	**< 0.001**	1	(Reference)	**< 0.001**
	Yes	2.02	1.50–2.73		2.26	1.67–3.07	
Familial ALS	No	1	(Reference)	0.711	1	(Reference)	0.679
	Yes	1.09	0.68–1.75		1.10	0.69–1.771	
EEC-R	Definite	1	(Reference)	**< 0.001**	1	(Reference)	**0.025**
	Probable	0.71	0.56–0.91		0.89	0.70–1.13	
	Probable lab. supported	0.43	0.31–0.61		0.68	0.49–0.95	
	Possible	0.62	0.47–0.82		0.68	0.51–0.92	
ALSFRS-R at diagnosis	Points	0.99	0.97–1.00	**0.032**	0.98	0.97–0.99	**< 0.001**
ALSFRS-R monthly decline	Points	1.32	1.27–1.38	**< 0.001**	1.24	1.10–1.39	**< 0.001**
ALSFRS-R monthly decline	< 1 point/month	1	(Reference)	**< 0.001**	1	(Reference)	**0.014**
	≥ 1 point/month	3.75	2.97–4.74		1.33	1.06–1.65	
BMI at diagnosis	kg/m^2	0.98	0.95–1.01	0.118	0.97	0.95–1.00	0.086
Weight variation between healthy state and diagnosis	kg	1.04	1.03–1.05	**< 0.001**	1.05	1.04–1.07	**< 0.001**
Riluzole treatment (no/yes)	No	1	(Reference)	0.39	1	(Reference)	0.703
	Yes	1.12	0.86–1.48		1.05	0.80–1.39	
Riluzole treatment duration/disease duration (onset to death/tracheostomy)	< 60%	1	(Reference)	**< 0.001**	NA	NA	NA
	≥ 60%	0.57	0.47–0.69		NA	NA	NA
Riluzole treatment duration/disease duration (onset to death/tracheostomy)	%	0.49	0.37–0.66	**< 0.001**	NA	NA	NA
Riluzole treatment duration/disease duration (diagnosis to death/tracheostomy)	< 75%	NA	NA	NA	1	(Reference)	**0.001**
	≥ 75%	NA	NA	NA	0.69	0.55–0.86	
Riluzole treatment duration/disease duration (diagnosis to death/tracheostomy)	%	NA	NA	NA	0.99	0.99–1.00	**0.010**
Delay from onset to riluzole intake	Months	0.96	0.95–0.97	**< 0.001**	0.98	0.97–0.99	**< 0.001**
Delay from onset to riluzole intake	< 10 months	1	(Reference)	**< 0.001**	1	(Reference)	**< 0.001**
	≥ 10 months	0.43	0.35–0.53		0.64	0.52–0.79	
Non invasive ventilation	No	1	(Reference)	**< 0.001**	1	(Reference)	**< 0.001**
	Yes	1.58	1.30–1.92		1.59	1.318–1.94	
Gastrostomy	No	1	(Reference)	**< 0.001**	1	(Reference)	**< 0.001**
	Yes	1.86	1.53–2.27		1.80	1.47–2.19	
Charlson Comorbidity Index	Points	1.21	1.15–1.29	**< 0.001**	1.24	1.17–1.31	**< 0.001**

Significant results in bold

EEC-R El Escorial Criteria-Revised, *ALSFRS-R* ALS Functional Rating Scale-Revised, *HR* hazard ratio, *CI* confidence interval

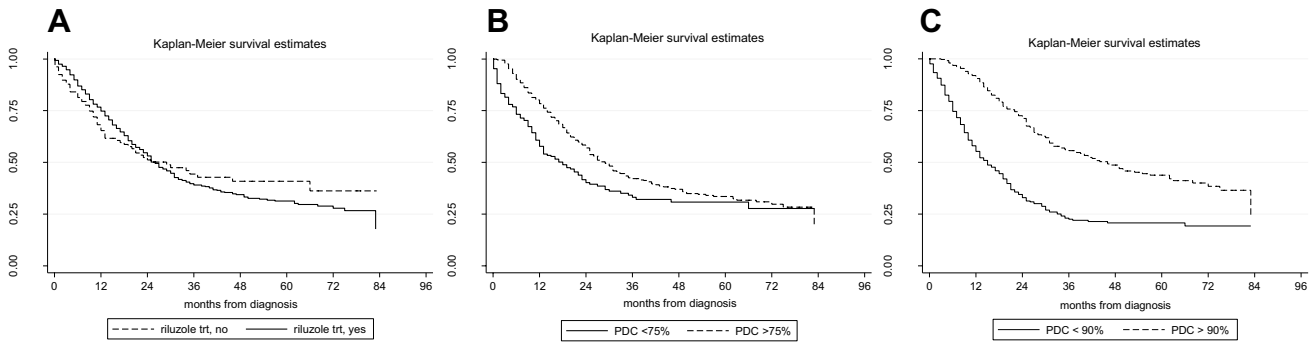

Fig. 1 **a** Tracheostomy-free survival from diagnosis of incident ALS cases in Emilia Romagna Region based on riluzole treatment; **b** tracheostomy-free survival from diagnosis of incident ALS cases in Emilia Romagna Region based on percentage of time of riluzole treatment in relation to disease duration (≥ 75 versus < 75% of time of disease duration); **c** tracheostomy-free survival from diagnosis of incident ALS cases in Emilia Romagna Region based on percentage of time of riluzole treatment in relation to disease duration (≥ 90 versus < 90% of time of disease duration)

Table 3 Clinical characteristics of patients with respect to % of riluzole treatment duration in relation to survival from diagnosis (> 90% versus 1–90% of time of disease duration, versus no treatment) ($N = 681$)

Explanatory variables	No riluzole treatment $N = 106$ *n* (%) *m* [SD]	Riluzole treatment ≤ 90% $N = 228$ *n* (%) *m* [SD]	Riluzole treatment > 90% $N = 347$ *n* (%) *m* [SD]	*p* value
ALS onset (Bulbar)	44 (41.51)	88 (38.59)	106 (30.55)	**0.043**
Sex (man)	51 (48.11)	125 (54.82)	195 (56.19)	0.340
Age at onset	69.85 [± 11.04]	68.33 [± 9.99]	64.27 [± 11.35]	**< 0.001**
Diagnostic delay	15.77 [± 17.98]	12.34 [± 13.30]	12.81 [± 10.85]	0.067
Phenotype				0.187
Bulbar	42 (39.62)	73 (32.02)	97 (27.95)	
Classic	41 (38.68)	100 (43.86)	150 (43.23)	
Flail arm and leg	14 (13.21)	32 (14.03)	73 (21.04)	
UMNp	6 (5.66)	13 (5.70)	19 (5.47)	
Respiratory	3 (2.83)	10 (4.38)	8 (2.30)	
Revised El Escorial criteria				0.562
Definite	27 (28.72)	71 (33.65)	91 (27.74)	
Clinically probable	37 (39.36)	71 (33.65)	108 (32.93)	
Probable lab-supported	12 (12.76)	26 (12.32)	54 (16.46)	
Possible	18 (19.15)	43 (20.38)	75 (22.86)	
Dementia	13 (12.26)	32 (14.03)	19 (5.47)	**0.001**
BMI at diagnosis (Kg/m^2)	24.00 [± 4.71]	24.16 [± 4.04]	24.64 [± 3.75]	0.290
Gastrostomy (yes)	24 (22.64)	78 (34.21)	105 (30.26)	0.101
Non invasive ventilation (yes)	28 (26.41)	77 (33.77)	141(40.63)	**0.019**
Invasive ventilation (yes)	8 (7.55)	49 (21.58)	46 (13.26)	**0.001**
ALSFRS-R monthly decline (points/month)	0.83 [± 0.69]	0.86 [± 0.85]	0.69 [± 0.67]	**0.016**
Days from diagnosis to riluzole intake	NA	78.98 [± 148.67]	17.62 [± 16.04]	**< 0.001**
Days from onset to riluzole intake	NA	442.37 [± 438.73]	401.91 [± 326.05]	**< 0.001**
Absolute duration of Riluzole treatment (days)	NA	361.38 [± 368.55]	1027.25 [± 611.16]	**< 0.001**
Charlson Comorbidity Index				**0.027**
<3	41 (38.68)	88 (38.60)	173 (49.86)	
3–5	59 (55.66)	127 (55.70)	165 (47.55)	
6–10	6 (5.66)	13 (5.70)	9 (2.59)	

Significant results in bold

UMN-p upper motor neuron-predominant, *BMI* body mass index, *ALSFRS-R* ALS Functional Rating Scale-Revised, *SD* standard deviation

Table 4 Independent prognostic factors (multivariable Cox analysis)

Variable	Survival from diagnosis to death or tracheostomy		
	Hazard ratio	95% CI	*p* value
Age at onset (years)	1.04	1.02–1.05	**< 0.001**
Dementia (yes/no)	1.56	1.05–2.32	**0.027**
Revised El Escorial Criteria classification	0.88	0.78–0.98	**0.021**
Weight variation between healthy state and diagnosis (kg)	1.05	1.03–1.07	**< 0.001**
Gastrostomy (yes/no)	1.46	1.14–1.88	**0.003**
Non invasive ventilation (yes/no)	1.43	1.12–1.82	**0.004**
Time from onset to diagnosis (months)	0.98	0.97–0.99	**0.004**
Riluzole treatment duration/disease duration (diagnosis to death/tracheostomy) (%)	0.98	0.98–0.99	**< 0.001**

Significant results in bold

ALSFRS-R ALS Functional Rating Scale-Revised

highest ALSFRS-R monthly decline (> 1.3 points/month), factors predicting survival were age at onset (HR 1.04, 95% CI 1.01–1.06, $p = 0.007$), dementia (HR 3.47, 95% CI 1.63–7.35, $p = 0.015$), weight loss at diagnosis (HR 1.06, 95% CI 1.01–1.10, $p = 0.009$), and gastrostomy (HR 2.47, 95% CI 1.35–4.50, $p = 0.003$).

Discussion

Riluzole is the only drug approved for the treatment of ALS by the European Medical Agency in Europe. After > 20 years from its discovery [28], a number of concerns about its therapeutic effects persist: the relatively unknown mechanism of action, the modest prolongation of survival (on average a few months), with concerns about cost effectiveness, the lack of benefit on some secondary measures of efficacy, and some controversies related to the drug efficacy in population-based studies and clinical practice [11]. Otherwise ALS has no cure, currently, and riluzole has a satisfactory safety profile, leading to recommend the drug to slow disease progression for patients with ALS [29].

Because ALS treatment (including all therapeutic interventions and drugs) is completely covered by INHS and considering that it has a relatively low cost, the percentage of ERR residents with ALS using riluzole is very high. In our study, 84% of patients received riluzole treatment for at least 1 month, a fraction that is higher than that reported in other studies carried on in Europe [30], USA [31] and Asia [17] probably due to the different socio-economic context. Given the widespread use of this drug in our country it is therefore important to determine whether and to what extent it is effective in the real-world ALS population, to improve patient counseling and the design of clinical trials.

In previous non-RCTs studies, the effect of riluzole on ALS survival was controversial, with some studies showing no gain of survival [32], and others showing an effect ranging from 6 to 12 months [3, 5, 12], a few studies documenting an effect for the first 6 months of treatment only, with a 15% reduction in mortality at 6 months [16]. Some investigators suggested that riluzole could only slow down motor neuron degeneration and it is more effective in early-stage patients [15, 33]; others suggest a possible beneficial effect only after long-term use of the drug [17]. This different effectiveness may be attributed to the different target populations (registry populations or referral cohorts). Moreover, in most studies riluzole use was analyzed as a categorical factor (use versus non use) [3, 5, 12, 15, 34–37], whereas only two recent studies considered the cumulative defined daily dose [16, 17] coming to opposite conclusions: a limited effect for the first 6 months in Austrian ALS patients [16], a possible effect due to long-term use in a Chinese cohort [17].

In our study, analysing survival in dependence of riluzole treatment showed a beneficial effect of the drug for the initial months of therapy only, because at 24 months after diagnosis the survival curves of riluzole-treated and untreated patients crossed with untreated patients showing an apparently better survival thereafter (Fig. 1a), as already reported [12, 16]. Although patients who took riluzole did not survive significantly more than patients who did not, prognosis of patients who took riluzole for more time (in relation to disease duration) was better. Our apparently inconsistent results, in agreement with a recent study [17], may reflect the hypothesis that drug efficacy can be confirmed only after long-term treatment. Moreover, patients enrolled in riluzole RCTs were perhaps more motivated to carry on the study treatment until the study end (hoping in a new therapy for ALS) than current real-world patients who may discontinue the drug because they saw themselves worsening or were aware of its limited effectiveness [37].

Nevertheless, since the duration of treatment with riluzole would be longer in patients living longer, this might be a

potential confounder of the dose-dependent effect of riluzole on survival, and the lack of difference between patients treated with riluzole and patients who were untreated may be due to the fact that more severely affected individuals do not take it or discontinue its intake because of a rapidly worsening condition. For this reason, we also adjusted our analysis for immortal time bias, but results were similar, suggesting that this potential bias did not substantially affect our findings.

We also eliminated from our sample patients died within 6 months from diagnosis, but the results were unchanged.

To test the hypothesis of a lack of efficacy of riluzole treatment in more severely affected patients at diagnosis, we also evaluated patients with a low ALSFRS-R score at diagnosis: in that group, the extent of treatment duration independently influenced survival.

Conversely, our analyses in patients who had a high ALSFRS-R monthly decline showed that in this small sample PDC did not influenced survival: in this rapidly progressive population riluzole, whenever given, has no effect on survival. As efficacy of riluzole seemed related to the duration of its intake, we hypothesized that the earlier it is given to patients the longer the patients will survive, as already suggested [38]. Therefore we studied the relationship between delay from disease onset and start of riluzole, and survival. We found that the earlier the patients took riluzole, the worse the prognosis, probably because of the well known negative prognostic role of a short diagnostic delay in ALS [3].

On the contrary, we identified the characteristics of patients who took riluzole for a longer period of time: these patients were younger, more frequently with spinal onset, without cognitive impairment, and with a lower CCI. Younger age and spinal onset, and absence of dementia, are well known factors related to a better survival [3]. These patients may be more compliant and more prone to comply with medical counseling and pharmacological treatment: we found that patients treated for a longer time with riluzole underwent more frequently NIV and IV, suggesting a general attitude to accept all therapeutic interventions which may influence survival [3]. These findings are similar to what reported by others [17].

The study has strengths and limitations. The major strength is its population-based design. In addition, we are fairly confident that case ascertainment in the study area was almost complete, as shown by the incidence rate of the disease [18].

A limitation of this study is the observational design, compared to the experimental context of RCTs. However, observational studies particularly if population-based, have the advantage of a longer follow-up than the RCTs, and also include participants who approximate real world population [39]. This also means that, since age at onset and disease severity are more heterogeneous in a population-based registry study than in RCTs [28, 40, 41], older and more severely affected patients were included in our study in comparison with RCTs. A recent Cochrane review [11] showed that analyzing pooled riluzole RCTs data, the effects of riluzole on survival were significant when the homogeneous group of participants in the first two trials were considered [28, 40], but when all three trials were analyzed [41], there was a high heterogeneity due to the addition of more seriously affected and older patients, and the combined treatment effect fell just short of significance [11].

Another possible limitation of the study is the fact that the vast majority of ALS patients received Riluzole [23]. In addition, we did not collect data on advance directives of patients in relation to nutritional and respiratory supports which can influence survival and we do not have data about reasons for riluzole treatment discontinuations or related side effects. Furthermore, we had genetic status only in a limited number of cases and this information would have impact survival especially in relation to C9orf72 hexanucleotide repeat expansion [42].

Finally, as discussed before, a specific and important weakness of our study is that, since the duration of treatment would be greater in patients living longer, this may be a potential confounder of the dose-dependent effect of riluzole on survival.

In summary, in our study patients who received riluzole for a proportionally longer period of time survived longer, thus suggesting that long-tem use of riluzole may prolong survival. Younger and cognitively normal ALS patients and patients with a spinal onset, a lower CCI, and lower decline of ALSFRS-R were more likely to use riluzole for a longer time and survived longer. However, further studies are needed to verify if long-tem use of riluzole prolongs survival.

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Compliance with ethical standards

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