Influence of cigarette smoking on ALS outcome: A population-based study

Influence of smoke and vascular risk factors on ALS outcome: a population-based study

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Abstract

Objective. To assess the prognostic influence of pre-morbid smoking habits and vascular risk profile on ALS phenotype and outcome in a population-based cohort of Italian patients.

Methods. A total of 650 ALS patients from the Piemonte/Valle d'Aosta Register for ALS, incident in the 2007-2011 period, were recruited. Information about premorbid smoke habits and chronic obstructive pulmonary disease (COPD) were collected at the time of diagnosis. A vascular risk profile of patients was calculated according to the Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice (JBS2).

Results. Current smokers had a significantly shorter median survival (1.9 years, interquartile range [IQR], 1.2-3.4) compared to former (2.3, IQR 1.5-4.2) and never smokers (2.7 years, IQR 1.8-4.6) (p=0.001). Also COPD adversely influenced patients' prognosis. Patients with a lower vascular risk profile had a better prognosis than those with intermediate and higher risk profiles. However, only smoking habits and CODP were retained in Cox multivariable model.

Conclusions. This study has demonstrated in a large population-based cohort of ALS patients that smoking is an independent negative prognostic factor for survival, with a dose-response gradient. Its effect is not related to the presence of COPD or to respiratory status at time of diagnosis. The understanding of the mechanisms, either genetic or epigenetic, through which exogenous factors influence disease phenotype is of major importance toward a more focused approach to cure ALS.

Keywords: Amyotrophic Lateral Sclerosis; Smoking; Vascular risk factors; Prognosis

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a fatal degenerative disorder of upper and lower motor neurons, in about 50% of cases ALS is also associated with cognitive impairment ranging from frontotemporal dementia to milder forms of executive or dysexecutive impairment.[1] In most cases ALS appears sporadically in the population; only about 10% of patients have a positive family history for ALS or frontotemporal dementia.[2]

ALS phenotype is quite heterogeneous for age and type of onset, and survival. Several factors have been found to influence ALS phenotype, including the genetic background, age and gender, pre-morbid diseases, life habits, and physical activity, but data are sparse and contradictory.[3,4] Some attention has been also devoted to the influence of pre-morbid vascular risk factors and cigarette smoking on ALS prognosis,[5-8] but no comprehensive study of these factor has been performed.

The aim of our study was to assess the prognostic influence of pre-morbid smoking habits and vascular risk profile on ALS phenotype and outcome in a population-based cohort of Italian patients.

Methods. The study design and the characteristics of the cohort have been reported in a previous paper.[9] In brief, all patients diagnosed with ALS during the period January 1st 2007 to December 31st 2011 (n=712) were eligible to be enrolled in the study. The patients were identified through the Piemonte and Valle d'Aosta Register for ALS[10] and diagnosed according to evised El Escorial diagnostic criteria.[11] Disease severity was assessed with the ALSFRS revised (ALSFRS-R) scale.[12] The decline rate for ALSFRS-R score was calculated as the mean monthly number of points loss from symptom onset to the time of diagnosis, calculated in months.

At time of diagnosis, for each patient we collected information about smoke habits and chronic obstructive pulmonary disease (COPD). Patients' smoking status was defined as current smoker (patient who was still smoking at the time of symptom onset), former smoker (patient who quitted

smoking before the onset of ALS) or never smoker. COPD was classified according to the GOLD guidelines.[13] Moreover, serum lipid profile (serum cholesterol, triglycerides, HDL, and LDL) and current and healthy BMI were also collected.[9,14] Pulmonary function tests (in particular forced vital capacity [FVC] percent of predicted and forced expiratory volume in the 1st second [FEV1]) were performed and annotated. FEV1/FVC ratio was calculated for diagnosing the severity of COPD.[14]

We also classified patients according to their overall vascular risk, calculated on basis of the Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice (JBS2),[15] with the University of Edinburgh Cardiovascular Risk Calculator (http://cvrisk.mvm.ed.ac.uk/index.htm). We used the risk based on the Framingham equation, namely the Cardiovascular Disease (CVD) risk. A time frame of 10 years has been utilized. Briefly, CVD equation for the calculation of cardiovascular risk is based on patients' gender, age, current smoking status (smoker/non-smoker), diabetes mellitus, systolic pressure, total cholesterol (mmol/L), and HDL cholesterol (mmol/L). Patients were subdivided in 3 groups according to their CVD 10-years risk, low risk (<10%), intermediate risk (10-19.9%) and high risk (≥20%) (Gray et al, 2014).

Genetic analysis. Genetic assessment was performed in 526 cases (80.9%).[16] All the coding exons and 50bp of the flanking intron-exon boundaries of *SOD1*, of exon 6 of *TARDBP*, and of exons 14 and 15 of *FUS* and exons 5, 9, 12 and 14 of *OPTN* and the only exon of *ANG* have been PCR amplified, sequenced using the Big-Dye Terminator v3.1 sequencing kit (Applied Biosystems Inc.), and run on an ABIPrism 3130 genetic analyzer. These exons were selected as the vast majority of known pathogenic variants are known to lie within these mutational hotspots.[2] A repeat-primed PCR assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9ORF72*.[17]

Statistical methods. Comparisons between means were made with Student's t-test or analysis of variance (ANOVA); comparison between categorical variables was made with $\chi 2$ test. All tests

were two-tailed. Levene's test was used to confirm the equality of variances. Since the distribution of the decline rate of ALSFRS-R score and its subscores did not follow a normal distribution, the correlation between this decline rate and smoking status was assessed with the Kruskall-Wallis test. Survival was calculated from onset to death, tracheostomy or censoring date (December 31st, 2015), using the Kaplan-Meier method, and compared with the log-rank test; when more than two ordinal strata were assessed the linear trend for factor level test was used. No patients were lost to follow-up. Multivariable analysis was performed with the Cox proportional hazards model (stepwise backward) with a retention criterion of $p<0.1$. A p level <0.05 was considered significant. Statistical analyses were carried out using the SPSS 22.0 statistical package (SPSS, Chicago, IL, USA).

Standard Protocol Approvals, Registrations, and Patient Consents. The study design was approved by the institutional Ethical Committees of the participating centers. Patients signed a written informed consent.

Results. Of the 712 incident patients, 650 (91.3%) were included in the study. The remaining 62 patients, not included because of incomplete data, did not differ as regards to any demographic or clinical variable with included patients (data not shown). The demographic and clinical characteristics of included patients, as well as of their pre-morbid smoking status and cardiovascular risk levels are reported in Table 1.

Smoking. In the present series, 121 patients (18.6%) were current smokers at the time of ALS onset, 182 (28.0%) were former smokers and 347 (53.4%) never smoked. Patients who were currently smoking at ALS onset had a younger age at onset (64.9 years, SD 11.6) than both former (67.6 years, SD 9.7) and never smokers (66.3 years, SD 10.7) ($p=0.07$). No differences in clinical presentation were found, although never smokers had more frequently a bulbar onset than all other categories (bulbar onset: never smokers, 34.9%, former smokers, 27.5%; current smokers, 26.4%; $p=0.10$); this difference was almost entirely due to the predominance of women with bulbar onset in the never smoker group. Current smokers had a significantly shorter median survival (1.9 years,

interquartile range [IQR], 1.2-3.4) compared to former (2.3, IQR 1.5-4.2) and never smokers (2.7 years, IQR 1.8-4.6) (p=0.001) (Figure 1). This difference was present both in men and women and was not modified stratifying by age at onset, type of onset and *C9ORF72* status (data not shown). Stratifying by FVC, FEV1 or FEV1/FVC ratio the negative effect of smoke on survival was still present (data not shown). Also, stratification for COPD did not modify the effect of pre-morbid smoking habits on survival (data not shown). Finally, smoking status was significantly correlated to the mean monthly decline of ALSFRS-R (p=0.033) and its gross motor (p=0.05) and respiratory (p=0.006) subscores; it was not correlated with BMI at diagnosis (p=0.233) (Kruskall-Wallis test).

COPD. A total of 44 patients (13 current smokers, 10.7%; 22 former smokers, 12.1%; and 9 never smokers, 2.6%) were affected by COPD at the time of ALS symptom onset. Patients with COPD had a similar age at onset as patients without COPD (68.1 [SD 10.2] vs. 66.3 [SD 10.6]; p=0.27). The median survival time of patients with COPD was significantly lower than that of patient without COPD (COPD, 1.7 years, IQR 0.9-2.5; non-COPD 2.6, IQR 1.5-4.3) (p=0.01) (Figure 2).

Vascular risk profile. Patients were subdivided according to their CVD-10 years risk profile. The frequency of different risk profiles was significantly different in the two genders (E-Table 1). Patients with a lower risk profile had a better prognosis than those with intermediate and higher risk profiles (men: low risk profile, median survival time 2.7 years, IQR 1.7-5.0; intermediate, 1.9 years, IQR 1.2-3.1; high 1.5 years, IQR 0.9-3.4; p=0.0001; women: low risk profile, 2.7 years, IQR 1.8-4.5; intermediate 1.9 years, IQR 1.2-2.9; high 0.8 years, IQR 0.7-1.3; p=0.001). However, when data were stratified by 10-years age groups at onset the effect of vascular risk profile disappeared in both genders.

Cox multivariable analysis. Cox model confirms that smoking status is an independent negative prognostic factor with an increased hazard ratio for current smokers vs. never smokers of 1.65 (95% c.i. 1.31-2.07, p=0.0001). COPD was also retained as an independent negative prognostic factor (hazard ratio 1.46, 95% c.i 1.06-2.01; p=0.02). The full model, with the list of the variables

included, is reported in Table 2. We performed an exploratory analysis including only the 526 patients for whom genetic analysis had been performed in order to verify the effect of the inclusion of *C9ORF72* expansion. In this model, smoking status remained significant, while *C9ORF72* resulted to be a significant modifier of survival (odds ratio 1.66, 95% c.i. 1.16-2.39, p=0.002). Lastly, in order to verify if the effect of pre-morbid smoking habits was mediated by respiratory status at time of diagnosis, a third model was performed including the 568 patients (87.4% of the whole cohort) for whom FVC at diagnosis was available; again, smoking status was retained in the model, while COPD was not retained. The complete exploratory models are reported in the E-Tables 2 and 3.

Discussion

We have assessed the effect of smoking and premorbid vascular risk factors on ALS phenotype and prognosis in a population-based cohort of patients. Premorbid smoke habits resulted a strong independent modifier of prognosis, with a decreasing gradient, current smokers having a reduction of overall survival of 10 months compared to never smokers, and former smokers having an intermediate survival. Also COPD resulted to be an independent prognostic factor. Patients with a high risk vascular profile, calculated according to the Framingham equations[15] had a worse prognosis than those with a low risk vascular profile in univariate analysis; however, this effect was not confirmed by the Cox multivariable analysis, since it was mostly due to the older age of patients in the highest cardiovascular risk group.

The role of smoking on ALS pathogenesis and survival is intriguing. Most published studies indicate that it increases the risk of developing ALS by 1.3-1.5-folds,[18,19] making smoking the only established environmental risk factor for ALS.[20] However, few studies have assessed its influence on ALS prognosis, with some inconsistencies.[7,21,22] In our population-based study, smoking habits displayed two effects on ALS phenotype: patients currently smoking at disease onset had a younger age at onset and a shorter survival. The effect of smoke on survival was independent from other

prognostic factors, including age, gender, site of onset, attending an ALS centre, El Escorial classification at diagnosis, *C9ORF72* status, COPD and respiratory function as measured by FVC, FEV1 and FEV1/FVC ratio.

The mechanisms at the basis of the biological effect of cigarette smoking on ALS are still uncertain. Several hypothesis have been raised, including: (a) the inhibition of paraoxonase, a family of enzymes contributing to reduce the damage of oxidative stress; (b) the inhibition of vascular endothelial growth factor signalling pathway; (c) a chemical effect of one of the component of smoke, formaldehyde;[23] (d) increased levels of lipid hydroperoxides, which are markers of oxidative stress, in serum and cerebrospinal fluid.[24] Smoking could also act at epigenetic level, through an aberrant methylation of DNA.[25] Although methylation effects of cigarette smoking are quite specific and reversible after smoking cessation, specific genes remain differentially methylated even 20 years after cessation,[26] explaining the persistent negative effect on ALS prognosis observed in former smokers in our series. These epigenetic affects could be also related to smoking habits of patients' parents, including an in utero exposure to maternal smoking.[27]

Among neurodegenerative disorders, smoking has been found to be a strong protective factor for Parkinson disease (PD).[28] While the mechanisms of smoking protective effects in PD remain to be fully elucidated, it appears that smoking acts differently on the neurodegenerative process ALS and PD.

COPD, a typical long term complication of cigarette smoking, has been also found to be an independent negative prognostic factor in ALS. However, its inclusion in the model did not decrease the effect of smoking habits on ALS outcome. The prevalence of COPD in our population is in keeping with recent epidemiological studies in the Italian population.[29,30] In our cohort COPD was also found in never smokers patients, though in a smaller percentage than in current and former smokers.[31]

Vascular risk profile, in particular lipid status, has been studied in ALS in several papers, but never systematically and with conflicting findings.[5,6,8,9,32-38] In the present population-based cohort, we utilized a validated algorithm which predicts the future vascular risk at a given time point;[39] we chose ten years, but results with a shorter timeframe were similar. This algorithm keeps into account the most relevant vascular risk factors, including age, gender, arterial hypertension, type 2 diabetes, cholesterol, HDL cholesterol, and cigarette smoking, derived from the Framingham study algorithm.[15] Our aim was to assess the interaction of the most important vascular risk factors instead of analyzing each one separately, as done in previous papers.[5,6,9,32-38] A high risk vascular profile was significantly more frequent in men, in keeping with previous studies on the general population.[39,40] We found that patients with a comparative high risk vascular profile had a worse prognosis than those with intermediate and low risk groups, but this was not confirmed by Cox multivariable analysis, probably because vascular risk profile is largely driven by patients' age, one of the strongest determinants of ALS prognosis *per se*.

A strength of our cohort is that it is highly representative of the general ALS population, since it includes ~90% of the patients who were diagnosed in the study period in Piemonte/Valle d'Aosta, and captured cases did not differ for any significant demographic or clinical parameters from non-captured patients.[9] Moreover, data were systematically collected at the time of diagnosis, using the same form during the whole study period.

Our population-based study on the influence of vascular risk factors on ALS phenotype found that smoking is a strong negative modifier of ALS prognosis, independent from age, gender and other known modifiers, including respiratory function, COPD and *C9ORF72* status. According to these findings, neurologists should consider to recommend to their ALS patients the cessation of smoking as a measure to significantly improve their outcome.

This study indicates that environmental factors and personal habits represent not only risk factors for ALS onset but can also influence its phenotype and prognosis. The discovering of the

mechanisms, either genetic or epigenetic, through which exogenous factors influence disease phenotype is of major importance toward a more focused approach to cure of ALS.

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Table 1. Demographic and clinical characteristics of patients

Factor	
Gender (female)	290 (44.6%)
Mean age at onset (years, SD)	66.4 (10.6)
Diagnostic delay (months, SD)	11.3 (10.9)
Site of onset (bulbar)	203 (31.2%)
El Escorial classification at diagnosis	Possible: 209 (32.1%) Probable Laboratory Supported: 69 (10.6%) Probable: 137 (21.1%) Definite: 235 (36.2%)
Mean ALSFRS-R score at diagnosis (SD)	40.2 (6.5)
C9orf72 positive §	33 (6.1%)
Mean BMI at diagnosis (SD)	24.3 (4.3)
Mean FVC at diagnosis (SD) *	82.6 (26.8)
Chronic obstructive pulmonary disease	44 (6.8%)
Smoking	Never 347 (53.4%) Former 182 (28.0%) Current 121 (18.6%)
Cardiovascular risk profile	Low: 462 (71.1%) Intermediate: 141 (21.7%) High: 47 (7.2%)

§ 526 patients; * 568 patients

Table 2. Multivariable model including all cases

Factor	**Levels**	**Hazard ratio (95% c.i.)**	**p value**
Age	18-49	1	
	50-59	1.44 (0.92-2.19)	0.092
	60-69	2.05 (1.38-3.03)	0.0001
	70-79	2.44 (1.66-3.68)	0.0001
	80+	3.06 (1.91-4.89)	0.0001
El Escorial	Possible	1	
	Probable Laboratory Supported	1.24 (0.84-1.83)	0.286
	Probable	1.91 (1.31-2.78)	0.001
	Definite	2.29 (1.57-3.34)	0.0001
Site of onset	Spinal	1	
	Bulbar	1.49 (1.23-1.81)	0.0001
Diagnostic delay	> 1 year	1	
	≤ 1 year	1.74 (1.44-2.10)	0.0001
ALS multidisciplinary center	Yes	1	
	No	1.35 (1.07-1.69)	0.001
Smoking status	Never	1	

	Former	1.25 (1.08-1.51)	0.01
	Current	1.64 (1.31-2-07)	0.001
ALSFRS-R decline	<0.75 points /month	1	
	≥0.75 points /month	1.32 (1.06-1.64)	0.012
COPD	No	1	
	Yes	1.46 (1.06-2.01)	0.021

Variables included in the model: age at onset (18-49, 50-59, 60-69, 70-79, 80+); gender (male, female); ALSFRS-R decline (<0.75 points/month, ≥0.75 points/months), BMI (underweight <18.5; normal weight, 18.5–24.99; overweight, 25–29.99; obese class I, II and II, ≥30), CVD 10-years risk (low risk, <10%, intermediate risk, 10-19.9%, high risk, ≥20%), diagnostic delay (>1 year; ≤ 1 year), smoking habits (never, former, current); El Escorial (possible, probable laboratory supported, probable, definite); ALS multidisciplinary centre (yes, no): Chronic obstructive pulmonary disease (COPD) (no, yes).

Figure legends

Figure 1. Kaplan-Meier curves by smoking status at time of ALS onset. The blue line represents never smokers, the green line former smokers and the red line current smokers.; p=0.001, linear trend.

Figure 2. Kaplan-Meier curves by co-morbid COPD at time of ALS onset. The blue line represents patients with COPD, the green line patients without COPD; p=0.01.