RESEARCH PAPER

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

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ABSTRACT

Objective To assess the prognostic influence of premorbid smoking habits and vascular risk profile on amyotrophic lateral sclerosis (ALS) phenotype and outcome in a population-based cohort of Italian patients.

Methods A total of 650 patients with ALS from the Piemonte/Valle d’Aosta Register for ALS, incident in the 2007–2011 period, were recruited. Information about premorbid cigarette smoking habits and chronic obstructive pulmonary disease (COPD) were collected at the time of diagnosis.

Results Current smokers had a significantly shorter median survival (1.9 years, IQR 1.2–3.4) compared with former (2.3 years, 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. Both smoking habits and COPD were retained in Cox multivariable model.

Conclusions This study has demonstrated in a large population-based cohort of patients with ALS that cigarette 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 towards a more focused approach to cure ALS.

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, premorbid diseases, life habits and physical activity, but data are sparse and contradictory.3,4 Some attention has been also devoted to the influence of premorbid vascular risk factors and cigarette smoking on ALS prognosis,5–8 but no comprehensive study of these factors have been performed.

The aim of our study was to assess the prognostic influence of premorbid cigarette smoking habits 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 1 January 2007 to 31 December 2011 (n=712) were eligible to be enrolled in the study. The patients were identified through the Piemonte and Valle d’Aosta Register for ALS10 and diagnosed according to revised El Escorial diagnostic criteria.11 Disease severity was assessed with the ALS Functional Rating Scale 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 their cigarette smoking 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 Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines.13 Pulmonary function tests (in particular forced vital capacity (FVC) per cent of predicted and forced expiratory volume in the 1 s (FEV1) were performed and annotated. FEV1/FVC ratio was calculated for diagnosing the severity of COPD.

Genetic analysis

Genetic assessment was performed in 526 cases (80.9%).14 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 BigDye Terminator v3.1 sequencing kit (Applied Biosystems), and run on an ABI Prism 3130 genetic analyser. These exons were selected as a 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

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the presence of the GGGGCC hexanucleotide expansion in the first intron of C9orf72.15

Statistical methods
Comparisons between means were made with Student’s t-test or analysis of variance (ANOVA); comparison between categorical variables was made with χ² 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 Kruskal-Wallis test. Survival was calculated from onset to death, tracheostomy or censoring date (31 December 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 V22.0 statistical package (SPSS, Chicago, Illinois, USA).

Standard protocol approvals, registrations and patient consents
The study design was approved by the institutional Ethical Committees of the participating centres. Patients signed a written informed consent.

RESULTS
Of the 712 incident patients, 650 (91.3%) were included in the study. The remaining 62 patients, were not included because of incomplete data, did not differ with regard 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 premorbid smoking status and cardiovascular risk levels are reported in table 1.

Cigarette 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, IQR 1.2–3.4) compared with former (2.3 years, 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 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 premorbid 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 body mass index (BMI) at diagnosis (p=0.233) (Kruskal-Wallis test).

Chronic obstructive pulmonary disease
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 years (SD 10.2) vs 66.3 years (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 years, IQR 1.5–4.3) (p=0.01) (figure 2).

Cox multivariable analysis
Cox model confirms that smoking status is an independent negative prognostic factor with an increased HR for current smokers versus never smokers of 1.65 (95% CI 1.31 to 2.07,

Table 1 Demographic and clinical characteristics of patients

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female)</td>
<td>290</td>
<td>(44.6%)</td>
</tr>
<tr>
<td>Mean age at onset (years, SD)</td>
<td>66.4</td>
<td>(10.6)</td>
</tr>
<tr>
<td>Mean diagnostic delay (months, SD)</td>
<td>11.3</td>
<td>(10.9)</td>
</tr>
<tr>
<td>Site of onset (bulbar)</td>
<td>203</td>
<td>(31.2)</td>
</tr>
<tr>
<td>El Escorial classification at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possible</td>
<td>209</td>
<td>(32.1%)</td>
</tr>
<tr>
<td>Probable laboratory supported</td>
<td>69</td>
<td>(10.6%)</td>
</tr>
<tr>
<td>Probable</td>
<td>137</td>
<td>(21.1%)</td>
</tr>
<tr>
<td>Definite</td>
<td>235</td>
<td>(36.2%)</td>
</tr>
<tr>
<td>Mean ALSFRS-R score at diagnosis (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C9orf72 positive*</td>
<td>33</td>
<td>(6.1%)</td>
</tr>
<tr>
<td>Mean BMI at diagnosis (SD)</td>
<td>24.3</td>
<td>(4.3)</td>
</tr>
<tr>
<td>Mean FVC at diagnosis (SD)*</td>
<td>82.6</td>
<td>(26.8)</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>347</td>
<td>(53.4%)</td>
</tr>
<tr>
<td>Former</td>
<td>182</td>
<td>(28.0%)</td>
</tr>
<tr>
<td>Current</td>
<td>121</td>
<td>(18.6%)</td>
</tr>
</tbody>
</table>

* Five hundred and twenty-six patients. † Five hundred and sixty-eight patients.

ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale revised; BMI, body mass index; FVC, forced vital capacity.

Figure 1 Kaplan-Meier curves by smoking status at time of amyotrophic lateral sclerosis onset. The blue line represents never smokers, the green line former smokers and the red line current smokers; p=0.001, linear trend.
p=0.0001). COPD was also retained as an independent negative prognostic factor (HR 1.46, 95% CI 1.06 to 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 was found to be a significant modifier of survival (OR 1.66, 95% CI 1.16 to 2.39, p=0.002). Finally, in order to verify if the effect of premorbid 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 1 and 2.

**DISCUSSION**

We have assessed the effect of smoking on ALS phenotype and prognosis in a population-based cohort of patients. Premorbid smoking habits resulted in a strong independent modifier of prognosis, with a decreasing gradient, current smokers having a reduction of overall survival of 10 months compared with never smokers, and former smokers having an intermediate survival. Also COPD resulted to be an independent prognostic factor.

The role of cigarette 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, making smoking the only established environmental risk factor for ALS. However, few studies have assessed its influence on ALS prognosis, with some inconsistencies. 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 smoking 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 on 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, and (d) increased levels of lipid hydroperoxides, which are markers of oxidative stress, in serum and cerebrospinal fluid. Smoking could also act at epigenetic level, through an aberrant methylation of DNA. Although methylation effects of cigarette smoking are quite specific and reversible after smoking cessation, specific genes remain differentially methylated even 20 years after cessation, explaining the persistent negative effect on ALS prognosis observed in former smokers in our series.

Among neurodegenerative disorders, smoking has been found to be a strong protective factor for Parkinson disease (PD). 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 prevalence of COPD in our population is in keeping with recent augmented and reversible after smoking cessation, specific genes remain differentially methylated even 20 years after cessation, explaining the persistent negative effect on ALS prognosis observed in former smokers in our series.

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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
Neurodegeneration

epidemiological studies in the Italian population.26 27 In our cohort COPD was also found in never-smokers patients, though in a smaller percentage than in current and former smokers.28

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.29 Moreover, data were systematically collected at the time of diagnosis, using the same form during the whole study period.

Our population-based study found that cigarette 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 patients with ALS the cessation of smoking as a measure to significantly improve their outcome.

This study indicates that environmental factors and personal habits represent risk factors for ALS onset and 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 towards a more focused approach to care of ALS.

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Contributors
ACal, GM, LM and ACH contributed to the study concept and design. ACan, DB, PC, LS, SC, MD, EBe, SC, AI, UM, CM, KM, EBo and FP were involved in the acquisition of data. ACal, MC, CM, EBo, FP, GM, LM and ACH conducted the analysis and interpretation of data. ACal, GM and ACH were responsible for drafting of the manuscript. ACan, ACal, DB, PC, LS, SC, MD, EBe, SC, AI, UM, CM, KM, EBo, FP, GM, LM and ACH performed the critical revision of the manuscript for important intellectual content. ACH obtained funding. DB, EBe, UM and KM provided administrative, technical and material support. ACal, GM, LM and ACH were involved in the study supervision. ACH had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the analysis. All authors have approved the submitted version of the manuscript. Members of the Piemonte and Valle d’Aosta Register for ALS (PARALS) are listed in an online supplementary appendix.

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Disclaimer
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Competing interests
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Patient consent
Obtained.

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Ethical Committee ‘Città della Salute e della Scienza’, Torino.

Provenance and peer review
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Data sharing statement
Additional unpublished data from the study can be obtained from the corresponding author.

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