Impact of occupational categories on the incidence of amyotrophic lateral sclerosis in Sardinia Island, Italy

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Impact of Activity At Work on the Incidence of Amyotrohic Lateral Sclerosis in Sardinia Island, Italy

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Abstract

Background. Activity at work is one of the potential risk factors for amyotrophic lateral sclerosis (ALS) for which previous controlled studies produced inconsistent results.

Objective. To assess the impact of several groups of activity at work on ALS incidence

Methods. ALS patients from the southern part of Sardinia who had onset during 2012–2021 and fulfilled El Escorial revised diagnostic criteria were included. The risk of ALS was estimated in relation to the occupation held in 2011, as obtained from the 2011 Census that classified working activities in ten groups. Each occupational group was compared with a reference category represented by all other occupations, and rate ratio were calculated. Additive interaction between activity at work and age at ALS onset/sex on ALS incidence was calculated.

Results and Conclusions. Employment in agriculture/breeding and in the armed forces were significantly associated with increasing ALS risk. None of the other assessed occupation groups was associated with change in the risk of ALS. Geographic analysis indicated that the effect of agriculture/breeding was particularly evident in the areas of higher risk for the general population. By contrast, an inverse pattern of spatial risk was associated with armed forces activity at work. The increased risk of ALS associated to agriculture/breeding was more evident in older people. No significant interaction was detected between working in the armed forces and older age/sex. The significant interaction between agriculture/breeding and age suggests that the mechanisms leading to ALS are complex and involve several factors.

Introduction

Amyotrophic lateral sclerosis (ALS) fits with the wide range of complex disorders influenced by both genetic and environmental factors (1). Over the last two decades, a great deal of new knowledge has leaded to the identification of several mutations associated to ALS,(2) whereas the systematic evidence of the association between environmental exposures (like lifestyle, associated medical conditions, activity at work, viral infections, electromagnetic fields, toxic substances) and ALS, besides being very limited, is inadequate or insufficient (1,3). To date, the only established non genetic risk factors for ALS are older age and male sex (1,3).

Attempts to identify non-genetic risk factors were mostly made by case-control studies,(3,4) a design often used in rare diseases that can be nevertheless biased by several factors, including defects in the collection of cases (as only selected cases, not representative of the general population, are included) and controls (that may not be drawn from the same frame as cases, or be equally motivated to provide accurate information) or incomplete information from clinical records. When searching for putative environmental causes of a disease, an alternative approach may be to study the incidence of the disease in those exposed to an environmental factor. An increased incidence of the disease in the exposed population would support the association between disease and exposure. Under this design, spatial epidemiology could also give important clues.(5)

Activity at work is one of the potential risk factors for ALS that received attention in the past years and for which previous controlled studies produced inconsistent and ambiguous results.(6,7) In this study, we assessed the impact of several groups of activity at work on the incidence of ALS using a 10-years population-based cohort of ALS incident cases from Southern Sardinia.

Methods

The study area covered 9768 km^2 (about one half of the whole island), hosted nearly two thirds of the whole Sardinian population , and included three administrative subdivisions, Cagliari, South Sardinia and Oristano provinces. In this area, a recent incidence study yielded an average crude annual incidence rate of 3.6 (95% CI, 3.2-4.1), with incidence being higher in men and in older people.(8) Geographic analysis revealed areas at different risk of ALS, with Oristano and South Sardinia provinces yielding higher incidence rates than Cagliari province (incidence rates per100,000 person-years: 3.9 [95% CI, 3.3–4.5] vs. 4.1 [95% CI, 3.3–5.3] vs. 2.4 [95% CI, 2.0–2.8]).(8)

The study population consisted of all people aged 15 years or more who resided in the study area and was recorded as active workers by the 2011 Italian census (www. istat.it). There were 335,138 individuals (198,949 men and 136,189 women), of whom 54,964 aged 55 or more. Subjects who retired in 2011 or before (n. 183,157, of whom 58 developed ALS during the follow up period) could not be considered for the study because the census did not collect their previous occupation.

The study population was retrospectively surveyed for ALS onset (defined as the time of motor symptoms onset) from 1 January 2012 to 31 December 2021. ALS onset was ascertained by the To identify individuals with ALS, we referred to the methodology used in a recent incidence study,(8) with cases being sought across all the neurological and genetic facilities from the study area. One neurologist from the University center (GB) reviewed medical charts and checked for duplicate cases. An incident case was defined as any individual fulfilling El Escorial revised diagnostic criteria (9) who resided in the study area in the incidence period. Clinical phenotypes were classified according to Chio' et al. (10). Most patients performed genetic analysis for C9ORF72 and TARDPB mutations, the most frequent causative ALS mutations in Sardinia.(11,12) Subjects with symptoms onset before the start of the observation period were excluded from the study.

The risk of ALS was estimated in relation to the occupation held in 2011. Information about demographics and activity at work in the general population was obtained from the 2011 Census that classified working activities in ten groups: 1. unskilled workers; 2. conductors of machinery, fixes and mobile machinery operators and assembly line workers; 3. Craftsmen, skilled laborers; 4. agricoltural activity and breeding; 5. occupations in sales and services for families; 6. intermediate (technical) occupations; 7. low-grade administrative and management occupations; 8. intellectual, scientific and high specialization accupations; 9. legislators, managers and entrepreneurs; 10. armed forces. Housewifes could not be considered for the study because this activity was not included in the ten groups of occupations. Nevertheless, we separately calculated Sla incidence even in this group. Demographic and clinical data of people who developed ALS as well as information about their activity at work in 2011 was retrospectively obtained obtained by medical records.

Statistical analysis was performed by STATA 11 package. Data were expressed as mean and standard deviations (SD) unless otherwise indicated. Differences between groups were test by the Mann-Whitney U test and the chi-square test, as appropriate. Crude incidence rates were calculated by the number of newly diagnosed cases per 100,000 inhabitants-year; the denominator was the working population from the study area aged over 15 years. Each occupational group was compared with a reference category represented by all other occupations, and rate ratio were calculated.

To assess additive interaction on ALS incidence between variables, we referred to the stratification technique as reported by Szklo and Nieto (13) and estimated the attributable risk for those screening positive to the first variable across the two strata from the second variable. The statistical significance of the difference between observed and expected joint effects was checked by the test of homogeneity of stratified estimates, as reported (13).

The study was approved by the local Institutional Review Board and conducted in line with the ethical rules for data collection.

Results

Study sample

Over the study period (2012 to 2021), 331 incident patients developed ALS. Sixty percent were men (n. 196) and 40% women (n. 135), with average annual incidence of 4.87 (95% CI, 4.25-5.59) and 3.14 (95% CI, 2.65-3.72) per 100,000 person-years respectively. Median age at symptoms onset was 65.3$\pm$11.3 years, with a higher ALS incidence in those aged 55 years or more (incidence rate: 8.64 [95% CI, 7.68–9.72] vs. 1.07 [95% CI, 0.82–1.39]). Thirty-two women who were housewifes for their lifetime were excluded from subsequent analysis as well as 58 patients who retired in 2011 or before (see methods). Likewise, 30 cases who lacked data on activity at work could not be analysed. The final study group included therefore 204 incident ALS cases. Patients who participated in the study and those who did not were similar for demographic and clinical features (Table 1).

Activity at work and Incidence of ALS

Based on data from the 2011 census, the working population in the study area amounted to 335,138 individuals (198,949 men and 136,189 women, 54,964 individuals aged $\geq$ 55 years). The distribution of the overall working population and of the 204 incident cases among the ten groups of occupations as well as the incidence of ALS in each occupational group vs. all other activities are shown in Table 2. Agriculture/breeding and Armed forces were the only occupational groups associated to a significant increase in the ALS incidence.

Agriculture/breeding and ALS

The agriculture/breeding group included 18 patients (14 men and 4 women, aged 68.7 ± 9.9 years at diagnosis) who showed clinical and genetic features similar to those detected in the overall sample (data not shown). Agriculture, breeding, and agriculture+breeding were the lifetime occupation for 9, 2 and 2 patients respectively. The remaining 5 patients changed work during their life, even though they were employed in agriculture for 5 to 10 years before ALS development.

The higher incidence of ALS in people who worked in agriculture/breeding was evident in both patients who carried C9orf72 and/or TARDPB causative mutations (incidence rate ratio: 3.84; 95% CI, 1.67-6.40; p = 0.008) and those who did not (incidence rate ratio: 2.52; 95% CI, 1.27-4.54; p = 0.006).

The higher incidence of ALS associated with agriculture/breeding was evident in both men (incidence rate ratio: 2.12; 95% CI, 1.13-3.68; p = 0.01). and women (incidence rate ratio: 6.61; 95% CI, 1.74-17.93; p = 0.004)

As compared with other occupations, agriculture/breeding was significantly associated with increasing risk of ALS in the provinces of Oristano and South Sardinia (incidence rate ratio: 2.99; 95% CI, 1.65-5.08; p = 0.0003) but not in the province of Cagliari (incidence rate ratio: 1.06; 95% CI, 0.13-3.9; p = 0.85).

Assessment of additive interaction (Table 3) showed that the effect of working in agriculture/breeding on ALS incidence was more evident in older people but not in men.

Armed forces and ALS

The armed forces group included 11 patients (11 men and 0 women, aged 61.9 ± 9.6 years at diagnosis). Their clinical and genetic features were similar to those detected in the overall sample (data not shown). Armed forces was the lifetime occupation for all patients.

The higher incidence of ALS in people who worked in the armed forces was not evident in patients who carried C9orf72 and/or TARDPB causative mutations (incidence rate ratio: 2.25; 95% CI, 0.45-6.99; p = 0.21) while a non significant trend was observed in those who did not (incidence rate ratio: 2.05; 95% CI, 0.87-4.154); p = 0.07). Activity at work in the armed forces was significantly associated with increasing risk of ALS in the province of Cagliari (incidence rate ratio: 2.83; 95% CI, 1.01- 6.45; p = 0.03) but not in the provinces of Oristano and South Sardinia (incidence rate ratio: 1.60; 95% CI, 0.51-3.85; p = 0.31).

No significant additive interaction could be detected between activity at work in the armed forces and older age (data not shown)

Discussion

In this population-based study on incident ALS patients from the southern part of Sardinia Island we found that employment in agriculture/breeding and in the armed forces significantly increased ALS risk as compared to the other occupations. Significant changes in the risk of ALS were not observed in the of the other occupation groups.

Occupation in agriculture has been previously described as a risk factor for ALS in several studies but the literature is controversial (3,4,6,7). Most studies dealing with this topic were retrospective/prospective case-control studies that could suffer from bias related to case and control selection. Our study design relied on incident cases and therefore limited such a bias. We also showed that the effect of agriculture/breeding on ALS incidence was independent of the causative C9orf72/TARDPB mutations and sex. Thus, agriculture/breeding may act as a risk factor for ALS independently of other known genetic and epigenetic risk factors. Increased exposure to agricultural chemicals (i.e. herbicides, insecticides, fungicides)

(14,15) or heavy work.(16) or might explain the increased ALS risk in agriculture/breeding workers. However, the lack of significant association between ALS risk and various physically demanding occupations, including skilled and unskilled workers, did not support heavy work as a factor contributing to ALS development.

While most previous studies tested the individual effect of single potential predictor of risk, our analysis provided further novel information indicating an additive interaction between employment in agriculture/breeding and older age. Explanation for the reported statistical interaction may point to an increased risk of developing ALS in agricultural workers aged ≥55 years. Although random variability or differential bias across strata cannot be excluded, our observation would drive attention toward factors increasing the risk for ALS in older people working in agriculture/breeding.

The increased incidence of ALS in people working in agriculture/breeding was particularly evident in the provinces of Oristano and South Sardinia where agriculture and breeding is to date more widespread. This area also hosts greater proportion of elderly people and was found to be at a greater risk for ALS than Cagliari Province (8), *in* agreement *with* literature data indicating older age as a risk factors for ALS (3). It is worth noting that, in the past, South Sardinia province hosted several mining sites for the extraction of carbon, iron and lead. Due to the closing of these mining sites in the 80s of the 20th century, it is possible that several workers changed their occupation from mining to agricultural and breeding activities. The role of co-exposure to metals and typical agricultural factors in the development of ALS needs to be investigated.

The significant additive interaction between agricultural/breeding and older age raised the possibility that variation in the age of study populations have contributed to the variable results of previous controlled studies on agriculture in ALS. More importantly, the additive interaction between the two exposures may provide insights into the mechanism through which these exposures operate. Positive interaction indicates that the joint presence of the two exposures enhances their independent effect. This may reflect a causal relationship between the two factors or shared pathophysiological mechanisms. Indeed several hallmarks of ageing correlate with susceptibility to neurodegenerative disease, (including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, mitochondrial dysfunction, cellular senescence, deregulated nutrient sensing, stem cell exhaustion and altered intercellular communication) and pesticides may contribute to some of these mechanisms.

The risk of ALS associated with general military service has been explored by several controlled studies that provided inconsistent results (6,7,17). Although our different methodologic approach showed an increased risk of ALS among armed forces workers, we could not provide enough information to consistently support this working activity as an independent risk factor for ALS. In fact, we could not check whether the effect of armed forces on ALS risk was also present in women. In addition, the effect of armed forces on ALS risk was no longer significant when patients were stratified by the most frequent causative genetic mutations in the study area, probably because of lack of statistical power. Interestingly, at variance with what observed for agriculture/breeding, the spatial profile of ALS risk among armed forces workers did not replicate the microgeographic variation of ALS risk in the study area.(8)

This study has strengths and limitations. The overall study population was relatively large, including more than 330 thousand people, thus providing good statistical power for examining the ALS risk in major occupational groups. Given the rarity of the disease, however, only a limited number of workers developed ALS during the 10 years observation period. Incomplete case ascertainment and selection bias inherent to study design would have occurred. However, relying on multiple data sources likely reduced ascertainment bias. The diagnostic accuracy was strengthened by the involvement of experienced field neurologists in the healthcare network referring to the tertiary center (6,7,8,18,19). Although it was not possible to collect the occupation for 90/331 ALS incident cases (26%), generalization of the results to the whole sample of ALS patients was likely because patients who participated in the study and those who did not were similar for demographic and clinical features. We also did not consider 32 women patients who were housewife for their lifetime; however, the risk for ALS in this group was 2.9 per 100,000 person-years (95% CI, 1.8 to 4.5), similar to the crude incidence of the study population (3.6/100,000 person-years [95% CI, 3.2–4.1]).(8) The low details of our classification system might have obscured significant association of ALS risk with occupations at the greatest details. By stratifying by sex and age we were able to evaluate potential confounding by known epigenetic factors for ALS. The information on occupation was derived from the 2011 census, i.e. at a time when ALS patients were not yet affected by the disease, so that is possible to exclude, in contrast with case-control studies based on self-reported information, that a recall bias affected the risk estimates. Taking into account only the occupation held in the 2011 census may be a weakness of the study, even though the occupations identified at risk were likely characterized by low mobility. Finally, we did not measure the length of employment, being therefore unable to evaluate dose-response relationships.

Despite the foregoing limitations, this retrospective study in a population living in a large area of Southern Sardinian showed an increased risk of ALS associated with agriculture/breeding and armed forces working activity. Our analysis took into consideration known genetic and epigenetic risk factors for ALS (age, sex, and the most frequent causative genetic mutations in the study area),(6,7,8) and supported agriculture/breeding as an independent risk factor for ALS. By contrast, we did not have enough information to evaluate the contribution of age, sex, and genetic mutations to the risk of ALS associated to work in the armed forces. The adherence of the risk carried by agriculture/breeding to the microgeographic variability of ALS incidence in the study area (8), and the inverse pattern of spatial risk associated with armed forces activity at work, raised the possibility that patients from different geographic areas have variable environmental risk factor profile. This hypothesis fits with the well established geographic variability in the genetic architecture of ALS.(2,6,7) Finally, the significant additive interaction between agriculture/breeding and age suggests that the mechanisms leading to ALS are complex and probably involve several factors, thus highlighting the importance of assessing interaction between exposures for future studies.

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