Amyotrophic Lateral Sclerosis spatial epidemiology in the Mount Etna region, Italy: further evidences for a pathogenetic role of volcanogenic metals.

Boumédiène F.[1*], Vasta R.[2*], Rascunà C.[3], Lo Fermo S.[3], Volanti P.[4], Patti F.[3], Ferrante M.[5], Giammanco S.[6], Zappia M.[3], Nicoletti A[3].

*These authors equally contributed to the work.

[1] Net, Unité Mixte de Recherche 1094, Université de Limoges, Limoges, France.
[2] ALS Center, 'Rita Levi Montalcini' Department of Neuroscience, University of Turin, Turin, Italy.
[3] Section of Neurosciences, Department GF Ingrassia, University of Catania, Catania.
[4] Intensive Neurorehabilitation Unit, ALS Centre, IRCCS 'Salvatore Maugeri' Foundation, Mistretta.
[5] Environmental and Food Hygiene Laboratory – LIAA, Department GF Ingrassia, University of Catania, Catania.
[6] Istituto Nazionale di Geofisica e Vulcanologia, Osservatorio Etneo, Catania.

Corresponding Author: Alessandra Nicoletti, Section of Neurosciences, Department GF Ingrassia, University of Catania, Catania. Mail: anicolet@unict.it, tel: 095378xxx

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Abstract

Background. Spatial epidemiology can give important clues on the etiology of a disease. Previously, we described a higher incidence of Amyotrophic Lateral Sclerosis (ALS) in the eastern flank of the Mount Etna when compared to the western one and intake of volcanogenic metals was proposed as a possible explanation.

Here we further investigated the spatial distribution of ALS cases in the Mount Etna region.

Methods. ALS cases in the residents of the province of Catania who had experienced the onset of symptoms during the 2005-2015 period were included. Address of residents at the moment of diagnosis was considered for each case. Considering the typical surface distribution of volcanic ashes during Mt. Etna eruptions, we divided the study region into an up- and a down-wind area. Furthermore, cluster analysis was performed using both Moran's Index and Kulldorff's spatial scan statistic.

Results. A total of 193 ALS cases were identified. The mean annual crude incidence rate was 1.6/100,000 person-years (95% CI 1.4-1.9). The down-wind area showed a significantly higher incidence compared to the up-wind area (respectively, SIR 1.2, 95% CI 1-1.4, and 0.7, 95% CI 0.5-1.0). Moran's I suggested a higher incidence cluster in the southeastern sector of the volcano. Kulldorff's statistics confirmed such suggestion by identifying a spatio-temporal cluster that includes 13 communalities. Here, 12.73 cases were expected whereas 33 were observed (SIR 2.6; 95% CI 1.8-3.6, *p-value* 0.003).

Discussion. Our study confirmed a higher ALS incidence in the area most exposed to fallout of volcanic ashes. Furthermore, a spatio-temporal cluster was found in the

southeastern flank of Mount Etna. Despite the retrospective nature of this study, adoption of two proper spatial analyses of data, independent on an *a priori* hypothesis, strengthens the validity of our results. These findings further suggest the possible role of volcanogenic metals in ALS pathogenesis.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder affecting motor neurons(1). It leads to a relentless paralysis of all voluntary muscles and eventually to death in 3 to 5 years from symptoms onset. Apart from 10% of patients for whom a genetic aetiology has been recognized, ALS causes remain unknown(2). Current hypotheses contemplate a multistep etiopathogenetic process in which both genetic and environmental factors are involved(3).

Metals have been proposed to play a role in the pathogenesis of many neurodegenerative diseases, amongst which ALS. Beyond specific pathways for each metal, oxidative stress is considered as a likely common pathogenic mechanism(4).

Volcanoes are a major source of metals(5) and Mount Etna is not only the largest active volcano in Europe, but also the main Mediterranean point source of volcanogenic metals in the atmosphere and, consequently, in the environment (6, 7). Previously, we analyzed the spatial distribution of ALS cases in the Mount Etna region, Italy (8). During a 5-years period, ALS incidence resulted almost three times higher in the eastern flank when compared to the western one. Since Etna ashes are normally blown toward the eastern and southern flank, we hypothesized that such distribution could be related to exposure to volcanogenic metals(8).

Here, we further explore the possible role of volcanogenic metal as risk factors for ALS by analysing the spatial distribution of ALS cases in the Mount Etna region during an 11-year period.

Materials and methods

Study population, study period and study area. The study was conducted in the province of Catania. It is located on the eastern part of Sicily (southern Italy) and its territory is divided into 58 communalities. According to the official 2011 Italian Census, the resident population is 1,078,665 inhabitants (520,731 males and 557,934 females) (9).

ALS patients diagnosed according to the El Escorial revised criteria (10) who were resident in the province of Catania and who had experienced the onset of symptoms during the 2005-2015 period, were considered as incident cases.

Cases ascertainment has been described elsewhere (8). Briefly, major hospitals, neurology wards of secondary hospitals, the ALS rehabilitative centre of Mistretta, the local Italian ALS association (AISLA) and local neurologists were asked for data on ALS cases. Furthermore, a trained neurologist reviewed charts in order to check diagnosis.

Spatial analysis. Crude incidence rate in the study area was calculated both for the entire population and separately for males and females. In order to allow for the comparison with other studies, the crude incidence rate was standardized both using the 2010 European population and through a direct method of standardization.

The home address at the moment of the diagnosis was considered for each ALS case. Standardized incidence ratios (SIRs) of each community were calculated using the population of the entire province as a reference through an indirect method of standardization. The ninety-five (percent?) confidence intervals (95% CI) were calculated assuming a Poisson distribution of data. Four communalities (Randazzo, Tremestieri Etneo, …) have an exclave. Based on the percentage of resident population (data not shown), we considered exclaves as a separated polygon only for the communality of Tremestieri Etneo (2633 inhabitants, 31.6 % of the total communality population).

In order to assess human exposure to volcanogenic elements, volcanic ash deposition during explosive eruptions was analyzed. Due to the prevalent direction of trade winds across the Etna area, gases emitted from the summit craters of the volcano are more frequently blown to its eastern and southern sectors(11). Thus, considering the map of likely crater plume dispersion(11), we divided the Etna area into an up-wind and a down-wind portions (figure 1). Communalities were considered as included either in the former or in the latter depending on the position of their centroid.

Finally, cluster analysis was performed using both local Moran's Index (also termed Local Indicator of Spatial Association, LISA)(12) and Kulldorff's spatial scan statistics(13).

LISA provides an estimate of the similarity of incidence among neighboring communalities. Briefly, statistical weights are assigned proportionally to the distance between communalities by constructing a spatial weight matrix. Thus, each communality SIR is related to the weighted mean of SIRs of its neighboring communalities. This will result in four types of association (low–high, high–high, high–low and low–low), with concordant values indicating stability in the incidence throughout the study area.

Kulldorff's analysis overlaps circular windows of variable radium to each point of the study area, each radium ranging from zero to a pre-defined maximum percentage of population at risk included in the window. We set this limit at 25%. The null hypothesis is that incidence does not differ between the inside and the outside of each window. The Monte Carlo simulation was then used to assess the statistical significance of the results (p-*value* was set at 0.05%).

Analysis was performed seeking purely spatial, purely temporal and spatio-temporal clusters. In order to better fit the real distribution of cases throughout the study area, only the surface of inhabited areas, rather than administrative boundaries, was considered when estimating centroids of each communality.

Statistical analysis. R 3.4 software(14) was used to perform basic statistics and data preparation for SatScan analysis. Both the difference between means and the difference between proportions were evaluated using the t-test and the chi-squared test, respectively. The significance level was set at 0.05. The Kulldorff's spatial scan statistic was implemented using the SatScan software, version 9.4.4(15).

Ethics statement. Approval from the local ethical committee (Azienda Ospedaliero-Universitaria Policlinico-Vittorio Emanuele, Catania) was obtained.

Results

Descriptive statistics. During the 11-years period under consideration, a total of 193 residents (96 males and 97 females, male/female ratio = 0.98) were diagnosed with ALS. The average age at onset was 62.4±10.8 years (61.9±14.6 years for males; 61.9±11.3 years for females). The appearance was spinal in 109 (56.5%) cases, bulbar in 45 (23.3%) cases, cognitive in three (1.6%) cases and respiratory in two (1%) cases. In 34 (17.6%) cases, it was not possible to retrieve the disease onset from medical records (table 1).

The mean annual crude incidence rate for the 2005-2015 period was 1.6 cases per 100,000 person-years (95%, CI of 1.4-1.9). When standardized for the 2010 European population, the ALS incidence rate did not change (1.6 cases per 100,000 person-years, 95% CI 1.4-1.9). For both males and females, the incidence rate peaked in the 65-74 years age class (respectively 6.2 and 4.9/100,000 person years), decreasing thereafter (table 2).

Spatial analysis. Address at the moment of disease onset was retrieved for all patients. Two communalities, namely Acireale and Gravina di Catania, both located on the south-eastern flank of the volcano, resulted with a higher-than-one SIR and a 95% CI not including one (table 3 and figure 2).

When considering the distribution of volcanic ashes, the down-wind area of Mt. Etna resulted with a significantly higher ALS incidence than the up-wind area (SIRs were respectively 1.2, 95% CI 1.0-1.4, and 0.7, 95% CI 0.5-1.0; SIR ratio 1.6, 95% CI 1.2-2.3).

Moran’s Index resulted, (figure 3)… therefore suggesting the presence of a cluster in the south-eastern flank of the Mount Etna (figure 3). [up to Farid]

SatScan confirmed the above indication by revealing a spatio-temporal cluster that included 13 communalities in the same area. Here, during the 2006-2010 period, 12.73 cases were expected whereas 33 were observed, therefore resulting in a SIR of 2.6 (95% CI, 1.8-3.6; *p-value* = 0.0029) (figure 4). Both demographical and clinical characteristics did not significantly differ between out- and in-cluster ALS patients (table 1).

Purely spatial analysis revealed a smaller cluster, embedded in the spatio-temporal one. This included four communalities, with 10.03 expected cases, 23 observed cases and a SIR of 2.3 (95% CI 1.5-3.4, p = 0.026) (figure 4). Finally, purely temporal analysis revealed an increase in ALS incidence in the 2007-2010 period. During this period, 68.48 ALS cases were expected, whereas 99 were observed, therefore giving a SIR of 1.5 (95% CI 1.2-1.8, p-value = 0.001).

Discussion

Recognition of clusters in the spatial distribution of cases can give useful hints about the etiology of a certain disease(16). The ALS incidence throughout the province of Catania was previously analyzed over a 6-years period. Based on the distribution of volcanic ashes during eruptions, the Etna area was tentatively divided into a western and an eastern flanks, discovering an incidence rate (IR) in the latter almost three-times higher than in the former (respectively, IR 0.9, 95% CI 0.5-1.4, and IR 2.4, 95% CI 2.0-2.9; RR 2.75; 95% CI 1.64–4.89; P < 0.001)(8).

Here, we further investigated the putative role of volcanic metals as a risk factor for ALS by performing an accurate spatial analysis of ALS cases in the Mt. Etna region over an 11-year period.

Based on the distribution of Etna ashes due to prevailing winds(11), we more appropriately divided the part of Catania province under study into an up- and a down-wind areas, being respectively the most and least exposed to fallout of volcanic ashes. We found SIR to be significantly higher in the down-wind area when compared to the up-wind one (SIR ratio 1.62, 95% CI). Moreover, when communalities SIRs were considered, Acireale and Gravina di Catania, both located in the down-wind area, showed a significantly higher ALS incidence (respectively SIR 1.78, 95% CI 1.04 - 2.85, and SIR 2.38, 95% CI 1.23 - 4.16).

Therefore, in order to overcome the possibility of biases deriving from the a-priori hypothesis, we carried out two independent cluster analyses. Performing both Moran's I and Kulldorff's statistics, we found a spatio-temporal cluster in the southeastern flank of Mt. Etna. During the 2006-2010 period, the number of observed ALS cases in the 13 communalities included in the cluster was more than double that expected. Occurrence of this cluster influenced the overall ALS incidence, causing an increase during the 2007-2010 period (thus forming also a temporal cluster). Finally, a purely spatial analysis revealed a smaller cluster embedded in the spatio-temporal one. Interpretation of this outcome is less intuitive, since the spatial cluster discovered could be either a legacy of the spatio-temporal cluster or its more focalized cause.

The putative relationship of such findings with human exposure to volcanic elements is further strengthened by the analysis of Mount Etna activity during the last decades. In fact, since 1961 a general increase in the frequency of Etna eruptions was observed. In particular, since 1971 a significant increase was observed both in the output of lava and in the number of explosive summit eruptions, mostly those named "subplinian"(17). Subplinian eruptions imply volcanic ash fallout dispersal that extends to distances of at least 100 km, with the ash deposit being continuous within several kilometres from the emission point and having mass in the order of 500–1,000 g per square metre(17). The above trend with increasing explosive activity at Mt. Etna continued during the period of the present study, with several violent paroxysmal summit eruptions that produced large emissions of volcanic ash, leading to the formation of a new summit crater, named New South-East Crater(18).

Such results confirm those from our previous study and further support the hypothesis of a possible role of volcanic metals in the ALS pathogenesis. The relationship between metals and ALS has been widely investigated. Recently, ecological, occupational and case-control studies evaluating the concentration of several metals in biological samples have been ruled out(4). Nonetheless, results of those studies appear to be incongruent. Methodological issues are likely to explain differences among studies. Indeed, ecological studies are prone to ecological fallacy(17), occupational studies do not take into account exposure outside of job places, and biological samples reflect actual exposure whether ALS pathogenesis is likely to begin years before diseases onset(2). However, firm evidences seem to exist for manganese, iron and especially for lead in assessing their role as ASL triggers(4). Beside specific mechanisms, oxidative stress has been proposed to explain such relationship(4). Indeed, metals are involved in many cellular reactions resulting in the collateral production of free radicals, normally drained by antioxidant systems. An excessive amount of metals can overwhelm the ability of such systems, therefore resulting in oxidative stress and finally in cellular disruption.

Worldwide, many spatial analyses have been ruled out due to inconsistent results, likely because of differences in the methods adopted(19–275). Among these, only one study proposed exposure to metals as a possible explanation for a spatial ASL cluster. Indeed, in Jefferson (Missouri, USA) a small cluster (3 observed cases, 0.47 expected cases; RR = 6.4, *p-value* =0.04) was found around a lead smelter. In addition to lead, the smelter produced other metals among which copper, zinc, chromium, cobalt, cadmium and nickel(20). However, the exiguous number of observed cases does not allow to rule out chance as a possible explanation. Other clusters have been explained either with exposure to beta-methylamino-L-alanine (BMAA)(19,28) or on the basis of genetics(21,27).

Several limits should be encountered when interpreting our results. First, the global incidence rate is lower than those reported from retrospective studies (median IR 1.52/100 000, 1.22 interquartile range 1.52-2.04)(29). Since age- and sex-adjustement did not modify the incidence rate, the difference is likely to be caused by a leakage in case ascertainment. Nonetheless, sources used for case ascertainment were spread throughout the study area and this makes a preferential out-cluster under-ascertainment less probable. Second, due to the retrospective nature of the study, information about genetics or familiarity is missing. Therefore, it is not possible to entirely rule out a genetic rather than environmental nature of the cluster. However, current hypotheses contemplate a multistep pathogenic process in which both genetic and environmental factors are involved. Thus, even in genetic cases it is still worth to investigate environmental factors as co-factors or triggers. Ecological fallacy should be considered as well. Indeed, exposure did not necessarily act on singular individuals. Still, as previously stated, it would be difficult to evaluate exposure to metals with approaches others than ecological. Finally, we did not take into account other environmental exposures that could have acted as confounders.

On the other hand, we performed two dedicated spatial analyses, which gave concordant results. Such analyses were independent from both a-priori hypotheses and administrative boundaries, therefore reinforcing the validity of our results.

In conclusion, we seemingly detected a spatio-temporal cluster of ASL cases in the eastern flank of Mt. Etna volcano. Because of the direction of prevailing winds in the Etna area, exposure to metals contained in volcanic ashes could be responsible for such finding. Nonetheless, further studies are needed in order to explore possible alternative hypotheses.

Disclosure of conflicts of interest. The authors declare no financial or other conflicts of interest.

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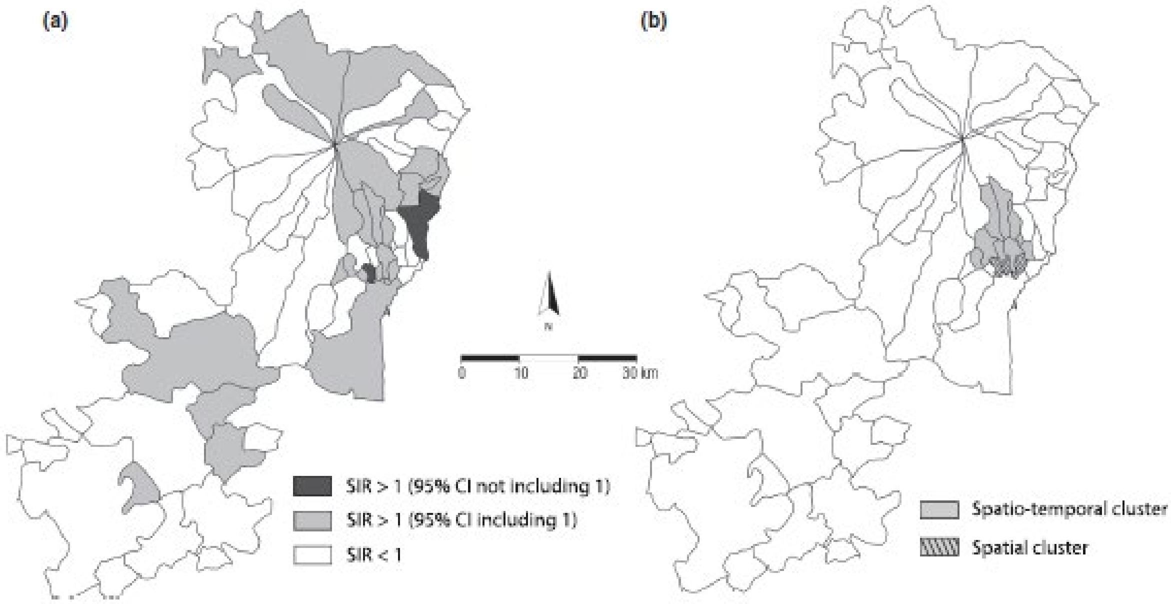

Figure 1 (a) Communalities' SIRs in the province of Catania. (b) The spatio-temporal and purely spatial high-incidence clusters revealed by the Kulldorff's statistic.