Metals analysis in a small cohort of ALS patients originating from a restricted geographical area: preliminary data

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Introduction:

Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder with an incidence of about 1/100.000 case per year. It is characterized by a selective degeneration of both upper and lower motor neurons in the brain, brainstem, and spinal cord (Fig.1), resulting in paralysis due to muscle weakness and atrophy, leading to death in 3-5 years since the first manifestations of symptoms[1]. In a small percentage of the cases, dementia is observed. Neurodegenerative disorders such as ALS have been linked to iron and metals metabolism in different studies through the years[2-4]. Transition metal induced toxicity has been proposed to be involved in ALS[5] and higher concentrations of metals and proteins that regulate metal homeostasis have been described in ALS patients[6]. Thiophylic metals have been shown to be capable of displacing iron from reduced FeS proteins[7]. This may represent a yet underexplored target in the context of ALS pathogenesis.

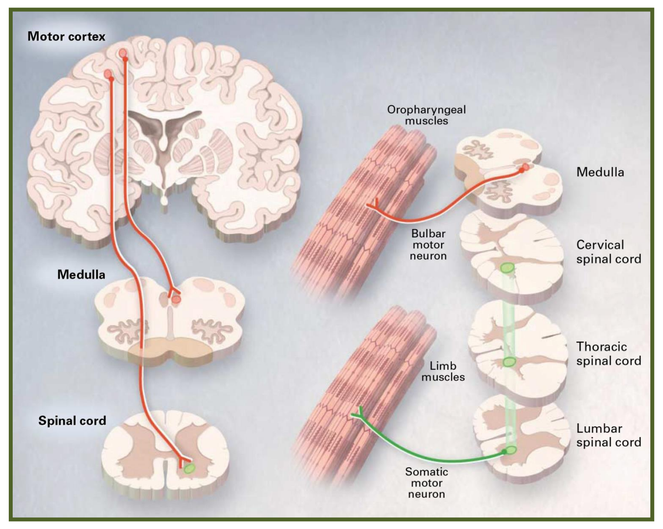

Fig.1 *Neuronal pathways affected in ALS*

Materials and Methods:

Samples of serum and urine were diluted (1:20 and 1:10, respectively) with 0.05% Triton X-100 in MilliQ water. Seronom™ Trace Elements Serum L-1 and Urine L-1 were used to build appropriate calibration curves. Samples were analyzed by ICP-MS (Bruker AURORA M90 ICP-MS). An internal standard solution (^{45}Sc, ^{89}Y, ^{159}Tb) was added to both samples and standards. Statistical analyses on the results have been carried out both with classical statistical elaborations (t-test and Principal Component Analysis) and with Auto CM algorithm, a special kind of Artificial Neural Network able to define the strength of the associations of each variable with all the others and to visually show the map of the main connections[8].

Results:

Analyses performed on serum samples highlighted elevated levels of Cr, Ni and Pb both in controls and in patients' group, if compared to literature data for the general population[9]. Higher concentrations of Ni and Pb were found in the patients' group, compared to the control's group (p-value = 0.0001 and 0.01). Surprisingly significant higher concentrations of As were found in the control's group (p-value = 0.05) (Fig. 2). Principal Component Analysis (PCA) confirmed these observations, and was able to discriminate between the two groups. The most important feature of the control group was the high concentration of As and a low concentrations of all the other metals analyzed. This observation was confirmed by Auto-CM analysis, that discriminated the two groups, clustering the control group with high levels of As (Fig. 3).

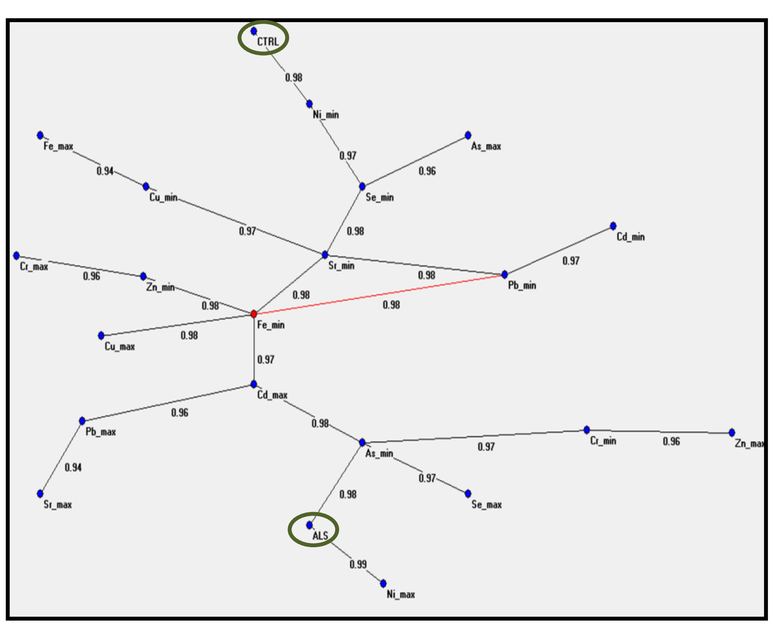

Fig.3 *Auto-CM analysis of metals' concentrations in serum*

Among the three metals that were significantly different, no one emerged as more relevant than the others in the discrimination of the control's group from the patient's group. Regarding urine analysis, all metals analyzed but Ni and Sr showed higher concentration than general population[9]. Intriguingly, Pb had low concentration both in patients' and in controls' urine, at contrast with its high levels found in serum analysis. Differences between patients and controls were significant for Fe (p = 0.01), Ni (p = 0.01), Zn (p = 0.008), As (p = 0.04), Sr (p = 0.02), Cd (p = 0.05). It must be noticed, by the way, that the control group consisted only of two subjects, different for age and geographical origin from the patients' group. PCA analysis discriminated the two groups: the control's group had low levels of metals, and the patient's group higher metal levels, with a patients' sub-group having high levels of As.

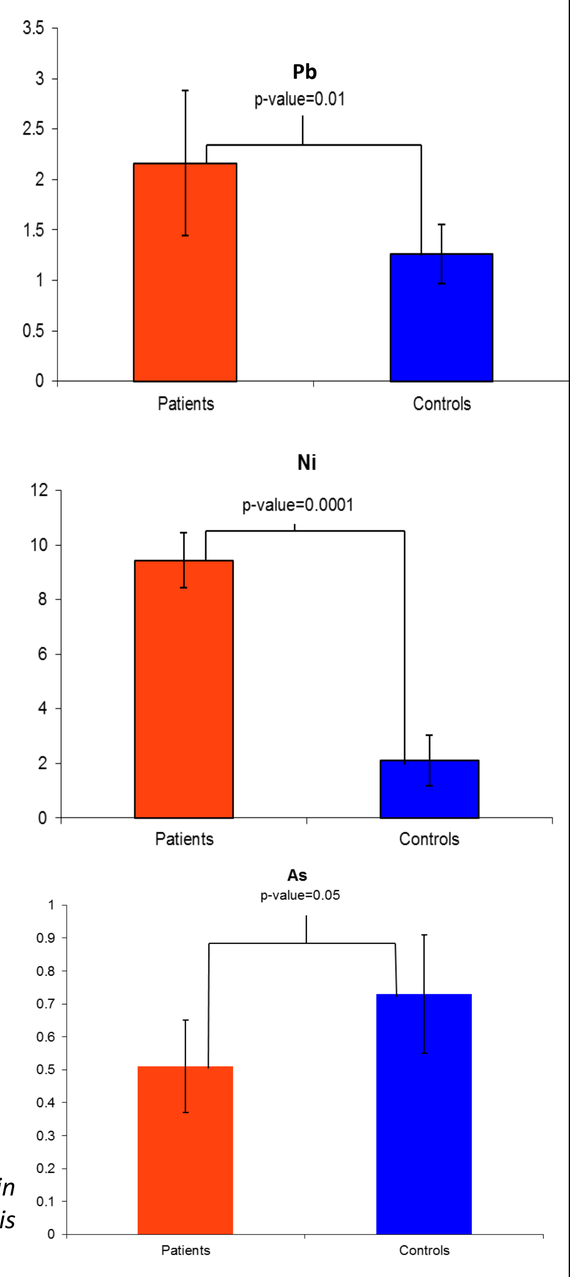

Fig.2 *Statistically significant elements in serum metal analysis*

Discussion:

Despite much research, the etiology of Amyotrophic Lateral Sclerosis still has to be clarified. Only a small percentage of the cases is attributable to genetic defects[10] and environmental factors could play a crucial role. Here we report the preliminary results of the analysis of metals' status in a small cohort of subjects originating from a restricted area, thus sharing environmental exposure. Our results confirm the hypothesis of a possible association between Pb exposure and ALS[11, 12] and provides further suggestions. The first one regards Ni, higher in ALS patients, and a second one, probably more unusual, is that high As levels have been found in the control's group. Further studies will confirm these results in other cohorts of subjects. We have also planned to perform an evaluation of serum proteins through a proteomic approach in order to expand the knowledge on the effect of dysregulated metals homeostasis on circulating proteins.

Connections to FeS proteins:

A great part of the lead damage in cellular physiology is caused by its ability to substitute for diverse polyvalent cations in their binding sites[13]. Pb and Ni have substantial affinity for protein thiols. Direct substitution of iron in various types of FeS clusters by thiophylic metals has been demonstrated [7]. Arsenic is known to interact with thiol-rich proteins, such as glutaredoxin, involved in FeS proteins biosynthesis, making it plausible that dysregulation of homeostasis of these metals could affect FeS-protein dependent events, opening a new and still unexplored area in ALS research.

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