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# Monitoring of chemical elements and oxidative damage in patients affected by Alzheimer's disease

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**Summary**. - The haematic concentration of 26 metals and the oxidative damage in 60 patients (20 males and 40 females) affected by Alzheimer's disease and 44 healthy individuals (33 males and 11 females) were compared. In patients, the following significant ($p \leq 0.05$) discrepancies were found: *i)* increment of Ca, Cd, Hg, Mg, Si and Sn, and decrement of Al, Co, Fe and Zn in serum; *ii)* higher concentrations of Cu, Li, Mn, Sn and Zr and lower of Fe, Hg, Mo in blood; *iii)* overproduction of oxidant species (SOS) and decrease of the anti-oxidant capacity (SAC) ($p \leq 0.001$, for both). Variables that, joined, better discriminated between patients and controls resulted to be Si, SOS, SAC, Co, Ca, Al in serum (94% of cases correctly classified) and Cu, Zr, Mo and Fe in blood (90% of cases properly categorized).

*Key words*: metals, oxidative status, Alzheimer's disease, serum, whole blood.

**Riassunto** *(Monitoraggio degli elementi chimici e del danno ossidativo in pazienti affetti dalla malattia di Alzheimer).* - Sono state confrontate le concentrazioni ematiche di 26 metalli e il livello del danno ossidativo in 60 pazienti (20 maschi e 40 femmine) affetti dalla malattia di Alzheimer e 44 individui sani (33 maschi e 11 femmine). Nei pazienti, sono stati riscontrati i seguenti squilibri significativi ($p \leq 0{,}05$): 1) nel siero, un incremento di Ca, Cd, Hg, Mg, Si e Sn ed una riduzione di Al, Co, Fe e Zn; 2) nel sangue, livelli più alti di Cu, Li, Mn, Sn e Zr e più bassi di Fe, Hg, Mo; 3) aumentata formazione di specie ossidanti (SOS) e ridotta capacità antiossidante (SAC) ($p \leq 0{,}001$ per entrambi). Le variabili che, insieme, meglio discriminavano fra pazienti e controlli risultavano essere Si, SOS, SAC, Co, Ca e Al nel siero (94% di casi classificati correttamente) e Cu, Zr, Mo e Fe nel sangue (90% di casi raggruppati esattamente).

*Parole chiave*: metalli, danno ossidativo, malattia di Alzheimer, siero, sangue intero.

## Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder manifested by loss of memory and other cognitive functions. The neuropathological characteristics include presence of neurofibrillary tangles and senile plaques, impaired synaptic function, oxidative stress and neuronal loss. In particular, senile plaques are closely related to the aggregation of β-amyloid (Aβ) within the neocortex [1]. Recently, there is accumulating evidence that Aβ precipitation and toxicity in AD are caused by abnormal interactions with neocortical metal ions, such as Cu, Fe and Zn [2, 3]. The fact that the homeostasis of these metals is perturbed in AD and that they concentrate in senile plaques, neurofibrillary tangles and cerebrospinal fluid (CSF) is rather supported [4]. Researchers hypothesized that the storage of metals might be the key to the damage of AD and perhaps to its treatment [5]. First, Cu and Fe can promote the *in vitro* aggregation into tinctorial Aβ amyloid [6]. Second and more importantly, the redox-active Cu(II) and, to a lesser extent, Fe(III) are reduced in the presence of Aβ with concomitant production of reactive oxygen species. These Aβ/metal redox reactions may lead directly to the widespread oxidation observed in AD brains [6]. Zinc role in amyloid plaque is complex. Zinc can squelch the formation of hydrogen peroxide

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and renders β-amyloid itself less toxic, but at the same time, abnormally high Zn levels can contribute to the formation of amyloid plaques [4]. Aluminium is one of the first metal ions though to be linked to AD. Patients with AD have been found to accumulate Al in hippocampal neurofibrillary tangles and at the centre of senile plaque cores [7, 8]. In few studies a high percentage of dementia of AD type was found to correlate with increased levels of Al in drinking water [9, 10]. Nevertheless, the proposed connection between Al and AD remains a controversial theory [11]. Manganese exposure may play an important role in causing Parkinsonian disturbances, but the relationship with AD is unclear. In fact, authors observed reduced Mn levels in the frontal and occipital lobes of AD individuals, but others have been unable to find differences between control and AD brain [8, 12]. Other authors showed incremented Mn levels in some brain regions, but diminished in the basal ganglia [13]. Alterations in other minerals, such as Ca, Hg, Mg, Pb and Si, have also been reported. Degeneration of the central nervous system (CNS) has been found to be associated to irregular metabolism of Ca and Mg [14, 15]. Both elements appear to interact with Al; in fact some forms of dementia are related to a depletion of these metals due to the high intake of Al [16]. Mercury is a known neurotoxin and seems to be able to be absorbed or accumulated in degenerative AD brain more readily than in those of controls [17, 18]. Moreover, the exposure to low levels of Pb may increase the risk of cerebral hypometabolism caused by direct inhibition of specific glucose-utilizing enzymes. In this context, Pb might be regarded as a risk factor in the abnormal glucose metabolism seen in AD [19]. Silicon, as alumino-silicate complexes, accumulates in the neurofibrillary tangles of AD, while, as silicic acid, seems to have a protective effect by reducing Al bio-availability [20]. As regards Cd, Patra *et al.* found that long-term exposure to this element increased lipid peroxidation and caused inhibition of superoxide dismutase (SOD) indicating oxidative stress [21]. Few papers have discussed the role of Cd in the brain, in any detail. Panayi *et al*. found decreased (at a low significance) levels of Cd in AD brain compared to normals, but Ward and Mason found no difference [8, 22]. On the contrary, no changes in the levels of other metals as Co, Cr, Ni and Sr have been so far detected in brain tissues of AD patients [8, 12].

Despite all, much more research is necessary to determine whether metals build-up or decrement is a cause or a result of AD and to better understand the exact role of metals in the development of the disorder. Most of the literature is based on metal imbalances in the CNS by means of a variety of *post mortem* tissue examinations, whilst, at present, there is a paucity of data on elemental alteration in human fluids of living AD patients. The goal of this study was to achieve concentration data on twenty-six metal ions (Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W, Zn and Zr) in serum and whole blood of patients affected by AD and of a control group. To date, this work presents the first comprehensive simultaneous multi-element study in these fluids for AD patients. Furthermore, in order to evaluate the potential relationship between oxidative stress and AD, the serum oxidative status (SOS) and the serum total anti-oxidant barrier (SAC) were also assayed.

## Materials and methods

### *Study population*

The AD group consisted in 60 consecutive patients (20 males and 40 females; mean ± age: 74.6 ± 6.39 yrs) affected by probable AD according to the NINCDS-ADRDA criteria. The mean duration of the disease was 3.9 ± 2.0 yrs. The severity of cognitive impairment was quantified by means of the Mini Mental State Examination, ranging from 28 to 2. The control group was constituted of 44 healthy volunteers (33 males and 11 females) older than 45 yrs, with no clinical evidence of neurological disease. Exclusion criteria for both population groups were the following: cardiological, respiratory, kidney or liver disorders; intestinal absorption abnormalities; active infections; assumption of thyroid hormones, lithium, vitamins or mineral integrators; any other psychoactive drug intake except for anti-Alzheimer medications. All subjects having metallic prostheses, surgical screws or intra-uterine inserts were also excluded. The study was approved by the Ethics Committee of the Neurological Science Department of the University of Rome "La Sapienza" and all subjects signed an informed consent form.

### *Pre-treatment and analysis of samples*

For each subject, venous blood was drawn with polyethylene syringes and transferred into 15 ml polystyrene tubes. Prior to the analyses, serum was simply diluted with deionized water, while blood was subjected to an acid-assisted microwave digestion. Aluminium, Ba, Be, Bi, Cd, Co, Cr, Hg, Li, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Tl, V, W and Zr quantifications were carried out by sector field inductively coupled plasma mass spectrometry. An inductively coupled plasma atomic emission spectrometer was used for the quantification of Ca, Cu, Fe, Mg, Si and Zn. The SOS and SAC were determined by means of a photometric

system. The SOS, as the sum of content of lipo- and hydro-peroxides, was expressed in U.CARR (1 U.CARR = 0.08 mg of peroxyl radicals for 100 ml of hydrogen peroxide). The SAC was the serum ability to oppose the massive oxidative action of HClO solution and was expressed as μmol $ml^{-1}$ of HClO. More details on sampling, pre-treatment of body fluids and quantification techniques were given elsewhere [23, 24].

*Statistical evaluation*

Results on the AD patients and controls were described in terms of mean, standard deviation (SD) and $25^{th}$-$75^{th}$ percentiles, and statistically compared by the Mann-Whitney test. Moreover, the discriminant analysis was applied to identify which variables better discriminate AD from controls. A forward step-wise procedure (SDA) to select the best variables in term of F value was carried out. The classification using the discriminant function was validated by a leave-one-out cross-validation approach. The SPSS 11.0 version statistical package was utilized (SPSS, Chicago, IL, USA).

## Results and discussion

Tables 1 and 2 report the basic statistics for elements, SOS and SAC in AD patients and control subjects, respectively. In serum of the AD group, Bi, Tl, V and W were at level below 0.1 ng $ml^{-1}$, with the lowest mean value for Bi (0.02 ng $ml^{-1}$). Concentrations between 0.1 and 1.0 ng $ml^{-1}$ were observed for Ba, Be, Cd, Co, Cr, Mn, Ni, Pb, Sb and Zr, and between 1.0 and 5.0 ng $ml^{-1}$ for Al, Hg, Li, Mo and Sn, with the highest mean value for Al (2.22 ng $ml^{-1}$). Among all the trace elements, the highest element was Sr with a concentration of 38.5 ng $ml^{-1}$. Major elements were between Si (223 ng $ml^{-1}$) and Ca (68.5 μg $ml^{-1}$) levels. In whole blood, again Bi, Tl and W were under 0.1 ng $ml^{-1}$; Be, Co, Cr, Sb, V and Zr were in the range 0.1-1.0 ng $ml^{-1}$; while Ba, Cd, Hg, Li, Mo, Mn, Ni and Sn were in the interval 1.0-10 ng $ml^{-1}$ with the highest concentration for Mn (8.93 ng $ml^{-1}$). The highest trace elements were Al (21.3 ng $ml^{-1}$), Pb (47.3 ng $ml^{-1}$) and Sr (26.1 ng $ml^{-1}$). Among the major elements, the lowest concentration was found for Si (165 ng $ml^{-1}$) while the highest for Fe (489 μg $ml^{-1}$).

In both matrices, there was substantially no evidence of differences in the distribution of Ba, Be, Bi, Cr, Ni, Pb, Sb, Sr, V, W and Tl between patients and controls. For almost all these elements (excluding Pb) the literature did not reported any clear indication of neurotoxicity. Effects of occupational Pb exposure on nervous system have been somewhat documented and it was the first metal suspected to be involved in amyotrophic lateral sclerosis [25, 26]. Nevertheless, unchanged plasma levels of Pb in AD patients were previously reported in support of the present results [27]. On the other hand, a number of elements apparently not implicated in neurodegeneration such as Li and Zr showed significant variations when the disease occurred. In particular, in AD, blood Li ($p \leq 0.001$) and blood Zr ($p \leq 0.05$) concentrations resulted to be raised. Differences between AD and controls have been revealed also for elements such as Mo and Co, for which only some evidences of neurotoxicity have been up to now reported. High concentration of Mo in soil seems to be responsible for a higher prevalence of multiple sclerosis, while Co is an inducer of oxidative stress and cell cytotoxicity increasing β-amyloid secretion [28, 29]. In this study, Mo level is 1.4-fold lowered ($p \leq 0.001$) in blood and Co is strongly reduced (1.7-fold) in serum of patients ($p \leq 0.001$). Mercury displayed an enhancement in the serum level and a depletion in blood of AD (always $p < 0.01$). Potential sources of Hg comes from environmental exposure, fish or seafood assumption or amalgam restorations but the individuals included in this study did not present any of these confounding factors. Preliminary reports noted blood Hg values in AD higher than in controls, but in contrast to these findings Fung *et al.* was not able to find any variation in blood of the same diseased population [27, 30, 31]. As regards Al, it displayed changes with the disease, with a depletion ($p \leq 0.05$) in serum of patients. This element is the main metal though to play a part in AD, although this link seems still quite tenuous and epidemiological studies on this evidence have been inconsistent [32]. Previous data indicated no changes in the Al content of AD when compared to controls, nor in serum neither in CSF [33, 34]. Another element considered to be strongly implicated in neurodegeneration is Mn. Excessive exposure to Mn has been reported to produce neurotoxicity [35]. At the cellular level, Mn reduces several intracellular antioxidants, and this decrease is compounded by the observation that Mn induces reactive oxygen species formation [36, 37]. These data and the increase of blood Mn content ($p \leq 0.05$) in our patients supported the involvement of Mn in AD. Basun *et al.* conversely observed Mn lowered in AD subjects and in a more recent investigation the two groups, AD and controls, were not different in serum Mn [27, 38]. In addition, differences were observed for Cd and Sn. Cadmium is a recognized neurotoxin in animal studies and there are also evidences in humans, with higher levels in serum of patients with neurological diseases [39]. In agreement with this observation, our patients had an increased ($p \leq 0.01$) concentration of serum Cd and this raised concentration could be significant in

**Table 1**. - Element concentrations (ng ml$^{-1}$) in serum of Alzheimer's disease (AD) patients and controls

| Element | AD patients | | Controls | |
|---|---|---|---|---|
| | Mean ± SD | 25$^{th}$-75$^{th}$ | Mean ± SD | 25$^{th}$-75$^{th}$ |
| **Al*(*)*** | 2.22 ± 1.24 | 1.29 - 2.59 | 2.79 ± 1.51 | 1.65 - 3.91 |
| **Ba** | 0.58 ± 0.26 | 0.36 - 0.71 | 0.69 ± 0.36 | 0.43 - 0.84 |
| **Be** | 0.31 ± 0.17 | 0.18 - 0.40 | 0.24 ± 0.14 | 0.10 - 0.35 |
| **Bi** | 0.02 ± 0.01 | 0.01 - 0.03 | 0.02 ± 0.01 | 0.01 - 0.02 |
| **Ca*(*)*** | 68,531 ± 5,265 | 65,238 - 72,245 | 61,378 ± 5,635 | 58,030 - 65,648 |
| **Cd*(*)*** | 0.14 ± 0.08 | 0.07 - 0.19 | 0.10 ± 0.05 | 0.07 - 0.12 |
| **Co*(*)*** | 0.11 ± 0.06 | 0.07 - 0.15 | 0.19 ± 0.10 | 0.11 - 0.25 |
| **Cr** | 0.20 ± 0.09 | 0.14 - 0.25 | 0.18 ± 0.07 | 0.13 - 0.22 |
| **Cu** | 967 ± 247 | 799 - 1,086 | 910 ± 197 | 755 - 1,062 |
| **Fe*(*)*** | 913 ± 429 | 542 - 1,220 | 1,596 ± 442 | 1,299 - 1,892 |
| **Hg*(*)*** | 1.78 ± 0.79 | 1.13 - 2.25 | 1.41 ± 0.73 | 0.95 - 1.72 |
| **Li** | 1.05 ± 0.77 | 0.55 - 1.46 | 1.01 ± 0.75 | 0.60 - 1.55 |
| **Mg*(*)*** | 18,314 ± 2,388 | 1,043 - 19,919 | 17,200 ± 1,781 | 16,466 - 18,550 |
| **Mn** | 0.63 ± 0.22 | 0.45 - 0.76 | 0.65 ± 0.24 | 0.48 - 0.80 |
| **Mo** | 1.18 ± 0.58 | 0.71 - 1.54 | 0.92 ± 0.49 | 0.52 - 1.24 |
| **Ni** | 0.59 ± 0.36 | 0.29 - 0.71 | 0.43 ± 0.36 | 0.17 - 0.56 |
| **Pb** | 0.44 ± 0.27 | 0.22 - 0.67 | 0.52 ± 0.22 | 0.36 - 0.61 |
| **Sb** | 0.10 ± 0.07 | 0.05 - 0.15 | 0.11 ± 0.09 | 0.05 - 0.15 |
| **Si*(*)*** | 223 ± 106 | 163 - 294 | 111 ± 55 | 74.1 - 136 |
| **Sn*(*)*** | 1.32 ± 0.67 | 0.80 - 1.79 | 0.78 ± 0.47 | 0.37 - 1.10 |
| **Sr** | 38.5 ± 14.1 | 29.0 - 49.1 | 38.7 ± 14.7 | 27.7 - 45.4 |
| **Tl** | 0.04 ± 0.02 | 0.03 - 0.06 | 0.05 ± 0.02 | 0.03 - 0.06 |
| **V** | 0.05 ± 0.03 | 0.03 - 0.07 | 0.06 ± 0.03 | 0.04 - 0.07 |
| **W** | 0.03 ± 0.02 | 0.02 - 0.05 | 0.03 ± 0.02 | 0.02 - 0.05 |
| **Zn*(*)*** | 685 ± 112 | 625 - 762 | 813 ± 135 | 704 - 910 |
| **Zr** | 0.14 ± 0.07 | 0.08 - 0.18 | 0.12 ± 0.05 | 0.09 - 0.15 |
| **SOS*(**)*** | 322 ± 31 | 305 - 347 | 273 ± 44 | 243 - 308 |
| **SAC*(**)*** | 321 ± 23 | 308 - 339 | 368 ± 74 | 340 - 378 |

*(*)*: $p \leq 0.05$; *(**)*: $p = 0.001$; SOS: serum oxidative status; SAC: serum anti-oxidant capacity; normal SOS values: 250-300 U.CARR and normal SAC values: > 350 µmol ml-1 of HClO.

inhibiting SOD activity and producing reactive oxygen species [21]. Tin showed enhancements in both serum and blood levels ($p < 0.001$) and this fact should be investigated more deeply to ascertain the organic aliquot of the total Sn, being only the organic species recognized to cause some effects on the brain. As regards the distribution of the major elements, marked unbalances ($p < 0.001$ in all cases) were found as a function of the disease: *i*) a decrement of Zn in serum; *ii)* a depletion of Fe in serum and blood; *iii*) an increment of Cu in blood; *iv)* an increment of Ca, Mg and Si in serum. As regards Zn, a possible role of Zn deficiency in the pathogenesis of AD has been reported and several authors found decreased concentrations in CSF and in serum of AD patients [38, 40]. Our patients had lower Zn and this deficit could account for a minor protection against the oxidative damage, being Zn the constituent of anti-oxidant SOD enzymes. Predominantly, Fe have been described to accumulate in different parts of the brain of AD subjects; this could fairly reflect its reduction in peripheral fluids, as found in our patients. Decreased plasma level of Fe was also found by Basun *et al.* but this evidence was not confirmed by other authors [27, 38]. A slight dysregulation of Ca seems to be present in AD and altered levels of reactive oxygen species and loss of glutathione were found to be dependent on elevated extracellular Ca [15]. Our patients had an increased concentration of serum Ca

**Table 2**. - Element concentrations (ng ml$^{-1}$) in blood of Alzheimer's disease (AD) patients and controls

| Element | AD patients | | Controls | |
|---|---|---|---|---|
| | Mean ± SD | 25$^{th}$-75$^{th}$ | Mean ± SD | 25$^{th}$-75$^{th}$ |
| **Al** | 21.3 ± 19.2 | 7.50 - 30.4 | 18.1 ± 10.8 | 9.20 - 23.1 |
| **Ba** | 1.24 ± 0.65 | 0.71 - 1.68 | 1.32 ± 0.68 | 0.84 - 1.72 |
| **Be** | 0.39 ± 0.18 | 0.27 - 0.52 | 0.41 ± 0.18 | 0.30 - 0.51 |
| **Bi** | 0.04 ± 0.01 | 0.03 - 0.05 | 0.03 ± 0.02 | 0.02 - 0.04 |
| **Ca** | 64,339 ± 7,877 | 59,504 - 69,005 | 62,870 ± 10,051 | 56,947 - 66,388 |
| **Cd** | 1.16 ± 0.79 | 0.58 - 1.68 | 1.06 ± 0.48 | 0.82 - 1.36 |
| **Co** | 0.15 ± 0.07 | 0.11 - 0.18 | 0.12 ± 0.09 | 0.05 - 0.16 |
| **Cr** | 0.63 ± 0.50 | 0.26 - 0.84 | 0.48 ± 0.33 | 0.25 - 0.55 |
| **Cu*(*)*** | 1,453 ± 469 | 1,074 - 1,785 | 946 ± 137 | 869 - 1,030 |
| **Fe*(*)*** | 489,534 ± 108,037 | 425,923 - 558,145 | 547,409 ± 49,050 | 524,453 - 574,841 |
| **Hg*(*)*** | 4.24 ± 3.22 | 1.95 - 6.35 | 5.85 ± 3.18 | 3.58 - 8.05 |
| **Li*(*)*** | 1.19 ± 0.52 | 0.84 - 1.59 | 0.74 ± 0.44 | 0.44 - 0.94 |
| **Mg** | 39,450 ± 7,600 | 34,197 - 43,393 | 40,235 ± 4,967 | 38,092 - 43,134 |
| **Mn*(*)*** | 8.93 ± 3.38 | 6.34 - 11.8 | 7.49 ± 3.48 | 5.41 - 9.49 |
| **Mo*(*)*** | 2.55 ± 1.05 | 1.71 - 3.21 | 3.47 ± 1.35 | 2.48 - 4.34 |
| **Ni** | 1.10 ± 0.72 | 0.57 - 1.44 | 0.97 ± 0.53 | 0.58 - 1.30 |
| **Pb** | 47.3 ± 22.5 | 32.0 - 60.6 | 50.9 ± 20.1 | 32.8 - 64.4 |
| **Sb** | 0.40 ± 0.23 | 0.21 - 0.54 | 0.41 ± 0.25 | 0.23 - 0.58 |
| **Si** | 165 ± 83 | 103 - 210 | 154 ± 63 | 112 - 181 |
| **Sn*(*)*** | 1.99 ± 1.12 | 1.14 - 2.77 | 1.39 ± 0.49 | 1.03 - 1.69 |
| **Sr** | 26.1 ± 10.2 | 18.7 - 32.3 | 28.2 ± 12.3 | 19.9 - 35.9 |
| **Tl** | 0.07 ± 0.05 | 0.03 - 0.09 | 0.08 ± 0.04 | 0.05 - 0.10 |
| **V** | 0.13 ± 0.08 | 0.06 - 0.18 | 0.09 ± 0.05 | 0.06 - 0.13 |
| **W** | 0.06 ± 0.03 | 0.03 - 0.06 | 0.07 ± 0.03 | 0.04 - 0.08 |
| **Zn** | 6,667 ± 1,475 | 5,539 - 7,628 | 6,810 ± 787 | 6,280 - 7,286 |
| **Zr*(*)*** | 0.63 ± 0.36 | 0.29 - 0.88 | 0.48 ± 0.25 | 0.31 - 0.60 |

*(*)*: $p \leq 0.05$

and this support the theory that alterations in Ca homeostasis could provide a marker of AD, as previously stated [41]. Also for Mg higher serum levels were here found, and this could mirror the occurrence of a relative deficit in brain regions during neurodegeneration, elsewhere reported [14, 16]. About Si, Hershey *et al.* found elevated CSF levels in the 80% of AD patients, in the same direction as the present results [42]. Another study reported an accumulation of this element as alumino-silicate complexes in the neurofibrillary tangles of AD patients and proposed that Si reduces the absorption of Al and increases its renal excretion [20]. The high content of blood Cu here found is rather supported by earlier literature [27, 43]. This observation of peripheral Cu alteration is in line with the theory of the involvement of Cu in AD both as catalyst in the free radicals production and by the interaction with the amyloid precursor protein that contains a Cu-binding site. With reference to the oxidative status of subjects, there was evidence that a situation of biological stress incurred in our patients as indicated by the increased SOS values respect to those of the control group ($p \leq 0.001$) (see Table 1). It must be taken in mind that the range for normal condition was 250-300 U.CARR, while higher values indicated a clear oxidative damage. Moreover, the antioxidant capacity was found to be lower in AD than in controls ($p \leq 0.001$) (normal condition, values > 350 μmol ml$^{-1}$ of HClO). This combination of augmented free oxidant species and depleted natural defence mechanisms leads to a clear condition of "oxidative stress" in our patients. Probably, the unbalances in neurotoxic elements (Al, Cd, Hg and Mn) in combination with not normal essential element concentrations (Ca, Cu, Fe, Mg and

Zn) found in our patients could favourite a greater likelihood for generation of free radical species or for decrement in antioxidant defences.

These results were confirmed by the SDA applied on the 26 independent variables. A little set of them resulted to have a discriminant role. In fact, six variables in serum, i.e., Al, Ca, Co, Si, SOS and SAC, considered together in the discriminant function, were able to appropriately classify 94% of cases and 92% after cross-validation. Aluminium, Ca, Co, Si and SOS had standardized coefficients > 0.30 and therefore contributed most to the prediction of group membership. In the case of blood, the variables Cu, Fe, Mo and Zr were selected by the model and all of them showed their good contribution to the categorization (standardized coefficients > 0.4). The discriminant function correctly classified 90% of cases (88% using a cross-validated method). The results obtained for blood would confirm the unbalance in essential elements such as Cu and Fe, whose role in the pathology has been early discussed. These data suggest that the SDA, together with other statistical procedures, could be successfully applied to study the AD also combining environmental factors and life styles, in order to grasp a more strict linkage between them.

## Conclusions

The present data sustain the mounting evidence of metals implication in AD. It is also confirmed that changes in the oxidative metabolism are primary aspects in AD neurodegeneration. There are reasons to suppose that a change in a single metal ion concentration is not restricted to this element but will affect the whole elemental distribution pattern. The possibility to use the levels of metals and the detection of oxidative stress at peripheral level might allow to manage studies aimed not only to therapeutic purposes but also to primary prevention.

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