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Correlation between metal ions and clinical findings in subjects affected by Alzheimer's disease

Giuseppe BOMBOI *(a)*, Francesca MARCHIONE *(a)*, Micaela SEPE-MONTI *(a)*, Antonella DE CAROLIS *(a)*, Valentina BIANCHI *(a)*, Emanuela MEDDA *(b)*, Anna PINO *(c)*, Beatrice BOCCA *(c)*, Giovanni FORTE *(c)*, Cristina D'IPPOLITO *(b)* and Franco GIUBILEI *(a)*

(a) Dipartimento di Neurologia, Ospedale S. Andrea, Università degli Studi "La Sapienza", Rome, Italy
(b) Centro Nazionale di Epidemiologia, Sorveglianza e Promozione della Salute;
(c) Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Rome, Italy

Summary. - There is a growing interest to evaluate metals in biological fluids in Alzheimer's disease (AD). There are numerous studies on this theme, but just few papers analyzed the relationship between haematic metal concentrations and the clinical features of the disease. In this study, possible associations between clinical features of AD and the variations in serum and blood concentration of some metals, as well as the serum oxidative status and the antioxidant capacity have been investigated. Sixty subjects with AD were enrolled. Some elements correlated with gender, depression and duration of the disease. However, the most significant result was the relationship between blood Ca and Fe levels and the severity of cognitive impairment. We hypothesize that Ca and Fe might play an important role in the pathogenetic mechanisms of AD.

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

Riassunto (*Correlazione tra ioni metallici ed aspetti clinici in soggetti con malattia di Alzheimer).* - C'è vivo interesse nel valutare i metalli nei fluidi biologici nella malattia di Alzheimer (AD). Esistono numerosi studi sull'argomento, ma solo pochi lavori hanno messo in relazione la concentrazione ematica dei metalli con gli aspetti clinici della malattia. In questo studio, abbiamo cercato di individuare possibili relazioni degli aspetti clinici della AD con le variazioni della concentrazione nel siero e nel sangue di alcuni metalli, nonché con lo stato ossidativo e la capacità antiossidante del siero. Sessanta soggetti con AD sono stati inclusi. Abbiamo trovato che alcuni elementi correlano con il sesso, la depressione e la durata della sintomatologia. Il dato più significativo è stato, comunque, la correlazione tra i livelli ematici di Ca e Fe e la gravità del deterioramento cognitivo. La nostra ipotesi è che il Ca ed il Fe possano svolgere un importante ruolo nella patogenesi della AD.

Parole chiave: malattia di Alzheimer, metalli, siero, sangue.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in aged population [1, 2]. Researches in the fields of genetic and molecular biology have produced many findings about this common disease [3]. However, its pathogenetic mechanisms remain unclear. The main clinical features of this neurodegenerative disorder are loss of memory and cognitive decline, which are the consequence of an important and progressive neuronal degeneration from various regions of the brain [4]. *Post mortem* neuropathological studies of affected AD brain show neurofibrillary tangles and senile plaques [5, 6]. Neurofibrillary tangles are cytoskeletal alterations resulting from the formation of an abnormally phosphorylated and aggregated τ protein within a few susceptible classes of neurons. In healthy nerve cells, the τ protein stabilizes microtubular components of the neuronal cytoskeleton that are involved in transporting substances between cellular compartments. Destabilization of the microtubules and obstruction of axonal transport owing to the formation of abnormal τ protein

Indirizzo per la corrispondenza (Address for correspondence): Franco Giubilei, Dipartimento di Neurologia, Ospedale S. Andrea, Università degli Studi "La Sapienza",Via di Grottarossa 1035-1039, 00189 Rome, Italy. E-mail: franco.giubilei@uniroma1.it.

probably result in inappropriate protein metabolism, synaptic malfunction, and impaired signalling by retrograde neurotrophic factors. Decline in these functions may contribute significantly to neuronal death. The initial product of the pathological phosphorylation is a soluble nonargyrophilic τ protein.

The other neuropathological feature, the senile plaque, is mainly composed by amyloid β-protein (Aβ), a peptide derived from the larger amyloid β-protein precursor (APP), an ubiquitously expressed transmembrane glycoprotein [7]. The "amyloid cascade" theory, which has dominated AD research for the past 20 years, proposes that Aβ is protein junk that spontaneously self-aggregates into amyloid fibrils that are somehow neurotoxic and cause dementia. The longer forms of Aβ (mainly Aβ 1-42) are regarded as particularly pathogenetic because they are overproduced as a result of familial gene mutations, and are more apparently self-aggregating than are shorter forms (e.g., Aβ 1-40) in *in vitro* studies [8, 9]. Although this amyloid hypothesis is feasible, it is still patently incomplete, particularly because there is still no proper understanding of the relations between amyloidogenesis and the development of the neurofibrillary tangles [10]. In particular, the self-aggregation hypothesis leaves important questions about the biology of AD unanswered. It is unknown, for example, why an ubiquitous protein, like Aβ, only precipitates in neocortex or why other mammalian species don't develop Aβ neuropathology with advancing age [11]. It is not clear, furthermore, why age and gender (elderly and female) are major risk factors. Chemical elements might play a role in these processes [12-14]. In fact, recently, neurochemical studies have suggested a role for metals in neurodegenerative disorders such as AD [15-20]. In particular, metals might play a major catalytic role in the production of free radicals [21, 22]. Considerable evidence is mounting that dyshomeostasis of the redox-active biometals and oxidative stress contribute to the neuropathology of AD [23]. A lot of data suggest that metals can interact directly with Aβ peptide [24]. The binding of metals to Aβ modulates several physiochemical properties of Aβ that are thought to be central to the pathogenetic process of the peptide [25]. In particular, studying the metallochemical properties, Bush has suggested an AD model that gives explanations to a lot of questions about the amyloid cascade theory [26]. According to Bush *et al.,* Zn seems to be the major neurochemical factor responsible for aggregating Aβ [27, 28]. Copper and Fe, instead, induce limited Aβ aggregation, which is exaggerated by slightly acid conditions. All these metals are constitutively found at high levels in the neocortical regions most prone to AD pathology, where they play important roles in normal physiology. Copper and Zn are released during neurotransmission. In particular, Zn release during synaptic transmission induces β-amyloid deposition in transgenic mice models for AD [29]. Less is currently known about extracellular Cu in the brain, but *in vitro* evidence suggests that cortical tissue depolarization releases Cu to achieve micromolar concentrations [30]. However, many studies imply a crucial role for Cu and Fe in Aβ aggregation [31]. Copper and Fe interaction with Aβ mediates the toxicity of the peptide in cell culture. Aβ catalyses H_2O_2 generation through the reduction of Cu and Fe, using O_2 and biological reducing agents (e.g., cholesterol, vitamin C, catecholamines) as substrates. According to these evidences, Bush *et al.* have hypothesized that Aβ and APP become corrupted in the biochemistry of their functions: to participate in metal-ion homeostasis and to control metal-ion-mediated oxidation [32]. APP has Cu- and Zn-binding sites in its N-terminal ectodomain, which modulate the adhesivity of the protein and the transfer of Cu [32, 33]. The exceptionally high affinity of Aβ for Cu and the selectivity of the Cu- and Zn-binding sites are compatible with physiological metal-ion binding. Aβ and APP might also participate in normal metal-ion homeostasis [34]. An inevitable age-dependent rise in cerebral Cu and Fe might hypermetallate the Aβ peptide, causing the catalysis of H_2O_2 production that mediates the toxicity and auto-oxidation of Aβ. The greater incidence of AD in females could be due to greater constitutive activity of the synaptic Zn transporter in this gender. On these bases, a new therapeutic strategy might include compounds that interdict metal-ion binding to Aβ reducing in this way the amyloid aggregation.

Despite the numerous pathophysiological researches, there are very few studies *in vivo* that have assessed the relation between the level of metal ions in serum and blood and the clinical status of AD patients.

The goal of our study is to evaluate the serum and blood concentration of metal ions in subjects affected by probable AD, as well as their oxidative status and anti-oxidant defences, and to correlate these values with the demographic and clinical features of the disease.

Materials and methods

Sixty subjects (40 females and 20 males; mean age ± SD, 74.6 ± 6.39, range 58-86 yrs) with a diagnosis of probable AD according to the NINCS-ADRDA criteria were evaluated [35]. The duration of the disease was calculated retrospectively from the onset of memory impairment, that was the first impaired cognitive domain in all the enrolled subjects. The mean duration of disease ± SD was 3.9 ± 2.0 yrs, range 10 months - 9 yrs. All subjects were consecutively recruited from our hospital

outpatient Memory Clinic from September 2003 to July 2004. The severity of cognitive impairment was quantified using the mini mental state examination (MMSE), corrected according to age and education [36]. The mean MMSE score ± SD was 17.12 ± 6.64 (range 28-2). Subjects with MMSE score > 23 were considered as affected from mild AD, while the subjects with MMSE score ≤ 23 were considered as affected from moderate to severe AD. All subjects underwent the screening for dementia, including a neuropsychological battery composed by the following tests: token, Raven, prose memory, phonological and semantic fluency, visual search and constructional apraxia. Mood was evaluated by a questionnaire that showed the presence/absence of depression. All the AD patients underwent a brain neuroimaging evaluation (MRI or brain CT). Patients and/or responsible caregivers gave informed consent for participation in the study.

For each subject, a venous blood sample was drawn with polyethylene syringes and transferred into 15 ml polystyrene tubes. Prior to analyses, serum was simply diluted with deionized water, while blood was subjected to an acid-assisted microwave digestion, as previously detailed [16, 37]. Analyses were performed by inductively coupled plasma atomic optical emission spectrometry for the quantification of Ca, Cu, Fe, Mg, Si and Zn and by sector field inductively coupled plasma mass spectrometry for the determination of Al, Ba, Be, Bi, Cd, Co, Cr, Hg, Li, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W and Zr [16, 37].

The serum oxidant status (SOS) and the anti-oxidant capacity (SAC) were determined by mean of a photometric system [15, 16]. The SOS was expressed in U.CARR, where 1 U.CARR corresponded to 0.08 mg of peroxyl radicals for 100 ml of hydrogen peroxide. The SAC was expressed in μmol ml^{-1} of HClO. The log-normal transformation was used for not normally distributed data and differences between mean values were evaluated by the Student's *t*-test.

Results

Serum Ca concentration showed significant differences between females and males (67,414 ± 5,171 *vs* 70,763 ± 4,861 ng ml^{-1}; $p = 0.04$). No difference was found in the serum concentration of the other elements in both genders (Table 1). Serum level of Be, Sr and V were significantly different in over 75 yrs old subjects compared to younger subjects (Be, 0.23 ± 0.12 *vs* 0.39 ± 0.19 ng ml^{-1}, $p = 0.001$; Sr, 42.2 ± 14.0 *vs* 34.7 ± 13.5 ng ml^{-1}, $p = 0.05$; V, 0.07 ± 0.04 *vs* 0.03 ± 0.01 ng ml^{-1}, $p = 0.001$). Mild AD subjects showed a lower serum concentration of Cd and Mo as well as a higher serum concentration of Mn than subjects with moderate-severe AD (Table 2). Depressed AD subjects had a significant higher Cd and Zr serum concentration compared to AD subjects without altered mood (Cd, 0.16 ± 0.08 *vs* 0.11 ± 0.07 ng ml^{-1}, $p = 0.03$; Zr, 0.15 ± 0.08 *vs* 0.11 ± 0.05 ng ml^{-1}, $p = 0.05$). On the contrary, SAC was significantly lower in depressed AD subjects (315 ± 24 *vs* 330 ± 20 ng ml^{-1}, $p = 0.02$). Finally, the serum Co and Sn level increased in subjects with a longer duration of disease (Co, 0.09 ± 0.05 *vs* 0.12 ± 0.06 ng ml^{-1}; $p = 0.04$; Sn, 0.94 ± 0.57 *vs* 1.45 ± 0.67 ng ml^{-1}; $p = 0.001$). With regards to blood, Al, Pb and Zn levels were significantly different in the two genders: females had a lower concentration of these ions compared to males (Table 3). Furthermore, blood concentration of Be, Si and Sr significantly differed in subjects over 75 yrs old respect to the youngest ones. In fact, blood levels of Si and Sr were higher in subjects over 75 yrs old (Si, 190 ± 92 *vs* 143 ± 66 ng ml^{-1}, $p = 0.04$; Sr, 29.3 ± 10.1 *vs* 23.1 ± 9.6 ng ml^{-1}, $p = 0.01$), whereas blood Be levels were lower (0.33 ± 0.16 *vs* 0.46 ± 0.19 ng ml^{-1}, $p = 0.007$). Mild AD subjects showed a different Ca and Fe blood levels compared to moderate to severe AD subjects (Table 4). Depressed AD subjects had lower Al and Sn blood concentrations than AD subjects without psychiatric disorders (Al, 14.2 ± 16.6 *vs* 26.0 ± 19.7 ng ml^{-1}, $p = 0.019$; Sn, 1.53 ± 1.05 *vs*. 2.29 ± 1.08 ng ml^{-1}, $p = 0.015$). Finally, AD subjects with a longer duration of disease showed a decrease in blood Ca, Fe, Mg, Zn, W and Zr concentrations than subjects with a shorter duration (Ca, 69,513 ± 8,355 *vs* 62,879 ± 7,041 ng ml^{-1}, $p = 0.007$; Fe, 558,884 ± 103,983 *vs* 465,482 ± 101,630 ng ml^{-1}, $p = 0.004$; Mg, 45,674 ± 8,052 *vs* 37,289 ± 6,357 ng ml^{-1}, $p = 0.001$; Zn, 7,449 ± 1,358 *vs* 6,384 ± 1,455 ng ml^{-1}, $p = 0.01$; W, 0.07 ± 0.03 *vs* 0.05 ± 0.03 ng ml^{-1}, $p = 0.006$; Zr, 0.86 ± 0.45 *vs* 0.55 ± 0.29 ng ml^{-1}, $p = 0.02$).

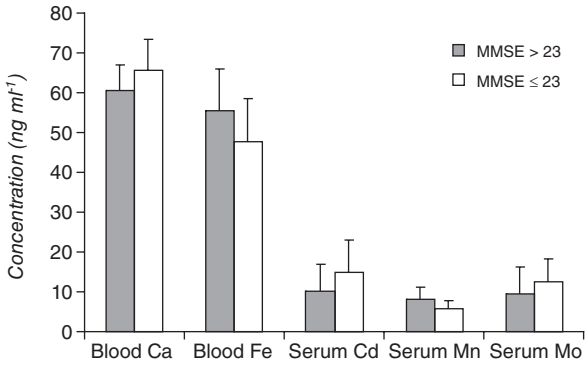

Fig. 1. - Haematological metal concentration (mean ± SD) in AD as a function of the MMSE score ($p \leq 0.05$). Values for Ca should be multiplied for 10^3 and Fe multiplied for 10^4; Cd should be divided for 100, and Mn and Mo for 10. MMSE: mini mental state examination.

Table 1. - Metals (ng ml^{-1}), SOS (U. CARR) and SAC (µmol ml^{-1} HClO) in serum of Alzheimer's disease (AD) subjects according to gender

Element	Females (no. 36)		Males (no. 17)		
	Mean	SD	Mean	SD	*p* value
Al	2.24	1.10	2.18	1.57	n.s.
Ba	0.53	0.21	0.68	0.34	n.s.
Be	0.30	0.18	0.33	0.18	n.s.
Bi	0.02	0.01	0.02	0.01	n.s.
Ca	67,414	5,171	70,764	4,861	0.04
Cd	0.14	0.08	0.13	0.07	n.s.
Co	0.12	0.05	0.11	0.07	n.s.
Cr	0.21	0.09	0.20	0.08	n.s.
Cu	9.88	2.42	925	259	n.s.
Fe	865	405	1.003	470	n.s.
Hg	1.68	0.76	2.01	0.84	n.s.
Li	1.10	0.85	0.91	0.54	n.s.
Mg	18,201	2,434	18,553	2,341	n.s.
Mn	0.63	0.20	0.63	0.26	n.s.
Mo	1.12	0.54	1.14	0.70	n.s.
Ni	0.54	0.32	0.70	0.42	n.s.
Pb	0.46	0.28	0.42	0.27	n.s.
Sb	0.11	0.07	0.09	0.07	n.s.
Si	214	100	250	121	n.s.
Sn	1.26	0.68	1.44	0.67	n.s.
Sr	37.9	14.3	39.8	14.0	n.s.
Tl	0.04	0.02	0.05	0.02	n.s.
V	0.05	0.03	0.05	0.03	n.s.
W	0.03	0.02	0.03	0.02	n.s.
Zn	673	115	709	108	n.s.
Zr	0.13	0.06	0.16	0.09	n.s.
SOS(*)	325	29	315	36	n.s.
SAC(**)	317	24	327	21	n.s.

n.s.: not significant; *(*)*: normal values of SOS: 250-300 U.CARR; *(**)*: normal values of SAC: > 350 µmol ml^{-1} of HClO; SOS: serum oxidative status; SAC: serum antioxidant capacity.

The relationship between cognitive status evaluated by MMSE score and ions concentration is shown in Fig. 1.

Discussion

The results of the present study show that there is a correlation between both clinical and demographic features of AD and the blood and serum concentration of some metal ions. In particular, blood Ca concentrations are directly, whereas blood Fe and serum Mn levels are inversely, correlated with the severity of cognitive impairment evaluated by MMSE. These findings are in according to previous results that reported an important role for these ions in the pathogenetic mechanisms of AD [25, 27, 38].

Although Fe has an important role in the normal physiological process, it was found at high levels in the neocortical regions of AD subjects. In normal situations, Fe induces limited Aβ aggregation, which is instead exaggerated by acid conditions. This interaction between Fe and Aβ mediates the toxicity of the peptide in cell culture. In fact, Aβ catalyses H_2O_2 generation through the reduction of Fe, using O_2 and biological reducing agents as substrates.

Table 2. - Metals (ng ml^{-1}), SOS (U. CARR) and SAC (μmol ml^{-1} HClO) in serum of subjects affected by mild (MMSE score > 23) and moderate-severe (MMSE score ≤ 23) Alzheimer's disease (AD)

Element	Mild (no. 8)		Moderate-severe (no. 43)		
	Mean	SD	Mean	SD	*p* value
Al	2.56	1.82	2.20	1.15	n.s.
Ba	0.69	0.30	0.55	0.25	n.s.
Be	0.28	0.17	0.32	0.18	n.s.
Bi	0.02	0.01	0.02	0.01	n.s.
Ca	72,459	4,961	68,173	5,159	n.s.
Cd	0.10	0.07	0.15	0.08	0.05
Co	0.08	0.04	0.12	0.06	0.06
Cr	0.19	0.09	0.21	0.09	n.s.
Cu	898	255	990	246	n.s.
Fe	1,044	382	883	437	n.s.
Hg	1.69	1.02	1.82	0.77	n.s.
Li	1.21	0.90	1.03	0.76	n.s.
Mg	19,195	3,183	18,179	2,264	n.s.
Mn	0.81	0.29	0.59	0.20	0.02
Mo	0.94	0.67	1.25	0.56	0.03
Ni	0.73	0.29	0.56	0.38	n.s.
Pb	0.50	0.29	0.43	0.27	n.s.
Sb	0.06	0.02	0.11	0.07	n.s.
Si	240	82.8	221	110	n.s.
Sn	1.40	0.65	1.33	0.69	n.s.
Sr	39.2	11.3	39.2	14.6	n.s.
Tl	0.04	0.02	0.04	0.02	n.s.
V	0.06	0.04	0.05	0.03	n.s.
W	0.03	0.02	0.03	0.02	n.s.
Zn	744	137	679	107	n.s.
Zr	0.14	0.09	0.14	0.07	n.s.
SAC	335	18	317	24	0.06
SOS	316	16	322	34	n.s.

n.s.: not significant; SOS: serum oxidative status; SAC: serum antioxidant capacity; MMSE: mini mental state examination.

The Ca hypothesis of neurodegenerative disorders, such as AD, suggested that altered cytosolic Ca concentrations and/or alterations in Ca homeostasis concerned cellular mechanisms underlying neuronal pathology. A previous study suggested that the measurement of Ca homeostasis could provide a very sensitive marker of AD [39]. Another research showed that Ca plays a pivotal role in mediating many important biological functions. The intracellular Ca concentration is regulated by a variety of systems and mechanisms. Alterations in Ca homeostasis might be critically implicated in brain aging and in the neuropathology of AD. In fact, one of the postulated mechanisms of Aβ toxicity seems to involve a Ca dysregulation accompanied with enhanced vulnerability to excitotoxic stimuli. According to this research, Ca disturbances and associated phenomena might represent an important approach to explore the cellular pathophysiology of AD [40]. The increase of Ca blood concentrations, as found in this study, might reflect a signal of this cerebral homeostasis alteration.

Other factors might have influenced the concentration of Ca and Fe, such as intakes, lifestyle, and drug administration. It is probable that the intakes of Fe might be reduced in severe AD because of lack of appetite and of behavioural disorders, such as delusions that are very

Table 3. - Metals (ng ml^{-1}) in blood of Alzheimer's disease (AD) subjects according to gender

Element	Females (no. 40)		Males (no. 20)		
	Mean	SD	Mean	SD	*p* value
Al	16.3	15.1	30.7	22.9	0.04
Ba	1.21	0.59	1.29	0.78	n.s.
Be	0.41	0.18	0.37	0.18	n.s.
Bi	0.04	0.01	0.04	0.01	n.s.
Ca	64,549	8,855	63,928	5,685	n.s.
Cd	1.04	0.73	1.39	0.89	n.s.
Co	0.15	0.07	0.14	0.07	n.s.
Cr	0.58	0.49	0.74	0.53	n.s.
Cu	1,486	486	1,387	440	n.s.
Fe	474,422	91,927	519,756	132,122	n.s.
Hg	3.90	3.36	4.96	2.86	n.s.
Li	1.20	0.49	1.19	0.60	n.s.
Mg	39,291	7,647	39,766	7,635	n.s.
Mn	9.01	3.22	8.76	3.77	n.s.
Mo	2.49	0.98	2.67	1.19	n.s.
Ni	1.14	0.79	1.03	0.58	n.s.
Pb	41.2	18.8	62.4	24.3	0.001
Sb	0.42	0.25	0.37	0.19	n.s.
Si	160	86	176	76	n.s.
Sn	1.93	1.09	214	1.20	n.s.
Sr	25.4	10.8	27.7	9.01	n.s.
Tl	0.06	0.04	0.08	0.05	n.s.
V	0.13	0.09	0.14	0.07	n.s.
W	0.05	0.03	0.06	0.03	n.s.
Zn	6,335	1,203	7,366	1,764	0.02
Zr	0.58	0.32	0.73	0.42	n.s.

n.s.: not significant.

common in the advanced phase of this disease. The immobilization and institutionalization that are common features of these subjects could amplify the above mentioned behavioural disturbances and, thus, influence ions homeostasis. Moreover, it is well-known that aging is one of the most important causes of Ca and Fe homeostasis alterations: in fact, endocrinological dysfunctions may occur in old people and these events might have influence on metal ion homeostasis.

In addition, we found that other metal ions are related to clinical and demographic features of AD. However, these findings are hard to interpret at the current knowledge of the implication of metals in this pathology. As regards the gender, females had lower values of Al, Ca, Pb and Zn than males, but no literature data supported these evidences. Finally, the oxidative metabolism changed in AD when compared with the healthy status, in line with previous observations [41]. In particular, excessive lipo- and hydro-peroxides levels have been found in our patients, together with a not sufficient antioxidant capacity. This more and more confirms the key role of the oxidative stress in the pathology of AD. Instead, no difference between the markers of the oxidative stress and demographic or clinical features of patients were found, except for a lower SAC values in depressed patients.

In conclusion, despite the speculation on the ability of peripheral markers to reflect changes that occur in the brain or on the possibility that ion alterations are an epiphenomenon of the AD, our data strongly suggest that Ca and Fe are involved during the pathogenetic mechanism of the disease. However, further studies are warranted to better understand the relationship between AD clinical features and alterations in metal ions, also considering lifestyle of subjects and their drug intake.

Table 4. - Metals (ng ml^{-1}) in blood of subjects affected by mild (MMSE score > 23) and moderate-severe (MMSE score ≤ 23) Alzheimer's disease (AD)

Element	Mild (no. 8)		Moderate-severe (no. 43)		
	Mean	SD	Mean	SD	*p* value
Al	24.9	25.4	21.2	17.9	n.s.
Ba	1.03	0.55	1.30	0.68	n.s.
Be	0.35	0.17	0.41	0.18	n.s.
Bi	0.04	0.01	0.04	0.01	n.s.
Ca	60,323	6,672	65,454	7,631	0.05
Cd	1.30	0.72	1.17	0.81	n.s.
Co	0.16	0.08	0.14	0.07	n.s.
Cr	0.67	0.33	0.63	0.54	n.s.
Cu	1,295	407	1,507	475	n.s.
Fe	552,606	104,172	477,498	107,455	0.05
Hg	5.87	3.79	3.91	3.12	0.07
Li	1.14	0.51	1.23	0.53	n.s.
Mg	42,166	8,342	38,845	7,552	n.s.
Mn	9.37	3.27	8.89	3.46	n.s.
Mo	2.72	1.17	2.55	1.03	n.s.
Ni	0.99	0.56	1.14	0.75	n.s.
Pb	51.4	17.2	46.7	23.9	n.s.
Sb	0.47	0.29	0.39	0.23	n.s.
Si	179	75.1	163	86	n.s.
Sn	1.76	1.22	2.07	1.10	n.s.
Sr	27.8	7.2	26.3	10.7	n.s.
Tl	0.07	0.04	0.07	0.04	n.s.
V	0.14	0.07	0.13	0.09	n.s.
W	0.07	0.03	0.05	0.03	n.s.
Zn	6,898	1,595	6,664	1,459	n.s.
Zr	0.70	0.42	0.62	0.35	n.s.

n.s.: not significant; MMSE: mini mental state examination.

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