Low aluminum levels in the human brain from controls and Alzheimer patients.

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Abstract

Aluminum is frequently mentioned as a potential risk factor of Alzheimer's disease (AD). There is an important literature related to this problem, with pros and cons, and this is still a matter of debate. Our goal was to address the problem directly and quantify this metal in the patient's brain from a prospective study that was fully characterized at the clinical, neuropathological and biochemical levels. In particular, the two degenerating processes that characterize AD were staged with a reliable and simple method, based on the quantification of pathological tau proteins and aggregated Abeta x-42 species. Moreover, we split the group of non-demented patients into two subgroups according to the absence or presence of Abeta deposits, corresponding to "controls" and incipient (prodromal) AD, respectively. Aluminum as well as other metals were quantified by two methods that are sensitive and specific: inductively coupled plasma (ICP) mass spectrometry and ICP- absorption emission spectrometry. The frontal cortices of 49 cases were analyzed. Results show that the levels of aluminum in the brain of controls are lower than expected, less than 1 microgram per gram of tissue, and are not significantly increased for degenerative disorders, including Alzheimer's disease.

Introduction

Aluminum is an abundant metal on earth, found in our food and in the kitchen, for cooking or storing. It has been suspected to be a risk factor for Alzheimer's disease (AD) a long time ago, when the disease was unknown at the molecular level. This is still a matter of debate, as is the etiology of AD (Wisniewski et al. 1966); (Edwardson et al. 1986); (Kasa et al. 1995); (Reusche et al. 2001); (Bush 2003) ; (House et al. 2004) ; (Zatta et al. 2003). Progress of analytical methodologies for the quantification of metals in biological tissues and progressive understanding of AD have led to modulation and adaptations of the "Aluminum" theory, with pros and cons. In fact the main obstacle to understand the role of aluminum salts as risk factors in AD has been the lack of a direct approach of the problem, generating possible pitfalls and uncertain conclusions.

Our present knowledge about AD shows we are dealing with a complex disease. This has opened the way for all types of theories related to risk factors.

Let us recall some fundamental facts. First of all, we know the molecular substrates of the two lesions that characterize AD:

1) amyloid plaques in the extra-cellular space of the cortical grey matter, resulting from the aggregation of a 39 to 43 amino-acid long peptide named Aβ (amyloid beta).

2) aggregated tau proteins that assemble into paired helical filaments in degenerating neurons. These two types of lesions reveal that AD is a complex neurodegenerative disorder, affected by two simultaneous, different but synergetic degenerating processes: Aβ and tau pathologies (Delacourte et al. 2002).

However, the sequence of events explaining AD etiology is still a matter of debate. Indeed, most of the molecular studies deal with the familial autosomic dominant (FAD) form of AD-which accounts for less than 0.5% of all cases (Campion et al. 1999). FAD mutations found on APP, the precursor of Aβ, or on presenilins (which cleave APP to generate Aβ), increase Aβ x-42 levels, and are pathogenic. These observations have generated the amyloid cascade hypothesis as the central cause of the disease (Hardy and Selkoe 2002). But the 99,5 % other AD cases, named "sporadic" cases exhibit neither mutations nor overexpression of Aβ.

Second, the correct and consensus criteria for a definite diagnosis of AD at the neuropathological level were published only in 1997, adding the quantification of tau pathology as an indispensable additional criterion, as defined by the Braak staging (Consensus 1997).

Third, and in the same way, models at our disposal are based mainly on transfected cells or transgenic mice with APP and presenilin mutations. They only reflect amyloidosis and are not fully relevant of

AD, since tau pathology is not observed. Therefore, neurotoxic events generated by high concentration of aluminum salts on these models do not guarantee a possible relevance to the disease.

Fourth, and most importantly, the biochemical quantification of brain lesions shows that the Alzheimer degenerating process may have been ongoing several decades before the clinical expression. Therefore a comparison between controls (non-demented patients) and AD (demented) patients is not appropriate to check a risk factor. Indeed, non-demented aged "controls" are frequently affected by an Alzheimer degenerating process. Therefore, a precise and sensitive detection of Aβ and tau pathologies in non-demented patients is absolutely necessary to select perfect controls (Delacourte et al. 2002).

At last, we demonstrated that the precise quantification of lesions, a necessary step to quantify the degenerating process and its possible relationship with aluminum salts, is strongly influenced by the choice of antibodies. Indeed, we showed that amyloid deposits at the first step of AD are not composed of Aβ 40 aggregates but instead of Aβ 42 species (Delacourte et al. 2002), that are in addition truncated in their amino-terminal part (Sergeant et al. 2003).

As a conclusion, we claim that the impact of an AD risk factor such as aluminum must be analyzed in the human brain tissue of numerous cases, demented and non-demented, of different ages, from a prospective and multidisciplinary approach. This ideal framework guarantees the quality of collected data at the clinical, neuropathological, biochemical and chemical levels.

Thanks to a fruitful collaboration with clinicians from Lille Hospital, we have been able to carry out such an approach, and to precisely quantify aluminum and other metals in the brain of patients with Alzheimer's disease versus controls.

Material and methods

Patients.

The 49 analyzed patients were randomly chosen among the 160 cases of our brain bank to represent 4 groups, as defined in Delacourte et al, 2002 (table I).

Quantification of Tau pathology :

Pathological tau proteins were semi-quantified, as described in Delacourte et al, 1999. They were detected and quantified on western blots, using a Pharmacia densitometric method. They were expressed as an arbitrary value in comparison to a « classical AD case » used as a positive standard (arbitrary value of 10), and a control without tau pathology as a negative control, as described in Delacourte et al, 1999.

Quantification of amyloid deposits :

Amyloid peptides were quantified by western blots, after their extraction by formic acid, as described in Delacourte et al, 2002. They were expressed in micrograms/ gram of tissue, in comparison to a synthetic Aβ 1-42 peptide (Biochem). In order to simplify the interpretation of the results, we also consider the following staging for the quantification of either Aβ 40 or Aβ x-42 aggregates that results from our biochemical analysis of Aβ species, as described in Delacourte et al, 2002:

Aβ quantification (μg/g tissue)	Stage
Trace < 2.5	1
2.5 to 5	2
5 to 10	3
10 to 25	4
25 to 50	5
50 to 100	6
100 to 200	7
200 to 400	8
400 to 800	9
Over 800	10

Each patient is ranked for Aβ deposits as Stage X for Aβ x-42 ; Stage Y for Aβ 1-40

Brain tissue samples :

They were dissected using teflon tools. Brain were stored at –80°C as well as samples that are stored before mineralization. Dissection of the tissue was undertaken so that the periphery of the brain tissue (meninges) was removed to avoid a possible contamination. The tissue sample was at the center of large pieces of neocortex.

Definition of groups:

The brain tissue from one young patient was used as a control to set up both ICP-AES and ICP-MS techniques and to check the reproducibility of these techniques. This patient was chosen because of the absence of tau and Aβ lesions.

The other groups were aged–matched in comparison to AD patients, and were therefore mostly older than 70 years old.

Group 1 corresponded to aged patients with a mild tau pathology but no Aβ deposits

Group 2 corresponded to infraclinical stages of AD (non-demented patients, or with mild cognitive impairment, with low to moderate levels of Aβ deposits and tau involvement below stage 7

Group 3: clinical AD patients (high level of Aβ and tau staging above 7

Group 4: patients with other neurodegenerative disorders (vascular dementia, Lewy body dementia (LBD), progressive supranuclear palsy (PSP) , frontotemporal dementia).

Tissue handling:

Half brains of our brain bank were stored in plastic boxes at -80°C. Brain samples were processed to avoid any possible contamination by metals. The frontal cortex was chosen, because this region is the most informative one for a diagnosis of a neurodegenerative disorder, and this brain area is spared in normal aging. Dissection and sampling were performed with teflon tools. The organic matrix of the brain tissue was solubilized in high quality grade nitric acid (Supra Pure nitric acid from Merck). A large piece of frontal cortex was removed (4g). The inner part of the neocortex was put in screwed teflon vials, weighted (150 mg approximatively) and homogenized in 6 ml of pure nitric acid. The solution was digested using an open-vessel hot plate at 150 °C for 2 hours until total digestion, cooled at room temperature and the resulting brain tissue lysate was diluted with MilliQ ultrapure deionised water (Millipore, USA) to a final volume of 10 ml prior to ICP metals analysis.

Different attempts led us to use 6 ml of nitric acid to mineralize 150 mg of brain tissue. The treatment was performed at 150°C during 1h30, in specific teflon potters. Then pure water was added to a total volume of 10 ml. This dilution is compatible with both analyses.

Concentration of metals in the brain tissue:

The results of metal concentrations are expressed in μg/g or mg/g of brain tissue.

Metal analyses

Aluminum and other metals were quantified using two reliable and sensitive methods:

- ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) (VARIAN Liberty Series II axial view) allowed to detect with an excellent sensitivity only one metal. Analyzed metals were Al, Ca, Cu, Fe, K, Mg, Na, P, Zn. Standards for calibration were those suggested by the manufacturer.

- ICP-MS (inductively coupled plasma mass spectrometry) (THERMO Electron Corporation X7 series) analyzed 20 different metals simultaneously. Quantified metals were: Li, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Sn, Sb, Pb. Semi-quantification was also performed for the following metals: Li, Be, B, Na, Mg, Al, Si, P, S, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, Ln, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Bkg, Th, U. For the instrument calibration, a mixture of metal standard solution (from 1 to 10 μg/l) was used. Concentration determined for each metal is the mean of three simultaneous analyses.

Reproducibility of these techniques was investigated by analyzing the same sample several times in one experiment and the same brain tissue sample was processed several times for each individual experiment, all along the study. A blank sample (nitric acid and water) was used to determine the baseline and contaminating signals were taken into account for the calculation of the concentration values.

Statistical analysis

Concentration of metals is given in microgram/gram of wet tissue. Statview and SPSS software were used for statistical analysis.

Results

Results obtained with ICP-AES and ICP-MS are reported in table II.

Reproducibility : Patient Dc.28, a young patient with neither tau nor amyloid pathology, was analyzed several times with ICP- AES and MS. Variation was low, below 5%, on different experiments which were performed on the same day and on experiments performed a week or a month later, and therefore using a new calibration for instruments. Reproducibility was correct for aluminum and all other metals.

Analysis of confounding factors

Age and post-mortem delay were two possible causes for bias in the study. This was not the case, in our data for most of the metals.

Post-mortem delay: No correlation was observed between post-mortem delay and metal levels, including aluminum except for Mn, Zn and Mg. These three metals were found to be slightly decreasing with post-mortem delay: p= 0.016, 0.044 and 0.009 respectively, with ICP-MS analysis. However no correlation was obtained with ICP-AES. The correlation values dramatically decrease for post-mortem delays over 20 hours.

Age: There was no significant difference of age between the different groups, and between the different classes of degenerating processes (tau; Aβ). This result shows that our groups were age-matched. A significant positive correlation of Cd and K with age was observed with ICP-MS (p=0,036 and 0,008 respectively) whereas there was a significant negative correlation with Na (p=0.029). On the other hand the ICP-AES analysis exhibited significant positive correlations of the age with the levels of Mg, K and P (p= 0.027, 0.021 and 0.010, respectively) and a negative correlation with Ca (p=0.006).

Relationship of metal levels with the Alzheimer pathology

The levels of different detected metals in the frontal cortex were compared to parameters that directly reflect the intensity of the degenerating process in Alzheimer's disease. These parameters are related to amyloidosis, with the quantification of Aβ aggregates x-42 (Delacourte et al. 2002), and to neurofirillary degeneration, with the tau stages and the quantity of abnormal tau in the frontal cortex used for the study (Delacourte et al. 1999).

Aluminum: The results obtained with both techniques were similar in that the levels of aluminum in all the groups of patients were found to be very low. However, they were two or three times higher with the ICP-AES method, but the signal to noise ratio demonstrated that this AES technique is not sensitive enough for the low levels of aluminum found in the nervous tissue. Therefore, for aluminum, only results with mass spectra were taken into account.

Other metals:

A significant decrease of K (p=0,0405) as a function of tau stages was observed using the ICP-AES technique. Other metals were found to be significantly decreasing with the tau pathology intensity in the frontal cortex : Cu, p = 0,0271 ; Mg, p = 0,0262 ; K, p = 0,0012.

Three metals were found to be decreasing with the aggregated Aβ x-42 peptides: Mg, p=0,034 ; K, p = 0,0061 ; P, p = 0,0385.

Comparing the aluminum level between groups

A one factor 3-level ANOVA was performed in order to compare the aluminum level between classes (after merging classes 2 and 3). First a Levene test of homogeneity did not show any difference between variances. Then an F-test gave a P-value 0.244 which indicates that no difference of aluminum levels between the classes.

However elementary statistics show that this conclusion is due essentially to a high variability since the aluminum level is in Classes 2 and 3 more than 2.5 times higher than its value for class one in our sample. Therefore, a more important sample may lead to an opposite conclusion.

Figure 1 : Elementary statistics of aluminum level between classes

Aluminum analysis

	n	Mean	Standard Deviation	Standard error
Class 1	6	,196	,2258	,0921
Classes 2 and 3	27	,499	,4569	,0879
Class 4	5	,318	,3580	,1601
Total	38	,427	,4260	,0691

We then performed *t*-tests in order to compare classes 1 (controls) and 2 (incipient Alzheimer) for the levels of the different metals and no difference was found (the least P-value was equal to 0.127 and was obtained for K). Note here that nearly all the levels of different metals were normal for both classes.

Comparing Classes 1 and 4 brought only one significant difference (for Pb with P-value = 0.006).

Conclusion

We quantified metals in the brain tissue of non-demented and demented patients with Alzheimer's disease or other neurodegenerative disorders. All these patients were fully investigated at the clinical, neuropathological and biochemical levels. Therefore it was possible to distinguish between non-demented patients that are controls from those that are already affected by incipient AD. We analyzed the concentration of metals in the frontal cortex because this brain area is the most informative one as far as neurodegenerative disorders are concerned. Furthermore, this brain area is not affected in aging, contrarily to the hippocampal area. Two complementary, sensitive and specific approaches were used for the detection of metals, namely ICP-MS and ICP-AES. First, we checked the possible biases

interfering with our results. Age did not change significantly the results, in controls and in demented patients. Post-mortem intervals had a significant effect after 30 hours.

Whatever the pathologies and the age, aluminum levels were always low, between 0.5 to 1 μg per gram of tissue. Levels of aluminum were very close to the background level. No correlation was observed between aluminum levels and tau or Aβ pathologies, nor with the age of patients, using ICP-MS values, n=37. Our analyses show that the normal levels of aluminum in a human brain tissue are below 1 μg/ gram of tissue. In the same way, Cu and Zn metals, mentioned as involved in the metabolism of APP, were not increased significantly.

No other metal was significantly correlated with Alzheimer pathology, using both ICP-MS and AES techniques.

Our study corroborates the study on aluminum levels in AD brains reported by Bjertness et al. 1996. Our quantification show even less aluminum levels in the CNS tissue, compared to Bjertness et al report. This is likely due to a lower contamination of our brain samples or to the improved technology since 1996.

Altogether, our study demonstrates that aluminum is not accumulating in the brain of Alzheimer patients. Therefore, there is no direct evidence that this metal is a risk factor for AD. However, our studies do not exclude that this metal at high concentration can be a neurotoxic, as observed for dialysis encephalopathy (Cannata-Andia and Fernandez-Martin 2002). But this is another problem, since these patients do not develop an accelerated Alzheimer pathology (Reusche et al. 2001). The significant decrease of Pb in class 4, which contains different types of non-Alzheimer neurodegenerative disorders, is likely non significant at the physiopathological level, and would necessitate larger subgroups to be confirmed. Also, we note that Cu and Zn that were reported as potential risk factors for AD (Bush 2003) were not spotted in our study.

Aknowledgment

The current study was funded by the International Aluminium Institute. We thank Pr Florence Pasquier, Dr Florence Lebert and Pr Claude-Alain Maurage for their contribution to the constitution of the brain bank (EA2691), which was supported by the integrated project APOPIS (Abnormal proteins in the pathogenesis of neurodegenerative disorders) funded by the European Community under the sixth framework programme.

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Table I

	Neocortical area	Age	Sex	Clinical diagnosis	Final diagnosis	Tau staging	Tau pathology	Aβ staging	Aβ x-42 pathology (μg/g)	*Post-mortem* delay (hours)	**GROUPS**
Dc.26	fr.	26	2	Control	Control	S0	0	S0,S0	0	8	Control
Bn.89	fr.	89	2	Control	Control	S2	0	S0,S0	0	16	1
Co.72	fr.	72	2	Control	Control	S2	0	S0,S0	0	19	1
Bl.93	fr.	93	2	Control	Control	S4	0	S1,S0	0	>12-24<	1
Le.69	fr.	69	2	Control	Control	S4	0	S0,S0	0	22	1
Hn.95	fr.	95	1	Control	Control	S4	0	S0,S0	0	>12-24<	1
Mu.88	fr.	88	2	Memory imp.	MCI	S4	0	S1	0	10	1
Rs.93	fr.	93	2	Memory imp.	MCI	S5	0	S1,S0	0	60	1
Dv.77	fr.	77	1		Control	S3	0	S5,S0	58	36	2
As.85	fr.	85	1	Control	Control	S3	0	S5,S2	7	5	2
Dl.98b	fr.	98	2	Control	Control	S4	0	S7,S1	192	22	2
Dl.98	fr.	98	2	Control	Control	S5	0	S6,S0	138	22	2
Lc.92	fr.	92	2	Memory imp.	MCI	S4	0	S3,S0	3	23	2
Rn.90	fr.	90	2	Memory imp.	MCI	S5	0	S7,S4	194	23	2
Fs.71	fr.	71	1	Control	Control	S6	0	S7,S0	153	25	2
Ai.87	fr.	87	2	Poss Vasc Dem	Alz+VaD	S7	1,6	S9,S0	237	22	3
Bu.84	fr.	84	2	Possible AD	Alz+VaD	S7	0,01	S9,S0	404	40	3
Ja.92	fr.	92	2	Mixed dementia	Alz+VaD	S7	0,1	S8,S0	256	23	3
Ag.79	fr.	79	1	Probable AD	Alz	S9	7	S9,S0	271	6	3
Ks.82	fr.	82	2	Probable AD	Alz	S9	5	S7,S3	125	25	3
Mn.86	fr.	86	1	Poss Vasc Dem	Alz+VaD	S9	7,5	S7,S5	106	24	3
Ci.89	fr.	89	1	Dementia	Alz+VaD	S9	0	S8,S1	299	>12-24<	3
Lu.91	fr.	91	2	Probable AD	Alz	S9	0,5	S7,S0	201	41	3
Rg.61	fr.	61	1	probable AD/LBD	Alz	S10	8,2	S8,S0	133	8	3
To.54	fr.	54	2	Probable AD	Alz	S10	18	S10,S7	1774	>12-24<	3
Tm.83	fr.	83	2	Probable AD	Alz	S10	0,2	S7,S2	214	6	3
Vn.81	fr.	81	1	Possible LBD	Alz	S10	2,5	S8,S4	130	>12-24<	3
Dn.90	fr.	90	2	Probable AD	Alz	S10	8	S8,S8	465	>12-24<	3
Mr.73	fr.	73	1	Probable Alzheimer	Alz+VaD	S10	10	S8,S6	396	>12-24<	3
Fu.74	fr.	74	1	Probable AD	Alz	S10	6,7	S7,S0	129	10	3
Pn.70	fr.	70	1	Probable AD	Alz	S10	10	S9,S7	588	24	3
Cl.76	fr.	76	2	Mixte dementia	Alz+VaD	S10	9	S9,S8	607	>12-24<	3
Nv.78	fr.	78	2	Atypical FTD	Alz	S10	10	S8,S4	360	>12-24<	3
Fa.85	fr.	85	2	atypical LBD	Alz+VaD	S10	10	S8,S8	368	5	3
Dm.83	fr.	83	2	Possible AD	Alz+VaD	S10	10	S8,S0	191	>12-24<	3
Du.90	fr.	90	2	Probable AD	Alz	S10	6	S8,S3	286	1	3
Gn.67	fr.	67	1	Probable AD	Alz	S10	1	S8,S0	173	>12-24<	3
Dl.75	fr.	75	2	Probable AD	Alz	S10	3,5	S8	509	4	3
Se.69	fr.	69	1	Probable AD	Alz	S10	8	S10,S5	1235	24	3
Dq.80	fr.	80	1	Probable AD	Alz	S10	1	S9,S0	387	12	3
Ao.79	fr.	79	1	Probable AD	Alz	S10	10	S8,S0	268	22	3
Rb.70	fr.	70	1	Probable AD	Alz	S10	10	S8,S0	440	5	3
Mt.72	fr.	72	1	Probable Alzheimer	Alz	S10	10	S9,S3	342	18	3
Dn.57	fr.	57	1	Probable Alzheimer	Alz	S10	10	S9,S4	513	>12-24<	3
Pd.66	fr.	66	1	FTD/PSP	DLDH	S0 Type0	0	S0,S0	0	>12-24<	4
Le.79	fr.	79	1	PSP or CJD	PSP	Type II	0,05	S6,S0	70	>12-24<	4
My.75	fr.	75	1	Vascular dementia	Vascular Dem.	<S7	0	S0,S0	0	>12-24<	4
Gr.56	fr.	56	2	PSP	PSP	Type II	10	S0,S0	0	>12-24<	4
Pl.79	fr.	79	2	Possible AD	AGD	S9	0,5	S4,S0	0	26	4
Lc.76	fr.	76	1	LBD/Park+AD	Familial LBD	S5	0	S0,S0	0	8	4

List of patients with clinical and neuropathological data.

Final diagnosis was established using a logical combination of clinical, neuropathological and biochemical data. Tau stages are determined as in Delacourte et al, 1999 and Aβ stages as in Delacourte et al 2002. The precise tau pathology and amyloid burden was determined in the frontal cortex used for the study, as described in Delacourte et al, 2002.

Group 1 corresponds to aged patients with a mild tau pathology and no Aβ deposits. Two patients had a memory impairment corresponding to mild cognitive impairment (MCI) due to tau pathology.

Group 2 corresponds to non-demented patients and two patients with MCI. Both can be considered at an infra-clinical stages of Alzheimer's disease (AD) since they have the two characteristic degenerating processes: tau and Aβ deposition.

Group 3 corresponds to demented patients with an Alzheimer degenerating process revealed by tau pathology in neocortical areas and important Aβ deposits. Some patients have also a vascular pathology and one had also overlapping clinical signs of Lewy body dementia (LBD).

Group 4 corresponds to other neurodegenetive disorders such as DLDH (dementia lacking distinctive histology), with clinical signs of FrontoTemporal Dementia (FTD) and progressive supranuclear palsy (PSP).

One PSP patient with a rapid evolution of dementia suggestion CJD (Creutzfeld Jacob disease), but in fact a real PSP with a specific tau profile type II (4R tauopathy).
One possible AD patient was diagnosed as a dementia with diffuse argyrophilic grains, and with the specific profile of 4R tauopathy.
One patient with parkinsonian features and dementia was affected by Lewy body disease.

Table II

			Arbitrary value of 0-10	Arbitrary value of 0-10	in µg/g of tissue																
			Tau	Tau	Aβ x-42		Al	Cu	Fe	Zn	Ti	V	Cr	Mn	Cd	Pb	23Na	24Mg	39K	44Ca	88Sr	P
Results	Age	pmi	stage	pathology	conc.		µg/g(tissue)	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	mg/l	µg/l	mg/l	µg/l	µg/l	mg/g
Group 1	88,3	28	3,5	0	0	mean	0,19	4,52	48,49	10,81	6,64	0,07	0,17	0,22	0,03	0,03	1,38	110,43	2,65	111,85	0,19	
n=6						std dev.	0,22	1,71	18,04	1,99	1,78	0,03	0,14	0,03	0,02	0,01	0,33	27,83	0,32	54,71	0,33	
Group 2	90,00	21,6	4,0	0,00	75,50	mean	0,35	3,94	55,06	10,63	7,03	0,06	0,11	0,26	0,03	0,05	1,19	120,82	3,06	102,78	0,20	
n=6						std dev.	0,39	1,14	14,90	1,32	1,58	0,03	0,04	0,05	0,02	0,05	0,24	19,89	0,51	25,29	0,24	
Group 3	79,76	19,2	9,3	6,47	385,33	mean	0,54	3,81	44,54	10,79	6,26	0,05	0,14	0,25	0,03	0,02	1,26	110,08	2,44	102,27	0,30	
n=20						std dev.	0,47	1,06	14,46	2,12	1,44	0,03	0,11	0,07	0,01	0,02	0,44	28,01	0,69	20,51	0,48	
Group 4	70,40	17		2,10	0,00	mean	0,32	3,83	43,34	10,20	5,32	0,05	0,11	0,23	0,02	0,00	1,38	98,01	2,80	123,08	0,06	
n=5						std dev.	0,36	1,10	15,67	1,59	0,88	0,03	0,09	0,03	0,01	0,01	0,35	12,15	0,64	36,18	0,05	

ICP-MS n=37

			Arbitrary value of 0-10	Arbitrary value of 0-10	in µg/g of tissue																
ICP-Aes																					
n=49			Tau	Tau	Aβ x-42		Al	Cu	Fe	Zn	Ti	V	Cr	Mn	Cd	Pb	23Na	24Mg	39K	44Ca	88Sr	P
Résultats	Age	pmi	stage	pathology	conc.		µg/g(tissue)	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	mg/l	µg/l	mg/l	µg/l	µg/l	mg/g
Group1	85,6	28	3,6	0	0	mean	1,76	4,92	47,51	8,64							1,51	116,64	2,22	57,81		1,82
n=7						std dev.	0,54	1,98	10,91	1,13							0,27	20,14	0,29	20,48		0,30
Group2	90,00	21,6	4,0	0,00	75,50	mean	1,56	3,72	52,29	9,56							1,25	130,40	2,20	59,88		2,14
n=6						std dev.	0,55	0,71	7,26	1,98							0,19	13,77	0,33	16,54		0,33
Group3	77,55	17,3	9,4	6,51	389,94	mean	1,24	3,49	45,13	8,61							1,57	110,27	1,83	75,48		1,80
n=30						std dev.	0,62	1,14	16,39	2,49							0,34	17,16	0,38	61,46		0,39
Group4	74,50	17	4,5	1,76	12,17	mean	0,82	3,43	34,36	9,55							1,62	105,19	1,71	61,02		1,50
n=6						std dev.	0,53	1,18	10,29	2,60							0,26	18,81	0,57	21,09		0,40

ICP-AES n=49

Results obtained with the ICP-MS analysis (top) and ICP-AES (bottom).

The concentration of the different metals are indicated. The values are averaged per group.
The average of the age, post-mortem interval (pmi), tau stages, tau pathology, Aβ pathology are indicated.
Concentrations are expressed in μg/g (micrograms per gram), or in mg/l (milligram/liter) or in mg/g (milligrams per gram of tissue) or per liter (mg/l).