Measuring aluminium in serum and tissues: overview and perspectives

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RESUMEN

Mediciones de aluminio en suero y tejidos: revisión y perspectivas.
El aluminio sérico es un índice útil de exposición reciente al aluminio; sin embargo, su valor como marcador de exposición crónica es muy limitado. La medición de aluminio en tejidos, no siempre asequible, da una información más exacta, si bien la relación entre acumulación y toxicidad es compleja.

SUMMARY

Measuring aluminium in serum and tissues: overview and perspectives.
Serum aluminium is a useful index of recent aluminium exposure; however, it gives little information about chronic exposure. Aluminium in tissues is a more reliable index, though the relationship between accumulation and toxicity is complex.

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Measurement of aluminium in biological material

Up to now different methods have been used for the measurement of aluminum. These are flame atomic absorption spectrometry, X-ray fluorescence, neutron activation analysis, inductively coupled plasma atomic emission and electrothermal atomic absorption spectrometry (ETAAS).

Due to its high sensitivity, specificity, relative simplicity and low-cost together with the ability to use small sample sizes ETAAS has become the method of choice in most laboratories.

In ETAAS the sample is atomized in a graphite tube, placed in the light path of a lamp emitting light with the specific absorption wavelength of the element to be determined. When the element is atomized a certain amount of the emitted light is absorbed. The absorbance is dependent on the concentration of the element of interest.

The literature shows great discrepancies between normal values for serum aluminum obtained in different laboratories [1]. This is partly due to extraneous addition of aluminum, which is ubiquitous in the environment. A lot of precautions must be taken to avoid contamination. In this regard, glassware, pipettes with metallic bodies, aluminum containing reagents and diluents should not be used. Sample pretreatment should be as simple as possible.

Measurement of aluminum in biological material is a critical element in the diagnosis and treatment of aluminum-related toxicity. Therefore, accuracy and precision of the analytical procedures should be verified at regular intervals by the laboratories concerned in an external quality control program.

It has been claimed recently [2] that ETAAS methods for aluminum measurement are worthless for clinical investigation since they are at best semiquantitative due to insuperable problems of interference. On the contrary our results [3] in studies dealing with problems of contamination, standardization and interference together with our laboratory performance obtained in the external quality control program organized by the university of Surrey (UK), show that aluminum can undeniably be measured accurately and precise. This does not exclude however that we do confirm the difficulty of the measurement. Therefore analysis for aluminum determination should only be carried out in specialized laboratories which do have the disposal of adapted material and personnel.

Distribution of aluminum in the human body

Despite the abundance of aluminum in the environment, tissue levels in man are quite low even in patients with renal failure showing aluminum accumulation. However, considerable relative differences exist between the latter and subjects with normal renal function.

Patients with chronic renal failure, especially those requiring dialysis treatment, also show a markedly different distribution of aluminum (Figs. 1, 2). In controls with normal renal function, the highest tissue levels are found in the lung [4], probably as a result of dust contamination. Only minute amounts of this aluminum seem to be transferred to plasma [5]. On the contrary, unselected chronic hemodialysis patients show elevated aluminum levels in bone, liver and spleen [6]. Due to its larger mass the total amount of aluminum accumulated in bone exceeds by far the quantities present in liver and spleen (Figs. 1, 2).

Consequently, bone aluminum levels provide the better estimates of the aluminum body burden in chronic renal failure. In this condition the ratio between bone and liver aluminum levels is variable [7] and the factors possibly affecting aluminum distribution (e.g. serum parathyroid hormone levels, vitamin D, fluoride, source of aluminum, rate of aluminum loading) have not been sorted out.

Besides the bone, liver and spleen, other tissues appear to selectively accumulate aluminum. For example, striking differences have been found between thyroid and parathyroid gland concentrations in patients with secondary hyperparathyroidism [8]. While the skeletal muscles account for the major part of the small amount of aluminum present in healthy subjects, their quantitative importance in the aluminum body burden of dialysis patients is relatively small (Fig. 1). In contrast to other investigators, we have found comparable levels of aluminum in heart and skeletal muscles of unselected chronic hemodialysis patients [8].

Brain levels of patients with aluminum accumulation are surprisingly low. However, they are higher than in controls, especially in patients showing dialysis encephalopathy. More and more it is appreciated that the concentrations of aluminum in different areas of the brain are highly variable. At present, the significance of this is not understood.

Studying bone aluminum levels (table I) we have observed important differences between chronic renal failure patients controlled by conservative treatment and those requiring hemodialysis therapy, even though dialysate aluminum levels were low (below 10 µg/l). Furthermore, higher bone levels are seen in cases of aluminum induced bone disease. Although the importance of bone aluminum levels has been denied by some [9], we have rarely observed aluminum-induced bone disease in patients with bone aluminum below 30 µg/g wet weight (complete transiliac bone biopsies) [10]. This association between elevated bone aluminum levels and aluminum-induced bone disease has been reported by several groups.

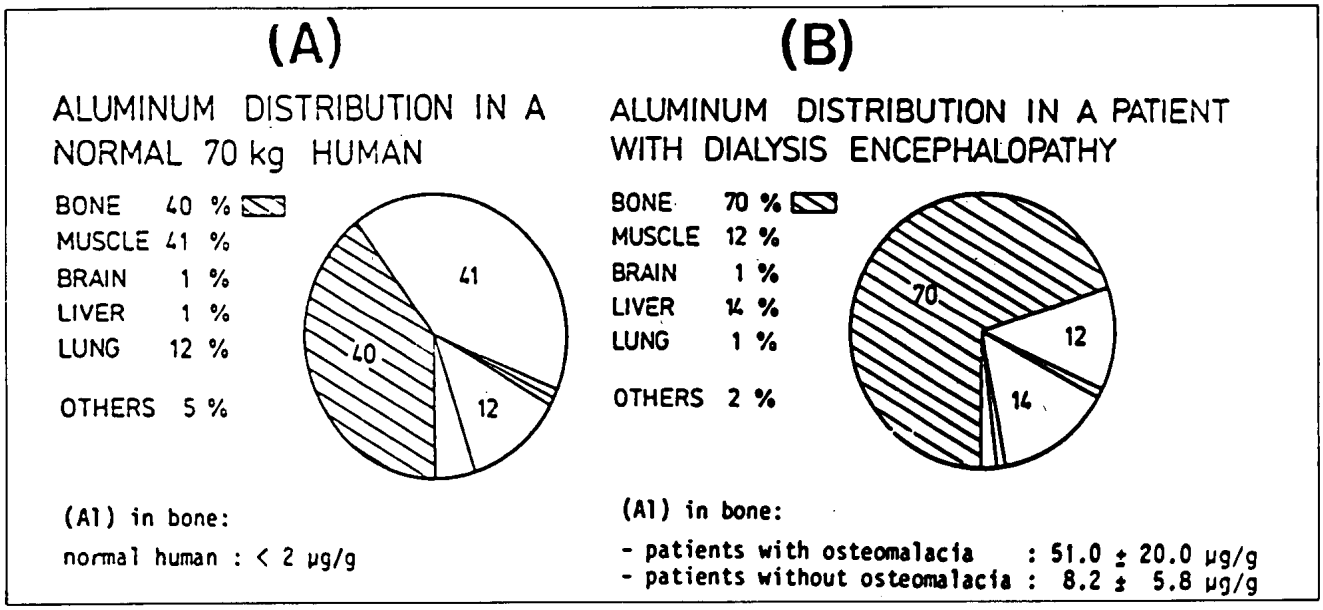


Fig. 1.—Aluminum distribution in a normal adult man (A) and in a dialysis patient with aluminum induced encephalopathy (B). Notice the striking differente in aluminum content of bone between normal subjects, and dialysis patients with and without aluminum induced osteomalacia.

Serum concentration of aluminum

The question how far serum aluminum is a reliable index for aluminum body burden, and useful for the monitoring of the dialysis patient necessitates a shaded answer.

Serum levels of aluminum are indeed higher in patients with aluminum-induced osteomalacia and dialysis encephalopathy [11]. However, several authors [6, 7, 12, 18] reported serum aluminum levels below 100 µg/l in an important percentage of the patients (up to 40 %) with encephalopathy or with clinically, histologically and histochemically proven aluminum induced osteomalacia.

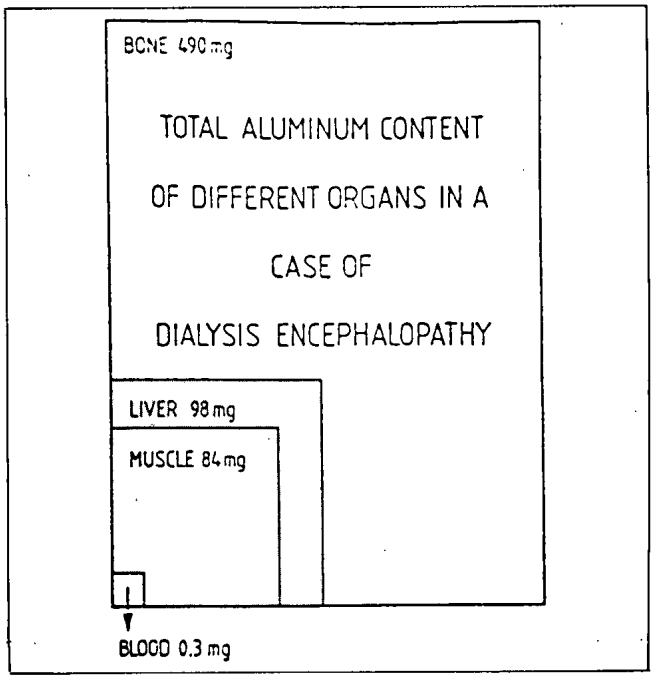
The limited value of the serum aluminum concentration was also stressed by Cannata et al [14]. These authors observed that increases of serum aluminum returned to preload values 5 to 6 weeks after an acute exposure in patients with continuous ambulatory peritoneal dialysis.

This clearly shows that if determinations of serum aluminum are performed at intervals of more than 4 months acute loads which may lead to important tissue deposition will be overlooked.

Recently, we could demonstrate that aluminum bone accumulation is frequently seen in dialyzed children even after recent start of the treatment and despite minimal oral intake of aluminum containing phosphate binders. These elevated aluminum bone concentrations could neither be predicted from serum aluminum levels nor from the results of a desferrioxamine (DFO) challenge test [15].

The observation that serum aluminum values normalizes within a few weeks following renal transplantation while brain aluminum remains high four years after the restoration of normal renal function by transplantation [16] provides a strong argument for the multicompartimental behavior of aluminum.

Fig. 2.—Total aluminum content of different organs in an ESRF patient with aluminum-induced encephalopathy.



Considering, moreover, the very small percentage (< 0.5 %) of the total body aluminum present in serum (Fig. 2) one can reasonably state that serum aluminum

Table I. Aluminum levels and degree of renal failure

	Number of individuals	Serum creatinine level (mg/dl)	Serum aluminum levels (μg/l)	Bone aluminum levels (μg/g)
Controls	10	< 1.2	2.0 ± 0.4	< 1.0
Severe renal failure conservative treatment	15	6.2 ± 2.7	29 ± 23	2.7 ± 2.0
Hemodialysis (60 ± 46 months)	27	—	140 ± 322	35 ± 29

will be a good indicator of acute exposure of the patient to aluminum and gives some indication of tissue aluminum (low sensitivity, high percentage of false negatives, table II).

It is therefore mandatory to perform aluminum determinations in the serum at regular intervals (e.g. every 4 months) in order to trace acute exposure to aluminum and as a guide to the risk of toxicity.

Tissue levels and aluminum toxicity

Comparing different tissues (e.g. brain, liver, bone, parathyroid gland, etc.), it appears that the relation between aluminum accumulation and toxicity is complex. While bone and liver both show elevated aluminum levels, toxicity has only been established in bone. On the contrary, aluminum toxicity has been demonstrated in brain although brain levels are relatively low (table III).

This suggests that, aluminum has accumulated at a critical ultrastructural site where it can interfere with an essential biochemical process before toxicity develops. Therefore, the elucidation of the mechanisms of aluminum toxicity will come from the demonstration of biochemical interactions and the detection of the ultrastructural distribution of the element.

In vitro experiments have demonstrated that aluminum may interfere with numerous biochemical processes [17]. For instance, it has been established that aluminum delays hydroxyapatite formation, the transformation of amorphous calcium phosphate to hydroxyapatite and the growth of hydroxyapatite seed crystals [18]. The significance of these observations for aluminum toxicity in vivo is however less clear.

Table II. Serum aluminum

— Parameter of acute exposure
— Limited correlation with total body aluminum
— Useful indicator for toxicity when determined regularly, e.g. every 4 months
— Monitoring as soon as exposure exists (aluminum hydroxide intake, dialysis)

Several histochemical [19, 20] and microanalytical techniques [31] have been used to determine the microscopic and ultrastructural localization of aluminum in tissues with excessive stores. The presence of aluminum at the osteoid/calcified-bone boundary in cases of aluminum-induced osteomalacia has been confirmed using histochemical staining with Aluminon, with laser microprobe mass analysis (LAMMA) and with secondary ion mass spectrometry [21]. It is evident that the ultrastructural localization of aluminum bears an important relationship with toxicity. The sequestration of aluminum in lysosomes of hepatocytes, Kupffer cells and spleen macrophages [21] might explain the absence of proven toxicity of aluminum for liver or spleen.

In contrast the presence of aluminum at the extracellular osteoid/calcified-bone boundary presumably may causes the mineralization defect seen in aluminum intoxication. Similarly the occurrence of aluminum in secretory granules of parathyroid cells [22] may inhibit PTH release resulting in the documented relatively low serum parathyroid hormone levels found in dialysis patients with aluminum-induced osteomalacia.

Using LAMMA, aluminum and iron have often been found at the same ultrastructural sites, e.g. in liver lysosomes [21], at the osteoid/calcified-bone boundaries [23] in parathyroid secretory granules [22] and in bone marrow cells with histiocytic appearance. It is worth mentioning that in subjects with massive iron overload and no aluminum accumulation osteomalacia as well as hypoparathyroidism have been reported [24]. It is possible that iron and aluminum interact with the same biochemical processes in causing these disease conditions.

In our studies microanalysis was applied to fresh tissue biopsies from chronic hemodialysis patients. Bone, liver, brain, thyroid and parathyroid glands have been studied using several techniques. These microanalytical procedures provide information on chemical composition of microvolumes in histological sections:

A) Electron Probe X-ray Microanalysis (EPXMA): An electron beam is focused on a selected region of the

Table III. Tissue levels and aluminum toxicity in end stage renal failure

Aluminum	Level	Toxicity
Bone	high	established
Liver	high	absent
Brain	relatively low	established
Parathyroid	relatively low	interference with release of PTH (?)

specimen; the image of this area is visualized in the electron microscope.

Characteristic X-rays are collected by crystal spectrometers or solid state detectors.

B) Laser Microprobe Mass Analysis (LAMMA): A high-power laser is focused on a histological section viewed by a light microscope. A selected area of a few μm^2 of the tissue is evaporated; the created ions are separated in a time-of-flight mass spectrometer and detected. A complete mass spectrum covering all elements present in the microvolume is produced for each laser shot.

C) Secondary Ion Mass Spectometry or Ion Microscopy (SIMS): A beam of primary ions is focused on the surface of the histological sample. As a result of the ion bombardment, the atoms of the first atomic layers are sputtered and ionized. These secondary ions reveal the atomic composition of the sample and produce a specific ion image. Selected images of the different ions can be displayed sequentially.

D) Electron Energy Loss Spectroscopy (EELS): A high-resolution electron energy spectrometer is combined with a conventional or scanning transmission electron microscope. Incident electrons are focused on a very small area of the section. After passing through the thin sample, the energy loss of the transmitted electrons due to interaction with the present atoms is measured.

E) Histochemical Staining (HS): The staining of tissues for aluminum involves the use of ammonium aurintricarboxylate (Aluminon). With aluminum this dye forms a bright-red colored complex. Another staining procedure utilizes acid solochrome azurine which reveals the aluminum as purple deposits. Routine histochemical methods usually lack sufficient specificity and suffer from interferences.

Despite present instrumental limitations of analytical sensitivity and spatial resolution of analysis, methodological improvements are expected. Sophisticated equipment e.g. (EELS imaging) combined with refined sample preparation techniques (cryofixation) will open a new perspectives to localize aluminum at the subcellular level. The development of analytical techniques for the microchemical characterization of trace and/or toxic elements in histological specimens is a subject of interdisciplinary research. Continuous improvement is needed to determine ever decreasing elemental concentrations with more refined spatial resolution.

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