

Metal release in patients with total hip arthroplasty by DF-ICP-MS and their association to serum proteins†

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A study on the level of metals released to body fluids from patients carrying metal-on-metal (based on Co–Cr alloys) total hip prosthesis and titanium alloys dental implants has been conducted. In the first part, total elemental determination of Co, Cr, Ti, Mo and Mn was done in whole blood and urine for both, control individuals and hip arthroplasty patients. Additionally, the work was extended to patients who carried dental implants. The samples, either acid digested (blood) or just diluted (urine) were analyzed using a double focusing inductively coupled plasma mass spectrometer (DF-ICP-MS). Both strategies are validated using the corresponding certified reference materials. The findings revealed an increased concentration of Cr and Ti and, to a lower extent, Co and Mn in the blood and urine of the patients. The second part of the work tries to explore the possible association of the released metals to human serum proteins. For this purpose, speciation of the above mentioned metals is accomplished using liquid chromatography (anion exchange) with ICP-MS detection. Such studies, firstly conducted in incubated standards and then in fresh serum from the patients, showed the elution of Mn associated to transferrin. Co eluted associated to albumin and Cr could not be detected. Spiking experiments showed that Cr(III) is clearly associated to transferrin and supports the theory that Cr is eliminated from the prosthesis as Cr(VI) that shows no interaction with the studied serum proteins.

Introduction

Total hip replacement surgery has increased enormously during the last decades and can be considered, nowadays, as a routine operation conducted in 0.16–0.2% of the population.¹ In total hip arthroplasty, the natural hip joint is replaced by an artificial one consisting of a femoral component (usually a polished metal ball mounted on a metal stem) and an acetabular component with a socket in which the metallic ball sits and swivels as the patient walks.² In the interest of implant longevity, several factors have to be considered in order to minimize the production of wear debris by the bearing surfaces, particularly the design and manufacture of the hip replacements and the materials employed. The metal-on-metal (M/M) total hip replacements, where the femoral and acetabular components are made of cobalt–chromium alloys, were widely used during the 60's. However, such materials were replaced in the 70's by the metal-on-polyethylene bearings, in which a metal femoral ball articulates on a polyethylene socket, due to observed high loosening rates and concerns about biological reactions of the metallic components of the M/M prosthesis.³ Curiously enough then, M/M

replacements were re-introduced by the late 80's once the polyethylene wear debris was suspicious of initiating undesirable biological reactions, such as osteolysis, that might lead to final failure of the implant.⁴ These second-generation metal-on-metal hip arthroplasties have been now in clinical use for more than a decade. However, initial concerns about the metal release from these prostheses are still a major drawback of their use. In this regard, several studies are available in the literature today about the levels of metals released from the M/M hip replacements in body fluids such as blood,⁵ serum⁶ or urine.⁷

Such studies have shown a wide range of metal levels from patient to patient. In general terms, Co and Cr levels in body fluids were found at increased levels in patients carrying M/M bearing (as compared to control individuals and patients with metal-on-polyethylene prosthesis).³ Indeed, those levels changed dramatically with the time elapsed from the surgery and the activity of the individual;⁸ but even four years after the arthroplasty, the patients have shown to contain whole blood concentrations of Cr and Co markedly above the immediate post-operative levels.⁵ Because it is not possible to measure radiographically wear in contemporary M/M hip systems, surrogate markers of bearing surface performance have been sought. In this regard, blood and/or urine concentrations of Co and Cr have been proposed as potential indicators of implant performance including bearing surface wear.

Once the metals get released from the prostheses, their transport, absorption, excretion and deposition in tissues is going to be strongly dependent on the type of biological binders to which they are associated.⁹ The association of the released metals to proteins, for instance, might increase the corrosion rate at the

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† This article is dedicated to Professor Jean-Michel Mermet, in recognition of his contributions to the field of atomic spectrometry.

implant/biology interface by increasing the dissolution of the surface passive (oxide) film formed on metallic materials especially of Cr and Co.¹ However, this topic has been very seldom tackled in the existing literature about M/M hip replacements¹⁰ and should be considered in more detail for a better understanding of the long-term toxic effects related to metal ions released by M/M arthroplasties. Some initial studies¹¹ revealed that the main components of the M/M alloys, Cr and Co, exhibit similar affinity for serum proteins, while such affinity is slightly different in the case of Ni. These interactions can be studied in detail by using hyphenated strategies that combine a powerful separation technique of the sought species (chromatographic-based) with sensitive and selective detectors of the metals (such as inductively coupled plasma mass spectrometry, ICP-MS).¹² ICP-MS offers excellent analytical performance characteristics for trace element determinations (total concentration)^{13,14} and also for speciation problems. In particular, different publications have demonstrated the almost ideal features of this type of detection for monitoring trace elements associated to human serum proteins.^{12,15}

Thus, in this work, we have used ICP-MS to illustrate, first, the total elemental concentration of the metals released by the prosthesis into the blood and urine of the patients with hip replacement (as compared to control individuals). Then, those elements of relative elevated concentration levels in the blood samples are studied by typical speciation strategies (HPLC-ICP-MS) in order to have a glimpse of their biological ligands.

Experimental

Instrumentation

For the total elemental determinations, a double focusing ICP-MS (DF-ICP-MS) instrument, ELEMENT II from Thermo Fischer Scientific (Bremen, Germany), was used. In the case of the speciation studies, the instrument used was a hexapole reaction system ICP-MS (ORS-ICP-MS) Thermo Fisher X SERIES^{II}. This hexapole is enclosed in a cell that can be pressurized with different gases (*e.g.*, H₂, He) in order to overcome polyatomic interferences. When the cell is not gas-pressurized, the hexapole works only as an ion guide lens. Both instruments were equipped with a Meinhard concentric glass nebuliser and with nickel sampler and skimmer cones. Details of the operation instrumental conditions and measurement parameters are given in Table 1.

A Milestone Ethos 1 microwave oven (Bergamo, Italy) equipped with a rotor for six PTFE vessels was used for digestion of human blood samples.

The high performance liquid chromatographic (HPLC) system used consisted of a dual piston HPLC pump (Shimadzu LC-10AD, Shimadzu corporation, Kyoto, Japan) equipped with a sampler injection valve, Rheodyne, Model 7125 (Cotati, CA, USA) fitted with a 100 μ L loop. Separations of human serum proteins were performed on an anion-exchange column Mono QTM 5/50 GL (Pharmacia, GE Healthcare, Spain). Chromatograms were obtained using a diode array detector (model 1100, Agilent Technologies, Waldbron, Germany). A scavenger column (25 \times 0.5 mm id) packed with Kelex-100 impregnated silica C₁₈ material (20 μ m particle size) was placed between the

Table 1 DF-ICP-MS, ORS-ICP-MS and MALDI-TOF operating conditions

DF-ICP-MS	
Instrument	Thermo Fischer ELEMENT II
Rf power	1275 W
Sample uptake flow rate	0.1 mL min ⁻¹
Plasma gas flow rate	15.99 L min ⁻¹
Auxiliary gas flow rate	0.80 L min ⁻¹
Nebuliser gas flow rate	0.96 L min ⁻¹
Resolution	3000
<i>m/z</i> monitored	⁴⁷ Ti, ⁴⁹ Ti, ⁵² Cr, ⁵³ Cr, ⁵⁵ Mn, ⁵⁹ Co, ⁹⁵ Mo, ⁹⁸ Mo
Acquisition mode	Scan
Mass window	125%
Sample per peak	10
Time per sample	0.009 s
Search window	150%
Integration window	200%
Number of scans	3
ORS-ICP-MS	
Instrument	Thermo Fisher X SERIES ^{II}
Rf power	1300 W
Plasma gas flow rate	13.0 L min ⁻¹
Auxiliary gas flow rate	0.70 L min ⁻¹
Nebuliser gas flow rate	0.92 L min ⁻¹
<i>m/z</i> monitored	⁵² Cr, ⁵⁵ Mn, ⁵⁹ Co
Collision cell parameters	
Hexapole bias	-9 V
QP bias	-7 V
Collision He gas flow	2 mL min ⁻¹
MALDI-TOF	
Instrument	Voyager-DETM STR BiospectrometryTM
Scan type	Positive
Instrument mode	Lineal
External calibration	10 μ M of bovine serum albumin
Matrix	Sinapinic Acid
Laser	2800 V
Spectrum acquisition	
Shoots	150
Scan range	500-4000
Acceleration voltage	25 000 V

pump and the injection valve to remove the metals of the HPLC buffers.

Reagents and materials

All calibration standard solutions were prepared from 1000 μ g mL⁻¹ single element standard solutions (Merck, Darmstadt, Germany) by dilution with Milli-Q water. Analytical reagent grade nitric acid 65% (w/v), additionally cleaned by sub-boiling distillation and hydrogen peroxide Suprapur 30% (w/v) (both from Merck), was used for the microwave digestion of the human whole blood samples. Two different reference materials were used in this study: Seronorm Trace Elements Whole Blood (Level 1, Ref 9067) and Seronorm Trace Elements Urine (Level 1, Ref. 201205), both from Seronorm (Nycomed AS, Oslo, Norway). These reference materials were kept in the refrigerator at 4 °C until analysis and were reconstituted following the procedures given by the manufacturer. Ultrapure deionized water

(≥ 18 M Ω) was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). All plastic containers were soaked in 10% (v/v) sub-boiling HNO₃ at least for 24 h and then rinsed copiously with Milli-Q water prior to use. All kinds of glassware were avoided to prevent metal contamination.

Blood and urine collection

The study group was made up of 15 patients (age 57.3 ± 17.2 yr, range 30–87), consisting of nine males (age 50.6 ± 17.9 yr, range 37–76) and six females (66.3 ± 20.3 yr, range 30–86) all of them with cementless M/M BiHapro total hip prosthesis (BiHapro-Biomet, Warsaw, Indiana, USA). The control group contained 10 subjects consisting of five females (age range 51–90) and five males (age range 50–85). Additionally, three patients carrying titanium-based dental implants that had undergone implant failure and showed metal hypersensitivity¹⁶ were also analyzed for total metal content in blood.

For this purpose, five millilitres heparinized vacutainer tubes (Greiner bio-one, Madrid, Spain) for trace elements were used for blood collection. To keep metal contamination under control, 10 mL of blood were drawn from each subject using a plastic cannula in 2 tubes. The first 5 mL were discarded. To obtain the serum, the blood was centrifuged at 1500 rpm after coagulation and the serum was introduced into a 1 mL vial (Eppendorf AG, Hamburg, Germany) decontaminated by the same procedure used for all plastic containers.

Morning urine samples were collected in polypropylene bottles (Soria Genlab, Madrid, Spain) that had been decontaminated using the procedure described above. To avoid the risk of metal adsorption on the surface of the plastic container, 1% (v/v) of sub-boiled nitric acid was added to the samples immediately after collection.

Samples of blood and urine were collected from all 15 patients with implants and from the 10 control subjects. All biological samples were stored at -20 °C until analysis.

Sample preparation and analysis

Urine samples were prepared by dilution 1 : 10 with ultrapure water. For whole blood analysis, aliquots of 1 mL were placed into the digestion Teflon vessels and mixed with 6 mL of sub-boiling nitric acid diluted (1 : 3) with Milli-Q water and 1 mL of high-purity hydrogen peroxide. After digestion and cooling, the samples were transferred into pre-cleaned polyethylene bottles and finally diluted 1 : 20 with Milli-Q water.

For speciation studies, the mobile phases contained: (A) 0.05 mol L⁻¹ TRIS-acetic acid buffer (pH 7.4) and (B) A + 1.5 mol L⁻¹ ammonium acetate and the separation of serum proteins was achieved using an ammonium acetate gradient of up to 50% B in 30 minutes at a flow of 1 mL min⁻¹. The injected volume was 100 μ L. The eluate from the column was passed through a UV detector, set at 280 nm, for protein monitoring and to an ORS-ICP-MS detector for metal detection using the operating conditions given in Table 1. The serum samples were diluted 1 : 1 (v/v) with buffer A and then they were filtered (0.22 μ m) before analysis. The spiked serum samples and standards were prepared incubating them with 60 ng mL⁻¹ of Mn(II) (from Mn(NO₃)₂),

Cr(III) (from CrCl₃·6H₂O), Cr(VI) (from Na₂CrO₄) and Co(II) (from Co(NO₃)₂), respectively. Then, those spiked samples were diluted 1 : 1 (v/v) with buffer A and filtered (0.22 μ m) before analysis.

Results and discussion

Determination of trace elements in blood and urine samples

The first aim of the work was the optimization and validation of an analytical strategy to allow the determination of the elements of interest (mainly Co and Cr but also Mo, Mn and Ti) in blood and urine samples of patients with M/M hip prosthesis. Thus, by following similar studies conducted in our research group,¹³ the blood samples were previously digested in a microwave oven and the urine samples were just diluted 1 : 10 with Milli-Q. In order to avoid polyatomic interferences, the samples were analyzed by DF-ICP-MS which can also provide adequate detection limits.¹³

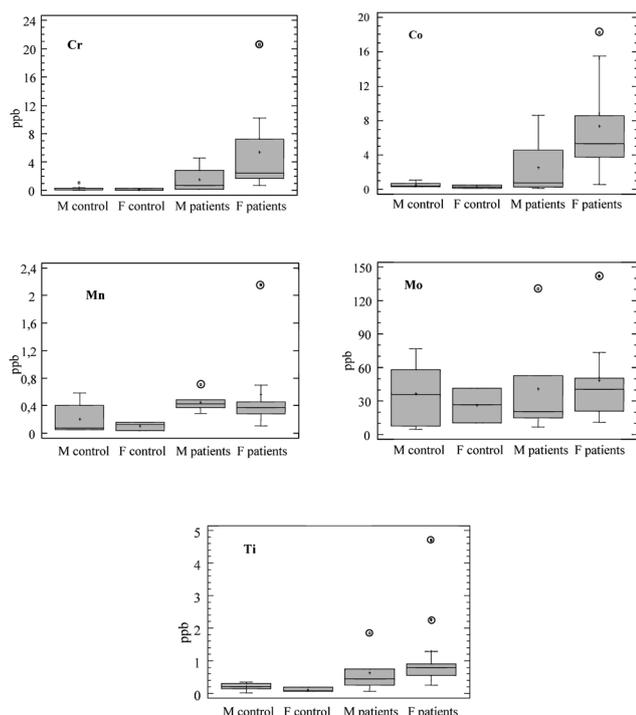
By using the instrumental conditions detailed in Table 1, it was possible to achieve limits of detection ranging from 1 to 5 ng L⁻¹ (using the IUPAC criterion and 2% sub-boiling nitric acid as blank solution) for all the elements of interest with precisions in terms of %RSD of about 2–4%. Such features allowed the validation of our methods for the desired elements in a reference material of urine (SeronomTM Level 1). The obtained results for such validation are summarized in the first column of Table 2. Of all the elements analyzed, only Ti and Mo are slightly out of the mean value (unfortunately, the standard deviations of the mean values in the reference material are not provided). The values obtained for the rest of the elements fit very well with the reference values. In the case of blood samples, it was possible to obtain a method detection limit ranging from 1 to 200 ng L⁻¹ (in this case, the used blank solution contained sub-boiling HNO₃ and H₂O₂). Similarly to the urine samples, our method was validated by analyzing a blood reference material containing the sought elements at the concentration levels expected in our control blood samples. The second column of Table 2 shows the obtained results for the reference material. In this case, all the elements (including Ti and Mo) show concentrations that are in good agreement with those provided with the reference material.

Once both sample types developed methodologies were validated, the analysis of the blood and urine of the patients with M/M hip prostheses was conducted. The obtained results for urine samples can be observed in Fig. 1 plotted as box diagrams independently for Cr, Co, Mo, Mn and Ti. On each diagram, the concentrations found in control individuals and in patients with M/M bearings (male and female, independently) are plotted in the form of rectangles whose upper and lower sides are given by the upper and lower quartile of the data distribution, respectively. The rectangles are divided into two parts by the value of the median, thus 50% of the data are above the median and 50% are below. The error bars at the edges of the rectangle represent the highest and lowest concentration encountered that are significant (this means values within 1.5 times the total length of the rectangle). The values considered as “abnormal” (within 1.5 to 3 times the total length of the rectangle) are represented with a small circle.

The box diagrams on Fig. 1 provide a visual idea of the concentration ranges in urine samples for Cr, Co, Mn, Mo and Ti

Table 2 Results obtained in the analysis of the two certified reference materials (Seronom Trace Elements Urine, Level 1, Ref. 201205 and Seronom Trace Elements Whole Blood, Level 1, Ref 9067) with the proposed methodologies

Element	Certified value urine/ $\mu\text{g L}^{-1}$	Found value urine/ $\mu\text{g L}^{-1}$ (mean \pm SD, $n = 3$)	Certified value blood/ $\mu\text{g L}^{-1}$	Found value blood/ $\mu\text{g L}^{-1}$ (mean \pm SD, $n = 3$)
Cr	0.56	0.56 ± 0.02	0.6	0.63 ± 0.03
Co	0.28	0.28 ± 0.01	0.15	0.16 ± 0.02
Mo	61.4	58.6 ± 0.3	0.5	0.4 ± 0.1
Mn	1.1	1.1 ± 0.01	10.6	10.6 ± 0.1
Ti	4.6	3.5 ± 0.2	2.3	2.1 ± 0.3

**Fig. 1** Box plots showing the observed distribution of Cr, Co, Mn, Mo and Ti in the analyzed urine samples of both male (M) and female (F) control and patient groups.

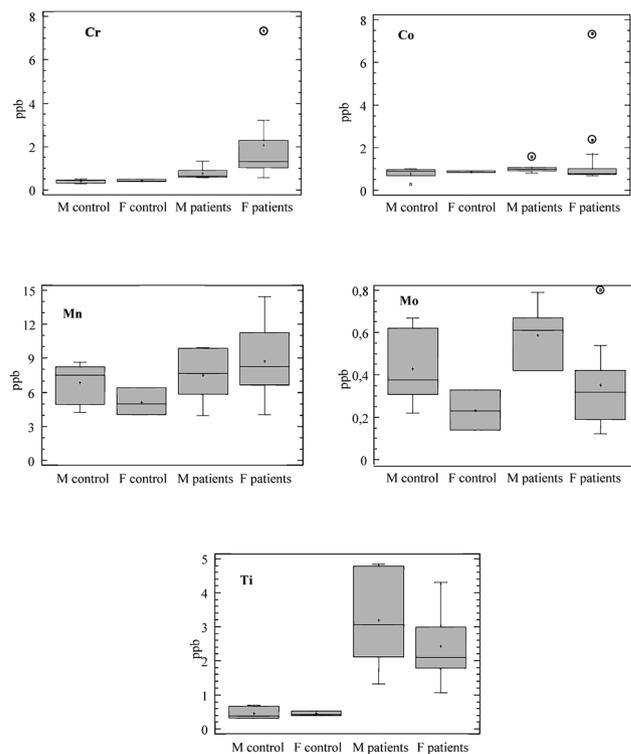
in both, control individuals and patients carrying M/M total hip replacements. As can be observed for Cr, although the dispersion of the data is quite high in the case of male patients, there is an important difference respect to the control individuals (there is not overlap between the two boxes) that occurs in the case of females as well. The control individuals showed concentrations (both male and female) below 0.3 ng mL^{-1} . Similar results can be observed also in the case of Co. For Mn and Mo, the boxes on Fig. 1 overlap almost completely showing that there are not significant differences in the concentration of these metals in control individuals and patients. Finally, in the case of Ti, there are some small differences in concentration between control and patients, especially in the case of female samples.

The analysis of these elements in circulating blood was also studied for the monitoring of possible losses of metals from the prosthesis. In this regard, Fig. 2 shows the results obtained for all the analyzed samples. In this case, it is also remarkable the low dispersion of the results obtained for Cr (mean about 0.4 ng mL^{-1}) and Co (mean about 0.8 ng mL^{-1}) in the control samples,

also similar to those reported by previous studies.¹⁷ Only in the case of Cr and Ti, (see Fig. 2) the patients have shown concentration values statistically significant with respect to those observed for the control individuals. As can be seen, these differences are especially important in the case of Ti in both, male and female patients. This is important since the prosthesis are composed of a cobalt–chromium alloy acetabular component and also a cobalt–chromium femoral head but mounted on a metal stem (placed inside the femur) made of titanium. Therefore, the liberation of metals could be primarily due to corrosion of the prosthesis by the biological fluids rather by direct friction of the fitting parts.

Total metal determination in implanted patients and hypersensitivity assays.

The determination of the metals released by titanium-based dental implants was also accomplished in a limited number of

**Fig. 2** Box plots showing the distribution of Cr, Co, Mn, Mo and Ti in the analyzed blood samples of both male (M) and female (F) control and patient groups.

blood samples from patients who showed rejection to the implants. Although reported success rates for dental implants are high, failures that require immediate implant removal do occur. Therefore, we studied the monitoring of implant components into blood to help on the diagnosis of the failure. Additionally, these patients were evaluated for metal sensitivity through the MELISA[®] assay. This is a type of lymphocyte proliferation assay used as a diagnostic tool in the evaluation of metal exposure in humans. Metal-induced allergy in humans is caused by the contact of the metal with the surface of memory lymphocytes. Upon re-exposure to the same metal (*in vivo*), the lymphocytes show an increased proliferation that reveals hypersensitivity to that metal.¹⁶ The measurement of such proliferation gives an indication about previous exposure to the metal even when it is not detectable at present.

The results obtained for the three patients analyzed are given in Fig. 3. As can be observed, all the patients carrying titanium alloy dental implants showed higher levels of this metal in the blood. However, only one of them gave positive results on the hypersensitivity test for this metal. The level of Cr, Co, Mn and Mo were similar or even lower than those obtained for the control individuals. Therefore, Ti biocompatibility should be revised since a number of cosmetics, together with prosthesis and dental implants carry this metal which is, in principle, completely inert.

Speciation of the released metals in serum samples of patients with M/M hip prosthesis

In general, cobalt–chromium alloys are reasonably inert in the body, especially over short time intervals. As shown previously, however, despite the *in vitro* evidence of good resistance to corrosion, Co and Cr can be released from M/M prosthesis and this release can cause health concerns. It is known that Cr(VI) is a potential carcinogenic in humans and there are studies proving that an increase in tumour incidence has been detected when Cr(VI) is present in the body.¹⁸ Similarly, on the bases of its mutagenicity, Co can be also considered a potential carcinogen

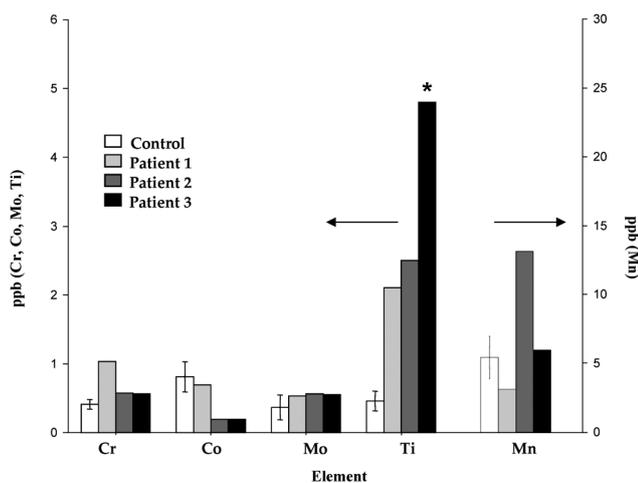


Fig. 3 Concentration of Cr, Co, Mo, Ti and Mn in the analyzed blood samples of three patients with titanium-based dental implants. The asterisk indicates positive results on the hypersensitivity test for Ti.

to humans. *In vitro* studies have also demonstrated that the presence of these two metals did reduce cell viability in osteogenic bone marrow cells.¹⁹ However, very little is known about the transport of these metals in the body once they are released from the prosthesis. In order to evaluate metal transport in the body by possible association of Co, Cr and Mn to human serum proteins in the blood samples, initial speciation studies were accomplished for samples with the highest detected concentrations of these metals. It is noteworthy that all the HPLC-ORS-ICP-MS chromatograms were obtained by using a scavenger column between the pump and the injection valve to avoid metal contamination²⁰ and all the chromatograms presented are blank (gradient) subtracted.

Fig. 4 shows the separation of the most abundant human proteins (immunoglobulin G (IgG), transferrin (Tf), albumin (Alb) and ceruloplasmin (Cer)) in a control human serum sample by anion-exchange HPLC with UV detection (280 nm) (Fig. 4A) and with ICP-MS detection (Fig. 4B) monitoring Fe, Cu and Zn. The proteins have been identified by matching their retention times with the corresponding standards and the presence of the essential metals in a given fraction is an additional proof (*e.g.*, Fe in transferrin or Cu in ceruloplasmin). The samples from the patients were analyzed using the same chromatographic

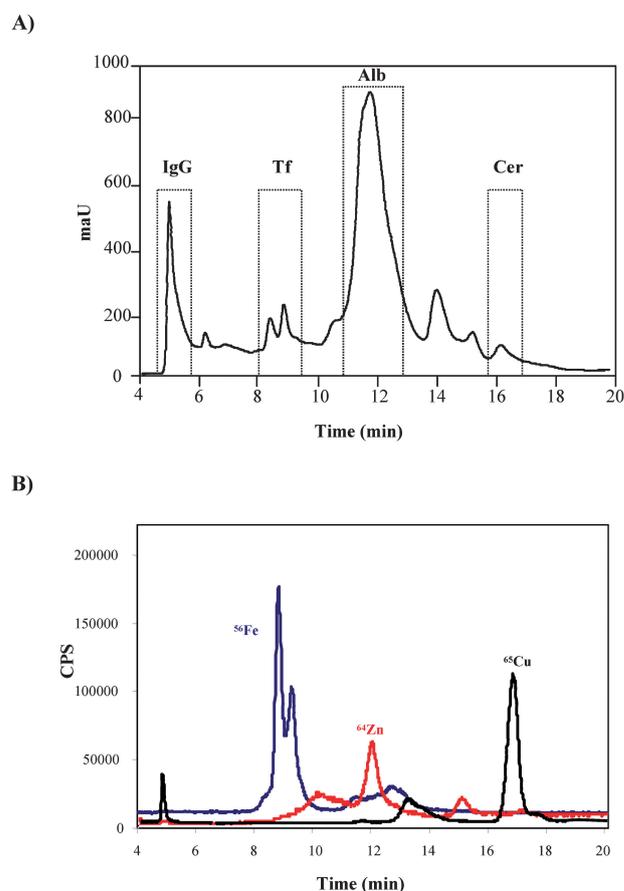


Fig. 4 Chromatographic profile of a serum sample from a control individual. (A) UV detection (280 nm), immunoglobulin G (IgG), transferrin (Tf), albumin (Alb), and ceruloplasmin (Cer); (B) ICP-MS detection monitoring Fe, Cu and Zn. Chromatographic conditions detailed in the text.

conditions. Thus, Fig. 5A shows the results for the HPLC-ORS-ICP-MS elution profile of ^{55}Mn from a serum sample corresponding to a patient with a concentration of Mn in whole blood of 8.3 ng mL^{-1} , as compared with that of a control individual containing 5.3 ng mL^{-1} . It is possible to observe that Mn shows several peaks: (i) a first fraction present at the void volume (probably as unretained Mn^{2+}); (ii) a second fraction at 9 minutes (coeluting with the Tf peak as shown in Fig. 4A); and (iii) a third fraction at 11 minutes that can be observed in all the chromatograms and that could be ascribed to anionic species of Mn with, for instance, citrate (LD about 1 ng mL^{-1}). Similar results have been documented by other authors²¹ and were confirmed here by spiking the control serum with 30 ng mL^{-1} of Mn(II) as Mn-citrate. As can be observed in the spiked serum sample of Fig. 5A, there is a clear preference of Mn(II) for transferrin and the column recoveries were above 90% in all cases. In order to verify this finding, the same solution of Mn-citrate was incubated with a standard of human serum transferrin and the results showed a single peak at about 11 minutes which did not match, specifically, with the protein chromatographic profile (see Fig. 5A). This could be probably ascribed to the formation of some anionic species of Mn, such as citrate, showing important retention in the column. In contrast, for the real serum sample, the interaction is with Tf, most likely due to the presence of

a third binding partner naturally present there (a synergistic ion that has proved to be also necessary in the binding of other metals to Tf, such as bicarbonate for Fe-Tf or Al-Tf complexes).

The speciation studies for Cr turned out to be slightly different. Although Cr, as Cr(III), seems to be associated to transferrin,²² results in the patient sample whose blood contained about 2 ng mL^{-1} of total Cr were negative (undetectable) with our HPLC-ORS-ICP-MS set-up. Fig. 5B shows the chromatographic profiles of control serum and patient serum together with the serum and a transferrin standard both incubated with 30 ng mL^{-1} of Cr(III). As can be observed, the Cr(III) elutes at the same retention time (9 min) as the transferrin peak (see Fig. 4A), in agreement with previous observations in our group.¹⁵ Contrary to the Mn profile, the transferrin standard incubated with Cr(III) showed very similar results to the incubated serum and the column recovery experiments revealed that $>80\%$ of the injected Cr eluted from the system. However, similar incubation experiments taken with Cr(VI) (at 30 ng mL^{-1}) instead did not provide detectable signals for Cr in our HPLC-ICP-MS experiments (LD 0.7 ng mL^{-1}). This result tends to support the idea of Cr released from the prosthesis as Cr(VI), potentially more toxic than the Cr(III), and that it is not bound to the serum components. Such Cr(VI) might be associated to the cellular components of blood (red blood cells, lymphocytes, *etc.*), since the total elemental concentration in whole blood is significantly high and it can not be detected in plasma. In fact, Cr is well known to generate DNA adducts and to diminish DNA synthesis so is expected to be found in the nucleus of cells such lymphocytes rather than in plasma.²³

Finally, the speciation of the Co present in human serum was similarly accomplished. Fig. 6 shows the HPLC-ICP-MS Co elution profile for control serum and serum from a patient with hip prosthesis (total Co content in whole blood 1.8 ng mL^{-1}). Fig. 6 also shows the Co elution profile for a control serum spiked with 30 ng mL^{-1} of Co(II). As can be observed, both control and patient serum showed a Co peak around 14 min that was also detectable in the blank gradient; however, in the case of the patient, an additional Co-containing peak appears at 12.5 min. This peak eluting at 12.5 min seems to correspond to the association of Co to albumin. This is in agreement with recent

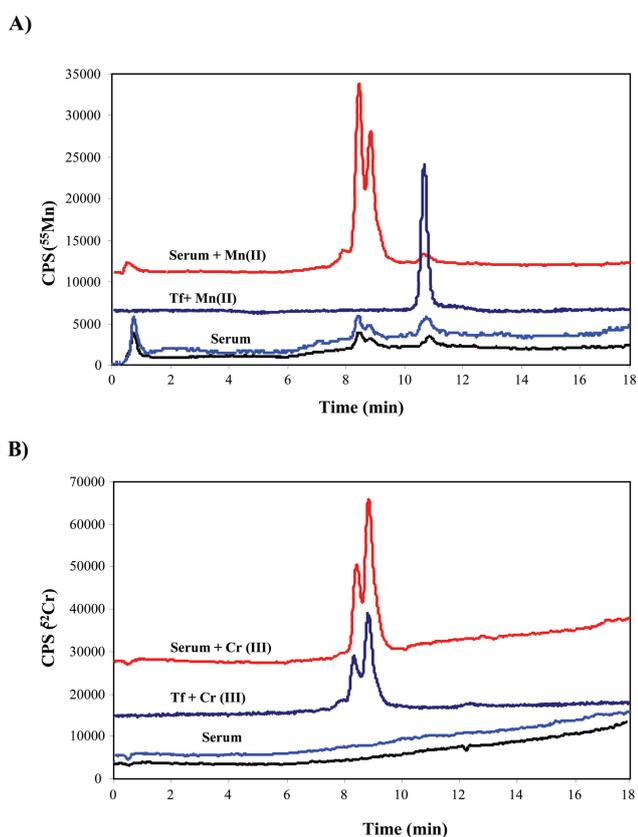


Fig. 5 Chromatographic profile obtained by HPLC-ICP-MS for: (A) Mn in serum from a patient, serum from a control individual, control serum incubated with Mn(II) and standard transferrin incubated with Mn(II); (B) ^{52}Cr in serum from a patient, serum from a control individual, control serum incubated with Cr(III) and standard transferrin incubated with Cr(III).

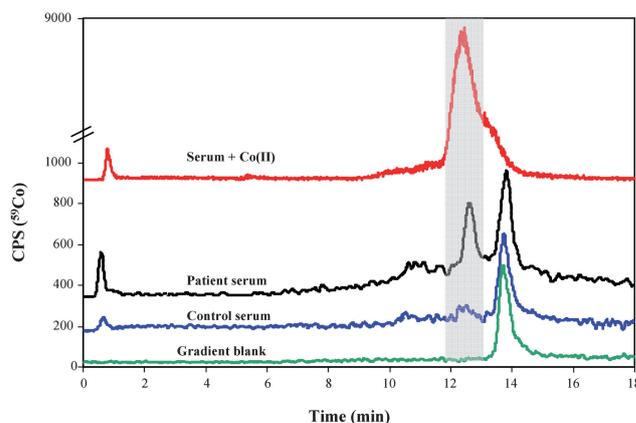


Fig. 6 Chromatographic profile obtained by HPLC-ICP-MS for ^{59}Co in serum from a patient, serum from a control individual, control serum incubated with Co(II) and blank gradient.

reports²⁴ that revealed very strong binding of Co to the *N*-terminus of albumin by ESI-MS. In fact, a species of albumin that is missing the first 2 *N*-terminal aminoacid residues did not form this adduct and the binding seems independent of the oxidation status of the most available cysteine residues.²⁴ The spiking experiments in serum provided similar results (see Fig. 6) to those described before for the patient showing a main Co peak at 12.5 minutes. However, the incubation of Co(II) with an albumin standard did not revealed any specific binding of the metal (data not showed). Therefore, it seems clear that the other serum components play a crucial role in the association of metals to proteins that can not be studied in protein standard models.

Finally, Ti speciation was conducted in the serum samples of the patients and also in the control individuals. According to existing literature,^{25,26} transferrin seems to be the main binder of Ti(IV) in human serum. However, the reported data have been obtained *in vitro* using “spiked” Ti(IV) serum samples. Unfortunately, our attempts to apply the developed HPLC-ORS-ICP-MS speciation methodology to Ti speciation failed to provide accurate results due to the presence of spectral interferences (namely, SO⁺).

Conclusions

A sensitive strategy for the total determination of trace elements potentially released from M/M total hip prosthesis to the biological fluids blood and urine has been developed and validated by means of DF-ICP-MS. The methodology has proved to be sensitive enough to precisely and accurately determine Co, Cr, Mn, Mo and Ti in two reference materials (at the level expected in the samples). Multi-elemental determinations in real samples have been accomplished in several samples of patients carrying such prosthesis and the levels compared to those of control individuals by means of box diagrams. Significant differences have been found for Cr and Ti, both in blood and in urine samples. Differences were also found in patients carrying titanium-based dental implants. Further on, speciation studies have been conducted in those samples where the level of Cr, Co and Mn was slightly higher than in control individuals. These results revealed the association of Mn to transferrin and of Co to albumin and the almost complete absence of Cr in the chromatograms. It is speculated that Cr might be released from the prosthesis as Cr(VI) and so it can not be detected under the proposed HPLC-ICP-MS experimental conditions.

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