Evaluation of the Method for Analyzing Chromium, Cobalt and Titanium Ion Levels in the Blood Following Hip Replacement with a Metal-on-Metal Prosthesis

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High-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) can be used to measure metal ion levels in whole blood, although the accuracy and repeatability of this method is not yet known in the clinical setting. In this study, chromium, cobalt and titanium ion levels were measured in three whole blood samples, each collected at the same moment from 101 patients undergoing total hip arthroplasty with a metal-on-metal (MoM) bearing. The first sample (purge sample) had direct contact with the metal needle used during insertion of the catheter, whereas the second sample (reference sample) and the third sample (reserve sample) did not. The absolute difference between reference samples and reserve samples was greater than the limit of quantification of the HR-ICP-MS device for all three ions (0.84 versus 0.35 µg/L for chromium, 0.74 versus 0.07 for cobalt, and 0.88 versus 0.70 µg/L for titanium), although the levels were very small in most cases, they exceeded the clinical significant threshold in 19 to 31% of the cases. No clinically significant difference was observed between reference samples and purge samples. Therefore, HR-ICP-MS is a clinically acceptable method to evaluate metal ion levels in blood following hip replacement with a MoM prosthesis, and it is not necessary to discard the purge sample to obtain repeatable results.

Introduction

Total hip arthroplasty (THA) is a common surgery that reduces pain and improves the quality of life in patients suffering from hip disorders (1–5). Several types of hip prostheses with different surface bearings have been used in THA, including metal-on-polyethylene, metal-on-metal (MoM) and ceramic-on-ceramic. Early artificial hip joints constructed with metal-on-polyethylene surface bearings resulted in the release of microscopic particles of polyethylene due to the metal femoral head rubbing against the polyethylene acetabular component. These particles are phagocytosed by macrophages, resulting in a chronic granulomatous inflammatory reaction (6). Inflammation leads to local osteolysis in the periprosthetic tissue and THA failure. Periprosthetic osteolysis is currently the dominant limiting factor in the longevity of hip prostheses.

Alternative bearing surfaces such as MoM have been developed in an attempt to reduce the incidence of osteolysis (7, 8). These newer bearings reduce both abrasive wear and the incidence of osteolysis at up to 10 years of clinical follow-up (9), although they are associated with the production of metallic debris, including chromium (Cr), cobalt (Co) (8, 10). Concerns have been raised about the effects of these metal ions in the body because their accumulation in body fluids has been linked to local inflammation, allergic reaction, carcinogenic effects and teratogenic effects (11).

Exposure to Cr and Co has been linked with both cutaneous and extracutaneous allergic reactions, cellular toxicity, cancer and inflammation (12–17). Studies in animals have demonstrated the genotoxic and teratogenic effects of Cr and Co (18, 19), although conclusive evidence linking Cr exposure to teratogenic effects in humans is not available (20). Excessive exposure to Co may lead to cardiomyopathy, possibly due to the disruption of cellular mitochondria, intracellular calcium homeostasis, β-adrenergic system or a specific allergic reaction (14).

Titanium (Ti) is another metal widely used in orthopedic implants. Not part of the bearing surfaces, its systemic release usually comes from passive corrosion. Less evidence in the literature links exposure to Ti ions with adverse effects. Concerns regarding elevated Ti ions in the blood arise from the ability of Ti dioxide, an agent commonly used in air and water purification, to induce pulmonary toxicity when inhaled (21). Furthermore, studies have shown that Ti nanoparticles play a role in the destruction of the periprosthetic bone matrix, and impair cell function by decreasing cell area, cell proliferation, mobility and the ability to contract collagen (22–24).

Although the toxicologic significance of local and systemic elevations in metal ions has not been definitively established, monitoring patients with MoM bearings for elevated metal ion concentrations in the blood can be useful in determining the performance of the bearing (25, 26). Blood concentrations of ions released from metal implants are low, often less than 1 µg/L (27). High-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) is one of the most sensitive and versatile techniques available for the measurement of metal ions in a wide range of sample matrices, including those of environmental and biological origin (28, 29). HR-ICP-MS was introduced in the laboratory in 1983 and allows for the quick and simultaneous analysis of several elements with good sensitivity (30, 31).

The primary objective of this study was to assess the repeatability of measuring metal ions between two blood samples collected at the same time from the same patient with a MoM hip prosthesis. The secondary objectives of this study were to (i) identify factors that may influence the repeatability of the measurement of metal ion concentrations in the blood; and (ii) assess the clinical impact of the practice of collecting a purge sample (i.e., discarding the first tube of collected blood to minimize any potential contamination due to the metal needle used during insertion of the catheter).

Methods and Materials

Study design

The data analyzed in this study were originally generated as part of a prospective study designed to determine the concentrations of Cr, Co and Ti ions in blood of patients who underwent total hip replacement (25, 32). The results presented here provide a retrospective analysis of the repeatability of the
measurement of metal ions in blood between purge samples, reference samples and reserve samples of whole blood.

**Study population**

Patients with degenerative hip joint disease who were scheduled to undergo unilateral MoM hip arthroplasty were recruited between July, 2003 and October, 2009. Patients were eligible for the study if they were 18 years or older, had a disease of the hip requiring total joint replacement, were eligible to receive a prosthesis made of metal alloy, were able to give informed consent and had at least two tubes of blood available from the same collection time. Patients were excluded from the study if they had other metal implants in the body (excluding dental fillings), underwent bilateral surgery, had a history of drug or alcohol misuse, were pregnant or women of childbearing age, were 70 years of age or older, had kidney failure or had a known or suspected allergy to the metal.

**Sample collection**

For each subject, three whole blood samples were collected pre- or postoperatively. One week before blood collection, the patients were asked to not modify their exercise routine or to engage in new, strenuous activities, take new medications or undergo other venous sampling (33). The vein was cannulated with a 22-gauge stainless steel needle (BD Vialon Biomaterial IV catheter, 0.9 × 25 mm, 35 cc/min; Beckton Dickinson, Mississauga, Ontario), and the outer plastic cannula was left in place while the needle was discarded. The catheter was used to collect three syringes (Luer-Lok graduated syringe, 10 cc capacity; Beckton Dickinson) of 5 cc of blood each. Each syringe was emptied into a separate polypropylene plastic tube (13 × 82 mm, 7 cc capacity; Sarstedt Inc., Montreal, Quebec) for storage of the samples and the identification numbers of the subjects were recorded directly on the tube. The blood of the first syringe was identified as the purge sample because it came into contact with the metal needle used to access the vein. The second tube was identified as the reference sample and was used to analyze the levels of metal ions. The third tube was identified as the reserve sample and was used to determine the repeatability of results. All blood collections were conducted by the same trained research nurse. The whole blood samples analyzed in this study were collected and stored at −20°C (25, 32). Metal ions, as inert elements, are known to have stability over a period of 28 days when the sample is stored at 4°C and indefinitely when frozen at temperatures below −15°C. Samples were randomly selected for analysis, regardless of the freezing time, and were frozen between 0 to 29 months. All analyses were performed in blind fashion by the Trace Elements Laboratory (London, Ontario, Canada).

**HR-ICP-MS**

The concentrations of Cr, Co and Ti ions in the whole blood samples were measured in an Element 2 High-Resolution, Sector-Field Inductively Coupled Plasma Mass Spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany). The detection limits were 0.1 µg/L for Cr, 0.02 µg/L for Co and 0.2 µg/L for Ti. The blood samples were exposed to concentrated nitric acid to digest protein and concentrated hydrogen peroxide to digest lipids. After dilution with water and internal standard yttrium 89, the final sample was introduced into the instrument and compared against aqueous standards with commercial blood controls to verify the results.

**Clinical significance**

The measurement of metal ion levels in blood is an indirect assessment of hip prosthesis performance because it is an indication of the friction between the surface bearings. When implants are working well, the average concentrations of Cr, Co and Ti vary between 0.5 and 3 µg/L in blood (25). According to the authors, a variation in concentration between different follow-up measurements of less than 1 µg/L for Cr and Ti and less than 0.5 µg/L for Co would not impact the clinical evaluation. In the event of a malfunction or clinical problem, the concentration of these ions usually increases dramatically from 2 to 20 µg/L. Therefore, a clinically significant change in the levels of Cr, Co and Ti is well above the quantification limit of the HR-ICP-MS instrument. Because no study has identified threshold values for clinical significance, concentration differences above 1 µg/L for Cr and Ti and above 0.5 µg/L for Co were considered to be clinically significant.

**Statistical analysis**

Statistical analysis was performed using SPSS software, version 14.0. Power analyses were performed using the software XLSTAT 2011. All patients with paired results were included in the study. Continuous values were presented as average ± standard deviation with minimum and maximum values. Differences between pairs of samples (in absolute values) were analyzed by a simple t-test with a confidence interval of 95%. Analysis by gender of patients was performed with a Student’s t-test for independent data. The impact of patient age was tested by simple linear regression and the impact of the type of prosthesis and delay before sample analysis were tested with an analysis of variance (ANOVA). Proportion tests were performed with a Chi-square test. Statistical significance was set at 0.05.

**Ethical considerations**

The research project was approved by the Scientific Committee and the Ethics Committee of Maisonneuve-Rosemont Hospital. The study was explained to patients and informed consent was obtained.
Results

Patient characteristics and blood samples available for analysis

A total of 434 pairs of blood samples from a population of 101 patients were analyzed in this study (Table I). The mean age of the patients was 51 (range 27–68 years), and there were 56 men and 45 women. Seventy-five patients underwent large diameter femoral head THA (LDH THA), seven underwent 28 mm THA, and 19 patients underwent hip resurfacing (HR). Delays in analysis between paired samples were between 0 to 12 months for 102 paired samples (34, 35 and 33 paired samples for Cr, Co and Ti, respectively), 13 to 24 months for 32 paired samples (13, 9 and 10 paired samples for Cr, Co and Ti, respectively), or >24 months for 31 paired samples (13, 9 and 9 paired samples for Cr, Co and Ti, respectively).

Repeatability between reference samples and reserve samples

The distribution of Cr, Co and Ti metal ion concentrations in reference samples and reserve samples are shown in Figure 1 and Table II. For Cr and Ti, the mean concentration in the reference sample was significantly different than the mean concentration in the reserve samples.

The mean absolute differences between pairs of reference samples and reserve samples were statistically significantly different for all three ions ($p < 0.001$; Figure 2 and Table III) and above the quantification limit of HR-ICP-MS for Cr (0.35 μg/L), Co (0.07 μg/L) and Ti (0.7 μg/L). Fifty-six percent of paired samples for Cr, 70% for Co and 35% for Ti had an absolute difference above the limit of quantification. Moreover, the mean absolute difference between pairs of samples was greater than the predetermined threshold of clinical significance for Co (0.74 versus 0.5 μg/L) (Table III), whereas it was lower for Cr and Ti (0.84 versus 1.0 μg/L and 0.88 versus 1.0 μg/L, respectively). Eighty-one percent of paired samples had an absolute difference in Cr concentration below the threshold of clinical significance, 69% had an absolute difference in Co concentration below the threshold of clinical significance and 70% had an absolute difference in Ti concentration below the threshold of clinical significance.

Table I

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sample</th>
<th>N</th>
<th>Minimum (μg/L)</th>
<th>Maximum (μg/L)</th>
<th>Average (μg/L)</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>Reference</td>
<td>78</td>
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<td>7.70</td>
<td>1.56</td>
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<td>Reserve</td>
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<td>0.20</td>
<td>5.70</td>
<td>1.12</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
<td>Reference</td>
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<td>0.25</td>
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<td>1.81</td>
<td>1.57</td>
<td></td>
</tr>
<tr>
<td>Titanium</td>
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<td>0.30</td>
<td>6.61</td>
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<td>0.012</td>
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<tr>
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<td>Reserve</td>
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<td>5.61</td>
<td>1.59</td>
<td>1.13</td>
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</tr>
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</table>

Table II

Distribution of the Concentrations of Cr, Co and Ti Metal Ions in the Reference Samples and reserve samples

![Figure 1](https://academic.oup.com/jat/article-abstract/37/2/90/857495/https://academic.oup.com/jat/article-abstract/37/2/90/857495)

Figure 1. Box plot of the concentrations of Cr, Co and Ti metal ions in the reference and reserve samples. Horizontal bars define the quartiles, with the second and third quartiles contained in the boxes. Circles represent outliers, defined as values that are 1.5 to 3 times higher than the values of the third quartile. The data identified with stars are extreme outliers, defined as values that are at least three times higher than the values of the third quartile.

![Figure 2](https://academic.oup.com/jat/article-abstract/37/2/90/857495/https://academic.oup.com/jat/article-abstract/37/2/90/857495)

Figure 2. Box plot of the absolute differences in concentrations of Cr, Co and Ti metal ions in the reference and reserve samples. Horizontal bars define the quartiles, with the second and third quartiles contained in the boxes. Circles represent outliers, defined as values that are 1.5 to 3 times higher than the values of the third quartile. The data identified with stars are extreme outliers, defined as values that are at least three times higher than the values of the third quartile.
Factors affecting the repeatability of measurements between reference samples and reserve samples

No statistically significant relationship was observed between the ages of the subjects and mean absolute differences (\(p = 0.052\) for Cr, \(p = 0.101\) for Co and \(p = 0.198\) for Ti), nor was there a significant relationship between gender and mean absolute differences (\(p = 0.722\) for Cr, \(p = 0.768\) for Co and \(p = 0.839\) for Ti). ANOVA did not show a significant relationship between the repeatability of measurements and the delay between sample analysis (0 to 12 months, 13 to 24 months or >24 months) for Cr (\(p = 0.139\)), Co (\(p = 0.112\)) or Ti (\(p = 0.057\)).

Repeatability between reference samples and purge samples

The distribution of Cr, Co and Ti metal ion concentrations in reference samples and purge samples are shown in Figure 3 and Table IV. Only for Ti was the mean concentration in the reference sample significantly different than the mean concentration in the purge samples. The 95% confidence intervals (CIs) of the average difference between pairs of reference and purge samples for was greater than 0 (0.30–0.78) for Ti and not significantly different than 0 for Cr (−0.16–0.31) and Co (−0.17–0.22). In theory, if the metal needle used to collect the blood samples contaminated the samples, the 95% CI of the average difference would be greater than 0, indicating that the purge samples had higher levels of ions than the reference samples.

The mean absolute differences between pairs of reference samples and purge samples were statistically significantly different for all three ions (\(p < 0.001\); Figure 4 and Table V) and above the quantification limit of HR-ICP-MS for all three ions (0.35 \(\mu\)g/L for Cr, 0.07 \(\mu\)g/L for Co and 0.70 \(\mu\)g/L for Ti).

Forty percent of paired samples for Cr, 56% for Co and 57% for Ti had an absolute difference in concentration above the limit of quantification. The mean absolute difference between pairs of samples was less than the predetermined threshold of clinical significance for Cr (0.46 versus 1.0 \(\mu\)g/L), Co (0.36 versus 0.5 \(\mu\)g/L) and Ti (0.84 versus 1.0 \(\mu\)g/L, Table V). Eighty-eight percent of paired samples had an absolute difference in Cr concentration below the threshold of clinical significance, 86% had an absolute difference in Co concentration below the threshold of clinical significance and 66% had an absolute difference in Ti concentration below the threshold of clinical significance.

Factors affecting the repeatability of measurements between reference samples and purge samples

No statistically significant relationship was observed between the age of the subjects and mean absolute differences, \(p = 0.252\) for Cr, \(p = 0.053\) for Co and \(p = 0.705\) for Ti). A significant relationship was observed between gender and repeatability of Cr (\(p = 0.029\)) and Ti (\(p = 0.026\)) ion measurements, but not for Co (\(p = 0.258\)). Measurements are less reproducible in women than men. ANOVA showed that the repeatability of results was significantly lower for samples that were analyzed 0 to 12 months apart than 13 to 24 months or >24 months apart for Co (\(p = 0.033\)) and Ti (\(p = 0.041\), but not for Cr (\(p = 0.462\)). However, there were few pairs of samples with delays of 0 to 12 months or >24 months.

Discussion

The primary objective of this study was to determine whether the measurement of Cr, Co and Ti ions in blood using HR-ICP-MS is repeatable between reference samples and reserve samples of whole blood collected in the same patients and at the same moment from patients undergoing MoM hip arthroplasty. A statistically significant difference was found in mean absolute differences between the levels of Cr (\(p < 0.001\), Table IV. Distribution of the Concentrations of Cr, Co and Ti Metal Ions in the Reference and Purge Samples

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sample</th>
<th>(N)</th>
<th>Minimum (\mu)g/L</th>
<th>Maximum (\mu)g/L</th>
<th>Average (\mu)g/L</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>Reference</td>
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<td>0.31</td>
<td>3.70</td>
<td>1.77</td>
<td>0.74</td>
<td>0.078</td>
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<td></td>
<td>Purge</td>
<td>72</td>
<td>0.16</td>
<td>5.09</td>
<td>1.32</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>Reference</td>
<td>72</td>
<td>0.06</td>
<td>6.94</td>
<td>1.61</td>
<td>1.49</td>
<td>0.784</td>
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<tr>
<td></td>
<td>Purge</td>
<td>72</td>
<td>0.06</td>
<td>6.94</td>
<td>1.61</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>Reference</td>
<td>74</td>
<td>0.05</td>
<td>5.31</td>
<td>2.51</td>
<td>1.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Purge</td>
<td>75</td>
<td>0.56</td>
<td>6.30</td>
<td>1.97</td>
<td>1.35</td>
<td></td>
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</table>

Figure 3. Box plot of the concentrations of Cr, Co and Ti metal ions in the reference and purge samples. Horizontal bars define the quartiles, with the second and third quartiles contained in the boxes. Circles represent outliers, defined as values that are 1.5 to 3 times higher than the values of the third quartile. The data identified with stars are extreme outliers, defined as values that are at least three times higher than the values of the third quartile.
Co ($p < 0.001$) and Ti ($p < 0.001$) ions in reference samples compared with reserve samples. The mean absolute difference was greater than the limit of quantification of the HR-ICPMS instrument, or 0.35 µg/L for Cr, 0.07 µg/L for Co and 0.70 µg/L for Ti. It exceeded the threshold for clinical significance for Co (>0.5 µg/L), but not for Cr and Ti (<1 µg/L).

This study could not identify the reasons why significantly different measurements of metal ions were obtained in two blood samples that were collected from the same patient at the same time and treated the same way. Possible explanations include contamination during sampling (although sampling-related effects should be minor because the samples were taken at the same time and subject to the same environmental conditions), contamination during storage or contamination during sample handling. Environmental contaminants can come from dust in the air, air pollution from factories that release metal particles, contact with metal surfaces and cigarette smoke (14, 34). Pineau et al. (34) demonstrated that Cr and Co levels can be up to 10 times higher in a laboratory without a controlled environment than in a laboratory with a controlled environment. Therefore, special care should be given to minimizing airborne contamination, strict decontamination methods, proper hand hygiene, selection of uncontaminated sampling equipment and monitoring the laboratory for contamination.

In this study, the samples may have been contaminated during storage, blood collection or laboratory testing. The laboratory, because it is certified for trace element analysis, was considered to be a negligible source of contamination. It is possible that the tubes were contaminated with metal ions prior to sample collection because they were not sterile or hermetically sealed. Unfortunately, it was not possible to quantify the risk of contamination of the tubes used to store the blood samples. Other materials were preserved in sterile packaging and were not considered to be sources of potential contamination.

In some cases, blood samples may have coagulated. Coagulation can cause significant variations in the analysis because the sample is less homogeneous. Some types of metals, such as lead and mercury, are particularly susceptible to significant variations in these conditions. However, the metals analyzed in this study, Cr, Co and Ti, are less subject to variation in results due to coagulation. It was not possible in this study to identify blood samples that had coagulated. There was a delay between the analysis of the reference and reserve samples for some of the patients in this study, but no correlation was found between storage time and results discrepancies.

The secondary objective of this study was to determine whether the levels of Cr, Co and Ti ions are greater in the purge sample than in the reference sample due to contamination by the metal needle used during vein puncture. The results demonstrate that the mean concentration differences of Cr, Co and Ti were above the limit of quantification of the HR-ICP-MS device, and were not significantly greater in purge samples than reserve samples for Cr and Co. Only Ti ion levels were significantly higher in purge samples (95% CI greater than 0 (0.30–0.78). These results indicate that purge samples may have been contaminated with Ti, however the metal needles used in this study did not contain any Ti metal. It is not possible to precisely identify any factors that may have influenced these results. Therefore, it is not necessary to discard the first 5 cc of blood collected.

**Limits of the study**
Because this study was a retrospective analysis, it was not possible to request that samples from the same patient be analyzed by the same technician on the same day and on the same test system. Because this was not the case in this study, it was not possible to determine the variability introduced into the results by these factors. Although metal ions show good stability in frozen blood samples, the time between tests for some of the pairs of samples was greater than recommended. Because the present study was conducted in a hospital, it was not possible to control for environmental contamination, and therefore, it was not possible to determine the influence of this factor on the results.

This study is the first to assess the precision and repeatability of whole blood measurements using HR-ICPMS in clinical practice. Measuring such low metal ion concentrations in biological fluids is recognized as a very challenging task. However, this evaluation, until recently kept for research purposes, is now
needed in the clinical field to better evaluate excessive joint wear in MoM arthroplasty and its associated complications (26, 35). To treat and follow these patients, clinicians and local/national toxicology laboratories will need to collaborate and expand their knowledge in this field.

Conclusions

The precision of Cr, Co and Ti metal ion measurements using HR-ICP-MS in blood samples from patients with MoM hip arthroplasty significantly exceeds the limits of quantification of the device. However, these differences are very small in most cases; they exceeded the clinical significant threshold in 19 to 31% of the cases. It was not possible to demonstrate the contamination of the purge samples with Cr or Co ions. Contamination, if it is real, would have a greater impact in blood samples from patients without MoM arthroplasty (pre-operative dosage, for example) because their levels of metal ions are very low in blood. From a clinical point of view, HR-ICP-MS has acceptable clinical repeatability and the exclusion of the first 5 cc of blood is not necessary for clinical monitoring of patients following an MoM arthroplasty.

References

analysis and in biology. *Annales Pharmaceutiques Francaises*, 64, 312–327.


