Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jor

Original Article

Differences in concentration of metal debris in blood, serum, and plasma samples of patients with metal-on-metal hip resurfacing arthroplasty

M. Khan^{a,*}, J.H. Kuiper^a, Christine Sieniawska^b, J.B. Richardson^a^a Institute of Orthopaedics, The Robert Jones and Agnes Hunt Orthopaedic and District Hospital, Oswestry, Shropshire SY10 7AG, United Kingdom^b Department of Clinical Biochemistry, University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, United Kingdom

ARTICLE INFO

Article history:

Received 24 June 2015

Accepted 4 October 2015

Available online 9 December 2015

Keywords:

Metal ions

Serum

Plasma

Chromium

Cobalt

ABSTRACT

Background/aims: Cobalt and chromium are released from MOM hips and their precise nature (ions/particles) is not known. Their distribution in serum, plasma, and whole blood may help to determine their nature.

Methods: We measured cobalt and chromium concentrations in plasma, serum, and whole blood samples of patients with resurfacing MOM hips.

Results: We found that chromium concentration was highest in plasma, followed by serum and whole blood. Chromium and cobalt concentrations were higher in serum and plasma, compared to whole blood.

Conclusion: We, therefore, suggest that in future cobalt and chromium concentrations shall be reported using plasma samples.

© 2015 Prof. PK Surendran Memorial Education Foundation. Published by Elsevier, a division of Reed Elsevier India, Pvt. Ltd. All rights reserved.

1. Introduction

Patients with metal-on-metal bearing hip arthroplasty have elevated levels of cobalt and chromium in blood and urine. Excess of both elements is toxic to humans, and concerns have been raised about their carcinogenic potential.^{1,2}

Several studies have reported cobalt and chromium levels in patients with metal-on-metal bearing hip replacement. The levels can be affected by differences in age, activity level,

time since implantation,³ analytical technique, and implant types.⁴

Most of the metal debris are produced as a result of wear. The commonest mode of wear at the bearing surfaces is adhesive wear, and therefore, it is expected that the wear debris will be in the form of particles. The literature mostly reports the circulatory cobalt and chromium debris as ion levels. We believe that this convention has resulted because of laboratory reporting of the results. In order to calculate the quantity of the individual element in an alloy, the alloy has to be broken down

* Corresponding author.

E-mail address: surgeonmunir@hotmail.com (M. Khan).

<http://dx.doi.org/10.1016/j.jor.2015.10.006>

0972-978X/© 2015 Prof. PK Surendran Memorial Education Foundation. Published by Elsevier, a division of Reed Elsevier India, Pvt. Ltd. All rights reserved.

into constituent elements (cobalt, chromium, etc.). This process occurs in an extremely hot plasma chamber of the machine, and thus at this stage, the elements are present in ionic states, which otherwise cannot be expected because cobalt and chromium are highly reactive, and therefore, do not exist in pure form.

If metal debris exists in the form of particles, then more of these are expected to be outside the red cells, which approximately make about 45 percent of the blood volume. In order to perform analysis of the whole blood concentration of metal debris, the red cells are hemolyzed. This process causes addition of the intracellular water to the extracellular compartment, and results in dilution of the concentration of metal debris in extracellular compartment, and will result in erroneous results. Similarly, some metal debris may be attached to plasma proteins, and therefore, elimination of those proteins can result in dilution of the results in serum samples. Hence, it is important to know which sample will give highest concentration of metal debris, and accordingly use it routinely.

We designed this study to measure the concentration of metal ion debris in whole blood, serum, and plasma, in order to identify the component of blood to which most of the wear debris is added from the hip joint. We tested the null hypothesis that there is no difference in whole blood, serum, and plasma cobalt and chromium levels of the same patient.

2. Material and methods

Patients who had metal-on-metal resurfacing hip replacement were identified from the hospital records. Patients with these implants were sent a postal invitation to take part in the study. The local Research Ethics Committee and the Research Panel in our hospital approved the study. All subjects were informed about the study and provided written consent.

2.1. Collection of whole blood, serum, and plasma samples

Plasma, serum, and whole blood samples were taken for analysis of cobalt and chromium levels from patients. The first 3 ml of each blood sample was discarded at collection and two samples (2 ml each) were then collected, one into a metal-free lithium heparin tube (Teklab, Durham, UK), and one into a plain metal-free container. The samples were then centrifuged at 3500 revolutions per minute for 5 min. Serum was isolated from the free metal container and plasma from the lithium heparin centrifuged tube.

2.2. Sample analysis

All blood analyses were performed at the SAS Trace Metal Unit at Southampton General Hospital (Southampton, UK). Cobalt was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and chromium was determined using Dynamic-Reaction Cell ICP-MS. The detection limits for each element were 1 nmol/L. To see differences between blood, serum, and plasma metal ion levels, the analysis was performed separately for each sample. To see the repeatability in sample measurements (precision of repeated measurements), measurements in each sample (blood, serum, and plasma) were taken in duplicate.

2.3. Statistical analysis

The two types of statistical analysis⁵ used for the purpose were intraclass correlations (ICCs),⁶ and Bland and Altman tests.⁷ Bland and Altman approach is based on the analysis of differences between measurements. The differences between two measures are plotted against average of the two measurements. The resulting graph shows size of each difference, the range of differences, and their distribution about zero (perfect agreement). Distributions on the graph will show if differences in samples are related to the size of the mean of two samples. In other words, as the mean of two samples increases, so does the difference. The results of Bland and Altman analysis include the mean difference between measures, the 95% confidence intervals, and the reliability coefficient (assuming that the mean difference is zero).

3. Results

There were seventeen patients with two different types metal-on-metal bearing resurfacing hip replacement (Birmingham Hip Resurfacing, Midland Medical Technologies, UK; and Cormet Hip Resurfacing, Corin Medical, UK). Ten participants were male and seven were females with the mean age of 55 years (range 42–80 years). The median time since implantation was 44 months (range 6–96 months).

3.1. Intersample analysis

Mean plasma, serum, and whole blood cobalt and chromium levels are shown in [Tables 1 and 2](#). Plasma and serum had high levels of cobalt and chromium, as compared to whole blood.

Table 1 – Mean and standard deviation of the cobalt and chromium levels in plasma, serum, and whole blood.

	Intersample reliability		
	Plasma (nmol/L) (Mean, SD)	Serum (nmol/L) (Mean, SD)	Blood (nmol/L) (Mean, SD)
Cobalt	40 (31.3)	42.2 (34.8)	33.8 (25.7)
Chromium	117 (52.2)	65.9 (39.7)	47.7 (24)

Table 2 – Mean and standard deviation of the cobalt and chromium repeatable measures (nmol/L) in plasma, serum, and whole blood.

Between-measurements repeatability in a sample (measurement 1 and measurement 2)					
Plasma cobalt (nmol/L) (Mean, SD)		Serum cobalt (nmol/L) (Mean, SD)		Blood cobalt (nmol/L) (Mean, SD)	
P1	P2	S1	S2	B1	B2
39.1 (31.1)	39.9 (31.2)	41.8 (34.2)	42.6 (34.5)	33.7 (24.2)	34 (25.3)

Table 3 – Results for intersample reliability and between-sample repeatability – ICCs and Bland and Altman tests.

	ICC		Bland and Altman			
	ICC coefficient	95% CI	Mean difference	SD nmol/L	Reliability coefficient	95% limits of agreement nmol/L
<i>Intersample reliability for cobalt and chromium measurements</i>						
Serum-blood (cobalt)	0.85	0.54–0.95	9.7	14	11.1	2.7–16.7
Plasma-blood (cobalt)	0.92	0.50–0.97	8.3	8.5	7.8	4–12.5
Plasma-serum (cobalt)	0.93	0.83–0.97	1.4	12.3	0.23	–7.5 to 4.7
Serum-blood (chromium)	0.60	0.09–0.86	17.2	28.5	34.8	–1.9 to 36
Plasma-blood (chromium)	0.11	–0.11 to 0.45	69.3	52	564	37.9–100
Plasma-serum (chromium)	0.54	–0.1 to 0.87	49	27.6	282	30.5–67.5
<i>Between measurement repeatability in cobalt measurements</i>						
Serum	0.99	0.99–1	0.7	1.5	0.3	–1.5 to 0.1
Plasma	0.99	0.99–1	0.22	1.04	0.005	–0.3 to 0.7
Blood	0.99	0.99–0.99	0.4	1.43	0.24	–1.1 to 0.4

The ICC coefficients and 95% confidence intervals for intersample reliability are shown in Table 3.

1. Cobalt measurements:

Plasma and serum cobalt measurements had better ICC as compared to whole blood and serum, and whole blood and plasma. Besides, the 95% confidence intervals were also wider for the ICC of whole blood and serum or plasma, as compared to serum and plasma (Table 3).

The Bland and Altman test results are shown in Table 3, and the distribution of values in Fig. 1 showed that plasma and serum had higher cobalt concentrations, as compared to whole blood levels. Plasma and serum cobalt levels were higher than whole blood levels (poor agreement), and this difference increased with increasing mean cobalt levels. On the other hand, serum and plasma cobalt levels had good agreement.

2. Chromium measurements:

For chromium measurements, plasma values were highest, followed by serum and whole blood (Fig. 2). Two

Fig. 2 – Bland and Altman plot for intersample reliability of chromium levels. '●' Comparison between serum and whole blood measurements. '○' Comparison between plasma and whole blood measurements. '▲' Comparison between serum and plasma measurements.

Fig. 1 – Bland and Altman plot for intersample reliability of Cobalt measurements. '●' Comparison between serum and whole blood cobalt measurements. '○' Comparison between plasma and whole blood cobalt measurements. '▲' Comparison between serum and plasma cobalt measurements.

distinct relationships can be seen. Firstly, there was a linear relationship between difference in serum/plasma chromium and the whole blood chromium, as the mean cobalt and chromium increased. Secondly, there was more chromium in plasma than serum and the difference ranged between 35 and 65 nmol/L. The difference between the two samples did not change with increasing mean chromium.

3.2. Intrsample repeatability of cobalt measurements

Intrsample repeatability was high for whole blood, serum, and plasma cobalt measurements. Results of the ICC coefficient are shown in Table 3. Results from the Bland and Altman analysis (Fig. 3) showed that agreement between repeated measures was highest in plasma. Most of the differences between the repeated measures were less than 1 nmol/L. There were only three outliers, in whom differences between repeated measures were more than 1 nmol. On the other hand, the agreement for repeated measures of whole blood and serum was poor, in comparison to plasma measurements. In other words, in comparison to plasma repeated measurements, the serum

Fig. 3 – Bland and Altman plot for repeatability of cobalt levels in different matrices. ● represents measurements of serum. ○ represents measurements of plasma. ▲ represents measurements of whole blood.

and whole blood repeated measurements resulted in more values lying outside the 1 nmol range.

4. Discussion

The results of our study showed that cobalt and chromium concentrations were higher in serum and plasma than whole blood. The difference is pronounced once the mean whole blood and plasma (or serum) cobalt is more than 20 nmol/L, and chromium is more than 50 nmol/L. This finding has important implication. Most of the body cells are exposed to extracellular fluid (serum/plasma), and therefore, whole blood measurements will underestimate the exposure of body tissues to cobalt and chromium concentrations.

Distribution of wear debris in blood and body fluids is not known precisely. Our results indicate that wear debris is not instantly internalized by circulating cells, and therefore, cobalt and chromium concentrations are high in serum/plasma, which is extracellular fluid. This pattern of distribution in which most of the wear debris is stored in extracellular (plasma/serum) fluid would occur if wear debris is composed mostly of free-floating particles derived from the parent alloy, or molecules bound to the extracellular proteins. Alternatively, intracellular storage capacity may be at best limited, and therefore, further additions of wear debris to the blood would result in elevation of only extracellular levels. This argument is supported by the finding of increasing difference between plasma/serum and whole blood measurements with increasing magnitude of mean cobalt and chromium (Figs. 1 and 2), which also show that additions of further wear-related chromium and cobalt wear debris is to the extracellular compartment. Consequently, the addition of intracellular fluid to the extracellular fluid happens when the whole blood sample is analyzed (because cells are lysed) and results in dilution of the cobalt and chromium concentrations, and hence lower whole blood concentrations.

Cobalt and chromium behave differently in plasma and serum. Unlike chromium, cobalt is identical in both samples.

The parent alloy contains approximately 2/3rd of cobalt and 1/3rd of chromium. Circulatory levels do not reflect on this distribution and often there is more chromium in circulation than cobalt. If the proportions of these elements were similar to the parent alloy, then all the debris would be composed of particulate matter. However, given the difference in the proportions of cobalt and chromium, it seems obvious that both the elements are not bound to each other, as they are found in the parent alloy lattice. Studies report that most wear particles are composed of oxidized chromium and a few particles appear to be of the alloy's carbide origin.⁸⁻¹⁰ The particular reason for this effect is not known, and it is thought that either chromium oxide particles are primarily produced at the bearing surfaces as a result of wear of passivation layer, or cobalt is readily dissolved out of these particles and removed in urine, leaving chromium oxide bound to proteins behind in the circulatory fluid. The earlier argument is supported by the presence of higher urinary cobalt than chromium in urine of patients with metal on metal hips.¹¹ Moreover, renal excretion capacity of cobalt has been shown to increase more than threefolds after metal-on-metal hip arthroplasty.¹² Another explanation could be removal of chromium by its binding to clotted blood, which is required for preparation of serum. In our study, the difference between plasma and serum chromium ranged from 35 to 65 nmol/L and this difference was not related to the magnitude of mean values. This observation suggests that there is either a fixed extrastorage capacity of plasma, or a certain fixed amount is indeed cleared off serum during the preparatory process by the clotting process.

In our study, there was no difference in plasma and serum cobalt concentrations. This can be seen from Bland and Altman plot values of difference lying on the zero (perfect agreement) line of the Bland and Altman plot.

Daniel et al.¹³ also found higher concentration of chromium and cobalt in serum compared to whole blood. The authors considered whole blood levels as gold standard because it was reported earlier that metal ions are stored inside the red cells. The group noted that for same whole blood (gold standard) cobalt and chromium there was variability in serum measurements, on the basis of which they suggested to perform whole blood levels. We do not agree with their approach because firstly, extracellular concentration is higher and also because circulating cobalt and chromium are products of wear, which is related to activity of patients, and therefore is expected to have variability.

We checked repeatability of cobalt in the samples because we previously used this element as an instant surrogate circulatory marker of prostheses wear. The rise in plasma cobalt was very small after an hour of exercise in patients with metal-on-metal resurfacing hip arthroplasty. This small rise in plasma cobalt could be affected by measurement error resulting from inadequate detection limits of the analytical technique or that of poor repeatability. Our analytical technique's detection limits were 1-2 nmol/L, and therefore acceptable. Repeatability error was, however, unknown. It is therefore reassuring to know that measurement error in plasma samples is the least and this provides confidence in estimation of the exercise-related rise in plasma cobalt as a marker of wear.

Based on the results of this study, we suggest to measure chromium and cobalt in plasma for the following reasons:

1. This sample has the highest concentration of metal debris.
2. Plasma chromium and cobalt levels represent the maximum exposure of tissues to metal ions. And,
3. Repeated plasma cobalt measures have least measurement error.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Davies AP, et al. Metal-specific differences in levels of DNA damage caused by synovial fluid recovered at revision arthroplasty. *J Bone Joint Surg Br.* 2005;87:1439-1444.
2. Brown C, et al. Consequences of exposure to peri-articular injections of micro- and nano-particulate cobalt-chromium alloy. *Biomaterials.* 2013;34:8564-8580.
3. Khan M, Kuiper JH, Richardson JB. Can cobalt levels estimate in-vivo wear of metal-on-metal bearings used in hip arthroplasty? *Proc Inst Mech Eng H.* 2007;221:929-942.
4. Khan M, et al. Current in vivo wear of metal-on-metal bearings assessed by exercise-related rise in plasma cobalt level. *J Orthop Res.* 2006;24:2029-2035.
5. Rankin G, Stokes M. Reliability of assessment tools in rehabilitation: an illustration of appropriate statistical analyses. *Clin Rehabil.* 1998;12(3):187-199.
6. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull.* 1979;86:420-428.
7. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307-310.
8. Catelas I, et al. Size, shape, and composition of wear particles from metal-metal hip simulator testing: effects of alloy and number of loading cycles. *J Biomed Mater Res A.* 2003;67:312-327.
9. Firkins PJ, et al. Quantitative analysis of wear and wear debris from metal-on-metal hip prostheses tested in a physiological hip joint simulator. *Biomed Mater Eng.* 2001;11:143-157.
10. Pourzal R, et al. Characterization of wear particles generated from CoCrMo alloy under sliding wear conditions. *Wear.* 2011;271:1658-1666.
11. Tiusanen H, et al. The effect of different bearing surfaces on metal ion levels in urine following 28 mm metal-on-metal and 28 mm metal-on-polyethylene total hip arthroplasty. *Scand J Surg.* 2013;102:197-203.
12. Daniel J, et al. Renal clearance of cobalt in relation to the use of metal-on-metal bearings in hip arthroplasty. *J Bone Joint Surg Am.* 2010;92:840-845.
13. Daniel J, Ziaee H, Pynsent PB, McMinn DJW. The validity of serum levels as a surrogate measure of systemic exposure to metal ions in hip replacement. *J Bone Joint Surg Br.* 2007;89(6):736-741.