

No need for immediate freezing of metal ion blood samples in patients with metal-on-metal hip articulations

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ABSTRACT

INTRODUCTION: A recent British medical device alert suggested monitoring patients with metal-on-metal (MoM) articulations using blood metal ions. These blood samples are usually frozen immediately and shipped frozen for analysis. Simply posting the samples in the mail would lower the costs and simplify logistics of metal ion testing. The aim of this study was to determine whether the metal ion value in the blood is stable when kept at room temperature.

MATERIAL AND METHODS: Eight patients with large-diameter MoM articulations were included. We compared levels of chromium (Cr) and cobalt (Co) in whole-blood samples frozen immediately, after four days and after 30 days.

RESULTS: We found Co ranging from 0.64 to 10.9 µg/l and Cr from 0.76 to 5.16 µg/l. There was no systematic reduction in the mean level of Cr and Co of the eight patients when we compared results from the blood frozen immediately with the blood frozen after four days and after thirty days. There was a tendency towards greater variation (limits of agreement) in the results of the individual blood samples over thirty days, but these increases were non-significant.

CONCLUSION: The variation of Co and Cr ions in blood kept at room temperature for up to thirty days is within clinically acceptable levels for the diagnosis of excess wear.

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TRIAL REGISTRATION: not relevant.

In recent years, reports of highly increased revision rates for both resurfacing and large-diameter head metal-on-metal (MoM) total hip arthroplasty compared with metal or ceramic on polyethylene articulations [1-3] has sparked debate on how to best monitor these patients.

Patients with total hip replacement of the MoM type may have increased levels of metal particles and metal ions, including cobalt (Co) and chromium (Cr), in the blood [4-6], and these elevated levels of Co and Cr ions can be associated with increased wear [7] of the MoM joint articulation. Increased metal-particulate exposure may cause local hypersensitivity reactions (ALVAL) [8], local toxic effects leading to tissue necrosis and formation of pseudo tumours [9-11]. These adverse

effects are multifactorial with some patients developing soft-tissue changes despite low metal ion concentrations, whereas others with high concentrations are resistant [8,12-14]. Metal ion concentrations alone therefore cannot be used as a single test to identify at risk patients, but the current recommendations are that concentrations be used as part of prospective monitoring [15].

Contrary to protocol-led studies where the blood samples are handled in a trace element lab and stored in the freezer until final analysis, the at-risk patients require the result within a short time frame. The usual procedure including shipping of frozen samples is work-intensive and costly for the individual samples. Simply posting the blood sample in the mail would simplify the logistics of metal ion testing thereby increasing the likelihood of a proper follow-up and also enabling the smaller hospitals without trace element labs to test their patients. In Europe, metal ion blood samples can be posted in the regular mail inside a protective cover and envelope marked "diagnostic specimen UN3373" [16].

Blood left at room temperature for several days degrades and it is important to validate that the Cr and Co concentrations are, indeed, valid before introducing a new method.

The aim of this study was to determine whether metal ion concentrations in the blood of a MoM population were stable at room temperature.

MATERIAL AND METHODS

The patients were recruited from a previously performed randomised controlled trial comprising 17 large-diameter MoM THA with a M2aMagnum/ReCap articulation in combination with a cementless, forged titanium Bimetric stem (Biomet, Briggde End, UK). All were contacted by letter and the first eight to respond positively were included in the study. The group counted four women and four men with a median age of 62 years (57-66 years) who were included in this study after informed consent. All patients underwent total hip replacement in the period from 2007 to 2009 with a mean follow-up of 25 months and a standard deviation (SD) of 12.0 months. The mean head size was 49.5 mm (SD = 3.0 mm). One of the includ-

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X-ray of metal on metal hip articulation.



ed patients had bilateral total hip replacement of the MoM type. All patients were healthy when the sampling was performed. One patient had hip pain that could be related to the MoM joint articulation at the time of sampling. The remaining seven included patients were painless at sampling.

Institutional board review was obtained on 13 October 2010 (project-ID: SJ-194).

The sample size was calculated to seven based on a Co and Cr concentration of 1 µg/l, an SD of 0.05 µg/l (information from analysing lab) and a minimum relevant difference of 0.2 µg/l (arbitrarily chosen as double the lab uncertainty in measurement which is 10% at 1 µg/l and deemed an acceptable deviation between samples in a MoM population). In this way we assured at least an 86% chance (power) to obtain a 90% normal region for the individual differences within the MIREDIFF (± 0.2 µg/l). One additional patient was added to safeguard against missing data. We thus obtained samples from eight patients in total.

Five blood samples were drawn from each patient. The first sample was discarded in each case to avoid chrome contamination from the steel needle [11, 17].

Powder-free vinyl gloves, Sempercare Vinyl (Sempermed, FL), were used during sampling. A cobalt-free steel needle (nickel 9.2%, chromium 18.4%, manganese 1.8%, iron 69.6%, and mixed listing 1%) and a Vacutainer "Safety-Lok Blood collection set" (21 G 0.8 × 19 mm × 304.8 mm) (BectonDickinson, NJ) were used. The blood was collected in trace element 6/7 ml Plus K2EDTA tubes (368381) (BectonDickinson). For each patient, two blood samples were frozen immediately. The remaining two blood samples were kept at room temperature and allowed to coagulate. These remaining blood samples were frozen after four days and 30 days, respectively. The blood samples were primarily frozen at minus 20 °C for the first hour and afterwards kept frozen at minus 80 °C, and all blood samples were finally sent frozen for analysis (ALS lab, Luleaa Sweden).

Analysis methods

The blood samples were analysed by an Inductively Coupled Plasma Sector Field Magnet Spectrometry Finnigan ELEMENT (Finnigan MAT, Bremen, Germany) with a limit of quantification for Cr of 0.4 µg/l and 0.1 µg/l for Co.

Statistical methods

First, raw means were calculated for samples frozen immediately and for samples frozen after four and 30 days to examine systematic changes in the metal ion concentrations over time. Second, we used each patient as his or her own control and evaluated the individual differences in concentrations between the two immediately frozen samples (A versus B) and between blood frozen immediately (sample A) and after four days and 30 days, respectively. These calculations were performed with a Bland-Altman 90% limit of agreement analysis (LoA). LoA is an expression of the mean of the differences between the two blood samples from the same individual. Following flushing of the sampling system, blood samples drawn and frozen at the same time should have the same ion concentrations. Any difference between them is random and reflects analytical variation, a risk of Cr from the needle (despite flushing) and possibly an in-

TABLE 1

Cobalt values in µg/l.

	Mean	Standard error	95% CI
CoA, immediate	2.71	1.10	0.10 to 5.32
CoB, immediately repeated	2.76	1.17	-0.01 to 5.52
Co-4 days	2.90	1.24	-0.03 to 5.83
Co-30 days	2.89	1.19	0.07 to 5.71

CI = confidence interval; Co = cobalt.

TABLE 2

Chromium values in µg/l.

	Mean	Standard error	95% CI
CrA, immediate	2.40	0.53	1.16 to 3.65
CrB, immediately repeated	2.49	0.53	1.24 to 3.75
Cr-4 days	2.41	0.50	1.22 to 3.59
Cr-30 days	2.36	0.50	1.16 to 3.55

CI = confidence interval; Cr = chromium.



TABLE 3

Individual differences of cobalt.

	90% LOA, µg/l	p-value
CoA, immediate, vs CoB, immediately repeated	-0.45 to 0.35	
CoA, immediate, vs Co-4 days	-0.85 to 0.46	0.21
CoA, immediate, vs Co-30 days	-0.64 to 0.28	0.71

LOA = limits of agreement; Co = cobalt.

homogeneous distribution of Cr and Co in the blood which will imply that all sample tubes do not contain exactly the same metal at baseline. The variability in concentration between two samples immediately frozen versus samples frozen after a four-day delay and between samples immediately frozen versus samples frozen after a 30-day delay was compared with the variability between two samples both frozen immediately with the equality of variance test. A p-value less than 0.05 was considered significant for the hypothesis tests. All analyses were performed using STATA 11.1. (StataCorp LP, College Station, Texas).

Trial registration: not relevant

RESULTS

We found Co values ranging from 0.64 to 10.9 µg/l and Cr from 0.76 to 5.16 µg/l.

The mean cobalt concentrations are presented in **Table 1**, which shows that there was no systematic reduction in the level of cobalt over thirty days. The mean chromium concentrations are shown in **Table 2**, which shows a small, non-significant reduction from 2.40 µg/l in immediately frozen samples to 2.35 µg/l in samples frozen after 30 days.

The individual differences are listed in **Table 3** and **Table 4**, which show that the individual differences in both Co and Cr increased after four and 30 days compared with baseline values. These increases were non-significant for both Co and Cr although the baseline-to-four-day Cr LoA was close to statistical significance with $p = 0.08$. There was no indication that the none significant increased variation depended on the actual ion concentration.

DISCUSSION

Consensus about the method of blood collection, the method of storage and the methods of analysis is desirable in the at-risk patient group [6] since both the medium used (serum, whole blood or erythrocytes) [18, 19], the analysing machines themselves inductively coupled plasma mass spectrometry (ICP-MS) or high resolution



TABLE 4

Individual differences of chromium.

	90% LOA, µg/l	p-value
CrA, immediate, vs CrB, immediately repeated	-0.33 to 0.14	
CrA, immediate, vs Cr-4 days	-0.49 to 0.48	0.08
CrA, immediate, vs Cr-30 days	-0.33 to 0.42	0.23

LOA = limits of agreement; Cr = chromium.

ICP-MS and the needle used in sampling [17] may influence the measured value of metal-ion concentrations.

To our knowledge, no publication has investigated whether the Cr and Co levels are constant during degradation of the blood.

In this study, we investigated blood ion levels in a group of eight MoM patients. The range of their individual blood ion levels of Cr and Co was wide, but no systematic reduction was observed in the mean level of blood frozen immediately compared with blood left at room temperature. There was a tendency towards an additional variability of about 0.3 µg/l over thirty days, but this variability was not statistically significantly different from the variability observed between two immediately frozen samples.

The variability did, however, exceed the 0.2 µg/l change we initially defined as the minimally relevant difference, but that was also true for the two samples frozen immediately. Without the extra sample frozen immediately that demonstrated a higher than assumed baseline variability, we would wrongly have concluded that leaving the blood at room temperature statistically significantly increased the variability.

The 0.2 µg/l was arbitrarily chosen for a 1 µg/l population and, in reality, our population had higher mean ion concentrations and higher standard deviations (0.12 µg/l for Co and 0.07 µg/l for Cr. In retrospect, the MIREDF should have been set at a higher level.

However, the inclusion of higher-wear patients strengthens the study as this population reflects reality where we now see increased wear in parts of the large head MoM population [20].

Lab uncertainty increases almost linearly with increased sample concentration and the variability in two immediately frozen samples; in particular. Co also reflects this fact. This means that the clinician will have to be extra critical if he or she uses the ion measurements for prospective monitoring of the samples from high-wear/high ions concentration patients to detect rising metal ion levels, where increases of 1 µg/l may simply reflect lab uncertainty. Our data do not indicate an additional, added variability at higher concentrations, but

the largest additional variability was seen for the patient whose level exceeded 7 µg/l. The sample size is a limitation of the study and larger sample size may have further clarified this aspect.

The results from this study indicate that easy handling of the samples comes with the cost of a non-significant additional 0.3 µg/l and the use of the method depends on which level of variability is deemed acceptable. The MRHA protocol aims to identify non-symptomatic at-risk patients for soft tissue imaging or revision [15]. The additional variability of the method ought to be acceptable if it is a question of identifying patients with high ion concentration (> 7 µg/l). The method will also be able to identify rising metal ion concentrations in the "normal" 1-3 µg/l patient in whom, for example, an increased 1 µg/l + since the last follow-up is observed. But when repeating a test of a "> 7 µg/l" patient three months later, lab uncertainty in itself may demand larger changes are ascertained to correctly identify an increase – regardless of the method – and the clinician needs to be aware of this.

It is a drawback of the present study that we only investigated blood kept at approximately 20 °C. In real life on a hot summer's day, the blood samples may be subjected to more extreme temperatures like 30-40 °C during storage and transport. Whether this would influence the value was not investigated in this study.

We do not know if these results would be valid for serum or erythrocytes as well, but the scope of the investigation was to find an easy logistic way for the clinician to test the patients. Once the blood components have to be pipetted into a separate container, a trace element lab is needed, and the logistic advantage disappears.

The object of sampling these at-risk patients is to detect signs of increased wear. A small increase change in variability will not mask any real wear. The observed tendency towards a greater variation (limits of agreement) in the results of the individual blood samples after four and after thirty days was non-significant and considered of small clinical significance in this population.

Whether samples for research trails could be handled this way depends on the accuracy demanded by the investigator.

We conclude that in a population of patients with MoM joint articulations, the variation of Co and Cr ions in blood kept at room temperature up to thirty days is within clinically acceptable levels for the diagnosis of excess wear and for monitoring at-risk patients.

There is no need for immediate freezing and shipping on frost when handling Cr and Co blood samples of whole blood.

CONFLICTS OF INTEREST: Disclosure forms provided by the authors are available with the full text of this article at www.danmedj.dk.

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