Chromium speciation in the blood of metal-on-metal hip implant patients

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\textbf{ABSTRACT}

The objective of this study was to determine the valence state of chromium (Cr) in the blood of individuals with Cr-containing metal hip implants. Serum and red blood cell (RBC) Cr concentrations from 52 patients with Cr-containing total hip arthroplasties were measured preoperatively and at 3, 12, and 24 months postoperatively. Geometric mean and median pre-surgery serum Cr concentrations were consistently below 0.2 mg/L, while geometric mean and median pre-op RBC Cr concentrations were typically about four- to six-fold higher than the serum values. A significant 5- to 13-fold increase was found in the mean and median serum Cr concentrations three months post-surgery, with an 8- to 18-fold rise at 12 and 24 months, respectively. Steady-state serum concentrations were reached between 3 and 12 months. In contrast, there were no marked differences in mean and median RBC Cr concentrations pre- and post-surgery. Slope regression analysis for our data was similar to those reported for Cr(III) in spiked blood samples. The analysis showed that Cr released from hip implants preferentially distributed into serum and not RBC, indicating that the form of Cr present in blood of hip implant patients was in the form of non-toxic Cr(III). Our findings indicate that blood Cr concentrations Cr(III) associated with metal implants do not pose an adverse health risk to patients, which is in agreement with findings published by most investigators.

\textbf{Introduction}

It is well established that biological implants composed of metal alloys release metal ions into the bloodstream of patients (Black et al. 1983; Coleman, Herrington, and Scales 1973; Jacobs et al. 1995). As noted in Michel et al. (1991, 61), “due to the action of the highly aggressive body fluids and to strong mechanical stress, all implant materials undergo processes of corrosion and destruction in the body.” Nickel (Ni), aluminum (Al), titanium (Ti), molybdenum (Mo), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), and zinc (Zn) as well as other metals have all been detected in blood of implant patients at levels higher
than those that occur in the general population (Dobbs and Minski 1980; Lux and Zeisler 1974; Michel et al. 1984; Nasser et al. 1990; Pazzaglia et al. 1983; Smethurst and Waterhouse 1977; Williams and Meachim 1974). Biological implants known to release metals include static devices such as rods, pins, plates, and screws, as well as articulating devices, including knee, hip, and elbow joint prostheses (Evans et al. 1974; Goldberg et al. 2008; Matthew and Frame 2000; Meachim and Williams 1973; Nasser et al. 1990; Schliephake et al. 1993).

Cr and Co-containing alloys have been used in hip and knee implants for over 70 years due to their biocompatibility, longevity, and resistance to corrosion (Friedman, Orland, and Greco 1994). The first CoCr combination was developed by Elwood Haynes in the 1920s (Friedman, Orland, and Greco 1994). Soon thereafter, a cobalt–chromium–molybdenum (CoCrMo) alloy (also referred to as “Vitallium”) was developed in 1936 (Friedman, Orland, and Greco 1994). Since that time, the articulating surfaces of most metal hip implants have been composed of various CoCr alloys (Gilbert 2007). Modern metal hip implants generate relatively low amounts of wear debris, and as a result, blood Co and Cr concentrations in hip implant patients are usually low and not markedly elevated above typical “background” levels. Blood Cr concentrations in the general population typically range from 0.2 to 2.8 μg/L (ATSDR 2012; Iyengar and Woittiez 1988), while reported mean concentrations in metal-on-metal (MoM) CoCr hip implant patients from 0.72 to 5.92 μg/L (Campbell et al. 2014; Jacobs et al. 1998; Kwon et al. 2010; Langton et al. 2009; Tkaczyk et al. 2010; Kerger et al. 2015). Similarly, blood Co concentrations in the general population are typically <0.5 μg/L (Alimonti et al. 2005; Daniel et al. 2009; Engh et al. 2009; MacDonald et al. 2003; Finley et al. 2013; Kerger et al. 2013; Tvermoe et al. 2014), while blood Co concentrations in MoM CoCr hip implant patients range from 0.2 to 10 μg/L (Antoniou et al. 2008; De Smet et al. 2008; Engh et al. 2009; Jacobs et al. 1996).

Occasionally, concerns have been raised regarding patient health risks associated with elevated blood Cr concentrations, particularly with respect to the valence state of Cr. Specifically, Cr(III) (trivalent chromium) is a naturally occurring, generally inert, essential element that is not toxic except at very high doses. In contrast, Cr (VI) (hexavalent chromium) is a biologically reactive, non-essential metal that may produce certain adverse health effects at elevated exposures (Shi et al. 1999; ATSDR 2012) depending on the route of entry. The oxidation and reduction kinetics of the ionic Cr species suggest that blood and tissue Cr in implant patients are likely to be in the form of non-toxic Cr(III). Specifically, the Cr within the alloy is in the elemental form, Cr(0), which in vivo readily oxidizes to Cr(III), but not Cr (VII). Oxidation of either Cr(0) or Cr(III) to Cr(VI) is thermodynamically favorable, and thus not normally expected to occur in biological tissues (De Flora et al. 1997; De Flora and Wetterhahn 1989). In addition, Cr(VI) in vivo is rapidly reduced to the more stable Cr(III) species due to the presence of reducing groups (De Flora et al. 1997; De Flora and Wetterhahn 1989). Nonetheless, while many investigators concluded that any Cr released from metal implants is most likely to be in the form of Cr(III) (Hart et al. 2010; Jacobs et al. 1995; Urban et al. 1994), others have suggested that Cr(VI) might be generated and released in vivo via implant corrosion and wear (e.g., Merritt and Brown, 1995).

There are many analytical difficulties and biological issues associated with quantification of specific Cr ion species in biological matrices, such as stabilization of the reactive Cr(VI) species in the extractant. However, separate analyses of serum and red blood cell (RBC) total Cr concentrations provide evidence of ionic speciation as a result of different partitioning characteristics of Cr(III) and Cr(VI) in whole blood. Specifically, while Cr(III)
generally does not penetrate the membrane of the RBC, Cr(VI) readily traverses the RBC membrane and becomes permanently bound to intracellular components (Figure 1) (Finley et al. 1997; Kerger et al. 1997, 2013; Miksche and Lewalter 1997). Accordingly, a sustained increase in RBC total Cr levels or RBC/serum Cr concentration ratios is viewed as evidence of Cr(VI) exposure, while an elevation in serum total Cr, with relatively little or no associated rise in RBC total Cr, is categorized as Cr(III) exposure (Finley et al. 1997; Kerger et al. 1997; Miksche and Lewalter 1997). This analytical fingerprinting technique was used previously to demonstrate that ingested Cr(VI) is reduced to Cr(III) in the gastrointestinal tract prior to systemic absorption (Finley et al. 1997).

The purpose of this study was to determine the valence state of Cr in blood of patients with Cr-containing hip implants. Serum and RBC Cr measurements were used as indicators of Cr speciation at pre-surgical and post-operative time points. To our knowledge, no such study has been published to date. This analysis is based upon an unpublished clinical investigation involving 71 patients who underwent total hip arthroplasties (THA) with ASR-XL or Pinnacle MoM CoCr implants. The time trends of serum and RBC Cr concentrations were assessed to characterize whether the results reflected the presence of Cr(III) or Cr(VI). A further aim was to compare Cr data arising from the two different devices ASR-XL or Pinnacle MoM CoCr implants.

**Materials and methods**

**General study description**

Data used in this analysis were collected as part of a randomized, comparative, multi-center clinical evaluation of the DePuy ASR-XL Acetabular Cup System and the Pinnacle MoM CoCr implants. The study was designed to compare the clinical performance of the DePuy ASR-XL and Pinnacle MoM CoCr hip implants, focusing on patient outcomes, implant survivorship, and the biological response to the implants.

![Figure 1. Cr(VI) and Cr(III) uptake in a red blood cell. This diagram depicts Cr(VI) readily entering a RBC via sulfate anion channels, reduction to Cr(III) via binding hemoglobin (Hb), and soluble ligands (L) such as glutathione and amino acids. The hemoglobin-bound Cr remains part of the RBC for its entire life (approximately 120 days). Water-soluble Cr(III) traverses the cell membrane via a slow diffusion process. © [Taylor & Francis]. Reproduced from [Kerger et al. (1997)] by permission of B.D. Kerger.](image-url)
Metal-on-Metal Total Hip System (Protocol Number 04062). Raw data were obtained for Cr concentrations in serum and RBC from two research sites, in addition to details regarding blood collection, processing, and analysis, from DePuy Orthopaedics. The patients in this study were undergoing THA for unilateral osteoarthritis of the hip. Patients with pre-existing arthroplasty were excluded from this analysis.

**Implant description and patient implant distribution**

The ASR-XL is a one-piece, CoCr acetabular component that both adheres to bone and articulates with a CoCr femoral head (ball) connected to a stem (Figure 2). The Pinnacle is a similar device, but the acetabular component is a combination of two pieces: a titanium acetabular cup that attaches to the bone, and a removable polyethylene or CoCr liner that is inserted into the acetabular cup, which provides the articular surface for the femoral head (Figure 2). In the present study, the liner was composed of CoCr. At both sites, all patients received a Summit™ Cementless Stem. Randomized patients from each site received either an ASR-XL™ Acetabular Cup System coupled with a 39–55-mm diameter metal femoral head, or a Pinnacle™ Metal-on-Metal Total Hip system coupled with a 28- or 36-mm diameter metal femoral head. Details regarding these devices are shown in Table 1. Seventeen ASR and 17 Pinnacle devices (34 total) were implanted at Site 9, and 16 ASR and 15 Pinnacle devices (31 total) were implanted at Site 1, yielding 65

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**Table 1. Hip implant component information.**

| Component                  | Pinnacle™                  | ASR-XL™                  |
|----------------------------|----------------------------|--------------------------|
| Femoral head               | Wrought, cobalt—chromium—molybdenum alloy | ASR-XL™; cast, cobalt—chromium—molybdenum alloy |
| Femoral head size          | 28 or 36 mm                | 39–55 mm                 |
| Acetabular cup             | Pinnacle™; cast titanium alloy (Ti6A14V), porous-coated | ASR-XL™ DuoFix™, one-piece |
| Acetabular cup size        | 55–66 mm with 28 and 36    | 44–62 mm                 |
| Acetabular liner           | Pinnacle™, Ultamet™; wrought, high-carbon, highly polished, cobalt—chromium—molybdenum alloy | None |
| Femoral stem               | Summit™ Cementless Stem, Porocoat® (standard and high offset) tapered, proximally coated, titanium | Summit™ Cementless Stem, Porocoat® (standard and high offset) tapered, proximally coated, titanium |
| Taper sleeve adaptor      | None                       | 12/14                    |
total implants. In addition, Cr concentrations were obtained for six patients without accompanied device information (accordingly these subjects were not utilized in the analyses).

**Blood collection and analysis**

Three vials each containing 7 ml blood were drawn at each sampling period. The first sample was discarded, the second vial was processed for Cr in RBC, and the third sample vial was processed for Cr in serum analysis. All blood samples were processed within 30 min and transported to the certified Trace Element Laboratory (TEL), London Health Sciences Centre, London (LHSC), Ontario, Canada. All serum and RBC samples were analyzed using high-resolution inductively coupled mass spectrometer (ICP-MS). The instrument limits of detection (LOD) were less than 1 ppt. Practical RBC LOD were 0.05 μg/L. The results were reported in SI Units (nmol/L); however, for reporting purposes, all data were converted to μg/L as follows: SI Unit multiplied by relative atomic mass of chromium (58.93) divided by 1000 = μg/L.

An initial run of two replicate samples each for serum and RBC for each patient were measured at each time point. An additional run of up to four samples were collected from some subjects if a large difference between the two first-run replicates was detected (Leopold 2011).

**Blood processing and analysis procedure for RBC samples**

Blood in 7 ml lithium heparinized BD Hemogard™ green-top BD Vacutainer® tubes (2) (Becton Dickinson) was mixed and centrifuged at 2000 g for 10 min (samples were spun and separated within 30 min of collection). The plasma and buffy coat were removed from the Vacutainer® tubes using polyethylene transfer pipettes, and remaining RBC was poured into 7 ml Sarstedt polypropylene tubes and stored at −70 °C.

RBC samples were prepared at a ratio of 1:100 in a solution of 0.1% nitric acid containing indium, lutetium, and yttrium. The samples were mixed by inversion, left to stand for 15 min, and then centrifuged at 2000 g for 15 min. Samples were subsequently analyzed by high-resolution sector field inductively coupled plasma mass spectrometry (ICP-MS) using Finnigan Element instrumentation. Instrument Cr RBC reference range was 0.4—3 μg/L.

**Blood processing procedure for serum analysis**

Blood in a 7-ml red-label blue-top Vacutainer® tube (Becton Dickinson) was allowed to clot for 20 min, then centrifuged at 2000 g for 10 min. Serum was removed from the Vacutainer® tube using a polyethylene transfer pipette and transferred to a 7-ml Sarstedt polypropylene tube and stored at 4 °C.

**Patient population evaluated in this analysis**

One of the assumptions of the statistical methods used in this analysis is that each subject has a serum or RBC measurement for each time period under consideration (Neter, Wasserman, and Kutner 1990). In order to optimize the sample size for this analysis, data collected at pre-op and at 3, 12, and 24 months post-op were used. The 36-month sampling point was not included because many of the patients did not participate in this
sampling event; similarly, a few patients had samples collected at 48 months post-op but these data were considered to be too sparse to merit inclusion. There was a small group of patients missing a single measurement from one of the three post-op sampling periods; for these patients, linear interpolation was utilized to estimate the measurement from the missing time period if the patient had values for the time period before and after. This yielded a sample size of contiguous serum and RBC values for 52 patients up to 24 months post-op. Individual serum and RBC Cr values for all participants at each time point are included in Supplemental Tables 1 and 2. As presented in Table 2, some patients did not have their blood collected precisely at the targeted times. For example, blood draws at post-op sampling time point #2, 12 months (365 days), were actually collected over a range of 297–428 days post-op. Therefore, the distribution of sampling times was examined and patient samples assigned to the closest targeted time. A summary of the target times, actual patient sample collection ranges, and our defined cut-off periods for each target time is shown in Table 2.

Identification of outlying replicate samples

For statistical analyses, the average of the replicated samples for each patient at each time period was used. A review of the replicate serum and RBC data for some patients indicated the potential presence of outlying measurements. For example, one patient, 09-0005, had pre-op RBC Cr replicate measurements of 0.4, 0.5, 2.9, and 234 μg/L. Clearly, the 234 μg/L value is anomalous and likely attributed to lab or analytical error. Accordingly, the Extreme Value Test, or Dixon’s Test, was employed to identify and eliminate outlier values (USEPA, 2006). Of 1248 total replicate values reported in the study, only 14 were identified as outliers. These outlying replicate values (identified in the Supplemental Tables 1 and 2, and presented in Supplemental Figures 1(A)–(J) and 2(A) and (B)) were excluded from subsequent statistical analyses. For example, for patient 09-0005, for pre-op time period, a mean replicate value of 1.27 μg/L RBC Cr (average of 0.4, 0.5, and 2.9 μg/L) was used.

Estimates of central tendency and statistical analysis

Two different estimates of central tendency were evaluated at each time point. Specifically, because serum and RBC Cr measurements for either device (Pinnacle or ASR) generally

| Sample period         | Actual range of days since surgery | Range of days used to define pre- and post-op sampling periods in this analysis |
|-----------------------|-----------------------------------|------------------------------------------------------------------------|
| Pre-op                | 0–29                              | 0–90                                                                   |
| Post-op #1 (target = 3 months = 90 days) | 90–353a                            | 0–225                                                                  |
| Post-op #2 (target = 12 months = 365 days) | 297–428                             | 226–540                                                                |
| Post-op #3 (target = 24 months = 730 days) | 674–956b                           | 540–900                                                               |
| Post-op #4 (target = 36 months = 1095 days) | 1003–1270                           | 900–1260                                                              |

aOne patient had serum and RBC post-op #1 samples collected at 353 days post-op; the 353-day measurements were assigned to the three-month (90 days) time period. Excluding this patient, the actual range for the post-op #1 sample period is 90–148 days.

bOne patient had serum and RBC post-op #3 samples collected at 956 days; the 956-day measurements were assigned to the 24-month time period. Excluding this patient, the actual range for the post-op #3 sample period is 674–877 days.
followed a lognormal distribution, data were log-transformed and geometric means utilized as one estimate of central tendency (see Supplemental Tables 3 and 4). The medians of non-transformed data were also determined. Both of these approaches are standard methods for estimating and comparing central tendencies for lognormally distributed data.

A two-step process was used to compare the central tendency estimates of serum and RBC Cr at different sampling time points. First, a repeated-measures ANOVA was used to evaluate the mean paired difference of the log-transformed data (log-transformed geometric means) for each time period, while a Friedman’s test was employed to determine the median paired difference of the non-transformed data (medians) for each time period (Neter, Wasserman, and Kutner 1990). These tests indicated whether or not a significant difference exists between mean or median differences within a given data distribution, but do not specifically demonstrate which values are significantly different from one another. In those cases where ANOVA or the Friedman’s test indicated that a significant difference between central tendency estimates existed, a post hoc analysis was conducted; the Holm–Bonferroni corrected paired Student’s t-test was used to identify significant differences between mean differences of the log-transformed data. The Holm–Bonferroni corrected paired Wilcoxon test was utilized to identify significant differences between median differences (Holm 1979; Neter, Wasserman, and Kutner 1990).

Regression analyses of RBC and serum Cr results

Sidaginamale et al. (2013) spiked whole blood samples with Cr(III) or Cr(VI) and determined the regression line slopes for Cr concentrations in RBC and serum. Similarly, serum and RBC data were converted from the present analysis at each time period (i.e., pre-op, 3, 12, and 24 months) to whole blood Cr concentrations for comparison. In brief, whole blood concentrations were estimated using volume data published in the 1975 International Commission on Radiological Protection “Reference Man” document and calculating the weighted average using serum and RBC Cr concentrations for each patient (ICRP 1975). Simple linear regressions were performed, and scatter plots were generated for each time point. These data were overlaid with regression lines reported by Sidaginamale et al. (2013), and the slopes of the regression lines were compared. Detailed methods regarding the estimation of whole blood concentrations and data analysis are included in the Supplemental material.

Results

Pre-op serum and RBC Cr values

As illustrated in Table 3, geometric mean and median pre-op serum Cr concentrations were consistently below 0.2 μg/L in each population evaluated, while the geometric mean and median pre-op RBC Cr concentrations were typically approximately four- to six-fold higher than serum values ranging from 0.57 to 0.69 μg/L (mean) and from 0.55 to 0.7 μg/L (median). These values are consistent with Cr concentrations reported elsewhere for background populations or pre-op patients scheduled to receive CoCr hip implants (Bartolozzi and Black 1985; Engh et al. 2009; Kerger et al. 1997).
Post-op RBC Cr values

The results presented in Table 3 indicate that geometric mean and median RBC Cr concentrations were not markedly altered at any post-op time point (3, 12, and 24 months) in the Pinnacle implant patients. Similarly, for ASR implant patients, median RBC Cr concentrations were not significantly affected at any post-op time point; the mean RBC Cr concentration was not changed at 3 and 24 months, but was significantly increased at the 12-month time period. There were no significant differences between the device types.

For comparative purposes, summary statistics for RBC Cr data with outliers included are shown in Supplementary Table 5. The results from both analyses were similar except that mean RBC Cr concentration was not significantly altered for the 12-month time period with the outliers included.

Post-op serum Cr values

Mean and median serum Cr concentrations were significantly increased at all post-op time points (compared to pre-op) for both Pinnacle and ASR patients (Table 3 and Figure 3). At three months post-op, geometric mean and median serum Cr concentrations rose 5- to 13-fold. At 12 and 24 months post-op, geometric mean and median serum Cr concentrations were elevated 8- to 18-fold compared to pre-op values. Mean and median serum Cr concentrations at 12 and 24 months were also higher than at 3 months, with no marked differences between 12- and 24-month values. Evidence indicates that the release of Cr reached a “steady state” at some point between 3 and 12 months. There were no significant differences in central tendency estimates for Pinnacle vs. ASR at any time point. For comparative purposes, summary statistics for serum Cr data with outliers included are given in Supplementary Table 5, although the results from both analyses were similar.

Table 3. Mean and median serum and RBC chromium concentrations (µg/L) over time for subjects with blood samples taken at pre-op, 3, 12, and 24 months corrected for outlying replicates.

| Device type | N  | Pre-op | 3 Months | 12 Months | 24 Months |
|-------------|----|--------|----------|-----------|-----------|
|             |    | Geometric mean serum Cr concentrations ± SD |          |           |           |
| Pinnacle    | 23 | 0.18 ± 2.5         | 0.84 ± 1.8a | 1.4 ± 2.3a | 1.4 ± 2.2a |
| ASR         | 29 | 0.14 ± 1.8         | 1.3 ± 2.2a  | 1.9 ± 2.8a | 2.2 ± 2.5a |
|             |    | Median serum Cr concentrations ± IQR |          |           |           |
| Pinnacle    | 23 | 0.12 ± 0.20        | 0.78 ± 0.60b | 1.2 ± 1.3b | 1.5 ± 1.6b |
| ASR         | 29 | 0.12 ± 0.12        | 1.5 ± 0.90b  | 1.8 ± 2.3b | 2.2 ± 2.7b |
|             |    | Geometric mean RBC Cr concentrations ± SD |          |           |           |
| Pinnacle    | 23 | 0.69 ± 1.6         | 0.47 ± 1.9   | 0.75 ± 2.5 | 0.69 ± 2.3 |
| ASR         | 29 | 0.57 ± 1.9         | 0.50 ± 1.8   | 0.98 ± 2.1a| 0.88 ± 2.3 |
|             |    | Median RBC Cr concentrations ± IQR |          |           |           |
| Pinnacle    | 23 | 0.70 ± 0.80        | 0.50 ± 0.38  | 0.56 ± 1.6 | 0.91 ± 1.5 |
| ASR         | 29 | 0.55 ± 0.55        | 0.50 ± 0.39  | 1.1 ± 1.3  | 0.91 ± 1.2 |

Sample size: serum (n = 52); RBC (n = 52).

aSignificantly different than pre-op concentrations (p < 0.05) based on post hoc Holm–Bonferroni adjusted paired t-test of natural log transformed data following repeated measures ANOVA.

bSignificantly different than pre-op concentrations (p < 0.05) based on post hoc Holm–Bonferroni adjusted paired Wilcoxon test following the Friedman’s test.
Regression analyses of RBC and serum Cr

A formal statistical analysis for comparison of slopes generated in this study vs. those reported by Sidaginamale et al. (2013) was not possible as the standard errors of the slope parameters were not reported in Sidaginamale et al. (2013). However, as can clearly be seen in Figure 4(a)–(c), the slopes of the regression lines associated with the present data for the 3-, 12-, and 24-month time points were similar (±0.18) and nearly parallel to Cr(III) spike slopes from Sidaginamale et al. (2013), but displayed no similarity to the Cr(VI) spike slope.

Discussion

In whole blood, Cr(VI) preferentially accumulates in RBC, whereas Cr(III) predominantly remains in the serum/plasma fraction. These partitioning characteristics provide a fingerprint of exposure to the different ionic forms of blood Cr. This study represents the first published analysis to rely on multiple pre- and post-op serum and RBC Cr measurements as indicators of Cr valence in patients with Cr-containing hip implants.

In general, mean and individual serum Cr concentrations remained elevated, but did not continue to increase significantly after the three-month sampling period. Data suggest that a “steady-state” condition was reached at some time between 3 and 12 months post-op. It is well documented that a “bedding-in” period typically occurs for a few months after the implant is placed which is followed by a stabilization in the wear rate (Dowson et al. 2004; Firkins et al. 2001; Silva et al. 2007; Wright and Goodman 2001). Hence, serum and RBC Cr concentrations measured at 12- and 24-month sampling periods in this study are considered to be representative of longer durations (i.e., the life of the implant).

Some of the blood draws did not occur within the target range of the days specified for that sampling period, but it is believed that this factor did not influence the main findings of this study (significant increases in serum Cr relative to RBC Cr). In addition, removal of the subjects that missed one sampling period (and thus had interpolated data for that period) did not markedly affect the outcome of the analyses (data not shown).

Engh et al. (2009) reported summary statistics (median and range) for serum and RBC Cr concentrations in 57 Pinnacle MoM total hip replacement patients pre- and post-op at 6, 12, and 24 months. In agreement with our observations, median serum Cr concentrations were found to be increased significantly at all time points (approximately eight-fold at 24 months), but there were no marked alterations in median RBC Cr concentrations.
(Engh et al. 2009). Engh et al. (2009, 103) noted that “[a]lthough serum chromium ion levels increased at 2 years for the two metal-on-metal groups … there was surprisingly no elevations in erythrocyte chromium ions.” However, it is worthwhile noting that the biological relevance with respect to the valence state of Cr was not addressed.

Similarly, Thuett et al. (2013) reported serum and RBC Cr concentrations for 22 Pinnacle MoM hip implant patients at pre-op, and 3, 12, and 24 months post-op. Median post-op serum Cr concentrations over time ranged from 0.78 to 0.88 μg/L, and median RBC Cr concentrations ranged from 0.85 to 1.8 μg/L. Compared to pre-op serum Cr concentrations (0.14 μg/L), a four-fold post-op rise in serum Cr concentrations was observed at 3 months, and a six-fold increase at 12 months. However, the RBC Cr concentrations did not change significantly after surgery.

Other clinical studies using less comprehensive methods reported similar results. Newton et al. (2012) evaluated post-op Cr concentrations in whole blood and plasma collected from patients with numerous different types of CoCr MoM implants from several manufacturers. Chromium was present at higher concentrations in plasma relative to whole blood, indicating blood Cr was preferentially distributed into the plasma compartment.
Newton et al. (2012, 1643) noted that the “fact that Cr does not appear to associate with blood cells suggests that the Cr species is the less toxic trivalent form.” Walter et al. (2008) measured serum and RBC Cr concentrations in 29 patients receiving Birmingham Hip Resurfacing prostheses (samples collected once between 5 and 59 months post-op). Serum Cr levels were increased post-op compared to controls (approximately four-fold), while RBC post-op Cr concentrations were similar to controls. Walter et al. (2008, 819) concluded that “the most likely explanation for most of the Cr being found in the serum is that it is in the trivalent form.”

Studies involving chemical analysis of Cr species in implants and tissues of implant patients also indicate the presence of Cr(III). Several studies reported that Cr(III)-carbides form on the surface of the bearing as a result of the manufacturing process (Catelas et al. 2003; Sims 1969; Wang et al. 1999). Both Cr(III)-oxide and Cr(III)-phosphate particles were detected at the articulating surface of the prosthesis (Catelas et al. 2004; Doorn et al. 1998; Hanawa, Hiromoto, and Asami 2001; Hart et al. 2010; Huber et al. 2009; Jacobs et al. 1995; Lewis et al. 2005; Urban et al. 1994; Walter et al. 2008). It was postulated that Cr-oxide particles originate from wearing of the passivation layer on the implant surface and possibly from oxidized Cr-carbides (Catelas et al. 2003; Silva et al. 2007). Hart et al. (2010, 4445) reported that “in all the patients where there were significant amounts of metal in the tissue we found Cr(III)PO₄ was much the most abundant and we observed no Cr(VI).” Furthermore, Cr corrosion products were analyzed from the modular heads and neck junctions of Al₂O₃-, CoCrMo-, and Ti₆Al₄V-containing devices retrieved during revision. Analysis identified three main corrosion products: mixed oxides of Cr, Mo, and Ti; chlorides of Co, Cr, and Mo; and, the most abundant product, Cr orthophosphate. Based on their analysis, Jacobs et al (1995, 98) reported: “that the chromium valence of the [Cr orthophosphate] corrosion product was 3+.”

The clinical and analytical findings described above are consistent with the fact that oxidation of Cr(III) to Cr(VI) is virtually impossible in vivo. Metal corrosion is essentially an electrochemical process, and various studies determined that the electrochemical potential at the implant surface is far too low to oxidize Cr(III) to Cr(VI). Contu, Elsener, and Böhni (2005) showed that the open circuit potential at pH 7 (human blood has a pH between 7.35 and 7.45) before, during, and after abrasion of the surface of a CoCrMo alloy in saline was −450, −725, and −220 mV, respectively. These investigators also reported an open circuit potential of −650 mV during abrasion of the alloy in serum at pH 7. Therefore, physiological conditions surrounding MoM implant junction and articulating surfaces likely range between −725 and −220 mV, even under wear situations. In addition, Haeri et al. (2012) noted that at least +500 mV is required to generate Cr(VI) at the surface of a CoCrMo biomedical alloy, and that cell death resulted at voltages greater than +300 mV. Thus, it appears that the +500 mV necessary to produce Cr(VI) release is not achievable in vivo; and even if it this process were to occur, cell death from the electrochemical current at or above +300 mV ensued prior to Cr(VI) release.

Finally, Hedberg and Wallinder (2013) found no apparent evidence for formation of Cr (VI) at static (no fretting) conditions, or in phosphate buffered saline (PBS) with physiologically relevant concentrations of hydrogen peroxide (representative of the hydrogen evolution that may occur in vivo as a result of the temporary fall in pH during the electrochemical events concomitant with destruction and repassivation of the metal alloy during abrasion). According to Hedberg and Wallinder (2013, 698), “[a]t open-circuit conditions
(no applied potential), Cr(III) was the only detectable form of Cr released … in PBS and PBS + H2O2.” Oxidation at an applied potential (0.7 V Ag/AgCl), a condition used in the literature (Merritt and Brown 1995; Merritt et al. 1983) to pre-age samples prior to in vivo tests, resulted in a 1000-fold higher release of Cr, predominantly as Cr(VI). This latter condition is considered irrelevant for the actual behavior of the alloys in vivo, unless the materials are oxidized at relatively high potentials prior to implantation (Hedberg and Wallinder 2013).

Eiselstein, Proctor, and Flowers (2007) postulated that, under highly acidic conditions, minute amounts of Cr(III) in solution might be liberated from the surface of the implant and participate in liquid-phase oxidation processes. Using “worst-case in-vivo conditions of low pH and high oxidizing potential” (702), and employing thermodynamic evaluations of Cr(III) oxidation, data suggested that there is a potential for Cr(VI) generation, noting that “it is thermodynamically possible for these reactions to occur in the body.” Similarly, Griffin et al. (2012) indicated that the acidic environment found with crevice corrosion at the head—neck taper may result in oxidation of Cr(III) to Cr(VI). However, as noted by Eiselstein, Proctor, and Flowers (2007, 702), any de novo Cr(VI) would be “very short lived and not circulated widely through the body.” Hence, the toxicological relevance of any particular in vivo Cr(III) oxidation, even if it does occur, is minimal. In summary, these studies are consistent with the views that electrical potential and acidic conditions required to oxidize Cr(III) to Cr(VI), and sustained presence of Cr(VI) in biological tissues, are not biologically plausible.

Merritt and Brown (1995) reported that 21 patients with CoCr hip implants displayed a mean RBC/plasma Cr ratio of 694:1, while 12 patients with TiCoCr hip implants had a mean RBC/plasma ratio of 1011:1. These values clearly conflict with the post-op RBC/plasma ratios observed in the study described in the current investigation (0.1—5.5), as well as those observed by others (0.1—1.3) (Engh et al. 2009; Walter et al. 2008). Merritt and Brown (1995, 631) concluded that “most patients had high ratios indicating hexavalent Cr release from corrosion or wear of these devices.” While it is conceivable that such highly increased RBC/plasma Cr ratios may indeed be suggestive of Cr(VI) release, there are some uncertainties and inconsistencies in the methodologies as well as interpretation of results in this study that raise questions regarding the validity of the findings. First, Merritt and Brown (1995) provided no details, nor any citation to any details, regarding the methods of serum and RBC sample preparation or analysis. There were no pre-op data and no observations from a control group without implants. Most importantly, the ratio values are not consistent with actual reported data. For example, for the CoCr hip implants, the mean RBC level was reported as 14.83 μg/L, while the mean plasma level was 8.31 μg/L. Hence, the ratio of mean RBC/mean plasma value is approximately 2:1, and it is therefore not clear the manner in which Merritt and Brown (1995) derived the exceedingly high elevated RBC/plasma ratio of 694:1 for this group.

Conclusions

The weight of evidence of numerous studies, including the findings presented in this investigation, indicate that only Cr(III) is apparently released from Cr-containing hip implants. Evidence indicates that the low levels of Cr present in blood and tissues present in MoM hip implant patients do not appear to pose any adverse health risk to the patient.
Disclosure statement

All the authors were employed by Cardno ChemRisk at the time of manuscript preparation. Cardno ChemRisk is a consulting firm that provides scientific advice to the government, corporations, law firms and various scientific/professional organizations. Cardno ChemRisk has been engaged by DePuy Orthopaedics, Inc., a manufacturer of prosthetic devices, some of which contain cobalt and chromium, to provide general consulting and expert advice on scientific matters, as well as litigation support. This paper was prepared and written exclusively by the authors, without comment by DePuy employees or counsel. Any significant review or input by other Cardno ChemRisk employees who are not authors is described in the References. It is likely that this work will be relied upon in medical research, nutrition research and litigation. One of the authors (D. J. Paustenbach) has previously testified on behalf of DePuy in hip implant litigation. It is possible that any or all of the authors may be called upon to serve as expert witnesses on behalf of DePuy. Funding for the preparation of this paper was provided by DePuy. The preparation of the paper, including conduct of the literature review, review of the individual papers, integration and synthesis of the findings, the conclusions drawn and recommendations made are the exclusive professional work product of the authors and may not necessarily be those of their employer or the financial sponsor of the review.

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