HIP

An analysis of metal ion levels in the joint fluid of symptomatic patients with metal-on-metal hip replacements

We retrospectively analysed concentrations of chromium and cobalt ions in samples of synovial fluid and whole blood taken from a group of 92 patients with failed current-generation metal-on-metal hip replacements. We applied acid oxidative digestion to our trace metal analysis protocol, which found significantly higher levels of metal ion concentrations in blood and synovial fluid than a non-digestive method. Patients were subcategorised by mode of failure as either ‘unexplained pain’ or ‘defined causes’. Using this classification, chromium and cobalt ion levels were present over a wider range in synovial fluid and not as strongly correlated with blood ion levels as previously reported. There was no significant difference between metal ion concentrations and manufacturer of the implant, nor femoral head size below or above 50 mm. There was a moderately positive correlation between metal ion levels and acetabular component inclination angle as measured on three-dimensional CT imaging.

Our results suggest that acid digestion of samples of synovial fluid samples is necessary to determine metal ion concentrations accurately so that meaningful comparisons can be made between studies.

Wear debris from metal-on-metal (MoM) hip replacements is now thought to be implicated in causing a local soft-tissue inflammatory response leading to premature failure of the implant. Higher rates of wear have been linked to this mode of failure than to other causes, and have been shown to result in raised systemic levels of chromium (Cr) and cobalt (Co) ions. Whether blood concentrations of these ions accurately reflect the levels in synovial fluid, and hence act as a surrogate marker of failure in the symptomatic MoM patient, remains ill defined, with only a few reports in the literature.

In the United Kingdom, with the advent of the Medicines and Healthcare products Regulatory Agency recommendation to all hip surgeons to investigate symptomatic MoM patients with blood metal ion analysis, the need to examine the relationship with joint fluid levels is more pressing. The clinical relevance of joint fluid analysis is not clear, and the resulting metal ion values are difficult to interpret.

Sampling fluid from the hip joint is more demanding than retrieving venous blood, and its value as a diagnostic investigation must therefore be carefully considered. Little information is available on the factors that might influence the relationship between the level of metal ions in synovial fluid and that in the blood. Such information might contribute to a better understanding of the mechanisms of failure in this group of patients.

Two studies involving a total of 43 patients have reported in vivo concentrations of Cr and Co ions in the synovial fluid of failed MoM hips. De Smet et al demonstrated a strongly positive correlation between rates of wear of the hip and synovial fluid concentrations, with serum metal ion levels in 26 patients (seven of whom had bilateral replacements). They also showed that patients at revision surgery had median joint fluid levels of Cr and Co ions approximately 20 times greater in those with metallosis than in those without. Langton et al examined the levels of metal ions in synovial fluid in 17 patients with adverse reaction to metal debris (ARMD), finding a wide range in Cr (~1000 μg/l to 46 000 μg/l) and Co (~1000 μg/l to 10 000 μg/l). Although both reports have contributed to the current knowledge in the field, the large variation in metal ion levels between the two studies and the low number of patients in both limits the applicability of the conclusions drawn.

In order to investigate precise relationships with metal ion levels, a method to ascertain and analyse synovial fluid that is robust and avoids inaccurate measurements is required.
First, intra-operative sampling of synovial fluid should be free of blood contamination from the surgical dissection. Secondly, metal ions should be liberated from their molecular bounds (for example Cr in red blood cells) into free ionic form through acid oxidative digestion in the laboratory.

The primary aim of this study was to validate a method of metal ion analysis in the synovial fluid of symptomatic MoM hip joints, and to investigate the relationship of these values to several clinical variables.

**Patients and Methods**

We retrospectively analysed the metal ion levels of synovial fluid collected from all patients who had been referred to our joint replacement service for problematic MoM hips over a two-year period. The study received ethical approval.

A history was obtained for all patients and an examination was undertaken by the senior author (AJH). An infection screen was performed using routine blood tests which included CRP as well as plain anteroposterior (AP) and lateral radiographs. Further investigation was done using three-dimensional (3D) reconstruction of CT data for anatomical orientation of the acetabular component, and metal artefact reduction sequence MRI to identify any soft-tissue abnormality. In order to exclude infection, aspiration (five patients). Samples were drawn using a stainless steel needle into a trace element vacutainer (Beckton-Dickinson, Oxford, United Kingdom) containing sodium ethylenediaminetetraacetic acid for blood, or sterile universal plastic containers for synovial fluid. Fluids were stored at -80°C until analysis, whereupon the sample was thawed to room temperature and re-suspended using a vortex mixer in preparation for further processing. We initially compared Cr and Co ion levels in whole blood and synovial fluid samples, using two different processing methods.

The first involved simple dilution of the sample in a diluent consisting of tetramethyl ammonium hydroxide (final concentration 0.5%, electronic grade; Alfa Aesar, Ward Hill, Massachusetts), Triton-X100 (final concentration 0.001%, ultrapure grade; Merck, Darmstadt, Germany) and butan-1-ol (final concentration 0.1%, SPS grade; Romil, Cambridge, United Kingdom). The diluent also contained inductively coupled plasma mass spectrometer (ICPMS) internal standard (gallium, 1 μg/l). Finally, 0.15 ml of the fluid was diluted to a final volume of 4.8 ml for ICPMS analysis. The isotopes measured were 52Cr and 59Co. Samples beyond the calibration range were diluted appropriately with de-ionised water and re-analysed.

The second method used acid oxidative digestion, a process that liberates metal ions bound to proteins in red blood cells, as well as insoluble material and metal nanoparticles found in the fluid of MoM hips. A 0.5 ml aliquot of synovial fluid was digested with 5 ml of nitric acid (SPS grade; Romil) at 100°C for 240 minutes, using a dry heating block (DigiPrep; SCP Science, Quebec, Canada). With each batch, two additional ‘blank’ samples were processed whereby the synovial fluid component was substituted with 0.5 ml of 18 ohm de-ionised water. After digestion, the remaining fluid in each tube was further diluted to 50 ml with de-ionised water and analysed with high-resolution ICPMS (Element2; Thermo Finnigan, Bremen, Germany). The blank samples were used to correct for any contamination in each batch. The concentration of metal ions was expressed as μg/l.

**Statistical methods.** For the initial 25 samples we performed Bland-Altman plots of Cr and Co values in order to analyse the level of agreement between the two methods of sample processing. Based on our findings, we used acid oxidative digestion for all 92 samples. This included the 25 samples initially analysed.

We used SPSS v 16.0 for Windows (SPSS Inc., Chicago, Illinois) for statistical analysis. Significance was set at p < 0.05. The Mann-Whitney U test was used to test for differences between synovial fluid ion levels in patients categorised according to cause of failure and femoral head size. Analysis of variance (ANOVA) with Tukey’s post hoc analysis was used to test differences between manufacturers.
We used scatter plots, Spearman’s rank correlation and linear regression to examine the relationship between synovial metal ion levels and acetabular component inclination angle and paired whole blood samples. A logarithmic (base 10) conversion of joint fluid values was performed to allow certain results to be illustrated as box plots.

**Results**

**Cobalt and chromium levels pre- and post-acid digestion.** Descriptive statistical results are summarised in Table I. Bland-Altman analysis (Fig. 1) demonstrated that the difference in the means between digested and undigested synovial fluid was significantly greater for Cr (12744 μg/l, p = 0.002) than for Co (1249 μg/l, p = 0.083). Regardless of processing method, Cr and Co levels in the synovial fluid of our control group were below the limits of detections of the mass spectrometer, which was 0.3 μg/l for both ions.

**Synovial fluid levels and category of failure.** A total of 64 patients who underwent acid oxidative digestion were categorised according to their cause of failure into ‘unexplained pain’ (n = 35) and ‘defined cause of failure’ (n = 29) (Table I and Fig. 2). When compared, there was no significant difference in either Cr or Co ion levels between the two groups (Mann-Whitney, p = 0.681 and p = 0.475, respectively).

**Synovial fluid levels and type of hip.** We investigated 92 samples which underwent acid oxidative digestion according to the manufacturer of the prosthesis. The three largest groups were composed of the Birmingham Hip Resurfacing in 36 hips (BHR; Smith & Nephew, Warwick, United Kingdom), Articular Surface Replacements in 18 (ASR; DePuy Ta...
International Ltd, Leeds, United Kingdom) and Cormet in 16 (Corin Group PLC, Cirencester, United Kingdom). The further types of hip were nine Durom (Zimmer Ltd, Swindon, United Kingdom), six Adept (Finsbury Orthopaedics, Leatherhead, United Kingdom), four Recap (Biomet UK Ltd, Swindon, United Kingdom) and three Mitch (Stryker Ltd, Newbury, United Kingdom). These latter 22 patients were combined into a single group labelled ‘Other’, to allow for statistical comparison (Table I and Fig. 3). There was no significant difference for either Cr or Co ion levels between manufacturers (ANOVA, p = 0.163 and p = 0.377, respectively).

Synovial fluid levels and femoral head size. A total of 87 component sizes which underwent acid oxidative digestion were available for analysis. In five patients the head size was not available. The mean head diameter was 47 mm (39 to 54). We grouped the sizes into those < 50 mm (n = 56) and those ≥ 50 mm (n = 31) (Table I and Fig. 4). There was no significant difference in either Cr or Co ion levels between the groups (Mann-Whitney, p = 0.610 and p = 0.620, respectively).

Synovial fluid levels and inclination angle of the acetabular component. We studied 56 patients with three-dimensional (3D) CT. We could not study the other 36 patients as they were referred to our centre and underwent revision surgery before our low dose CT scan protocol was available. The correlation between inclination angle and joint fluid metal ion levels was weakly positive for Cr (Spearman’s ρ = 0.213, p = 0.114) and Co (Spearman’s ρ = 0.286, p = 0.032) (Fig. 5).

Relationship between synovial fluid and blood metal ion levels. Of the 92 samples of synovial fluid analysed, 30 had paired whole blood samples. There was a significant and
moderately positive correlation between the levels in whole blood and joint fluid for Cr (Spearman’s $\rho = 0.48$, $p = 0.007$) and Co (Spearman’s $\rho = 0.412$, $p = 0.024$) (Fig. 6). The linear regression equation for cobalt ion levels was:

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\text{Synovial fluid Co ion level} = 4.1 \times \text{Blood Co ion level} + 0.01 \\
\hspace{1cm} (95\% \text{ CI -4.1 to 12.2})
\]

**Discussion**

We have demonstrated that acid oxidative digestion causes a significant increase in metal ions measured in both blood and synovial fluid samples, thereby providing a more accurate reflection of actual concentrations. Interestingly, the effect of protein digestion on the liberation of metal ions was greater for Cr than for Co, suggesting that more chromium is bound to protein. Depending on the processing used, clinical studies in the current literature may be under- or over-reporting these levels, and this demonstrates the need for a standardised laboratory protocol that is universally applied. It is only then that meaningful comparisons between studies examining metal ion concentrations can be made.

We have found a wider range of Cr and Co levels than other prominent studies in this field (Table II). De Smet et al\(^1\) analysed the synovial fluid of failed MoM hips, differentiating between those with and without metallosis observed at revision surgery. In patients with no metallosis, median Cr and Co levels of 179.5 $\mu$g/l and 106.25 $\mu$g/l, respectively, are reported. These values appear an order of magnitude lower than our ‘unexplained pain’ cohort (Cr 1137 $\mu$g/l; Co 1127 $\mu$g/l). With regard to gender distribution, manufacturer type, femoral head size and mean period to revision, their study was similar to our own. It would therefore seem reasonable to assume that the differences in these results relate to the laboratory processing of fluid samples, or possibly the effect of blood contamination quoted in their paper. Further interpretation is limited, as
they categorised their results by the term ‘metallosis’, defined as a ‘grey discolouration’ at the time of surgery, which was open to observer variations. Langton et al. reported a series of 17 failed MoM hips diagnosed with ARMD (encompassing the term ‘metallosis’) with metal levels approximately six times greater than either of our two diagnostic groups. This again might be related to the laboratory treatment of their synovial fluid samples, or may also represent a group of outliers, as the predominant hip type in their study sample was the ASR. The results of both studies do, however, echo ours in identifying a wide range of metal ion concentrations in the synovial fluid of a failed MoM hip.

**Synovial fluid metal ions and category of failure.** The examination of synovial fluid metal ion levels by modes of failure did not identify a significant difference between those patients who presented with unexplained pain and/or those who failed due to a definable cause. We did note that the highest median synovial fluid Cr ion levels were in patients in whom the failure was attributed to a size mismatch between head and acetabular component (log Cr 3.8 μg/l) or acetabular component malorientation (log Cr 4 μg/l), compared with the unexplained group (log Cr 3.1 μg/l) (Fig. 2). Although the numbers of patients in these groups were too few to draw any definitive conclusions, it is likely that these increased levels were due to higher rates of wear where the mechanics of the MoM bearing were suboptimal. Irrespective of the cause of failure, the range of Cr and Co levels in the synovial fluid of failing MoM hips is too wide to establish a discrete threshold that would indicate a harmful level. A limitation of this analysis is that we did not establish a discrete threshold that would indicate a harm level. A limitation of this analysis is that we did not establish a discrete threshold that would indicate a harm level. A limitation of this analysis is that we did not establish a discrete threshold that would indicate a harm level. A limitation of this analysis is that we did not establish a discrete threshold that would indicate a harm level. A limitation of this analysis is that we did not establish a discrete threshold that would indicate a harm level. A limitation of this analysis is that we did not.

**Correlation with blood metal ion levels.** Synovial fluid ion levels in a failing implant are higher for Cr than for Co, an unexpected finding given that hip resurfacing designs are composed of a metal alloy composition of 1:2 Cr to Co. The concentrations we found reflect findings of in vitro studies suggesting that raised levels of Cr and Co inhibit osteoblastic activity,21 induce osteocytic apoptosis22 and upregulate osteoclastic cells,23 and may ultimately contribute to component loosening as the mode of MoM failure. However, in blood analysis the reverse trend is true, where Co is the predominant ion (Fig. 6). This pattern has been recognised previously4 and suggests that the Cr is sequestered within the joint at far higher concentrations than Co, which appears to be more readily disseminated into the circulation.

### Table II. Comparison of the median (range) metal ion concentrations between this study and previous studies

| Authors (number of patients in analysis) | Cause of failure | Number of patients | Synovial fluid levels (μg/l) | | | |
|---|---|---|---|---|---|
| This study (n = 64) | Unexplained pain | 35 | 1337 (0 to 190 416) | 1127 (2 to 14 285) | |
| Defined cause of failure | 29 | 1512 (0 to 263 298) | 1014 (1 to 12 444) | |
| De Smet et al3 (n = 26, of which 7 are bilateral) | Without metallosis | N/A* | 179.5 (19 to 661) | 106.25 (13 to 769) | |
| | With metallosis | N/A | 5136.5 (155 to 29 080) | 2185 (110 to 5120) | |
| Langton et al4 (n = 17) | Adverse reaction to metal debris | 17 | ~8,000 (1000 to 46 000) | ~5,000 (0 to 10 000) | |

* N/A, not available
In both synovial and blood samples we noted several outliers, with four patients having high Cr levels and two patients with high Co levels (Fig. 6). All had ASR components in situ, with two failures attributed to acetabular component malorientation and two to unexplained pain.

In general, we found that synovial fluid levels had a wide range in vivo and were only moderately correlated with blood ion analysis. The association is not as strong as previously reported by De Smet et al, who demonstrated a high correlation coefficient between serum and synovial fluid samples for Cr \( r = 0.92 \) and Co \( r = 0.88 \). However, there are several differences between our study and theirs. First, we used whole blood, rather than serum, in order to provide a more complete analysis of the concentrations of circulating ions. Daniel et al demonstrated the variability and lack of agreement between values in concurrent serum and whole blood samples, recommending the latter as a more accurate measure of systemic metal ion levels. Walter et al investigated the differences in distribution of Cr and Co ions in various blood fractions after MoM hip resurfacing. They found the concentration of Cr ions was far greater in serum than blood, which may explain the far higher correlation found by De Smet et al. Secondly, it is not clear from De Smet et al whether an acid oxidation protocol was used during sample processing, which, as we demonstrated earlier, will increase the variation in the results.

We also noted several patients who had relatively high synovial fluid levels, despite normal blood levels. This raises the possibility that some patients have an increased excretion rate. These findings highlight the need to measure metal ion levels in the synovial fluid of symptomatic patients, particularly in the presence of other normal investigations, in order to identify a poorly functioning hip.

**Limitations.** We recognise the limitations of our study. First, in examining the amount of Cr and Co in the synovial fluid we did not necessarily quantify the amount of physiologically active material. The acid digestion step releases metal ions from particulate debris thus providing a measure of total ion content within the synovial fluid. This value is likely to be higher than the metal ion concentrations that peri-prosthetic tissues are exposed to in the hip joint. The acid digestion used in our assay procedure may not be directly comparable to the phagocytic uptake and digestion process of particle debris seen in vivo. The metal ion levels in synovial fluid measured in acid-digested samples in the unknown. Although cobalt ions have been implicated, the trigger for soft-tissue inflammatory reactions remains unknown. Although cobalt ions have been implicated, the relative contributions of the different types of debris remain speculative. Secondly, we did not examine all of the factors likely to influence synovial fluid levels, such as total fluid volume within the hip, making it impossible to determine the total amount of ions present in the joint. We assumed that ion concentrations are uniform throughout the joint, and that this is reflected in the aspirate obtained. It is likely that if the differences in volume were accounted for, a smaller variation in total metal ion levels would be found and a stronger correlation between these levels and those in the blood, or category of failure, could be determined.

Thirdly, we failed to detect a significant difference in metal ion levels in several of our analyses, for example between type of failure or head size. Despite being the largest study of its type to date, it may have been insufficiently powered to demonstrate a difference. Fourthly, although all our patients had haemoglobin and haematocrit values within the normal range, we did not take into account the impact the variations in these parameters could have on the concentration of ions measured by our whole blood analysis. Finally, corrosion, as well as wear, can contribute to metal ion levels in the synovial fluid of failing MoM hips. Both fretting and passive corrosion are evident in modular MoM total hip components at the taper junction. A limitation of our study is that we did not distinguish between hip resurfacing devices and modular components, although only nine of the latter were included in our sample of 92 analysed hips.

Our study on synovial fluid levels in the hip following MoM hip replacement reveals firstly that acid digestion of the samples is required to determine metal ion concentrations accurately, so that meaningful comparisons can be made between clinical studies adhering to a standardised laboratory protocol, and secondly, that there is a wide variation of synovial fluid levels and they are not as strongly correlated with whole blood levels as was previously thought.


