Circulating levels of cobalt and chromium from metal-on-metal hip replacement are associated with CD8⁺ T-cell lymphopenia

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We carried out a cross-sectional study with analysis of the demographic, clinical and laboratory characteristics of patients with metal-on-metal hip resurfacing, ceramic-on-ceramic and metal-on-polyethylene hip replacements. Our aim was to evaluate the relationship between metal-on-metal replacements, the levels of cobalt and chromium ions in whole blood and the absolute numbers of circulating lymphocytes. We recruited 164 patients (101 men and 63 women) with hip replacements, 106 with metal-on-metal hips and 58 with non-metal-on-metal hips, aged < 65 years, with a pre-operative diagnosis of osteoarthritis and no pre-existing immunological disorders.

Laboratory-defined T-cell lymphopenia was present in 13 patients (15%) (CD8⁺ lymphopenia) and 11 patients (13%) (CD3⁺ lymphopenia) with unilateral metal-on-metal hips. There were significant differences in the absolute CD8⁺ lymphocyte subset counts for the metal-on-metal groups compared with each control group (p-values ranging between 0.024 and 0.046). Statistical modelling with analysis of covariance using age, gender, type of hip replacement, smoking and circulating metal ion levels, showed that circulating levels of metal ions, especially cobalt, explained the variation in absolute lymphocyte counts for almost all lymphocyte subsets.

New technology has led to a dramatic increase in the use of metal-on-metal (MOM) hip replacement and resurfacing, so that in the United Kingdom it has become the most commonly used type of procedure for those patients with osteoarthritis who are < 60 years of age.^{1,2} Approximately 50 000 patients worldwide have had the latest type of MOM replacement in the last three years, but the rate of increase in countries such as the United States is projected to be 100% per annum since the Food and Drug Administration (FDA) approved the use of two of these devices in 2006³ and 2007.⁴ The FDA approval is provisional, subject to prospective monitoring of the blood levels of cobalt (Co) and chromium (Cr), which is typically tenfold greater than in normal healthy individuals, and has been linked to DNA damage of lymphocytes.⁵ In a pilot study, we observed lower peripheral lymphocyte counts in patients with MOM hip replacement compared with a group with metal-on-polyethylene (MOP) articulations.⁶

In this study we describe the levels of Co and Cr ions in whole blood and the lymphocyte subsets (total, CD3⁺, CD4⁺, CD8⁺, CD19⁺, CD16⁺) of a large series of patients with either MOM or ceramic-on-ceramic (COC) hip replacement.

The data were also compared with the results from our previous report on MOP hip replacement.⁶ Our aims were to determine whether the finding of reduced T-lymphocyte counts in patients with MOM hip replacement was repeatable when an unstudied series of such patients was compared with those with COC or MOP hips, to explore the influence of potential confounding factors including age, smoking, gender and time since operation and to establish whether circulating Co and/or Cr levels were related to any differences in lymphocyte counts.

Patients and Methods

Our study included consenting, consecutive patients resident in the South East of England, and were therefore easier to recall, who were aged < 65 years and who had received a successful hip replacement between January 2003 and January 2005, following the diagnosis of osteoarthritis in one or both hips, at our institutions. We recruited 100 patients with unilateral MOM hip replacement, 25 with bilateral MOM hip replacement, 34 with MOP and 25 with COC hip replacements. The study was approved by the ethics committee of the Royal National Orthopaedic Hospital.

We excluded patients with known causes of reduced blood lymphocyte counts such as those receiving cytotoxic or immunosuppressive therapy, previous high-dose radiation, chronic infection such as HIV and environmental immunotoxicity. With regard to factors which might influence the blood level of Co and Cr, we excluded patients if they had been exposed to these metals from other sources, for example, from other hip implants or plates, from inhalation in metal foundries or from drugs such as unusual dietary supplements. Applying these criteria resulted in the exclusion of 12 unilateral and seven bilateral MOM hip patients. There were no COC patients excluded. One MOP patient was excluded because the blood sample had been lost. This last group of patients was from our previously published study,⁶ but all the blood samples were re-analysed for metal levels, using more advanced techniques. The final numbers of hip replacements in each group were 88 unilateral MOM, 18 bilateral MOM, 25 unilateral COC and 33 unilateral MOP.

We recorded whether patients smoked,⁷ which is known to stimulate macrophages and increase the CD4⁺ count, their age⁸ and gender.⁹ In addition, because of a reported small effect of seasonal¹⁰ and circadian¹¹ variation on the lymphocyte count, blood was collected within the same two-hour period in the morning throughout the months of February and March.

There were also factors which might affect the rate of wear of hip replacements which, in the case of MOM hips, might also affect blood metal levels. These could be grouped into patient-related factors such as activity,¹² implant factors including the design and materials¹³ and the 'bedding-in' phase during which there is high wear,¹⁴ and surgical factors such as the position of the components.^{15,16} The effects of implant factors were reduced by excluding patients in whom the replacement had been performed less than six months previously thereby avoiding the high-wearing, bedding-in phase, and including only one type of hip replacement for each group. These were as follows: MOM, the Birmingham Hip Resurfacing (Smith & Nephew, Warwick, United Kingdom) with both bearing surfaces made from American Standard Testing Materials (ASTM) F75 Co-Cr; COC, the Exeter (Stryker, Newbury, United Kingdom) femoral stem (stainless steel) and ABG acetabular component (Stryker) with alumina ceramic-bearing surfaces (CeramTec, Plochingen, Germany); and MOP, Co-Cr ASTM F75 femoral head and polyethylene acetabular component (Biomet, Bridgend, United Kingdom) and Depuy, Leeds, United Kingdom with the Stanmore femoral component (Biomet).

The unilateral MOM group was considerably larger than the 34 hips presented in our previous study.⁶ Power calculations were used to determine the size of the bilateral MOM and COC groups. We assumed that patients with a COC replacement would have a similar T-cell count to those with a MOP replacement or a healthy control group, and that the bilateral MOM patients would have a reduced

T-cell count in comparison with unilateral MOM patients. Detection of the difference of 0.3 in log-transformed T-cell counts with an SD of 0.4 in each group was seen in our original study⁶ between the MOM and the MOP patients using a two-sample two-sided *t*-test at a level of significance of 5% and given a 3:1 allocation ratio, required 24 patients (18) in the smaller group to achieve a power of 90% (80%).

Demographic and clinical data were collected from the patients' notes and outpatient consultations. We defined a smoker as any patient who had smoked within the preceding three months. Blood samples were taken using a plastic cannula to avoid metal contamination from the needle.

Analysis of Co and Cr. Samples were analysed at the MRC Human Nutrition Research Unit (Cambridge, United Kingdom) for the level of Co and Cr in whole blood according to a recently validated method¹⁷ using inductively-coupled plasma mass spectrometry (Perkin Elmer Elan+ 6100, Perkin Elmer, Waltham, Massachusetts). Homogeneity of the sample solutions was ensured by the appropriate preparation of samples and gentle agitation immediately before analysis. A double-type spray chamber (Perkin Elmer) and wide-bore V-groove nebuliser (Jobin Yvon Horiba Ltd, Stanmore, United Kingdom) were used. The rate of flow for aspiration of the samples was 0.9 ml/min. A dynamic reaction cell gas of argon and 5% hydrogen was used for analysis of Cr but not for Co. Three different Seronorm (Stasjonsveien, Billingstad, Norway) whole-blood-certified reference materials, showing low, moderate and high levels of Co and Cr, were used to prepare calibration curves and as quality controls within batches of samples. Samples were analysed in seven separate batches over a period of five weeks. Samples were analysed blindly but we ensured that each batch contained a representative number of samples from each group of patients. The samples from the previously reported MOP group⁶ were re-analysed with the batches and only the new analytical data were used. The total levels of the metal ions were measured.

Analysis of lymphocyte populations. A standard method was used, involving lymphocyte staining with monoclonal antibodies and counting using a fluorescence-activated cell sorter (FACS) at the Royal Free Hospital (London, United Kingdom). This had two laser beams allowing the use of four-colour staining. The monoclonal antibodies used recognised the following populations: T-cells (CD3⁺), T-helper cells (CD4⁺), T-cytotoxic cells (CD8⁺), B-cells (CD19⁺) and natural killer cells (CD16⁺, CD56⁺).

Statistical analysis. The data on the lymphocyte counts and Co and Cr levels were skewed with many outliers. Therefore we determined statistical significance using non-parametric methods such as the Kruskal-Wallis test for continuous variables, and the chi-squared test for categorical variables. This allowed assessment as to whether the two MOM groups or the two non-MOM (COC and MOP) groups could be pooled together. Pair-wise comparisons of lymphocyte numbers using the two-sided Mann-Whitney U test were used to determine which lymphocyte subtypes

Fig. 1

Box plot of CD3⁺ T-cell counts against the type of hip replacement. Variable means and the upper and lower limits of the laboratory reference range are shown (COC, ceramic-on-ceramic; MOP, metal-on-polyethylene; MOM; metal-on-metal).

Fig. 2

Box plot of CD8⁺ T-cell counts against the type of hip replacement. Variable means and the upper and lower limits of the laboratory reference range are shown (COC, ceramic-on-ceramic; MOP, metal-on-polyethylene; MOM; metal-on-metal).

Table I. Details of the results giving the median (interquartile range) for continuous, and the number and *percentage* for categorical variables

	Overall (n = 164)	MOP* (n = 33)	COC† (n = 25)	Unilateral MOM‡ (n = 88)	Bilateral MOM (n = 18)	p-value [§]
Gender (%)						
F	63 (38)	14 (42)	16 (64)	26 (30)	7 (39)	0.016
M	101 (62)	19 (58)	9 (36)	62 (70)	11 (61)	
Smoking (%)	23 (14)	9 (27)	7 (28)	4 (5)	3 (17)	0.002
Age in yrs (range)	58 (51 to 63)	64 (55 to 67)	59 (54 to 60)	56 (51 to 61)	56 (51 to 61)	0.004
Body mass index (kg/m ²)	26 (24 to 29)	28 (26 to 28)	26 (25 to 33)	26 (23 to 28)	25 (23 to 27)	0.003
Months after surgery	37 (25 to 48)	25 (18 to 20)	25 (20 to 33)	43 (37 to 52)	29 (24 to 34)	< 0.001
Cobalt (parts per billion)	1.29 (0.49 to 2.01)	0.44 (0.32 to 0.748)	0.21 (0.17 to 0.25)	1.71 (1.29 to 2.33)	2.45 (1.56 to 4.30)	< 0.001
Chromium (parts per billion)	1.77 (0.63 to 2.61)	0.65 (0.21 to 1.10)	0.32 (0.24 to 0.45)	2.33 (1.79 to 2.91)	2.35 (1.70 to 3.36)	< 0.001
Total lymphocyte count (× 10 ⁹ /l)	1.77 (1.52 to 2.24)	1.76 (1.53 to 2.51)	2.15 (1.76 to 2.65)	1.76 (1.48 to 2.15)	1.6 (1.30 to 1.86)	0.004
CD3 ⁺ count (× 10 ⁹ /l)	1.28 (1.00 to 1.63)	1.37 (1.01 to 1.79)	1.65 (1.29 to 2.07)	1.12 (0.86 to 1.39)	1.12 (0.86 to 1.39)	0.001
CD4 ⁺ (× 10 ⁹ /l)	0.82 (0.63 to 1.12)	0.8 (0.62 to 1.1)	1.16 (0.82 to 1.37)	0.80 (0.62 to 1.02)	0.81 (0.63 to 1.04)	0.007
CD8 ⁺ (× 10 ⁹ /l)	0.37 (0.27 to 0.57)	0.49 (0.32 to 0.64)	0.50 (0.31 to 0.65)	0.34 (0.26 to 0.49)	0.31 (0.23 to 0.46)	0.024
CD19 ⁺ (× 10 ⁹ /l)	0.20 (0.14 to 0.29)	0.24 (0.14 to 0.33)	0.24 (0.19 to 0.42)	0.19 (0.13 to 0.28)	0.16 (0.14 to 0.20)	0.003
CD16 ⁺ (× 10 ⁹ /l)	0.29 (0.19 to 0.37)	0.31 (0.19 to 0.37)	0.25 (0.18 to 0.35)	0.31 (0.21 to 0.42)	0.20 (0.13 to 0.32)	0.038

* MOP, metal-on-polyethylene

† COC, ceramic-on-ceramic

‡ MOM, metal-on-metal

§ Kruskal-Wallis test for continuous variables and/or the chi-squared test for categorical variables

Median lymphocyte subset counts from other reported studies: Schaberg et al⁷ and Huppert et al⁸

differed between each pair of groups. We adjusted for potential confounders in a multivariate analysis of covariance (ANCOVA) model to determine the effect of the demographic, clinical and laboratory characteristics of the groups of patients. A p-value ≤ 0.05 was assumed to be significant.

Results

Figures 1 and 2 and Tables I and II illustrate and quantify the intergroup differences. There were significant differences in the levels of metal ions in whole blood and in the absolute lymphocyte counts due mainly to CD4⁺/CD8⁺ differences in the groups, as defined by the type of hip replacement. A total of ten patients of the 106 (9.4%) with MOM

hips had circulating levels of Cr which were greater than 5 parts per billion (ppb). The total lymphocyte and CD3⁺ counts merely represented the sums of their sub-populations.

The median CD8⁺ counts (Table I) were markedly reduced in both the unilateral (0.34) and bilateral MOM (0.31) groups when compared with the COC group (0.5), the MOP group (0.49) and data from the studies of Schaberg et al⁷ and Huppert et al.⁸ Laboratory-defined T-cell lymphopenia was present in 13 patients (15%) (CD8⁺ lymphopenia with absolute counts < 0.2 × 10⁹/l and 11 patients (13%) (CD3⁺ lymphopenia with absolute counts < 0.8 × 10⁹/l) with unilateral MOM hips. The only lymphocyte subtype which differed significantly when the uni-

Table II. P-values (two-sided Mann-Whitney U test) for pair-wise comparisons of circulating metal ion concentrations and absolute lymphocyte counts

	MOP* vs unilateral MOM†	MOP vs bilateral MOM	MOP vs COC‡	Unilateral MOM vs bilateral MOM	Unilateral MOM vs COC	Bilateral MOM vs COC
Whole blood Co	0.000	0.000	0.000	0.046	0.000	0.000
Whole blood Cr	0.000	0.000	0.021	0.966	0.000	0.000
Total lymphocyte count	0.239	0.003	0.066	0.166	0.003	0.001
CD3 ⁺ count	0.141	0.000	0.026	0.482	0.000	0.001
CD4 ⁺ count	0.679	0.001	0.013	0.528	0.001	0.010
CD8 ⁺ count	0.024	0.024	0.939	0.593	0.024	0.046
CD19 ⁺ count	0.101	0.005	0.326	0.156	0.005	0.001
CD16 ⁺ count	0.227	0.157	0.673	0.007	0.158	0.184

* MOP, metal-on-polyethylene

† MOM, metal-on-metal

‡ COC, ceramic-on-ceramic

lateral MOM cohort was compared with both non-MOM comparison groups (MOP and COC) was CD8⁺ ($p = 0.024$ and $p = 0.024$ for MOP and COC, respectively; Table II). The bilateral MOM cohort was also different for the CD8⁺ count when compared with the COC ($p = 0.046$) and the MOP control group ($p = 0.024$) with additional differences for total lymphocytes CD3⁺, CD4⁺, and CD19⁺.

The median CD4⁺ count was increased in the COC group when compared with all other groups (Table I), and historical data,^{7,8} which may be a confounding effect of smoking. This was found most frequently in the COC group and is known to increase the CD4⁺ count.⁷

The median value of the body mass index (BMI) of all groups was within the same category of overweight (BMI 25 to 30). There was a significant difference in age between the groups which probably reflects the current, widely used practice of implanting COC or MOM hips in patients aged < 65 years and of MOP in those over 65 years ($p = 0.003$, chi-squared test). Similar differences in gender probably reflected the current practice of restricting MOM hip resurfacing in female patients to those aged < 55 years,¹⁸ unless a bone mineral density scan is particularly favourable, because of the increased risk of fracture of the femoral neck in the resurfaced femoral head.

Accordingly, we incorporated factors such as age, gender, the type of hip replacement, smoking and circulating metal ion levels into our later ANCOVA statistical modelling to determine their effects on absolute lymphocyte numbers.

We transformed the lymphocyte subset counts logarithmically and undertook ANCOVA to determine whether differences in lymphocyte subset counts in the groups could be explained by differences in the type of hip replacement when adjusting for gender, months after surgery and smoking as potential confounding factors. We used lymphocyte subset counts as the dependent/outcome variable, with the type of hip replacement, gender and smoking as the factors, and months after surgery and age as covariates. Backward selection was used to reduce the final models to include only significant predictors. The

Fig. 3

Scatter plot of circulating metal ion levels in all patients showing log transformations of Co vs Cr ion levels (ppb, parts per billion) (MOP, metal-on-polyethylene; MOM, metal-on-metal; COC, ceramic-on-ceramic).

p-values from these final models are given below. The type of hip replacement was strongly predictive of all lymphocyte subset counts (p-values varied between 0.003 for CD19⁺ and 0.046 for CD16⁺), broadly in agreement with results of the Mann-Whitney U test shown in Table II. Months after surgery and age were not significant factors. Gender was significant for CD3⁺ ($p = 0.018$) and CD4⁺ ($p = 0.018$). Smoking had a significant predictive effect only for the CD16⁺ count ($p = 0.024$). Thus, the type of hip replacement was the most consistent factor for the differences in lymphocyte subset counts.

Having ascertained the differences in lymphocyte subset counts between the groups, we sought to explain the findings in relation to the circulating blood levels of metal ions. Those were highly correlated (Spearman correlation 0.892; $p < 0.001$). Figure 3 shows the distribution of both metals by the type of hip replacement (the four lowest blue points correspond to values below 0.05, the limit of quan-

Table III. Cobalt (Co) category by hip replacement

Quartile group of Co	Cohort type	MOP*	Unilateral MOM†	Bilateral MOM	COC‡	Total
1	Count	17	0	0	24	41
	% within cohort type	51.5	0.0	0.0	96.0	25.0
2	Count	16	21	3	1	41
	% within cohort type	48.5	23.9	16.7	4.0	25.0
3	Count	0	37	4	0	41
	% within cohort type	0.0	42.0	22.2	0.0	25.0
4	Count	0	30	11	0	41
	% within cohort type	0.0	34.1	61.1	0.0	25.0
Total	Count	33	88	18	25	164

* MOP, metal-on-polyethylene

† MOM, metal-on-metal

‡ COC, ceramic-on-ceramic

Table IV. Chromium (Cr) category by hip replacement

Quartile group of Cr	Cohort type	MOP*	Unilateral MOM†	Bilateral MOM	COC‡	Total
1	Count	16	0	0	25	41
	% within cohort type	48.5	0.0	0.0	100.0	25.0
2	Count	16	20	5	0	41
	% within cohort type	48.5	22.7	27.8	0.0	25.0
3	Count	1	34	6	0	41
	% within cohort type	3.0	38.6	33.3	0.0	25.0
4	Count	0	34	7	0	41
	% within cohort type	0.0	38.6	38.9	0.0	25.0
Total	Count	33	88	18	25	164

* MOP, metal-on-polyethylene

† MOM, metal-on-metal

‡ COC, ceramic-on-ceramic

tification). In order to analyse further predictive factors for lymphocyte subset counts, we categorised patients by quartiles of metal ion levels (Tables III and IV).

Patients who had hips without Co or Cr in either bearing surface (COC group) had the lowest levels of metal ions in whole blood. MOP hips had the next highest levels, and the bilateral MOM hips had the highest (Fig. 4). Smoking was associated with low metal ion levels (Mann-Whitney U test, $p = 0.005$), but when adjusted for type of hip replacement this association disappeared. Thus, smoking was a confounder for metal levels because there was a greater frequency of smokers in the COC cohort (which had a low level of metal ions).

We undertook ANCOVA using lymphocyte subset counts as the dependent variable, categorised Co and Cr levels, gender and type of hip replacement as the factors and months after surgery and age as covariates. Neither age nor time after surgery was significant and they were there-

fore excluded from the models. The final models given in Table V include only gender, Co category because this was marginally more predictive than Cr and the type of hip replacement. Women had higher lymphocyte counts. Co category was the most significant factor for the differences in lymphocyte subset counts (Fig. 5). The type of hip replacement was not significant after adjusting for gender and Co category.

Discussion

Our results showed that patients with MOM hips had reduced peripheral blood counts of T-lymphocytes in particular and B-lymphocytes when compared with control subjects with hip replacements which did not produce metal wear debris. All pair-wise comparisons of lymphocyte subset counts of any MOM cohort (either total, unilateral or bilateral) with both control cohorts (COC or MOP) revealed that the effect was consistent only for

Fig. 4

Box plot of whole blood Co level vs type of hip replacement (ppb, parts per billion; (COC, ceramic-on-ceramic; MOP, metal-on-polyethylene; MOM, metal-on-metal).

Fig. 5

Box plot of CD8⁺ T-lymphocyte counts vs whole blood Co category (COC, ceramic-on-ceramic; MOP, metal-on-polyethylene; MOM, metal-on-metal).

Table V. The final analysis of covariance statistical models for log lymphocyte subset counts. Cobalt (Co) category, type of hip replacement and gender are the only factors included in these models

Log lymphocyte subset counts						
Predictive factor	CD16	CD19	CD3	CD4	CD8	Total lymphocytes
Gender	0.787	0.312	0.018	0.018	0.141	0.022
Co category p-value*	0.194	0.001	0.001	0.004	0.039	0.007
Type of hip replacement p-value*	0.087	0.699	0.304	0.225	0.645	0.239
Correlation coefficient (R ²) (adj)	0.027	0.073	0.112	0.094	0.032	0.085
Significant differences in Co categories†		1 to 2, p = 0.020, 1 to 3, p = 0.001, 1 to 4, p = 0.017	1 to 3, p < 0.001, 1 to 4, p = 0.065	1 to 3, p = 0.002	1 to 3, p = 0.070	1 to 3, p = 0.004

* the p-values for Co category are adjusted for gender; those for the type of hip replacement are adjusted for gender and Co category (type I sum of squares)

† the Tukey honest significant difference method was used to adjust for multiple comparisons

CD8⁺ T-lymphocytes. Since CD3⁺ cells are largely CD4⁺ plus CD8⁺, the differences in the CD3⁺ count between cohorts may have been predominantly due to differences in the CD8⁺ count. We found that, when all the patients were analysed together, the level of Co or Cr (both constituents of the wear debris from MOM hips) in the blood inversely correlated with the absolute counts of T- and B-lymphocytes.

In a small previous study, we reported a reduction of 30% in the CD8⁺ T-cell count in peripheral blood in patients with MOM hips when compared with those with MOP hips.⁶ This observation was confirmed in this follow-up study using larger numbers of patients and two different groups, both with different non-MOM bearings, for comparison.

Moreover, unlike before, this study was designed to minimise the effect of potential confounding variables, known to influence lymphocyte subset counts in healthy persons. These included infection, immunosuppressive medication, circadian variation,¹¹ seasonal variation,¹⁰ smoking⁷ and gender.⁹ There were still some differences between the groups, but our statistical modelling allowed us to adjust for these and still show a statistically signifi-

cant reduction of T-cell counts in MOM patients. In fact, for unilateral MOM patients, 11 (13%) had CD3⁺ counts and 13 (15%) had CD8⁺ counts which were below the lower laboratory reference ranges (0.8 and 0.2 × 10⁹/l, respectively) used to define lymphopenia.

There are laboratory reference ranges for immunological tests, but there is some inter-laboratory variation, and therefore these cannot be wholly relied upon. Hence, we compared patient groups which differed only in the type of hip replacement. The robustness of our findings was therefore influenced by our control subjects, who showed similar median and interquartile ranges for lymphocyte subset numbers to those reported by other groups.⁸ Importantly, our study has shown that the lymphocyte count reduction may not be limited to CD8⁺ T-cells only, since there was also some reduction in CD19⁺ (B-cell) counts. This appeared to be related to the level of Co and Cr in whole blood, so that higher levels were associated with a reduction of both CD8⁺ and CD19⁺ counts.

One other study has reported absolute lymphocyte counts after hip replacement.¹⁹ It showed significant differences in total lymphocytes and CD16⁺ lymphocyte subsets, but not T-cell subsets CD3⁺, CD4⁺ or CD8⁺ when

22 patients with loose MOP hip replacements were compared with 20 healthy control subjects. In a more recent study the same authors reported lymphocyte subset counts from patients with the following types of hip replacement: MOM in 15, MOP in 13 and COC in 13.²⁰ They found no differences in lymphocyte subset numbers in these groups. They did, however, find a reduction in T-cell lymphocyte counts when they compared either the MOM or MOP groups with a healthy control series. The patients' ages and time from operation were similar to those of our patients, but the small numbers in each group probably resulted in a study that was underpowered.

An important contribution of our present study was a demonstration of a close relationship between circulating levels of Cr and particularly Co and lymphocyte counts. Metal wear debris, generated at the rate of 1 million, 40 nm particles per step walked²¹ (active patients walk up to 3 million steps per year²²) may reduce circulating lymphocyte numbers, possibly by diverting these cells out of the circulation, although more likely by interfering with antigen presentation to lymphocytes and their subsequent expansion. The wear debris is generated as a nanoparticle with a high surface area²³ which is likely to be in equilibrium with its constituent metal ions in solution (Co and Cr). Indeed, some preliminary evidence suggests that there is linearity between whole blood and urine levels of these metal ions indicating that, in the circulation, the metal is present as a soluble species or particles which are much smaller than when first generated, initially 40 nm in diameter although the renal glomeruli have a pore size of 5 nm. The plasmarisation step of inductively-coupled plasma mass spectrometry in which the sample may be heated to 9000° Kelvin, helps to ensure that the sample reaching the inductively-coupled plasma is reduced to ionised atoms before reaching the detector. Thus metal nanoparticles and soluble species are not distinguished by this technique. Support for soluble metal species as the active moiety affecting lymphocyte counts comes from *in vitro* studies showing that they are toxic to lymphocytes.^{24,25}

In spite of variation in previous reports on values for circulating Co and Cr levels in MOM patients, recent carefully-controlled analyses agree that median levels of 1.7 ppb (Co) and 2.3 ppb (Cr) are typical for MOM whole blood, and thus tenfold higher than the median levels in control subjects.¹⁷ The blood sampling method, laboratory equipment and standard operating procedures used in both the original series and in this large follow-up study were identical with the exception of the method of analysis for blood Co. The relationship between whole-blood Co and Cr is not linear but follows a second-order relationship in that the ratio between Co and Cr is different at low levels from that at high levels. This could be a result of differential rates of metabolism and saturation of elimination mechanisms.

We currently have no obvious clinical evidence that the reduction in the T-lymphocyte count found in MOM

patients is detrimental, although this has yet to be investigated. In fact, it is possible that a reduction in certain T-cell subsets, such as expansions of certain CD8⁺ T-cells to common persistent viral infections, for example cytomegalovirus, may relatively increase overall mortality in the elderly.²⁶⁻²⁸ It has been proposed that MOM patients have reduced mortality compared with healthy control subjects.²⁹ Moreover, there is evidence in rodents that very low-dose Cr nanoparticles are beneficial.³⁰ Overall, however, no study has determined a safe or toxic dose of Co or Cr in man. Further work is required to determine the long-term implications, whether beneficial or detrimental, of elevated Co and Cr levels in patients with MOM articulations and to establish safe upper limits.

This is the first report in man of a direct link between the level of Co and Cr ions in blood and the numbers of circulating lymphocytes. Long-term studies are needed to determine whether the moderate lymphopenia associated with 'high' levels of Co or Cr after the insertion of metal implants are detrimental or even beneficial to longevity. An urgent analysis of current registries of patients with hip implants should provide a useful preliminary answer to this question.

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