Changes in Metal Levels and Chromosome Aberrations in the Peripheral Blood of Patients After Metal-on-Metal Hip Arthroplasty

Dariusz Ladon, PhD,* Ann Doherty, PhD,* Roger Newson, PhD,† Justine Turner, PhD,‡ Manjit Bhamra, MD,§ and C. Patrick Case, MD*

Abstract: A prospective study was performed to investigate changes in metal levels and chromosome aberrations in patients within 2 years of receiving metal-on-metal hip arthroplasties. There was a statistically significant increase of cobalt and chromium concentrations, with a small increase in molybdenum, in whole blood at 6, 12, and 24 months after surgery. There was also a statistically significant increase of both chromosome translocations and aneuploidy in peripheral blood lymphocytes at 6, 12, and 24 months after surgery. The changes were generally progressive with time, but the change in aneuploidy was much greater than in chromosome translocations. No statistically significant correlations were found in secondary analyses between chromosome translocation indices and cobalt or chromium concentration in whole blood. Although the clinical consequences of these changes, if any, are unknown, future epidemiological studies could usefully include direct comparisons of patients with implants of different composition. **Key words:** hip arthroplasty, chromosomal translocation, aneuploidy, metal ions, cobalt, chromium, molybdenum.

The metal alloys that are used in orthopaedic surgery include potential mutagenic metals such as chromium, cobalt, nickel, and vanadium. It is not known whether there are any adverse clinical effects after very long term exposure to these metals in the alloys used in total joint arthroplasties. In a previous study we showed that there was an increase of chromosome aberrations consisting of aneuploidy (gain or loss of chromosome) and chromosome translocations- (exchange of part of chromosome arm from 1 chromosome to another) in the peripheral blood of patients at revision surgery of metal-on-polyethylene prostheses [1].

It was therefore of interest to test whether similar changes were occurring with metal-on-metal implants. In this study we have performed a prospective study of individual patients before and after total hip arthroplasty using metal-on-metal bearings, in order to assess changes in metal concentrations and chromosome aberrations over time more exactly. We have used the same cytogenetic biomarkers such as chromosome aberrations (ie, aneuploidy and chromosomal translocations) as they have been suggested to be useful as indicators of exposure to environmental mutagens [2]. As in our previous study [1] we used the technique of chromosome

*From the *University of Bristol, Orthopaedic Surgery, BIRC-Bristol Implant Research Center, Bristol, UK, the †Department of Public Health Sciences, King's College, London, UK, ‡LGC Ltd., Teddington, UK, and the §Rotherham General Hospital, Rotherham, UK.*

Benefits or funds were received in partial or total support of the research material described in this article. These benefits or support were received from Zimmer GmbH, Winterthur, Switzerland.

Reprint requests: C. Patrick Case, MD, Department of Orthopaedic Surgery, Bristol Implant Research Centre, Winford Unit, Southmead Hospital, University of Bristol, BS10 5NB Bristol, UK

0883-5403/04/1908-3016$30.00/0
doi:10.1016/j.arth.2004.09.010

painting (Fluorescence *in situ* hybridization), which is known to be an improvement over classical cytogenetics [3] to score these aberrations.

Materials and Methods

Patients

A total of 95 patients with the Metasul® (Zimmer GmbH, Zimmer, Winterthur, Switzerland) metal-on-metal total hip arthroplasty (head and articulating surface: wrought cobalt chrome high carbon; acetabular cup: polyethylene; stem: protasul S30 stainless steel) were recruited to provide the data for this study with patient informed consent and with Institutional Ethical Committee approval. There were 20 patients from Colchester, Essex and 75 from Rotherham, Yorkshire, UK. The patients, median age 69.3 years (range, 50.9–83) had received their implants for osteoarthritis and all implants were functioning well. Patients with an existing prosthesis or previous radiotherapy or chemotherapy were excluded from the study. Ten ml of peripheral blood was withdrawn into plastic vials containing lithium-heparin for metaphase spreads. Blood samples were obtained not only immediately prior to operation but also 6 months after (80), 1 year after (89), and 2 years after (54) total hip arthroplasty to allow a successful comparison. Only samples with optimal preparations were used. Another 5 ml of blood was collected into a Teflon plastic container (Teklab, Inc, Durham, UK) suitable for trace metal analysis. These samples were immediately frozen at −80°C. Preoperative samples were used as the internal control in this study. Cultures for cytogenetics were set up within 24 hours of sample collection.

Chromosome Preparation

Whole blood cultures were initiated by the addition of 5 ml RPMI 1640 medium containing10% Fetal Bovine Serum, (Sigma Ltd, Gilliaman, UK) 25mM Hepes, penicillin (100 U/ml) and streptomycin (100 U/ml), 25mM L-glutamine and phytohemagglutinin (1.25%). Duplicate cultures for each patient were carried out for 72 hours at 37° C. Colchicine (final concentration 10 μg/ml) was added and incubated for 45 minutes before the end of the culture. The cells were harvested and slides prepared in standard conditions as previously described [4].

Chromosome Painting

Fluorescent *in situ* hybridization was performed on freshly made slides "aged" with ethanol at 94° C for 20 sec as described by Henegariu et al. [5] Each slide was evaluated for chromosomal aberrations (translocations and aneuploidy) by simultaneous painting of chromosomes 1, 2, and 3. The method was performed with the use of commercially available human painting probes (Cambio, Cambridge, UK) according to the supplier's protocol. Three hundred metaphase spreads were examined per patient. The evaluation was performed with the use of an Olympus BX41 microscope equipped with single band-pass filter (Olympus, Southall, UK) to visualize the green and red fluorochromes and a tri band-pass filter, which allows the simultaneous detection of DAPI, FITC, and Texas Red. A Cytovision digital-imaging system was used to capture multicolor images (Applied Imaging Ltd., Richmond, UK). Two observers performed the cytogenetic analysis. One of these observers performed a more detailed study of chromosome loss and gain.

Metal Analysis

Whole blood metal measurements, in nanograms/ml (ng/ml), were obtained using high-resolution inductively coupled plasma mass spectrometry of chromium (Cr), cobalt (Co), and molybdenum (Mo) as described previously [6]. The detection limits were 0.2 ng/ml for all 3 metals.

Statistical Methods

For the purposes of statistical analysis of metal levels, we recoded the measurements that were below the detection limit to the detection limit. This is conventional practice for this type of analysis. For each postoperative measurement, we measured the difference between the postoperative measurement and the corresponding preoperative measurement, and estimated the median difference, with conservative 95% confidence limits calculated by the inverted binomial method [7].

Loglinear Poisson regression models were used to analyze the repeated aberration index measurements on the patients, using Huber variances clustered by patient to calculate confidence intervals for mean aberration indices and ratios between these mean indices. For each combination of chromosomal aberration index (translocation or aneuploidy) and postoperative measurement time, we identified patients with measurements (made by the same scorer) both at that time and at the preoperative-operative baseline, and fitted to these measurements a log-linear Poisson regression model whose parameters were a mean preoperative baseline index and a ratio of mean postoperative index to mean preoperative baseline index.

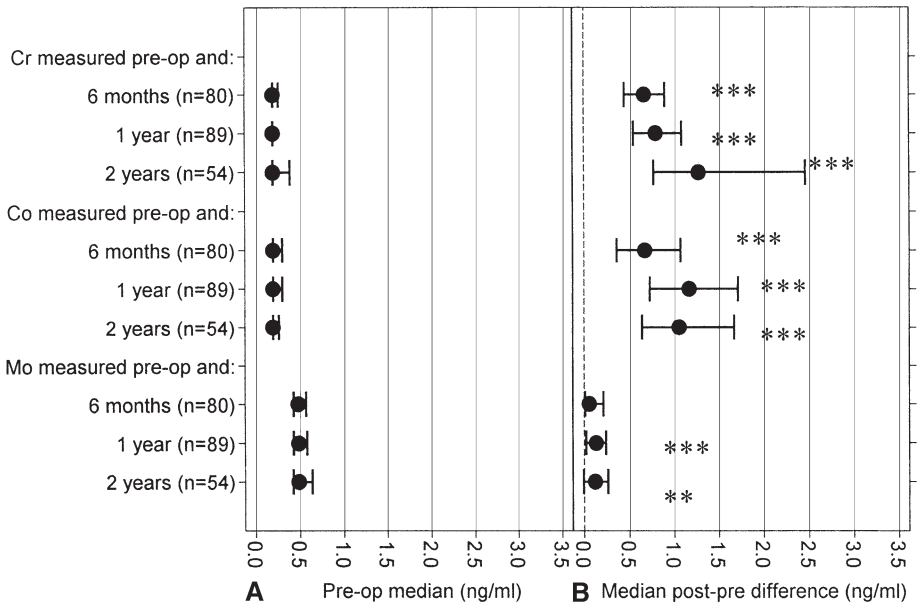

Fig. 1. Median differences of metal concentration (with 95% confidence limits) between postoperative levels after 6 months, 1 year, and 2 years and the corresponding preoperative levels (right side). The median preoperative values for each set of patients that generated the postoperative measurements at 6 months, 1 year, and 2 years, respectively, are shown on the left hand side (**$P<.01$; ***$P<.001$).

In a secondary set of analyses, we measured the tendency for patients with higher metal levels to have higher aberration indices than patients with lower metal levels when assessed at the same time after the operation by the same scorer. This was done by fitting a model for each combination of index (translocation or aneuploidy gain) and metal (Cr, Co, and Mo). Each model contained a baseline mean index for preoperative measurements, 3 mean index ratios for the 3 postoperative times (6 months, 1 year, and 2 years), and a mean index ratio per doubling of metal concentration. The per-doubling ratio was calculated by including in the model, as a continuous covariate, the binary logarithm of the metal concentration divided by its detectability limit.

Results

The changes in metal concentration in whole blood are shown in Figure 1. Preoperative values for chromium, cobalt, and molybdenum are shown in Figure 1A and postoperative changes in Figure 1B. The levels of chromium and cobalt were significantly altered at all time intervals up to 2 years postimplantation. The level of chromium was highest at 2 years and the level of cobalt was highest at 1 year. For example at 1 year the median increase of Cr level was from .2–1.23 ng/ml and of Co from 0.2–1.51 ng/ml. There was a smaller increase of molybdenum.

Two observers performed the cytogenetic analysis in this study. Both observers obtained similar and statistically significant results (Figs. 2 and 3). The mean preoperative measurements for these patients are shown in the Figures 2A and 3A, and the ratios between mean postoperative and preoperative aberration indices, with their 95% confidence limits, are shown in Figures 2B and 3B. There was a significant increase of chromosome translocations postoperatively compared to baseline values taken immediately prior to operation (Fig. 2). There was also a statistically significant increase of aneuploidy compared with preoperative values (Fig. 2). The aneuploidy involved both chromosome loss and gain (Fig. 3). The increase in aneuploidy was much greater than that of chromosome translocations and both were progressive with time.

The levels of aberrations were compared with metal concentration. Patients with higher molybdenum concentrations had higher chromosomal translocation indices than patients with lower molybdenum concentration score by the same scorer and at the same time during follow-up. There were no statistically significant correlations between chromosome translocation indices and cobalt or chromium concentrations (Table 1).

Discussion

Our study has shown an increase in chromium and cobalt levels (at least 6- to 7-fold) and an increased incidence of chromosomal translocations (1.5-fold) and aneuploidy (2- to 4-fold) in the peripheral blood of patients within 2 years after metal-on-metal joint arthroplasty compared with

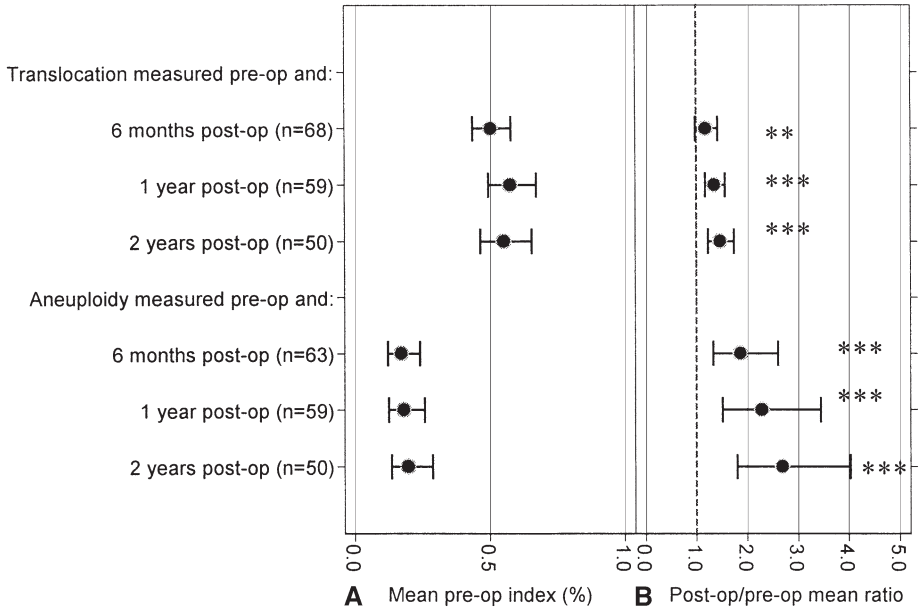

Fig. 2. Combined cytogenetic data of the 2 observers: The ratios (95% CI) of the post/preoperative mean aberration indices (chromosome translocations, aneuploidy gain) for the patients who were assessed at 6 months, 1 year, and 2 years postoperatively are shown on the right side. The mean preoperative levels for each set of patients who generated data at 6 months,1 year, and 2 years postoperatively is shown on the left hand side (**P<.01; ***P<.001).

preoperative values. Our previous research investigated the effect of metal-on-polyethylene joint arthroplasty at revision arthroplasty at an average of 11.5 years (range, 3–21) after primary arthroplasty [1]. There was an increase of asymmetrical (lethal) aberrations (breaks and gaps, 2- to 3-fold) in bone marrow [8] and an increase of symmetrical (nonlethal and cumulative) aberrations in peripheral blood, whose type depended on the metal alloy. Patients with worn cobalt chrome implants showed a 2- to 3-fold increase in both chromosome translocations and aneuploidy whilet patients with worn titanium implants showed a 5-fold increase of aneuploidy with no increase in translocations [1]. The increase of metal levels and chromosome aberrations for metal-on-metal implants in this study is not quite as great as that which we previously reported for the metal-on-polyethylene prostheses [1], but the postoperative intervals are much shorter and observations were made from patients

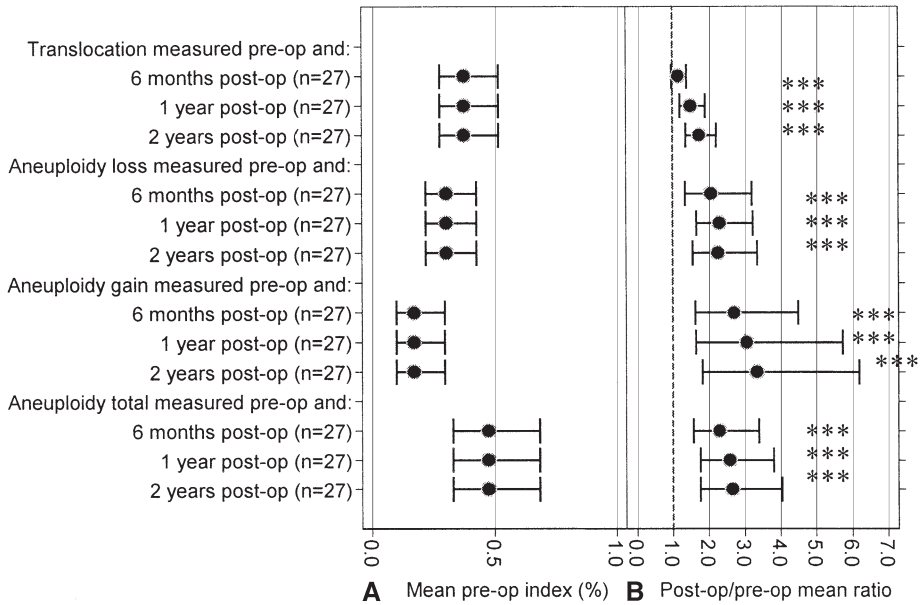

Fig. 3. Cytogenetic data of the single observer who recorded both aneuploidy gain and loss: The ratios (95% CI) of the post/preoperative mean aberration indices (chromosome translocations and aneuploidy gain and losses) for the patients who were assessed at 6 months, 1 year, and 2 years postoperatively are shown on the right side. The mean preoperative levels for each set of patients who generated data at 6 months,1 year, and 2 years postoperatively is shown on the left side (**P<.01; ***P<.001).

Table 1. Index Ratios per Doubling of Element Concentration From the Secondary Models (Adjusted for Time and Scorer)

Association	Sample Size	Index Ratio	(95% CI)	*P* Value
Translation index and:				
Cr	95	1.02	(0.97, 1.07)	.43
Co	95	1.06	(1.00, 1.13)	.071
Mo	95	1.10	(1.02, 1.19)	.013
Aneuploidy index and:				
Cr	95	1.01	(0.95, 1.07)	.69
Co	95	1.03	(0.95, 1.11)	.53
Mo	95	1.01	(0.88, 1.14)	.94

Abbreviations: Cr, chromium; Co, cobalt; Mo, molybdenum.

with well-functioning implants rather than worn implants at revision surgery.

The cause for the increased aberrations is not understood. Any effects due to ageing (from a mean age of 69–71) years during this prospective study would be minimal [9]. Potential additional effects of diagnostic x-rays have been discussed theoretically [8] and practically in previous publications and are not likely to explain the magnitude of these changes. Smoking habits showed very little change during the confines of this study. There are 4 reasons why the increase in chromosomal aberrations might in theory be caused by the particulate and soluble metal wear debris from the prosthesis. First, the type of chromosome aberration is different *in vivo* in patients with worn cobalt chrome compared to titanium alloy metal-on-polyethylene prostheses and is therefore less likely to be a nonspecific effect of surgery [1]. Second, the same difference between cobalt chrome and titanium vanadium aluminium implants is noted *in vitro* if wear debris is extracted from periprosthetic tissues and applied to cells in tissue culture [10]. Third, an increase of both chromosome translocations and aneuploidy appears to be present in patients with cobalt chrome prostheses (unlike titanium alloy implants) whether they are metal-on-metal or metal-on-polyethylene. Finally the types of aberrations can be theoretically explained by an action of the metals Cr, Co, and V. Cr(VI) leads to DNA adduct formation [11], DNA-DNA cross-links, DNA-protein cross-links [12], and DNA strand breaks while Co(II) inhibits the incision and polymerization step of excision DNA repair [13]. Both Cr(VI) and Cr(III) lead to chromosome breakage [14]. The combination of Cr and Co(II) would in principle be sufficient to cause the DNA damage that underlies the chromosomal translocations that we have observed. Likewise V(V), Cr(VI), and Cr(III) have been reported to cause aneuploidy in higher concentrations [14,15].

However, and this is a very important however, this study has shown only a statistically significant correlation between molybdenum concentration and chromosome translocations in the blood and no correlation between metal levels and aneuploidy. If chromosome aberrations were indeed caused by metals, it would have to be argued that measurements of chromosome aberrations and metal in the blood offer only a limited view of changes in the bone marrow (from where these types of aberrations in the blood originate). The metal in the bone marrow is particulate and soluble but the measurements in the blood are likely to grossly emphasize the soluble fraction. Absolute concentrations of metals may not be meaningful. Metal ions and particles may have quite different effects [18]. Low concentrations of metals may induce genomic instability [16,17], which would lead to aberrations in the blood. High concentrations might have additional toxic effects, which would not [8]. Cobalt and chromium ions may act in synergy [11–14] so that single measurements do not reflect their activity.

In summary these results have shown an increase of chromosome aberrations and metal level in the peripheral blood within 2 years of receiving a metal-on-metal hip arthroplasty. Only the molybdenum level could be correlated with chromosome translocations. To what extent chromosome aberrations were increased by soluble or particulate metal [18] in the bone marrow (not measured in this study) or to some other aspects related to joint arthroplasty surgery is not known and has not been addressed in this study. The long-term effects of joint arthroplasty are uncertain [19]. The clinical consequences of the increase in chromosome aberrations are unknown [20]. These results re-emphasize the need for epidemiological studies in which direct comparisons can be made between patients with metal-on-metal and metal-on-polyethylene prostheses as well as between patients with implants of different

metal alloy composition and at long postoperative survivals [19,20].

References

1. Doherty AD, Howell RT, Ellis LA, et al: Increased chromosome translocations and aneuploidy in peripheral blood lymphocytes patients at revision arthroplasty of total hip prostheses. J Bone Joint Surg Br 83:1075, 2001
2. Collins AR: Molecular epidemiology in cancer research. Mol Aspects Med 6:359, 1998
3. Lichter P, Cremer T, Borden J, et al: Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridisation using recombinant DNA libraries. Hum Genet 80: 224, 1988
4. Ladon D, Pieczonka A, Jolkowska J, et al: Molecular follow-up of donor lymphocyte infusion in CML children after bone marrow transplantation. J Appl Genet 42:547, 2001
5. Henegariu O, Heerema NA, Lowe Wright L, et al: Improvements in cytogenetic slide preparation: controlled chromosome spreading, chemical aging and gradual denaturing. Cytometry 43:101, 2001
6. Case CP, Ellis L, Turner JCFairman B: Development of a routine method for the determination of trace metals in whole blood by magnetic sector inductively coupled plasma mass spectrometry with particular relevance to patients with total hip and knee arthroplasty. Clin Chem 47:275, 2001
7. Conover WJ: Practical nonparametric statistics, 3rd ed. John Wiley and Sons, New York, 1999
8. Case CP, Langkamer VG, Howell RT, et al: Preliminarily observations on possible premalignant changes to bone marrow adjacent to worn total hip arthroplasty implants. Clin Orthop 329(Suppl):S269, 1996
9. Collins AR: Molecular epidemiology in cancer research. Mol Aspects Med 19:359, 1998
10. Doherty AT, Lewis B, Howell RT, et al: Wear debris from adjacent to failed total hip arthroplasty induces metal specific aberrations in the *in vitro* micronucleus assay. Mutagenesis (in press), 2004
11. Singh J, McLean JA, Pritchard DE, et al: Sensitive quantitation of chromium-DNA adducts by inductively coupled plasma mass spectrometry with a direct injection high-efficiency nebulizer. Toxicol Sci 46:260, 1998
12. Xu J, Bubley GJ, Detrick B, et al: Chromium(VI) treatment of normal human lung cells results in guanine-specific DNA polymerase arrest, DNA-DNA cross-links and S-phase blockade of cell cycle. Carcinogenesis 17:1511, 1996
13. Hartwig A: Carcinogenicity of metal compounds: possible role of DNA repair inhibition. Toxicol Lett 235:102, 1998
14. Seoane AI, Dulout FN: Genotoxic ability of cadmium, chromium and nickel salts studied by kinetochore staining in the cytokinesis-blocked micronucleus assay. Mutat Res 490:99, 2001
15. Ramirez P, Eastmond DA, Laclette JP, Ostrosky-Wegman P: Disruption of microtubule assembly and spindle formation as a mechanism for the induction of aneuploid cells by sodium arsenite and vanadium pentoxide. Mutat Res 386:291, 1997
16. Coen N, Kadhim MA, Wright EG, et al: Particulate debris from a titanium metal prosthesis induces genomic instability in primary human fibroblast cells. Br J Cancer 88:548, 2003
17. Glaviano A, Mothersill C, Campisi J, et al: Genomic instability caused by metal ions. Mol Biol Cell 14S: 370a, 2003
18. Shins RP: Mechanisms of genotoxicity of particles and fibers. Inhal Toxicol 14:57, 2002
19. McGregor DB, Baan RA, Partensky C, et al: Evaluation of the carcinogenic risks to humans associated with surgical implants and other foreign bodies. A report of an IARC monographs programme meeting. International Agency for Research on Cancer. Eur J Cancer 36:307, 2000
20. Case CP: Chromosomal changes after surgery for joint replacement. J. Bone Joint Surg Br 83:1093, 2001