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This is the author's manuscript				
Original Citation:				
Availability:				
This version is available http://hdl.handle.net/2318/119148 since				
Publisher:				
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# Ion Release and Chromosomal Damage from Total Hip Prostheses with Metal-on-Metal Articulation

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Received 11 November 2002; revised 14 April 2003; accepted 21 April 2003

Abstract: A prospective multicentric study was carried out in patients having metal-onmetal METASUL components (Sulzer Medica, Winterthur, Switzerland) in order to check the following null hypotheses:

H1: The concentration of Co, Cr, Ni, and Mb in blood and urine is not modified by the implant of a hip prosthesis with METASUL components at 6 months.

H2: The incidence of markers of chromosomal damage [sister chromatid exchanges (SCEs) and micronuclei (Mni)] in lymphocytes is not modified by the implant of METASUL components at 6 months.

H3: The concentrations of Co, Cr, Ni, and Mb in blood and urine did not correlate with the incidence of the markers of chromosomal damage.

The measurements showed a 2-fold increase of Co in blood, a 10-fold increase of Co in urine, a 1.5-fold increase of Cr in the blood, and a 3-fold increase of Cr in the urine at a follow-up of 6 months from the operation; there was also a significant increase in the Ni blood concentration at the 7 day checkup. The study cohort did not show any modification in the frequency of markers of chromosomal damage in the peripheral lymphocytes at any of the observation times. The amount of the SCEs and Mni recorded at all the observation times did not correlate with each other or with any of the ion levels measured in the blood and in the urine. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 67B: 750–757, 2003

Keywords: genotoxicity; ions, METASUL; hip; prosthesis

# INTRODUCTION

The degradation and wear of the components used in orthopedic prosthetic surgery result in the release of metal ions that both accumulate in the tissues surrounding the implant and enter the bloodstream. Some of these ions were found to have the ability to induce chromosomal damage (genotoxicity or mutagenicity) and/or to induce the development of cancer (carcinogenicity). In the CoCr alloys, Cr and Ni have established carcinogenic compounds, and Co is classified as a possible carcinogen itself.<sup>1</sup>

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Evidence of these effects in experimental studies in vitro and in vivo raised a question about the possible increased risk of local and remote neoplasm in patients who underwent joint replacement. Several authors compared the incidence of neoplasm in the general population against that in patients with joint prostheses. While the risk of incidence of local neoplasm in the site of the implant was found to be negligible, these studies were unable to give an unequivocal answer regarding the incidence of remote neoplasm. Gillespie et al. first reported a slight increase of tumors of the lymphatic and hemopoietic systems in patients with hip prosthesis.<sup>2</sup> This result was confirmed by Visuri and Koskenvuo in subjects with metal-on-metal McKee-Farrar hip prostheses.<sup>3</sup> Two further studies, comparing the overall cancer risk in operated-on cohorts with rates derived from national data in Sweden could not support the hypothesis of an increased risk in patients

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with hip replacement.<sup>4,5</sup> In a later study, Visuri et al. compared a cohort of patients with McKee–Farrar prostheses with patients with metal-on-polyethylene articulation: Both cohorts showed a lower incidence of cancer with respect to the general population, but the risk was lower in the metal– polyethylene group than in the metal–metal group.<sup>1</sup> The release of metal particles and ions from the McKee–Farrar prostheses was hypothesized to be the cause of the increased risk of lymphoma and leukemia.<sup>6,7</sup>

The interest about the McKee–Farrar study was renewed since, metal-on-metal articulation from cobalt–chromium– molybdenum alloy was reintroduced into hip arthroplasty as an alternative to polyethylene and ceramic components in 1988.<sup>8,9</sup> The newer design, material, and fabrication technology of this second generation of metal–metal components are the basis of their good clinical results at medium-term follow-up reported in literature.<sup>10–12</sup> On the other hand, the increased concentration of cobalt and chromium in the body fluids found in patients who underwent the implant of such metal-on-metal components led to some concern about the possible long-term carcinogenic effect of the exposure to such high metal concentrations.<sup>13</sup>

A prospective multicentric study was carried out in patients having metal-on-metal METASUL components (Sulzer Medica, Winterthur, Switzerland) with the aim of rejecting the following null hypotheses:

**H1**: The concentration of cobalt (Co), chromium (Cr), nickel (Ni), and molybdenum (Mb) in blood and urine is not modified by the implant of a hip prosthesis with METASUL components at 6 months.

**H2**: The incidence of markers of chromosomal damage in lymphocytes is not modified by the implant of METASUL components at 6 months.

**H3**: The concentrations of Co, Cr, Ni, and Mb in blood and urine did not correlate with the incidence of the markers of chromosomal damage.

The sister chromatid exchanges (SCEs) and the micronuclei (Mni) were used as markers of chromosomal damage for the determination of the genotoxicity.

# PATIENTS AND METHODS

## **Patient Enrollment**

Between 1999 and 2001 thirty patients were consecutively chosen from among those selected for the implant of an uncemented hip prosthesis with METASUL components (Figure 1). The patients fit the following inclusion criteria:

- Age below 65 years
- Diagnosis of primary osteoarthritis, congenital hip dysplasia, or avascular necrosis
- Smoking habit below five cigarettes/day

**Figure 1.** The ARMOR acetabular component with METASUL insert (Sulzer Medica, Winterthur, Switzerland).

- Absence of history of occupational exposure to Cr, Co, and Ni dusts
- Absence of history of diseases able to induce DNA damage
- Absence of known systemic diseases
- Absence of history of pharmacological treatments able to induce an increase in the amount of the markers of genotoxicity (cytostatic, antianxiety, antipsychotic, and antimaniacal drugs, estrogens, anticonvulsants)<sup>14</sup>
- Acceptance of the study protocol.

The study cohort was formed by 10 males and 20 females; the mean age was 52 years (range 34-64 years).

The implanted components are reported in Table I. Posterior-lateral surgical approach was used for all implants. The postoperative rehabilitation protocol was the same in all cases: two days of bed rest and subsequent walking recovery; weight bearing with two crutches for the first month, one crutch for the second month, full weight bearing after the 2-months checkup.

# **Blood and Urine Sampling**

Blood and urine samples were taken previous to surgery, at 7 days and at 2 and 6 months after surgery; three blood samples

Stem	Composition	No. Implanted	Cup	Composition (Metal Back)	No. Implanted
Alloclassic Zweymüller	Protasul <sup>®</sup> -100 (Ti-6Al-Nb)	24	Alloclassic Zweymüller	Protasul®-Ti (Ti)	4
CLS	Protasul®-100 (Ti-6Al-Nb)	4	Armor	Protasul®-Ti (Ti)	20
Stelcor	Protasul®-100 (Ti-6Al-Nb)	2	Artek	Protasul®-100 (Ti-6Al-Nb)	4
			Marburg	Protasul®-Ti (Ti)	2

TABLE I. Details of the Components Implanted. The Artek Cup has an Internal Diameter of 38 mm, and a few All the Other Acetabular Components Have an Internal Diameter of 28 mm. Because Only Artek Cups are Present in the Study Cohort, the Diameter of the Head Was Not Considered as a Variable for the Statistical Analyses

(5 ml) and one urine sample were taken at each observation time.

Peripheral blood samples were taken by using a venflon canula inserted into a vein in the forearm. The first blood sample was placed in a Hemogard<sup>®</sup> Vacutainer<sup>®</sup> plastic container with lithium-heparin as anticoagulant (Becton Dickinson Vacutainer Systems, Meylan), and was used for the analysis of the chromosomal damage; the second and third blood samples were placed in a Hemogard<sup>®</sup> Vacutainer<sup>®</sup> plastic container with K2-EDTA as anticoagulant, and were used to measure the ion level in the whole blood. This sequence was aimed at reducing the metal contamination in the samples for the ion measurement by washing out metal residuals from the needle with the blood in the first sample.

#### Ion-Level Measurement

The concentrations of cobalt, chromium, nickel, and molybdenum were measured in whole blood and urine by furnace Zeeman atomic absorption spectrophotometry (Perkin Elmer 4100 ZL). The blood samples were analyzed after dilution 1:1 with Triton X-100 0.1% solution and subsequent direct analysis, and the urine samples were directly analyzed without dilution. The validation of the measurements was performed by comparison with certified standards (NYCOMED Seronorm Trace Elements). The detection limits of the spectrophotometry were: 0.10  $\mu$ g/l for Cr in blood and for Cr and Co in urine, 0.20  $\mu$ g/l for Co and Ni in blood and Ni in urine, and 0.50  $\mu$ g/l for Mb in blood and urine.

#### Sister Chromatid Exchange and Micronucleus Tests

For the analysis of sister chromatid exchange (SCE) blood was cultured, within 24 h of drawing, in medium RPMI 1640 (Sigma Chimica, Milano, Italy) supplemented with 30% fetal bovine serum (FBS, Sigma Chimica) and 10  $\mu$ g ml<sup>-1</sup> phytohemagglutinin (PHA, Sigma Chimica), 100 U ml<sup>-1</sup> penicillin, and 50 U ml<sup>-1</sup> streptomycin. The cultures were incubated for 72 h at 37 °C: after 24-h culture, 5  $\mu$ g ml<sup>-1</sup> bromodeoxyuridine (Sigma Chimica) was added, and 1 h before cell harvesting 0.25  $\mu$ g ml<sup>-1</sup> colchicine (Colcemid, Sigma Chimica) was added to each culture to arrest mitosis.

After hypotonic treatment (KCl 0.56%) and cell fixing by methanol and acetic acid (3:1), preparations were stained with 1.5% Giemsa and microscopically examined. SCEs were scored in 40 metaphase cells from each culture where the most striking feature of metaphase is that all the chromosomes become aligned with their centromeres in a single transverse plane. In first-division cells (M1) all the chromosomes were stained dark, whereas in third or subsequent divisions (M3) they were lightly stained. In second-division cells (M2) all the chromosomes showed differential staining.<sup>15,16</sup>

The same lymphocyte technique employed for SCEs was used for the micronucleus (Mni) test.<sup>17</sup>). After 44 h of incubation Cytochalasin B (Cyt-B, Sigma Chimica) was added at a final concentration of 6  $\mu$ g ml<sup>-1</sup>and the cells were cultured for 72 h at 37 °C. Cyt-B, inhibited cytokinesis, and binucleated cells are accumulated in the first division cycle. After hypotonic treatment (KCl 0.56% and NaCl 0.9% 1:1) at room temperature the cells were fixed in methanol and acetic acid (3:1) and stained in Giemsa (5%). The frequency of Mni was estimated by blind scoring of 1000 binucleate cells at a × 1000 magnification. Briefly, micronuclei were scored in cytokinesis-blocked cells easily recognizable given their binucleate appearance indicating they must be dividing cells having completed nuclear but not cytoplasmic division.

## **Statistical Analysis**

All data were recorded in a custom-made database and analyzed with a software program (SAS ver. 8.1, SAS Institute Inc., Cary, NC). Statistical significance was stated at 5% for all the tests performed ( $\alpha = 0.05$ ).

In order to check null hypotheses H1 and H2 the ion level in blood and urine and the amount of SCEs and Mni measured at each postoperative observation time were compared with the preoperative values by paired t test (normal distribution of the differences) or by the sign rank test (nonnormal distribution of the differences).

The analysis of variance (ANOVA) for repeated measures was used to analyze the trend of the measured parameters during the observation period. The correlation between the ion levels and the markers of chromosomal damage (null hypothesis H3) was studied by Pearson correlation coefficient

Variable	Pre-op Mean [DS] Median P (Normality)	7 Days Mean [DS] Median P (Normality) P (t test) P (Sign-Rank Test)	2 Months Mean [DS] Median P (Normality) P (t test) P (Sign-Rank Test)	6 Months Mean [DS] Median P (Normality) P (t test) P (Sign-Rank Test)
Co (blood)	1.23 [1.03]	1.43 [1.52]	2.13 [2.72]	2.32 [1.45]
	0.96	1.25	1.45	2.05
	< 0.001	< 0.001	< 0.001	0.02
		0.47	0.12	<0.001
		0.49	0.29	<0.001
Co (urine)	1.13 [0.88]	2.32 [1.96]	6.41 [7.35]	10.07 [13.83]
	1.04	1.47	3.80	5.20
	0.01	< 0.01	< 0.01	< 0.01
		0.01	<0.001	0.01
		<0.001	<0.001	<0.001
Cr (blood)	1.14 [1.03]	1.57 [1.28]	1.34 [0.73]	1.70 [1.04]
	0.75	1.40	1.32	1.38
	< 0.001	< 0.001	0.55	0.01
		0.16	0.49	0.03
		0.05	0.22	0.03
Cr (urine)	0.86 [1.88]	2.62 [4.54]	2.10 [1.18]	2.81 [2.98]
	0.41	1.19	1.89	1.65
	< 0.001	< 0.001	0.27	< 0.001
		0.05	0.02	<0.001
		<0.001	<0.001	<0.001
Mo (blood)	1.05 [0.87]	1.09 [1.03]	1.13 [0.89]	1.71 [1.82]
	0.80	0.60	0.80	1.15
	< 0.001	< 0.001	< 0.001	< 0.001
		0.94	0.78	0.14
		0.80	0.83	0.42
Mo (urine)	5.91 [4.94]	4.38 [4.84]	9.58 [9.59]	10.49 [9.68]
	4.25	2.50	7.00	5.90
	0.01	< 0.001	< 0.001	< 0.001
		0.04	0.11	0.07
		0.01	0.22	0.19
Ni (blood)	2.58 [4.09]	2.01 [4.51]	3.71 [7.91]	3.00 [4.83]
	1.40	1.20	1.10	1.60
	<0.001	<0001	<0.001	<0.001
		0.09	0.58	0.73
	2 41 56 003	0.17	0.67	0.53
Ni (urine)	3.41 [6.89]	3.88 [3.63]	2.41 [3.06]	2.38 [2.10]
	1.36	3.28	1.75	1.67
	< 0.001	< 0.001	<0.001	< 0.001
		0.89	0.69	0.45
		0.05	0.33	0.37

TABLE II. Results of the Ion-Level Measurement. The *t* Test or the Sign-Rank Test were Used Alternatively, Depending on the Normality of the Results, To Compare the Preoperative Results with Those at the Different Postoperative Observation Times. The Ion Concentration Is Expressed in  $\mu g/I$ 

analysis. In order to check the congruency of the sample size, the  $\beta$  error and the power of each test performed  $(1 - \beta)$  were calculated.

# RESULTS

The overall results of the assays are reported in Tables II and III.

#### Ion-Level Measurement

**Cobalt.** There was an increase in the Co blood levels during the observation period: The difference between the preoperative level (median 0.96  $\mu$ g/l) and the 6-month level (median 2.05  $\mu$ g/l) was statistically significant (p < 0.001). As to the urinary excretion of Co the concentration recorded preoperatively (median 1.04  $\mu$ g/l) was significantly lower than that recorded at 7 days (median 1.47  $\mu$ g/l; p < 0.001),

Variable	Pre-op Mean [DS] Median P (normality)	7 Days Mean [DS] Median P (Normality) P (t Test) P (Sign-Rank Test)	2 Months Mean [DS] Median P (Normality) P (t Test) P (Sign-Rank Test)	6 Months Mean [DS] Median P (Normality) P (t Test) P (Sign-Rank Test)
Mni	6.12 [3.20]	6.59 [3.28]	5.26 [3.10]	5.72 [3.55]
	5.50	6.00	5.00	5.00
	0.52	0.15	0.11	< 0.01
		0.49	0.70	0.54
		0.39	0.65	0.34
SCEs	6.75 [4.06]	6.28 [1.75]	5.80 [1.56]	5.60 [1.22]
	6.01	5.80	5.60	5.43
	< 0.01	0.13	0.47	0.28
		0.76	0.29	0.20
		0.90	0.64	0.40

TABLE III. Results of the SCEs and Mni Tests. The Statistic Analysis Was Performed as for Table II

at 2 months (median 3.80  $\mu$ g/l; p < 0.001) and at 6 months (median 5.20  $\mu$ g/l; p < 0.001) (Figure 2, Table II).

**Chromium.** There was a significant increase in the amount of blood Cr concentration at 7 days (median 1.40  $\mu g/l$ ; p = 0.05) and at 6 months (median 1.38; p = 0.03) compared to the preoperative level (median 0.75  $\mu g/l$ ). In urine samples, the amount of Cr was preoperatively significantly lower than that recorded at all the observation times (pre-op: median 0.41  $\mu g/l$ ; 7 days: median 1.19  $\mu g/l$ , p < 0.001; 2 months: median 1.89, p < 0.001; 6 months: median 1.65  $\mu g/l$ , p < 0.001) (Figure 2, Table II).

**Molybdenum.** The blood level of Mb did not undergo any significant modification during the observation period; the

same was found for the urinary levels with the exception of a significant decrease at 7 days (preop: median 4.25  $\mu$ g/l; 7 days: median 2.50  $\mu$ g/l, p = 0.04) (Figure 2, Table II).

**Nickel.** The blood levels of Ni recorded postoperatively did not differ significantly from the preoperative level, whereas urine levels showed a significant increase of Ni at the 7day measurement (pre-op: median 1.36  $\mu g/l$ ; 7 days: median 3.28, p = 0.05) (Figure 2, Table II).

### Sister Chromatid Exchange and Micronucleus Tests

The amount of SCEs slightly decreased during the observation period from a preoperative mean of 6.01 to 5.43 at 6 months; however, the statistical analysis did not reveal any



Figure 2. Median concentrations of Co, Cr, Mb, and Ni in blood and urine.

significant difference between pre-operative values and those recorded at all the postoperative observation times (Table III). The power of the test performed was 94.1%, assuming a differential threshold of 2 SCEs between the preoperative and the 6-months values (99.7% with a threshold of 3 SCEs).

The Mni did not show any significant difference between the values recorded at the different observation times (Table III). The power of the test was 75.5%, assuming a differential threshold of 2 Mni between the preoperative and the 6-months values (96.2% with a threshold of 3 Mni).

## Correlation between the Ion Levels and the Indicators of Genotoxicity

The amount of the SCEs and Mni recorded at all the observation times did not correlate with each other nor with any of the ion levels measured in the blood and in the urine.

### DISCUSSION

The ions that were measured (Co, Cr, Mo, Ni) are all defined as *essential* metals for the human organism: This means that they are, at a given amount, an essential part of at least one enzyme and that there is a mechanism of homeostasis to avoid their accumulation. This mechanism is represented by the transportation system from the local tissues to the blood and by the subsequent elimination through the urine; this clearance mechanism is very effective for Co, Mo and Ni, which are almost completely excreted within 24–48 hours.<sup>18</sup>

As for the Cr, it has been shown that the urinary excretion is not fully effective and that it tends to accumulate in the tissues and in red blood cells. In order to measure the Cr in the red blood cells as well, the present study was performed on whole blood rather than on serum.

The study of the ion concentration in blood and urine showed that the implantation of metal-on-metal prostheses determines a significant increase in the concentration in the blood and, more significantly, in the urine, of the main components of the implanted metal alloys, Co and Cr. Measurements showed a 2-fold increase of Co in blood, a 10-fold increase of Co in urine, a 1.5-fold increase of Cr in blood and a 3-fold increase of Cr in urine at a follow-up of 6 months from the operation; there was also a significant increase in the Ni blood concentration at the 7-day checkup. These findings are in agreement with previous reports and lead to the rejection of the null hypothesis H1.

Gleizes et al. reported a 10-fold concentration of Co concentration in serum at a mean follow-up of 12.9 months in 41 patients with metal-on-metal articulation.<sup>19</sup> Jacobs reported a 3-fold increase of Co concentration in serum, a 9-fold increase of Cr concentration in serum, and a 35-fold increase of Cr concentration in urine in eight patients implanted with McKee–Farrar prostheses in comparison with control subjects at a follow-up of more than 20 years.<sup>7</sup>

Cobalt in serum does not necessarily originate from the metal-on-metal articulation alone: Kreibich reported a 2-fold

increase in Co serum concentration in 14 patients with aseptic loosening of porous-coated anatomic (PCA, Howmedica International) hip replacement with metal-on-polyethylene coupling against a group of patients with the same stable components.<sup>20</sup> Brien et al. found a significant increase of Co and Cr in the synovial fluids of loose Co–Cr cemented stems with metal-on-polyethylene coupling at revision against stable components.<sup>21</sup>

The clinical interest in the release of metal from CoCr components derives from the evidence that a significant proportion of the Cr released as ions in patients with total hip replacements is  $Cr^{6+}$  rather than the dietary form  $Cr^{3+}.^{22}$  The  $Cr^{6+}$  can be reduced to  $Cr^{3+}$  inside the cells by biological reductants in reactions that give rise to  $Cr^{4+}$  and  $Cr^{5+}$  unstable intermediates; the ability of such intermediates to induce DNA damage determines the carcinogenic ability of  $Cr^{6}$ . Wu described an increase in SCEs in peripheral lymphocytes in workers exposed to chromium compounds.<sup>23</sup> Other epidemiological studies have shown a strong association of occupational exposure to chromate particles and the incidence of nasal and lung cancer.<sup>24</sup>

To date, no consensus has been reached on possible carcinogenicity of cobalt ions, while their mutagenic properties have been proven in *in vitro* experimental models.<sup>25</sup>

Nickel was found to be both genotoxic *in vitro* and carcinogenetic *in vivo* (lung and ethmoidal bone) with a considerable latency after the exposure;<sup>26</sup> no description was found in the literature regarding the genotoxic or carcinogenetic effects of molybdenum.

Despite the increased concentration of Co and Cr, the present study cohort did not show any modification in the frequency of sister chromatid exchanges and micronuclei in the peripheral lymphocytes at any of the observation times. The sister chromatid exchanges (SCEs) and the micronuclei (Mni) were used as endpoints for the determination of the genotoxicity because of their sensitivity to the actions of carcinogenic and mutagenic substances.<sup>23,27</sup> The Mni are small extranuclear bodies that are formed in mitosis from acentric chromosomal fragments or chromosomes that are not included in either daughter nucleus; the SCEs result from the interchange of DNA replication products at apparently identical loci of the sister chromatid of a chromosome in response to a damaged DNA template. Even if a direct association between their frequency and the risk of cancer is not described, they are thought to reflect genomic instability.<sup>27</sup>

The study of the power of the tests of genotoxicity showed that the sample size was adequate; therefore the null hypothesis  $H_2$  is accepted: The incidence of markers of chromosomal damage in lymphocytes was not modified by the implant of METASUL components at 6 months.

The absence of any correlation among the SCEs, the Mni and the ion concentration in the blood and urine (null hypothesis H3) can be explained by the fact that the measured concentrations of Co, Cr, and Ni did not exceed in any case the threshold for exposed workers defined as nontoxic by several authors.<sup>25,28</sup>

In summary, the present study has shown that the implant of prostheses with METASUL components determines an increase in the concentrations of mainly Co and Cr in blood and urine, but that this increase has no genotoxic effects on the peripheral lymphocytes in the selected group at a follow-up of 6 months.

In contrast to these findings, other studies have shown an increase of chromosomal changes in patients who underwent hip replacement. For example, Stea and co-workers found a significantly higher number of SCEs in peripheral lymphocytes in patients with prostheses with metal-on-polyethylene gliding coupling made mainly by Ti-Al-Va alloy or cemented than in a control group of untreated patients.<sup>14</sup> Recently, Doherty and co-workers described a significant increase of genetic changes in blood lymphocytes at revision arthroplasty. Their study has shown a correlation between the type of chromosomal change and the metal composition of the implanted prosthesis.<sup>29</sup> The difference between these finding and the results of the present study can be probably explained by the differences in the study design (prospective vs. case control), in the composition of the implanted components (cemented or uncemented Ti-Al-Va alloy), in the criteria of enrollment of the patients, and in the length of the follow-up; furthermore, Doherty used different endpoints to determine the chromosomal damage (aneuploidy and chromosome translocations).

The criteria of enrollment of the patients adopted in this study were aimed at obtaining a homogeneous group of patients with no history of exposure to other metal sources, to factors able to modify the amount of indicators of genotoxic damage of the lymphocytes, without known systemic diseases and therefore with normal mechanisms of clearance of the metal ions. It is therefore not possible to exclude that the implanting of metal-on-metal components could determine an increase at pathological ion concentrations in patients exposed to other metal sources. Furthermore, a deficiency in the mechanisms of ion clearance, as occurs in chronic renal insufficiency, can determine by itself a pathologic increase of the concentrations of the metals released.<sup>30</sup> Therefore, in the presence of such conditions, the implanting of prostheses with metal-on-metal gliding contact should perhaps be avoided.

The length of the follow-up is another critical point: Some metals are reported to rise to their highest concentrations in the body fluids shortly after an operation, due to a possible "running in" effect.<sup>7</sup> This assumption should be not true for chromium, which tends to partially accumulate in the tissues. Furthermore, the cementless metal-on-metal prostheses are particularly implanted in young adults. Hence, the long life expectancy of those patients corresponds to a long-term exposure to increased metal concentrations. Even if in the literature we could not find any report correlating the length of the follow-up and the amount of genetic changes or the development of neoplastic diseases, this aspect makes it mandatory to further investigate the long-term risk after joint implantation.<sup>3</sup>

The authors would like to thank Mr. Paolo Spinelli and Mr. Valerio Bergo for their fundamental help in the atomic absorption spectrophotometry.

## REFERENCES

- Visuri T, Pukkala E, Paavolainen P, Pulkkinen P, Riska EB. Cancer risk after metal on metal and polyethylene on metal total hip arthroplasty. Clin Orthop Relat Res 1996;329S:280–289.
- Gillespie WJ, Frampton CMA, Henderson RJ, Ryan PM. The incidence of cancer following total hip replacement. J Bone Joint Surg [Br] 1988;70B:539–542.
- 3. Visuri T, Koskenvuo M. Cancer risk after Mc-Kee/Farrar total hip replacement. Orthopaedics 1991;14:137–142.
- Nyrén O, McLaughlin JK, Gridley G, Ekbom A, Johnell O, Fraumeni JF Jr, Adami HO. Cancer risk after hip replacement with metal implants: A population-based cohort study in Sweden. J Natl Cancer Inst 1995;87:28–33.
- Mathiesen EB, Ahlbom A, Bermann G, Lindgren JU. Total hip replacement and cancer. A cohort study. J Bone Joint Surg [Br] 1995;77:345–350.
- Gillespie WJ, Henry DA, O'Connell DL, Kendrick S, Juszczak E, McInneny K, Derby L. Development of hematopoietic cancers after implantation of total joint replacement. Clin Orthop 1996;329(S):290–296.
- Jacobs JJ, Skipor AK, Doorn PF, Campbell P, Schmalzried TP, Black J, Amstutz HC. Cobalt and chromium concentrations in patients with metal on metal total hip replacements. Clin Orthop Relat Res 1996;329S:256–263.
- 8. Weber BG. Metal-metal pairing in total hip replacement: back to the future. Z Orthop 1992;130:306–309.
- 9. Muller ME. The benefits of metal-on-metal total hip replacements. Clin Orthop Relat Res 1995;311:54–59.
- Wagner M, Wagner H. Medium-term results of a modern metal on metal system in total hip replacement. Clin Orthop Relat Res 1996;329S:123–133.
- Dorr LD, Wan Z, Longjohn DB, Dubois B, Murken R. Total hip arthroplasty with use of the METASUL metal-on-metal articulation. J Bone Joint Surg [Am] 2000;82:789–798.
- Delaunay C. Couple de friction métallique de seconde génération en arthroplastie totale primaire non cimentée de hanche. Rev Chir Ortop Reparatrice Appar Mot 2000;86:809–824.
- Jazrawi LM, Kummer FJ, Dicesare PE. Alternative bearing surfaces for total joint arthroplasty. J Am Acad Orthop Surg 1998;6:198–203.
- Stea S, Visentin M, Granchi D, Savarino L, Dallari D, Gualtieri G, Rollo G, Toni A, Pizzoferrato A, Montanaro L. Sister chromatid exchanges in patients with joint prostheses. Biomaterials 2000;15:772–777.
- Bigatti P, Lamberti L, Cannas M, Rossi E. Lack of sisterchromatid exchange induction by polymethyl methacrylate bone cement in human lymphocytes cultured in vitro. Mutat Res 1989;227:21–24.
- Lamberti L, Bigatti P, Ardito G. Cell kinetics and sister chromatid exchange frequency in human lymphocytes. Mutat Res 1983;120:193–199.
- Fenech M, Morley A. Measurement of micronuclei in lymphocytes. Mutat Res 1985;147:29–36.
- Merrit K, Brown SA. Distribution of cobalt chromium wear and corrosion products and biologic reactions. Clin Orthop Relat Res 1996;329S:S233–S243.
- Gleizes V, Poupon J, Lazennec JY, Chamberlin B, Saillant G. Value and limits of determining serum cobalt levels in patients with metal-on-metal articulating prostheses. Rev Chir Orthop Reparatrice Appar Mot 1999;85:217–255.
- Kreibich DN, Moran CG, Delves HT, Owen TD, Pinder IM. Systemic release of cobalt and chromium after uncemented total hip replacement. J Bone Joint Surg [Br] 1996;78B:18–21.

- Brien WW, Salvati EA, Betts F, Bullough P, Wright T, Rimnac C, Buly R, Garvin K. Metal levels in cemented total hip arthroplasty. Clin Orthop Relat Res 1992;276:66–73.
- Rogers GT. *In vivo* production of hexavalent chromium. Biomaterials 1984;5:244–245.
- Wu FY, Tsai FJ, Kuo HW, Tsai CH, Wu WY, Wang RY, Lai JS. Cytogenetic study of workers exposed to chromium compounds. Mutat Res 2000;464:289–296.
- Langardt S. One hundred years of chromium and cancer; a review of epidemiological evidence and selected case reports. Am J Ind Med 1990;231–237.
- World Health Organization. International Agency for Research on Cancer. Chromium, nickel and welding. In: IARC, editors. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon, IARC; 1990. Vol. 49, p 150–157.
- Christie NT, Tummolo DM, Klein CB, Rossmann TG. Role of Ni(II) in mutation. In: Nieboer E, Nriagu JO, editors. Nickel and human health: Current perspectives. Toronto: Wiley; 1992. p 305–315.

- 27. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG, Tice R, Waters MD, Aitio A. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutat Res 2000; 463:111–172.
- Langard S, Norseth T. Chromium. In: Friberg L, Nordberg GF, Vouk V, editors. Handbook on the toxicology of metals (2nd ed.) Paris: Elsevier; 1986. p 185–209.
- Doherty AT, Howell RT, Bisbinas I, Learmonth ID, Newson R, Case CP. Increased chromosome translocations and aneuploidy in peripheral blood lymphocytes of patients having revision arthroplasty of the hip. J Bone Joint Surg [Br] 2001;83B:1075–1081.
- Brodner W, Grohs JG, Bitzan P, Meisinger V, Kovarik J, Kotz R. Serum cobalt and serum chromium level in 2 patients with renal failure after total hip prosthesis implantation with metalmetal gliding contact. Z Orthop Ihre Grenzgeb 2000;138:425– 429.
- Case CP. Chromosomal changes after surgery for joint replacement. J Bone Joint Surg [Br], 2001;83B:1093–1095.