The potential role of metal ion release as a marker of loosening in patients with total knee replacement

A COHORT STUDY

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We investigated the role of ion release in the assessment of fixation of the implant after total knee replacement and hypothesised that ion monitoring could be a useful parameter in the diagnosis of prosthetic loosening. We enrolled 59 patients with unilateral procedures and measured their serum aluminium, titanium, chromium and cobalt ion levels, blinded to the clinical and radiological outcome which was considered to be the reference standard. The cut-off levels for detection of the ions were obtained by measuring the levels in 41 healthy blood donors who had no implants. Based on the clinical and radiological evaluation the patients were divided into two groups with either stable (n = 24) or loosened (n = 35) implants.

A significant increase in the mean level of Cr ions was seen in the group with failed implants (p = 0.001). The diagnostic accuracy was 71% providing strong evidence of failure when the level of Cr ions exceeded the cut-off value. The possibility of distinguishing loosening from other causes of failure was demonstrated by the higher diagnostic accuracy of 83%, when considering only patients with failure attributable to loosening.

Measurement of the serum level of Cr ions may be of value for detecting failure due to loosening when the diagnosis is in doubt. The other metal ions studies did not have any diagnostic value.

The number of total knee replacements (TKR) presenting for revision continues to increase.[1-3] For successful revision the cause of failure should be understood. This is currently established by clinical and radiological examination. Aseptic loosening is the main problem, with periprosthetic bone loss being the principal factor limiting the survival of the implant. Osteolysis results from a particle-induced foreign-body response to wear of the implant and corrosion. The metal surfaces may be subject to wear and corrosion, with particle and ion release, which may be involved in the process of failure. A bacterial presence may also contribute to the degradation of the implant and enhance the inflammatory process.[4]

The relationship between the systemic concentration of metal ions and failure in TKR has received little attention. Some reports have shown that failure in TKR is associated with elevated ion levels,[5-8] but the diagnostic use of these data has not been described.

We hypothesised that serum ions could be surrogate markers in the diagnosis of loosening in TKR. To explore this we measured the serum concentrations of aluminium (Al), titanium (Ti), chromium (Cr) and cobalt (Co) in patients with unilateral TKR. The clinical and radiological course was used for reference and the diagnostic accuracy of the tests was evaluated.

Patients and Methods

The Institutional Ethics Committee on human research approved the design of the study. A pre-test power calculation was performed in order to define the sample size. For an effect size, one SD from the control previously analysed in a pilot study was used because a biologically or clinically meaningful difference in outcome was not known. This represented 2.3 mg for Al, 0.72 mg for Ti, 0.15 mg for Cr and 0.14 mg for Co. We found that 90% power for all the elements corresponded to a sample size of 22 subjects for each group, with statistical significance level of $p < 0.05$.

The study included 69 patients with TKR admitted for a programme of pain management. A personal history was obtained from each patient, including details of other potential sources of metal ion release. Thus, ten patients with hip or contralateral implants were excluded. Ingestion of drugs containing metal ions, and renal impairment were also exclusion criteria, but no patient had these

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characteristics. Accordingly, 59 patients with unilateral TKRs were enrolled and underwent blood sampling. A clinical and radiological evaluation was performed by an orthopaedic surgeon (DT) and radiologist independently of one another and was applied as the reference for the diagnosis of failure. Using the classification of Baré, MacDonald and Bourne[9] the patients were divided into two groups comprising 24 with stable (group I) and 35 with failed loose implants (group II). The laboratory staff were blinded as to which group the patients had been allocated.

All the patients were assessed by the Knee Society clinical rating system[10] which gives a total knee score depending on pain, range of movement and stability, and a function score based on walking and stair-climbing ability. Although in hip replacement the level of activity has not been considered to be a confounding factor,[11] we obtained a score for the level of activity following a method described previously.[12] The radiological analysis was made on anteroposterior, lateral and skyline views according to the method of Ewald.[13] A change in position or subsidence of the implant as seen on sequential radiographs also indicated loosening, as well as progressive widening of the cement-bone or bone-prosthesis interface and fragmentation of the cement under a component.[14] An algorithm, described in a previous paper, was used to diagnose septic loosening pre-operatively.[15]

Group-I patients had a good clinical outcome with the ability to walk for more than 3 km per day and no radiological evidence of failure. After testing, they were followed for at least two years and did not show any sign of loosening. Group II patients had undergone revision for septic and aseptic loosening, infection, rotational malalignment, patellofemoral problems or instability.[9,16,17] The groups were matched for age, gender and duration of follow-up. Those patients with malalignment or instability without loosening had symptoms from the early post-operative period and those with loosening had symptoms from six months post-operatively to a few years from surgery.

The implants had either Co-based alloy for the femoral and tibial components (n = 22) or a Co-based femoral component and a TiA1V alloy tibial component (n = 37) (Table I).

A group of 41 normal volunteers (group III, blood donors), who in the last three months had not taken any medication and had not received any metal implants were recruited in a pilot study in order to obtain cut-off values for each ion.

Ion measurement. All testing procedures were validated according to the quality assurance standard (EN ISO 9001).[18] Blood samples were obtained from the antecubital vein of fasting patients, collected in metal-free Vacutainers (Becton Dickinson and Co, Meyland, France) and coded so that the examiner did not know the source of the sample. In order to avoid contamination from the needle, the first 5 ml of blood withdrawn were discarded. Serum was separated by centrifugation at 400 *g* for ten minutes at 4°C.

The ion content was measured using a graphite furnace atomic absorption spectrometer (GFAAS) equipped with double-background correction deuterium/Zeeman (Unicam Model Solaar 939 QZ; Unicam, Cambridge, United Kingdom) after calibration using certified standard solutions for each element. The furnace thermal programs and spectrometer parameters have been reported previously.[19-21] The specimens were diluted with 0.1 vol% HNO_3 and 0.05 vol% Triton X100 and analysed as 15 µl aliquots in triplicate. For analysis of Cr and Co magnesium nitrate was added as a matrix modifier. All the results were expressed as ng/ml, equivalent to µg/l and parts per billion. The sensitivity of the method was established by using detection limits for the sample matrix, which were 1.36 ng/ml for Al, 2.91 ng/ml for Ti, 0.06 ng/ml for Cr and 0.08 ng/ml for Co. All ion levels which were below the detection limit were assigned the detection level values. The accuracy and precision of the methods were validated using SRM 1598 NIST (National Institute of standards and technology, standard reference program, Gaithesburg, Maryland) human serum for all the elements and trace elements UTAK (UTAK Laboratories Inc, Valencia, California) for Cr. Results with a related deviation standard % greater than 10% were rejected to ensure repeatability of the test.

Statistical analysis. Quantitative results were expressed as the mean and range and the median value. Specific differences between groups were evaluated by the Mann-Whitney U test to compare one continuous and one nominal variable or Fisher's exact test to compare two nominal variables.

Table I. Details of the patients with stable TKRs (group I) and failed implants (group II)

Parameter	Group I (n = 24)	Group II (n = 35)
Gender		
Male	5	9
Female	19	26
Mean age in years (range)	69 (59 to 84)	67 (47 to 79)
Alloy		
CoCrMo	7	15
TiA1V + CoCrMo	17	20
Indication for primary surgery		
Osteoarthritis	23	34
Rheumatoid arthritis	1	-
Post-traumatic arthritis	-	1
Indication for revision surgery		
Septic loosening		11
Aseptic loosening		16
Infection	-	2
Rotational malalignment		3
Patellofemoral problems		1
Instability		2
Mean follow-up in months (range)	39 (10 to 108)	30 (8 to 74)

Table II. Serum ion levels (ng/ml) expressed as the mean (range) in both groups

	Group I	Group II	Control	p-value
Al	3.30 (1.36 to 7.95)	4.34 (1.55 to 8.99)	3.98 (1.36 to 8.22)	0.14
Ti	2.91 (2.91 to 3.73)	3.05 (2.91 to 6.43)	3.20 (2.91 to 5.4)	0.991
Cr	0.24 (0.06 to 1.39)	0.45 (0.06 to 1.44)	0.24 (0.06 to 0.50)	0.001
Co	0.44 (0.08 to 4.65)	1.10 (0.08 to 8.80)	0.26 (0.08 to 0.59)	0.75

Table III. A comparison of the ion levels between all failed implants (Group II) and those failed implants with loosening to assess the performance of Cr ion levels in detecting loosening

	Implants with failure	Implants with loosening
Mean (SEM) Cohen's kappa	0.446 (0.15)	0.653 (0.15)
Pearson chi-squared value	9.1	15.31
Fisher's exact p-value	0.0022	0.0004
Diagnostic performance (95% confidence interval)		
Sensitivity	0.56 (0.37 to 0.74)	0.74 (0.54 to 0.93)
Specificity	0.91 (0.79 to 1.03)	0.91 (0.79 to 1.03)
Diagnostic accuracy	0.71 (0.59 to 0.84)	0.83 (0.71 to 1.04)
Positive predictive value	0.88 (0.73 to 1.04)	0.88 (0.71 to 1.04)
Likelihood ratio test +	6.11 (4.75 to 7.47)	8.11 (6.76 to 9.45)
Negative predictive value	0.52 (0.09 to 0.94)	0.80 (0.64 to 0.96)
Likelihood ratio test -	0.49 (0.05 to 0.94)	0.29 (-0.47 to 1.05)

The area-under-receiver-operating characteristic (ROC) curve (AUC) measure was used as the predictivity model. The 75th percentile of ion values measured in the pilot study were chosen as the cut-off value and Cohen's kappa[22] was used to evaluate the agreement between cut-offs and failure. The relation between the results and diagnostic grouping was described according to the Standards for Reporting of Diagnostic Accuracy[23] using probabilistic measures such as sensitivity, specificity, likelihood ratios and predictive values.[24] A likelihood ratio positive test above 10 and a likelihood ratio negative test below 0.1 have been noted as providing convincing diagnostic evidence, whereas those above 5.0 and below 0.2 give good diagnostic evidence.[25] Multivariate logistic regression analysis, considering cut-off, age, gender, follow-up, activity level, type of fixation and bearing, was undertaken to find the best model for predicting failure. The correlation between ion values and follow-up and age was calculated using the Spearman rho correlation test. The data were analysed using SPSS software version 15.0 (SPSS Inc, Chicago, Illinois).

Results

Patients with failed implants (group II) showed a significant increase in the mean Cr level, in comparison with the control group (Mann-Whitney U test, p = 0.03) and those with stable implants (group I, Table II). By contrast, group I did not show any significant difference in their mean Cr levels compared with the control group (Mann-Whitney U test, p = 0.55). Logistic regression applying Cr cut-off, age, follow-up, gender, activity level, type of fixation and type of bearing showed the evaluation of Cr to be the best model for predicting failure (odds ratio 9.5; 1.27 to 70.97 95% CI; Mann-Whitney U test, p = 0.028). In addition, the ROC curve showed that measurement of Cr was a good predictivity marker (AUC 0.78; 0.63 to 0.88 95% CI; Mann-Whitney U test p < 0.0001). In order to establish the diagnostic accuracy of testing of the Cr level to predict failure, the 75th percentile of the mean ion concentration in control subjects of 0.35 ng/ml was designated as the upper reference limit (cut-off). Cohen's kappa showed the agreement between cut-off and failure with a mean of 0.446 and SEM of 0.15 (Mann-Whitney U test, p = 0.008). The differences between the clinical outcome of success or failure of the TKR and the value of ion analysis in predicting failure were evaluated using Fisher's exact test (Table III).

A significant correlation was found between the Cr and Co values (r = 0.50; Spearman's rho, p = 0.009) in patients with a failed TKR, whereas there was no correlation between ion levels and follow-up and between ion levels and age (Al age R = 0.14, p = 0.49; Ti age R = 0.01, p = 0.94; Cr age R = 0.08, p = 0.68; Co age R = 0.11, p = 0.55) (Al follow-up R = 0.50, p = 0.99; Ti follow-up R = 0.46, p = 0.25, Cr follow-up R = 0.018; Co follow-up R = 0.20, p = 0.55).

A further analysis was undertaken excluding implants which had failed because of rotational misalignment, instability, patellofemoral problems or sepsis without loosening. After the exclusions, no differences were found between patients with failure because of septic or aseptic loosening (Mann-Whitney *U* test, p = 0.46), concerning Cr and they were therefore analysed together. Cohen's kappa and the diagnostic accuracy were recalculated in order to evaluate the ability of Cr levels to predict failure exclusively due to loosening. A better diagnostic accuracy was demonstrated

to distinguish patients with loosening from patients with other mechanisms of failure (Table III).

Ti, Al and Co concentrations did not show a significant variation between groups and were not further analysed (Ti $p = 0.95$, Al $p = 0.89$, Co $p = 0.75$).

Discussion

A differential diagnosis should be obtained first when planning revision TKR.[16] Generally, this can be established by clinical and radiological assessment, but sometimes the cause is not apparent. Supplementary investigations may be of value. The estimation of the serum level of cross-linked N-terminal telopeptide and osteoprotegerin has been proposed as surrogate markers,[26-28] but these indices have not shown good diagnostic accuracy.

We hypothesised that the serum ion concentrations would be a good surrogate marker for diagnosing a malfunctioning TKR and that such monitoring could contribute to the differential diagnosis of loosening. There have been a few reports which have shown an increase in Ti release in patients with failure of metal-backed patellar components.[6-8,29-31] Dissemination of metal ions to synovial tissue, lymph nodes, liver and spleen in failed implants has been described.[32-34] Luetzner et al[35] studied the levels of serum Co, Cr, and molybdenum by GFAAS, but only in patients with stable unconstrained TKRs, and demonstrated, differently from us, an increase in ion levels in comparison with control patients without implants. Liu et al[5] evaluated ion values by GFAAS in two groups of patients with failed and stable cementless TKR, but the measurements were performed on whole blood. Nevertheless, they found that the level of Cr ions was significantly increased in patients with loosened implants in comparison with those with stable TKRs. This suggested that there was an association between an increase in the ion level and failure. Sarmiento-González et al[36] used Inductively-Coupled Plasma Mass Spectrometry for ion determination in whole blood of patients with TKR, but did not find any significant difference between those with stable implants and healthy subjects. However, the diagnostic accuracy of this method has not been assessed.

We preferred graphite furnace atomic absorption spectrometer to ICP-MS because although the latter is advantageous for multi-element analysis, we were measuring only a few and the sample volume required was much smaller for GFAAS than for Inductively-Coupled Plasma Mass Spectrometry. Additionally, GFAAS uses simpler and less costly instrumentation.[37]

A limitation of our study was that we did not consider the ion content of red blood cells since there has been some evience that Cr accumulates in these cells. However, the literature is divided on this topic.[38,39] We followed the recommendations of MacDonald, Brodner and Jacobs[40] in relation to hips and measured ion levels in the serum.

Concentrations lower than the cut-off were associated with stable implants, except in two cases, and a clinico-radiologically stable condition was still present at follow-up at two years. By contrast, high levels of Cr were detected in patients with failure. A high specificity and positive predictive value for Cr ion levels confirmed that this measurement gave good diagnostic evidence for loosening. In regard to the implants included in our study, multivariate analysis showed that neither the type of fixation nor the type of bearing was correlated with the outcome of the prosthesis and levels of Cr ions. When failure was not due to loosening, the Cr ion level was not increased whereas in most implants with septic and aseptic loosening an increase in the Cr ion level occurred. This supports our hypothesis that the test could be used to distinguish loosening from other mechanisms of failure when the radiological signs remain in doubt. Values for Co levels did not show any significant differences between groups.[37] This could be dependent on the kinetics associated with the Co ions which are transported from the site of the implant and eliminated in the urine, whereas Cr ions are stored in the tissues and slowly excreted.[41] However, the significant correlation between Cr and Co concentrations in patients with failed implants showed also that Co had a tendency to increase, but when considered alone this was not statistically significant. Concentrations of Al ions did not appear to be relevant probably because of the low proportion within the alloy. Evaluation of Ti was probably underestimated because of the high instrumental detection limit.

In conclusion, measurement of the level of Cr ions appeared to be a good surrogate marker of loosening whereas that of other metal ions did not show significant changes. When the clinical and radiological signs are unclear estimation of the serum Cr level could be of value.

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