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Sensitivity to implant materials in patients with total knee arthroplasties

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Abstract

Materials used for total knee arthroplasty (TKA), may elicit an immune response whose role in the outcome of the arthroplasty is still unclear. The aim of this study was to evaluate the frequency of sensitization in patients who had undergone TKA, and the clinical impact of this event on the outcome of the implant. Ninety-four subjects were recruited, including 20 patients who had not yet undergone arthroplasty, 27 individuals who had a well-functioning TKA, and 47 patients with loosening of TKA components. Sensitization was detected by using patch testing including haptens representative of cobalt-based alloys (CoCrMo), titanium-based alloys (TiAlV), and bone cements. The frequency of positive skin reactions to metals increased significantly after TKA, either stable or loosened (No Implant 20%; Stable TKA 48.1%, $p = 0.05$; Loosened TKA 59.6%, $p = 0.001$, respectively). We found a higher frequency of positive patch testing to vanadium in patients who had a Stable TKA with at least one TiAlV component (39.1%, $p = 0.01$). The medical history for metal allergy seems to be a risk factor, because the TKA failure was fourfold more likely in patients who had symptoms of metal hypersensitivity before TKA. The prognostic value was supported by survival analysis, because in these individuals the outcome of the implant was negatively influenced (the logrank test Chi square 5.1, $p = 0.02$). This study confirms that in patients with a TKA the frequency of positive patch testing is higher than in the normal population, although no predictive value is attributable to the sensitization because patch testing was not able to discriminate between stable and loose implants. On the contrary, the presence of symptoms of metal allergy before implantation should be taken into account as a potential risk factor for TKA failure.

Keywords: Knee replacement; Hypersensitivity; Cobalt alloy; Titanium alloy

1. Introduction

Total knee arthroplasty (TKA) has been a major advance in the treatment of knee disabilities, predictably achieving excellent results at a long-term follow-up with relatively low perioperative morbidity [1,2]. In over 95% of patients TKA relieves pain, improves functional status, and restores most of their normal activities of daily living. In spite of the excellent outcome, TKA can fail over time, and the revision rate over 5 or more years is 2% of knees and 2.1% of patients [1]. TKA failure is generally multifactorial even though the main reasons may be ascribed to mechanical and biological causes [3,4]. Chronic inflammation following the generation of wear particles has been recognized as the main biological mechanism leading to implant failure [5]. Moreover, if degradation products are able to interact with the immune system, undesirable immunotoxic effects may be induced, including a delayed-type hypersensitivity reaction (DTH) [6–9]. Metals and acrylic cements represent the main components of TKA: in contact with biological fluids they undergo corrosion and wear, and metal ions or other molecules may induce a DTH [10]. Nickel, cobalt, and chromium are known to be the most common sensitizers, [9,11] but also hypersensitivity to titanium and vanadium has been described [12,13]. Polymeric biomaterials, namely acrylic bone cements, are not easily chemically degraded and immunogenic reactions to poly-methyl-methacrylate or other constituents have been occasionally reported [14]. The possible correlation between hypersensitivity and

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implant failure has been previously investigated, but the main question about the cause–effect relationship between these events has not yet been solved.

One of the major difficulties in understanding the clinical implications of hypersensitivity to the implant components is the lack of universally accepted testing methods [15]. Several *in vitro* tests based on the reactivity of immune cells to metal–protein complexes have been proposed in diagnosing a systemic hypersensitivity to the implant components [11,16,17], but they have some faults that limit large-scale application, including the high costs and the need to be performed in qualified laboratories. In addition, the number of immune cells recruited from biological samples does not allow an exhaustive assay of all the immunogenic substances contained in metal alloys and bone cements, and the elevated toxicity of some chemicals often hampers their *in vitro* testing [11,18]. Alternatively, the *in vivo* approach, i.e. the epicutaneous skin-testing (patch testing), is cheap and allows to estimate simultaneously several haptens [19]. Although patch testing is the most common method used to diagnose contact allergy to metals, its validity in determining a deep-tissue hypersensitivity lets some doubts [10,11]. Nevertheless, its usefulness in detecting the sensitization to implant materials may be improved if patients are tested with an appropriate series of haptens according to the prosthesis components [19,20].

An additional limit in establishing the role of the sensitivity to the implant components is the paucity of clinical studies providing clear data of a connection between metal sensitivity and outcome of the implant [15]. The majority of investigators suggest that hypersensitivity can be a contributing factor to implant failure, because of the high proportion of metal DTH in patients with prosthesis loosening [10], the shorter lifespan of the implant in patients having positive patch testing [19], and the histological findings of hypersensitivity-like reaction in tissue around the artificial joint, especially in metal-on-metal bearings [21–25]. Most studies have been performed in cohorts of patients undergoing a total hip arthroplasty (THA) [10], while few data are available for patients with TKA [9]. From a theoretical point of view, the proportion of positive skin reactions in patients with TKA could differ from those observed in THA for several reasons, including the biomechanics of the joint influencing the metal ion release.

The main goals of our pilot study were to evaluate (i) the frequency of skin sensitization in patients who had undergone TKA, and (ii) the clinical impact of this event on the outcome of the implant. For this purpose we chose to use patch testing by applying a panel of haptens representative of cobalt-based alloys (CoCrMo), titanium-based alloys (TiAlV), and bone cements.

2. Patients and methods

2.1. Study design

Ninety-four Caucasian individuals were enrolled in this retrospective case–control study. The study design was approved by the institutional ethical committee on human research and signed informed consent was obtained from the patients. The sample size was calculated on the basis of the literature data considering the prevalence of sensitization in general population and the mean value of the sensitization frequency recorded in patients undergoing total joint replacement [10,11,19]. A personal history was collected from each individual, including details concerning diseases that had led to TKA, other diseases, presence of other bone or joint implants, previous orthopaedic surgery, and drug intake. A medical history of hypersensitivity to metal before TKA was documented in 10 patients (10.6%): it was verified by reports of previous skin-testing or investigated through a questionnaire aiming to verify the presence of contact dermatitis symptoms. Nobody of our patients presented cutaneous symptoms of metal allergy at time of the study inclusion. Patients who were using corticosteroid or other immunosuppressive drugs were excluded from the study.

Consecutive patients were recruited among individuals who were admitted to the orthopaedic department for pre- and post-operative medical examination planned for the TKA surgery. Three groups of patients were evaluated: the first group (No Implant) included 20 patients who were candidates for TKA; the second group consisted of 27 individuals who had already undergone TKA that was clinically and radiographically stable (Stable TKA); the third group consisted of 47 patients who had already undergone total knee arthroplasty but showed clinical and radiographic evidence of failure. More details on the groups are shown in Table 1. The implantation of knee prosthesis in both pre- and stable TKA groups, as well as the replacement of failed TKA, was performed by one orthopaedic surgeon who is specialized in knee surgery. The knee performance was valued using the 'Knee Society Clinical Rating System' [26] that establishes a 'total knee score' depending on pain, range of motion, and stability, and a 'function score' based on walking and stair climbing. Radiographic diagnosis of failure was made on the results of anteroposterior, lateral and skyline views according to the roentgenographic evaluation scoring system of the 'Knee Society' [27]. A shift or subsidence of implant position on sequential radiographs also indicates loosening [28], as well as progressive widening of the cement-bone or bone prosthesis interface and cement fragmentation under a component [29]. The total-body bone scan with technetium-99m-labeled hydroxymethylene diphosphonate was

Table 1
Data on the patients

	No Implant ($n = 20$)	Stable TKA ($n = 27$)	Loosened TKA ($n = 47$)
Gender			
Males	6 (28.7%)	5 (18.5%)	16 (34.0%)
Females	14 (71.3%)	22 (81.5%)	31 (66.0%)
Age (years)			
Mean ± standard deviation	65.2 ± 8	66.1 ± 10	70.4 ± 6
Median	69	68	70
Range	42–84	42–84	57–79
Indication for arthroplasty			
Idiopathic OA[a]	18 (90.0%)	23 (85.1%)	42 (89.4%)
Post-traumatic OA	2 (10%)	1 (3.7%)	3 (6.4%)
Other causes[b]	–	3 (11.1%)	2 (4.2%)
Positive medical history for metal allergy	2 (10%)	1 (3.7%)	7 (14.9%)
Follow-up (months)			
Median	–	18	24
Range	–	9.6–120	4.8–132
Other implants	–	8 (29.6%)	11 (23.4%)
Metal composition of the implant(s)			
CoCrMo alloy	–	3 (11.1%)	16 (34.0%)
TiAlV alloy	–	1 (3.7%)	2 (4.3%)
CoCrMo/TiAlV alloys	–	23 (85.2%)	27 (57.4%)
Unknown	–	–	2 (4.3%)
Cemented TKA		26 (96.3%)	43 (95.6%)

[a] Osteoarthritis.
[b] Other causes were fractures (2), septic OA (2), rheumatoid arthritis (1).

performed to confirm the radiographic suspicion of loosening. In patients who had an increase in inflammation markers, either hexamethylpropyleneamine oxide-labeled granulocyte scintigraphy or aspiration were performed to prove the presence of infection [30].

Nineteen TKA patients (25.7%) had an additional bone or joint implant at another site, including hip prosthesis and fixation devices. The type of implant was known in 97% of cases, since two patients arrived to our observation with an antibiotic-loaded bone cement spacer, and neither information nor radiographic documentation of the previous implant were available.

2.2. Patch testing

Hypersensitivity to metals was tested before the surgery by using the following haptens: 5% nickel sulphate, 1% cobalt chloride, 2% chromium trichloride, 0.5% potassium dichromate, 2% ferric chloride, 2% molybdenum chloride, 2% manganese chloride, 2% vanadium trichloride, 1% aluminium chloride, 1% niobium chloride, and 2% titanium dioxide. The last hapten was chosen as an oxide, rather than salt, to mimic the host reactivity of titanium-based devices, which form on their surface a titanium dioxide film. Haptens for bone cements were the following: 5% methyl-methacrylate, 2% butyl-methacrylate, 2% triethylene glycol dimethacrylate, 2% ethylene glycol dimethacrylate, 2% *N*,*N*-dimethyl-paratoluidine, 5% hydroxy-ethyl-methacrylate, 2% benzoyl-peroxide, and 1% hydroquinone monobenzyl ether (F.I.R.M.A SpA, Florence, Italy). Vaseline, which was the carrier in the patch testing, was assayed as a negative control. A drop of each hapten was smeared on Haye's chamber test, which was applied to the back of the patient. After 48–72 h, patch testing was evaluated by observers who were unaware of clinical information or the composition of the implant. Skin reactions were graded according to their intensity as 0 (no reaction), plus or minus reaction (week erythema only, doubtful), 1+ (erythema with edema covering at least 50% of the patch test site), 2+ (erythema and papules covering at least 50% the patch test site), and 3+ (vesicles or bullae covering least 50% of the patch test site) [31].

2.3. Calculations and statistical analysis

Quantitative results were expressed as mean plus and minus standard deviation. The nonparametric analysis of variance (Kruskal–Wallis) was applied to detect the effects of the various clinical variables on the quantitative results, and the Mann–Whitney test was applied to detect specific differences between groups. In each group, the frequency of positive patch testing was calculated, and the Chi square test with Fisher's exact test were used to highlight differences attributable to clinical variables. Probabilistic measures, such as sensitivity, specificity, and likelihood ratios, as well as their 95% confidence limits (95% CL), were used to describe the 'diagnostic accuracy' of our series of haptens in diagnosing DTH patients who had a previously documented metal hypersensitivity [32]. The same method was used to assess the relationship between patch testing result and TKA status. In this context, specificity and sensitivity signified the effectiveness of the patch testing at excluding or at detecting TKA failure, and positive and negative likelihood ratios described the discriminatory properties of positive and negative test results. Positive likelihood ratios above 10 and negative likelihood ratios below 0.1 provide convincing diagnostic evidence, whereas those above 5 and below 0.2 give good diagnostic evidence [33]. The Kaplan–Meier product-limit method was used to estimate the 5-year survival frequency of the TKA, in our case series since revision was considered as the end point of interest; the logrank ''Mantel–Cox'' test was applied to highlight significant differences among the survival curves of groups [34]. Differences were considered significant if the p value was less or equal to 0.05.

3. Results

3.1. Sensitization and status of the implant

The frequency of skin positive reaction was not influenced by age or presence of other implants, but in all groups females showed a higher percentage of positive patch testing (data not shown). The frequency of positive skin reactions to at least one hapten (metal or cement) was significantly higher in group of patients tested after TKA (No Implant 20%, TKA 55.4%; Chi square 7.9, $p = 0.005$), and no differences were observed between stable and loosened implants (Stable TKA 48.1%, Loosened TKA 59.6%; Chi square 0.91, $p = 0.46$). No differences were found with regards to the intensity of skin reaction: the proportion of 1+, 2+, and 3+ patch testing was similar in all groups and no doubtful reactions were observed.

In 'No Implant' group, the frequency of positive patch testing for at least one metal hapten was 15%, and differed significantly from that observed in patients with Stable TKA (44.4%, Chi square 4.6, $p = 0.05$) and Loosened TKA (57.4%, Chi square 10.2, $p = 0.001$). When the reactivity against each hapten was considered, we found a higher frequency of positive patch testing to vanadium which resulted significant in patients with Stable TKA ($p = 0.02$) (Table 2). Niobium chloride was tested only in half of the patients and it was never found positive.

The frequency of positive reaction to at least one of the bone cement components (Table 3) was similar in all patient groups. No significant difference was found when positive skin reaction for each hapten was considered.

The group of patients with Stable TKA was split according to the presence of clinical symptoms (Table 4), and the 14 patients who complained of a moderate pain related to the implant showed a high frequency of sensitization to metal haptens, mostly to vanadium. The proportion of positive patch testing was higher than that found in patients without clinical symptoms, and significantly higher than that observed in patients who were candidated for TKA. These findings did not depend on the different length of follow-up in the two groups, because it was identical in patients with and without clinical symptoms (median time 18 months).

Patients who had a Loosened TKA were divided according to the cause of the failure, i.e. septic ($n = 17$) and aseptic loosening ($n = 21$), and mechanical failure including malrotation ($n = 5$) associated with patellar maltracking ($n = 3$) and stiffness ($n = 1$) (Table 5). The 21 patients who had an aseptic

Table 2
Frequency (%) of positive skin reactions to metal haptens in patient groups

	No Implant TKA ($n = 20$)	Stable TKA ($n = 27$)	Loosened TKA ($n = 47$)
At least one hapten	15.0[b,c]	44.4	57.4
Nickel sulphate	10.0	7.4	23.4
Chromium[a]	5.0	7.4	19.1
Cobalt chloride	5.0	11.1	12.8
Ferric chloride	0	0	2.2
Molybdenum chloride	0	0	4.3
Manganese chloride	5.0	3.7	23.4
Titanium dioxide	5.0	0	2.1
Aluminium chloride	0	0	0
Niobium chloride	0	0	0
Vanadium trichloride	5.0[b]	33.3	19.1

[a] Positive patch testing for at least one of the two tested haptens.
[b] Chi square p value < 0.05 in comparison to the 'Stable TKA' group.
[c] Chi square p value < 0.005 in comparison to the 'Loosened TKA' group.

Table 3
Frequency (%) of positive skin reactions to bone cement components in patients with cemented TKA

Cement haptens	No Implant ($n=20$)	Stable TKA ($n=26$)	Loosened TKA ($n=43$)
At least one hapten	10.0	20.5	20.9
Methyl-methacrylate	5.0	4.0	9.3
Butyl-methacrylate	5.0	4.0	2.3
Triethylene glycol dimethacrylate	10.0	4.0	2.3
Ethylene glycol dimethacrylate	5.0	4.3	0
N,*N*-Dimethyl-paratoluidine	0	0	0
Hydroxy-ethyl-methacrylate	0	4.0	2.3
Benzoyl-peroxide	0	0	9.3
Hydroquinone monobenzyl ether	0	0	4.7

loosening showed the highest frequency of sensitization to metal haptens, followed by septic loosening and mechanical failure. The proportion of positive patch testing in septic and aseptic loosening was significantly higher than that observed in candidates to TKA ($p=0.007$ and $p=0.004$, respectively). No statistically significant differences were found among subgroups of failed TKA, except for the absence of sensitization to nickel in patients with mechanical complications. As above, the length of follow-up did not influence the frequency of positive patch tests because it was identical in all subgroups (median time 24 months).

3.2. Metal sensitization and implant composition

According to the metal composition of the implant, four groups were considered: (i) implants with CoCrMo only, (ii) those with TiAlV only, (iii) those with both alloys (CoCrMo/TiAlV), and (iv) those with unknown materials (Table 1). Contingency tables were recalculated for each group with more than five individuals. Subgroups with less than five individuals were not considered, i.e. Stable TKA made of CoCrMo ($n=3$) or TiAlV ($n=1$), Loosened TKA made of TiAlV ($n=2$), and TKA with unknown composition ($n=2$). In all groups the frequency of sensitization to at least one metal hapten was significantly higher than in 'No Implant' patients (Table 6). Patients with stable CoCrMo/TiAlV implants showed a higher percentage of positive patch testing to vanadium in comparison to individuals of the pre-implantation group (Chi square 7; $p=0.01$). In the group of Loosened TKA the frequency of positive patch testing to metals did not change according to the composition of the implant, even though patients who had a failed CoCrMo prosthesis showed a high frequency of positive reactions to manganese (Chi square 4.4; $p=0.06$), and reactivity to vanadium was found more frequently in the group with failed CoCrMo/TiAlV implant.

Table 4
Frequency (%) of positive skin reactions to metal haptens according to the presence of clinical symptoms (moderate pain) in stable TKA group

	No Implant TKA ($n=20$)	Stable TKA with clinical symptoms ($n=14$)	Stable TKA without clinical symptoms ($n=13$)
At least one hapten	15.0	57.1 ($p=0.02$)[a]	30.8
Nickel sulphate	10.0	7.1	7.7
Chromium	5.0	0	15.4
Cobalt chloride	5.0	21.4	0
Manganese chloride	5.0	7.1	0
Vanadium trichloride	5.0	42.9 ($p=0.01$)[a]	23.1
Follow-up (median months)	–	*18*	*18*

[a] Chi square p value vs 'No Implant TKA'.

Table 5
Frequency (%) of positive skin reactions to metal haptens according to the cause of TKA failure

	No Implant ($n=20$)	Aseptic loosening ($n=21$)	Septic loosening ($n=17$)	Mechanical failure ($n=9$)
At least one hapten	15.0	61.9 ($p=0.004$)[a]	58.8 ($p=0.007$)[a]	44.4
Nickel sulphate	10.0	23.8	35.3 ($p=0.05$)[b]	0
Chromium	5.0	28.6	11.8	11.1
Cobalt chloride	5.0	19	5.9	11.1
Manganese chloride	5.0	19.0	23.5	33.3[a]
Vanadium trichloride	5.0	23.8	17.6	11.1
Follow-up (median months)	–	*24*	*24*	*24*

[a] Chi square p value vs 'no implant TKA'.
[b] Chi square p value vs 'mechanical failure'.

3.3. Diagnostic and prognostic value of sensitization

The ability of patch testing to demonstrate metal DTH in patients with a positive medical history of contact hypersensitivity, was considered (Table 7). In the two-by-two cross table the positive and negative medical history for metal DTH matched the positive and negative patch testing. As expected, the very high sensitivity (1.00; 95% CL = 0.87–1.00) indicated that the probability to have a metal sensitization was null in patients who showed negative patch testing (negative

Table 6
Frequency (%) of positive skin reactions to metal haptens according to the metal composition of the implant

	No Implant	Stable TKA[a], CoCrMo/TiAlV ($n=23$)	Loosened TKA[a]	
			CoCrMo ($n=16$)	CoCrMo/TiAlV ($n=27$)
At least one hapten	15.0	52.2 ($p=0.02$)[b]	56.3 ($p=0.01$)[b]	42.4 ($p=0.003$)[b]
Nickel sulphate	10.0	8.7	8.3	29.6
Chromium	5.0	8.7	25.0	25.0
Cobalt chloride	5.0	13.0	12.5	14.8
Ferric chloride	0	0	6.3	0
Molybdenum chloride	0	0	0	3.7
Manganese chloride	5.0	4.3	31.3 ($p=0.06$)[b]	18.5
Titanium dioxide	5.0	0	0	3.7
Aluminium chloride	0	0	0	0
Vanadium trichloride	5.0	39.1 ($p=0.01$)[b]	12.6	25.0

[a] Subgroups with five individuals or less were not considered, i.e. Stable TKA made of CoCrMo ($n=3$) or TiAlV ($n=1$), Loosened TKA made of TiAlV ($n=2$), and TKA with unknown composition ($n=2$).
[b] Chi square p value vs 'No Implant TKA'.

Table 7
Measures of test performance

	Sensitivity	Specificity	Likelihood ratio positive test	Likelihood ratio negative test
Patch testing result and medical history of metal allergy[a]				
At least one metal hapten	1.00 (0.87–1.00)	0.64 (0.62–0.64)	2.83 (2.27–3.52)	0
Patch testing result and outcome of TKA[b]				
At least one hapten	0.60 (0.46–0.74)	0.52 (0.33–0.71)	1.24 (0.78–1.95)	0.42 (0.26–0.59)
Metal haptens	0.57 (0.43–0.72)	0.56 (0.37–0.74)	1.29 (0.79–2.10)	0.77 (0.48–1.23)
Bone cement haptens	0.19 (0.08–0.30)	0.85 (0.71–0.98)	1.24 (0.42–3.66)	0.96 (0.77–1.18)
Medical history for DTH and outcome of TKA[c]	0.15 (0.05–0.25)	0.96 (0.89–1.03)	4.02 (0.52–31.0)	0.88 (0.77–1.01)

Sensitivity = TP/(TP + FN), specificity = TN/(TN + FP), where TP is True Positive, FN is False Negative, TN is True Negative, and FP is False Positive. Likelihood ratio positive test = sensitivity/(1 − specificity). Likelihood ratio negative test = (1 − sensitivity)/specificity.

[a] Matrix of the two-by-two cross table: 'allergic-positive test' (TP), 'non allergic-positive test' (FP), 'allergic-negative test' (FN), and 'non allergic-negative test' (TN).

[b] Matrix of the two-by-two cross table: 'failed TKA-positive test' (TP), 'Stable TKA-positive test' (FP), 'failed TKA-negative test' (FN), and 'Stable TKA-negative test' (TN).

[c] Matrix of the two-by-two cross table: 'failed TKA-allergic' (TP), 'Stable TKA-allergic' (FP), 'failed TKA-non allergic' (FN), and 'Stable TKA-non allergic'(TN).

likelihood ratio = 0); on the contrary, specificity (0.64; 95% CL = 0.62–0.64) and positive likelihood ratio (2.81; 95% CL = 2.28–2.81) were not equally cogent, because a large number of patients with TKA had a negative medical history of DTH and positive patch testing.

With regards to the status of the TKA, i.e. stable or failed implant, the probability measurement of diagnostic accuracy showed that no predictive value may be ascribed to the patch testing results. The positive medical history for metal DTH seemed to be related to the TKA failure showing a high specificity value (0.96%; 95% CI = 0.89–1.03). The positive likelihood ratio meant that TKA failure is fourfold more likely in patients who had symptomatic metal allergy before the implantation (likelihood ratio: 4.02; 95% CI = 0.52–31.0), even if the large confidence interval did not validate completely the predictive value.

In our case series, the presence of skin reactions to at least one hapten did not influence the 5-year survival of the implant (positive patch testing: 35.6%; 95% CL = 18.8–52.5%; negative patch testing: 31.0%; 95% CL = 9–53.1%). Similar results were found when the chemical type of sensitizing agent, namely metal and bone cement, was considered.

The lowest survival rate was observed in patients who had a positive patch test and positive medical history of metal hypersensitivity (12.5%; 95% CL = 0–35.4%) and differed from the survivorship rate of patients who had a negative medical history and positive patch testing (40.9%; 95% CL = 21.6–60.1%) or no evidence of sensitization (31.0%; 95% CL = 9–53.1%). The statistical comparison of survival curves showed significant differences (the logrank test Chi square: 5.1, $p = 0.02$) (Fig. 1).

4. Discussion

In this study, we evaluated the frequency of sensitization in patients undergoing TKA. In absence of universally accepted tests to diagnose hypersensitivity to the implant components [15] we chose the *in vivo* approach by using patch testing, which presents some evident benefits, i.e. no specific facilities are necessary to perform the analysis, it is suitable for large-scale screening, and it allows the simultaneous evaluation of all the immunogenic substances which may be contained in the artificial joint [19]. This is a clear advantage in the preoperative planning of TKA, as the surgeon can choose the best composition of the implant in order to avoid substances that may induce undesirable effects. That does not exclude the reliability of the *in vitro* methods, which offer quantitative results and are advisable in doubtful cases, when the reactivity to few haptens has to be determined. There are some doubts about the validity of patch testing as a method to determine deep-tissue hypersensitivity [10], as the immune reactivity in dermal contact is likely to differ from that in the peri-implant tissues [11]. Nevertheless, we have to consider that corrosion

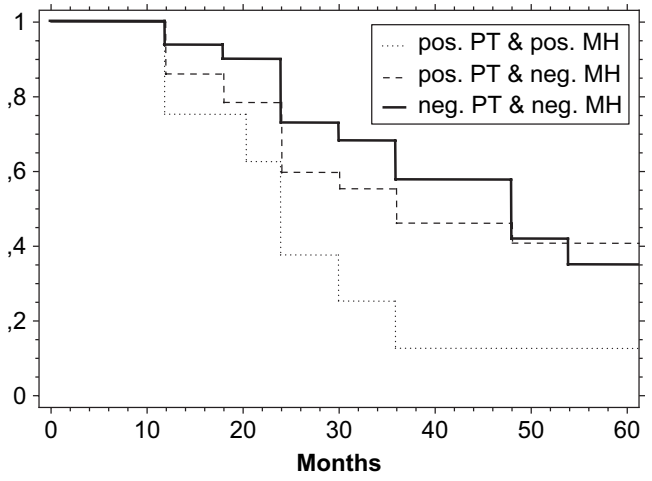

Fig. 1. Kaplan–Meier cumulative survival plot for follow-up (>12 months) in three groups of patients: positive patch testing and positive medical history (MH) for metal hypersensitivity (pos. PT and pos. MH, $n = 8$), positive patch testing and negative MH (pos. PT and neg. MH, $n = 30$), negative patch testing and negative MH (neg. PT and neg. MH, $n = 32$). The logrank (Mantel–Cox) test: Chi square 5.1; $p = 0.02$.

products released from the implant circulate in body fluid and migrate to remote organs, including the cutaneous annexes [35]. In addition, the anatomical location of TKA may favour the accumulation of degradation products in superficial strata close to the skin. Moreover, sensitization occurs in genetically predisposed subjects [7], and it is reasonable to assume that a positive skin reaction reflects the susceptibility to the immunopathological response irrespective of the source of the immunogens. An appropriate panel of haptens taking into account the prosthesis components may improve the helpfulness of patch testing in detecting the sensitization to implant materials [19,20]. With this aim, we used a series of 20 haptens, including the most important components used for the manufacturing of knee arthroplasty. In previous experience, the type and the concentration of the haptens included in patch testing showed a good diagnostic accuracy, and in a case series of patients who had undergone total hip arthroplasty the frequency of positive reactions was similar to that obtained by other authors using the lymphocyte transformation test [11].

In patients who had not yet undergone a TKA the frequency of sensitization to the metals was similar to that of general population [10], and nickel was the most common metal sensitizer, followed by cobalt, chromium and manganese, while sporadic responses to vanadium were found. The frequency of positive skin reactions increased significantly after TKA, either stable or loosened. When the reactivity against each hapten was considered, we found a higher frequency of positive patch testing to vanadium in patients with Stable TKA, while no significant difference was found when positive skin reaction for each cement hapten was considered. In agreement with the findings of previous works, the high frequency of positive skin reaction to vanadium was observed in patients who had at least one TiAlV component [13,19]. The highest frequency of manganese-positive skin reaction was found in patients who had a loosened implant made of CoCrMo, even if in the Co-based alloy the amount of this ion is very low, i.e. less than 1%. In spite of the lack of vanadium in Co-based alloy, the proportion of positive reaction to this metal is higher in patients implanted with CoCrMo prosthesis in comparison to 'No Implant' group, even if the difference was not statistically significant. An explanation for this unexpected result could be the cross-sensitization between vanadium and other metals, such as manganese, which is responsible for a high percentage of positive patch tests. These results confirm that the sensitivity reaction mainly depends on the genetic predisposition of the individual, rather than on the concentration of the immunogen [7]. The exposure to vanadium and manganese compounds may induce a contact dermatitis [36,37] and it is reasonable to assume that their continuous release from the implant may induce an immune response in patients with TKA.

The good diagnostic accuracy of patch testing is proved by sensitivity value 'one' together with a null negative likelihood ratio, which denote the ability of the assay to detect a cutaneous metal hypersensitivity when it is present [31]. On the contrary, the unsatisfactory values of specificity and positive likelihood ratio meant that a large number of patients who had no symptoms of metal allergy before TKA showed a positive skin reaction, suggesting that metal ions released from implants are able to enhance the reactivity to metals. Specificity and positive likelihood ratio ameliorated when only strong positive reactions with +2/+3 grading were considered (data not shown). The +1 reactions are classified as a possible contact allergy, and some caution should be exercised in interpreting the results [29]; nevertheless, they reflect the capability of developing a response to the antigen challenge and cannot be disregarded.

The second goal of the study was to analyse the clinical impact of the sensitization on the outcome of the implant. Metal-specific lymphocyte responses could be related with poor implant performance, because activated-T lymphocytes generate pro-osteoclastogenic factors able to modify bone homeostasis [38]. The theory formulated for the total hip arthroplasty suggests that the sensitivity to implant components cannot be considered as the only cause of implant failure, but a considerable event in a chain of processes promoting the prosthetic loosening [10,19,21–25]. Even though the presence of the implant is related to an enhanced reactivity to metals, a cause–effect relationship between sensitization and TKA failure is unlikely, because we found a high frequency of sensitization in patients with stable implant. The high frequency of sensitization in Stable TKA correlated to the presence of clinical symptoms: individuals who complained of moderate pain showed a higher frequency of sensitization to metals, especially to vanadium. The presence of positive skin reaction also correlated with the cause of the failure: the frequency of positive patch testing in septic and aseptic loosening was higher than that observed in patients with mechanical complications. However, no predictive value was attributable to the presence of sensitization, as likelihood ratios showed that patch testing was not able to discriminate between stable or failed implants. The question whether metal sensitivity is a cause or a result of prosthesis loosening is not yet solved, because sensitization could be the consequence of metal ions released from loosened prosthesis. Nevertheless, it is reasonable to assume that a pre-existing metal allergy is able to facilitate the onset of events leading to the implant failure. Actually, the medical history for metal allergy seemed to be a risk factor for TKA failure. Our results indicated that TKA failure was fourfold more likely in patients who had symptoms of metal hypersensitivity before the implantation, and the prognostic value was supported by survival analysis, because in these individuals the outcome of the TKA was negatively influenced.

5. Conclusions

Materials used for TKA are well known for their good biocompatibility, but the corrosion of the implant components and metal ion release may elicit an immune response in patients undergoing TKA. The frequency of positive skin reactions increased significantly after TKA, but the clinical impact of this event on the implant failure has not been proved, because no significant differences were found between stable and loosened prostheses. The only risk factor in our analysis was the

presence of a positive medical history for DTH, which influences negatively the implant lifespan, and increases fourfold the likelihood to have a TKA failure. Finally, our results show that patch testing may be a suitable method to evaluate simultaneously and rapidly the immunogenic substances contained in the artificial joint. The usefulness of patch testing does not exclude the reliability of the in vitro methods which are advisable in doubtful cases and/or when the reactivity to few haptens has to be confirmed.

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