Ther Adv Musculoskel Dis
2022, Vol. 14: 1–15
DOI: 10.1177/1759720X221092263

Evaluation of local tissue peri-implant reaction in total knee arthroplasty failure cases

Ava Brozovich, Terry Clyburn, Kevin Park, Katharine D. Harper, Thomas Sullivan, Stephen Incavo and Francesca Taraballi

Abstract

Introduction: Implant-related hypersensitivity is emerging as a causative factor as a potential source of total knee arthroplasty (TKA) failure. Mechanistically, this type IV hypersensitivity reaction (T4HR) is mediated by effector T-cells, macrophages, and leukocytes that infiltrate to the site of implant and react to metal exposure and induce inflammatory tissue damage.

Methods: A case–control study was performed where cortical bone was taken at the time of revision surgery for all patients operated on for primary TKA in which metal allergy was suspected and for revision TKA cases done for presumed metal allergy. Cytof was used to determine the cell density of inflammatory cells, specifically Th1, Th2, M1, and M2 cells.

Results: Comparing the mean cell density of primary *versus* revision TKA, revision TKA patients had significantly higher number of Th2 cells compared with Th1 cells ($p = 0.0043$). Among revision cases, there were significantly more M1 *versus* M2 macrophages ($p = 0.034$) within a patient. When comparing mean cell density of M1 *versus* M2 macrophages, there was a significant difference in both primary and revision TKA surgeries ($p = 0.0041$ primary, $p < 0.001$ revision). Among revision patients who had a predominance of Th2 cells, four (44%) of nine patients had a negative LTT/patch test.

Conclusion: These data support metal hypersensitivity, mediated by a T4HR, for some cases of TKA failure. Current methods to screen patients for metal hypersensitivity prior to primary TKA have been inclusive. This study demonstrates the need for a more sensitive screening test from specimens in the knee joint, to more accurately identify patients who will exhibit a T4HR to metal.

Keywords: metal hypersensitivity, metal implant, total knee arthroplasty, type IV hypersensitivity

Received: 7 October 2021; revised manuscript accepted: 28 February 2022.

Correspondence to:
Francesca Taraballi
Center for Musculoskeletal Regeneration, Houston Methodist Research Institute, R10-123 6670 Bertner Avenue, Houston, TX 77030, USA

Orthopedics and Sports Medicine, Houston Methodist Hospital, Houston, TX, USA
ftaraballi2@houstonmethodist.org

Ava Brozovich
Texas A&M College of Medicine, Bryan, TX, USA

Center for Musculoskeletal Regeneration, Houston Methodist Research Institute, Houston, TX, USA

Orthopedics and Sports Medicine, Houston Methodist Hospital, Houston, TX, USA

Terry Clyburn
Kevin Park
Thomas Sullivan
Stephen Incavo
Orthopedics and Sports Medicine, Houston Methodist Hospital, Houston, TX, USA

Katharine D. Harper
Department of Orthopedic Surgery, Washington DC Veterans Affairs Medical Center, Washington, DC, USA

Introduction

Total knee arthroplasty (TKA) is a common orthopedic procedure, with dramatic increases projected over the next 30 years.[1] Due to the rapidly expanding elderly population, TKA is expected to continue to rise. Elderly patients who present with stage III/IV osteoarthritis (OA) often have unrelenting pain that is not relieved by anti-inflammatories, physical therapy, or steroids. To relieve pain associated with bone-on-bone exposure, TKA is performed. During TKA, surgeons remove the diseased articular surfaces of the knee and insert polyethylene and metal prosthetic components (CoCr and TiAlV typically).[2] Currently, surgeons rely on a variety of metals (nickel, cobalt, chromium, molybdenum, zirconium, and titanium alloys) for total joint implants.[3] Given the dramatic increase expected for TKA, correlated with the aging population, significant rise in complications, such as infection, pulmonary embolism, pain, and stiffness (restricted range of motion), is expected.[4,5]

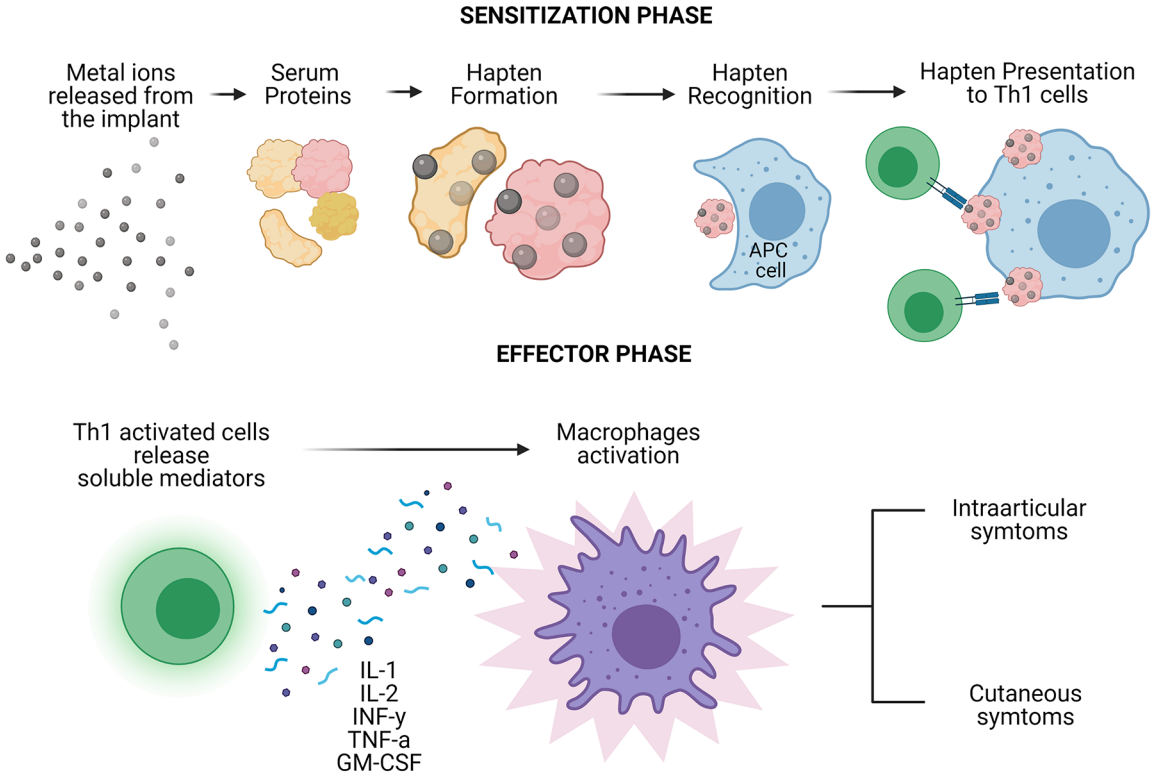


Figure 1. Mechanism of action for type IV hypersensitivity reaction (T4HR) that occurs within the joint in primary TKA. T4HR involves a delayed cell-mediated response following the activation of T-lymphocytes. Following the activation of the T-lymphocytes, inflammatory cytokines are released causing a systemic response, which includes the recruitment of activated macrophages at the level of implant.

However, although TKA is increasingly common as a method to decrease pain associated with OA, patient dissatisfaction is not uncommon.[6–8] As implant design and surgical technique continue to improve, implant-related metal hypersensitivity has emerged as another plausible explanation for TKA failure and subsequent revision surgery.[9–16] Current screening tests are unreliable and inconclusive, and the vague, non-specific symptoms attributed to metal hypersensitivity make it difficult to elucidate the exact role metal hypersensitivity plays in TKA.[2,11–18] In addition, most cutaneous patch testing involves metals in the aqueous form, which is a different exposure from the antigen form of the metal in the oxide layer of the metal implant.[19,20]

There is some evidence that supports that a delayed type IV hypersensitivity reaction (T4HR) can occur within the joint following primary TKA. Mechanistically, this reaction is mediated by effector T-cells, macrophages, and leukocytes that infiltrate at the site of implant, get exposed to the metal, and induce inflammatory tissue damage (Figure 1).[18] Prior studies have found that the prevalence of T4HR is 25% among patients with a well-functioning implant and rises to 60% among patients with poorly functioning implants.[3] Furthermore, cell analysis from blood samples revealed that patients with metal hypersensitivity had increased cell surface markers found on T-cells (CD3+ CD45RO+) compared with patients presenting with debris-synovitis (increased CD14+ cell surface markers) or infection (CD16+ cell surface markers) at the time of TKA revision.[18,21]

Currently, there are two tests commonly performed to detect metal hypersensitivity: cutaneous patch testing or lymphocyte transformation test (LTT).[18,22] Patch testing exposes the skin to metals; however, it is doubtful that the cutaneous response is the same process as the response within the articular joint.[9,10] LTT is a blood test that exposes the patient's lymphocytes and monocytes to metal salts to measure their proliferation using a radioactive nucleotide (^{3}H-thymidine).[18] Although LTT is a quantitative assay, it has not

been validated, is not covered by most insurances, and is not readily available, and increased LTT has been shown to not correlate to a hypersensitivity reaction within the joint.[23]

The clinical issue is whether some cases of TKA failure are due to a localized T4HR, which may render systemic tests falsely negative. To address this, it is necessary to demonstrate an intraarticular T4HR reaction. To do so, we have used the cell type analysis known as Cytof to compare the inflammatory reaction with the standard of care of hypersensitivity testing (LTT or patch testing). This technique is commonly used in immunology and differentiates macrophage cell types into M1 and M2 and T-cells into Th1 and Th2. In general, M1 macrophages indicate pro-inflammatory, pathogen clearance, and tissue damage; M2 macrophages are present in tissue remodeling, fibrosis, anti-inflammatory processes, and phagocytosis. Th1 cells indicate the presence of intracellular pathogens and Th2 cells indicate extracellular parasites, hypersensitivity (allergy), and asthma.

With this information, we can compare intraarticular T4HR with patch and LTT results, as well as determine whether a combination of screening tests allows for a deeper level of understanding toward this complex reaction. The primary purpose of this study is to determine the inflammatory cells present in response to metal implants. The secondary purpose is to compare these results with LTT and patch test results.

Materials and methods

Institutional review board (IRB) approval for the collection of patient data, results of the LTT and patch test, and procurement of patient samples has been obtained (IRB Pro0002133) at Houston Methodist Hospital. Informed consent was obtained.

We evaluated two patient populations: patients scheduled for (1) revision TKA (11 patients) and (2) primary TKA with or without LTT or patch testing (eight patients). The first group of patients was being operated on for primary TKA implant failure. These patients had acceptable alignment and stability on physical examination and radiographs and no other likely cause of failure. Failure was defined by unacceptable pain, swelling/effusion, stiffness/poor range of motion, or functional dissatisfaction. Among patients in the revision TKA group, X-rays [anteroposterior (AP) and lateral] were obtained and reviewed by the surgeon prior to revision TKA to rule out sub-optimal alignment issues. In the second (primary) group, patients received hypersensitivity testing for (1) concern for metal hypersensitivity, (2) patient having a history of repeatable and noticeable skin reaction to inexpensive jewelry, or (3) prior patch test result. Some, but not all, had an LTT performed. If the LTT was not obtained, it was due to expense to the patient.

At the time of surgery, bone marrow and bone specimens were obtained. Specimens included the femur cortex, taken from the distal femur freshening cut. Specimens were specifically evaluated for the relative prevalence of Th1 *versus* Th2 T-cells and M1 *versus* M2 macrophages.

Processing of human specimens

Following collection of bone and synovium specimens, samples were placed in 10% formalin at room temperature with gentle agitation for 24–48 h. Samples were then placed in 70% ethanol overnight. Specifically, bone samples were decalcified using ethylenediaminetetraacetic acid (EDTA) and then cut and embedded in paraffin, while synovium specimens did not go through the decalcification step. Specimens were processed and stained using hematoxylin and eosin (H&E). H&E staining exhibited inflammatory cellular infiltrates in all samples.

Cytof antibody staining

Cytof staining was utilized to identify different inflammatory cell populations within specimens. The antibodies used in Cytof attach to various cell surface markers, allowing for the identification of specific subpopulations of T-cells, macrophages, and fibroblasts.

Metal-labeled antibodies were prepared according to the Fluidigm protocol.[24] Antibodies were obtained in carrier/protein-free buffer and prepared using the MaxPar antibody conjugation kit (Fluidigm). After determining the percent yield by absorbance measurement at 280 nm, the metal-labeled antibodies were diluted in Candor PBS Antibody Stabilization solution (Candor Bioscience) for long-term storage at 4°C. Antibodies used in this study are listed in Figure 3(e).

Samples were baked at 60°C overnight, then dewaxed in xylene, and rehydrated in a graded

series of alcohol (ethanol absolute, ethanol: deionized water 90:10, 80:20, 70:30, 50:50, 0:100; 10 min each) for imaging mass cytometry. Heat-induced epitope retrieval was conducted in a water bath at 95°C in Tris buffer with Tween 20 at pH 9 for 20 min. After immediate cooling for 20 min, the sections were blocked with 3% bovine serum albumin in Tris-buffered saline (TBS) for 1 h. For staining, the sections were incubated overnight at 4°C with an antibody master mix (Figure 3(e)). Samples were washed 4 times with TBS/0.1% Tween 20. For nuclear staining, the sections were stained with Cell-ID Intercalator (Fluidigm) for 5 min and washed twice with TBS/0.1% Tween 20. Slides were air-dried and stored at 4°C for ablation.

The sections were ablated with Hyperion (Fluidigm) for data acquisition. Imaging mass cytometry data were segmented by ilastik and CellProfiler. Histology topography cytometry analysis toolbox (HistoCAT) and R scripts were used to quantify cell number, generate T-distributed Stochastic Neighbor Embedding (T-SNE) plots, and perform neighborhood analysis. For all samples, cellular densities were averaged across two images per specimen.

Statistical analysis. All statistics were performed using SPSS. Cell counts will be compared between groups using a two-tailed *t*-test at a 95% significance level. Using power analysis, with a power of 90%, an effect size of 0.5, and an alpha of 0.05, we determined that 15 patients need to be recruited for our study.

Results

H&E staining exhibited inflammatory cellular infiltrates in all samples (Figure 2). However, H&E was inadequate to distinguish between types of T-cells and macrophages. Due to these limitations, Cytof staining was performed.

Cytof analysis yielded T-SNE plots, allowing for the assessment of cell population diversity. Only one patient (P8) was not able to have Cytof performed due to poor DNA staining. T-SNE plots provide a visual representative of the cell density of specific cell populations based on cell surface markers identified by antibodies utilized.[24] T-SNE demonstrated that revision TKA cases demonstrated a significantly higher number of inflammatory cells compared with primary TKA (Figure 3). In general, M1 macrophages indicate pro-inflammatory, pathogen clearance, and tissue damage; M2 macrophages are present in tissue remodeling, fibrosis, anti-inflammatory processes, and phagocytosis. Th1 cells indicate the presence of intracellular pathogens and Th2 cells indicate extracellular parasites, hypersensitivity (allergy), and asthma.

When determining whether there was a significant difference in Th1 *versus* Th2 cells (allergy) within each patient among primary TKA, there was no significant difference in Th2 (cluster 12) ($p = 0.478$) or Th2 (cluster 14) ($p = 0.25$) *versus* Th1 cells. Among revision cases, there was no significant difference: Th2 (cluster 12) ($p = 0.41$) or Th2 (cluster 14) ($p = 0.31$) *versus* Th1 cells (Figure 4). However, when looking at mean cell density, revision TKA had a significantly higher cell density compared with primary TKA (Figure 4(c) and (d)). In addition, revision TKA patients had a higher raw number of Th2 cells *versus* Th1 cells except for one patient (R4, Figure 4(b)). When evaluating mean of cell density among primary *versus* revision TKA, revision TKA patients had a significantly higher number of Th2 (cluster 12) cells compared with Th1 ($p = 0.0043$) (Figure 4(d)). The increased prevalence of Th2 T-cells among revision TKA strongly indicates the presence of a T4HR among revision TKA cases.

The relative prevalence of macrophage sub-types was evaluated. There was no significant difference between M2 and M1 macrophages within individual patients who had a primary TKA ($p = 0.17$). Among revision cases, there was a statistically significant difference within a patient: M1 *versus* M2 ($p = 0.034$) (Figure 4(e) and (f)). However, when comparing the cell density of M1 and M2 among revision and primary TKA cases, there was a significant difference in both primary and revision TKA surgeries ($p = 0.0041$ in primary, $p < 0.001$ in revision) (Figure 4(g) and (h)). This indicates there is a higher prevalence of M1 macrophages among revision TKA, suggesting a T4HR.

When examining the number of inflammatory cells in each femoral cortical sample, the number of cells in revision TKA had a 1000-fold higher total number of cells compared with primary TKA cases (Figure 3). This indicates that although a T4HR is present, there are multiple inflammatory processes that are occurring in tissue surrounding the implant.

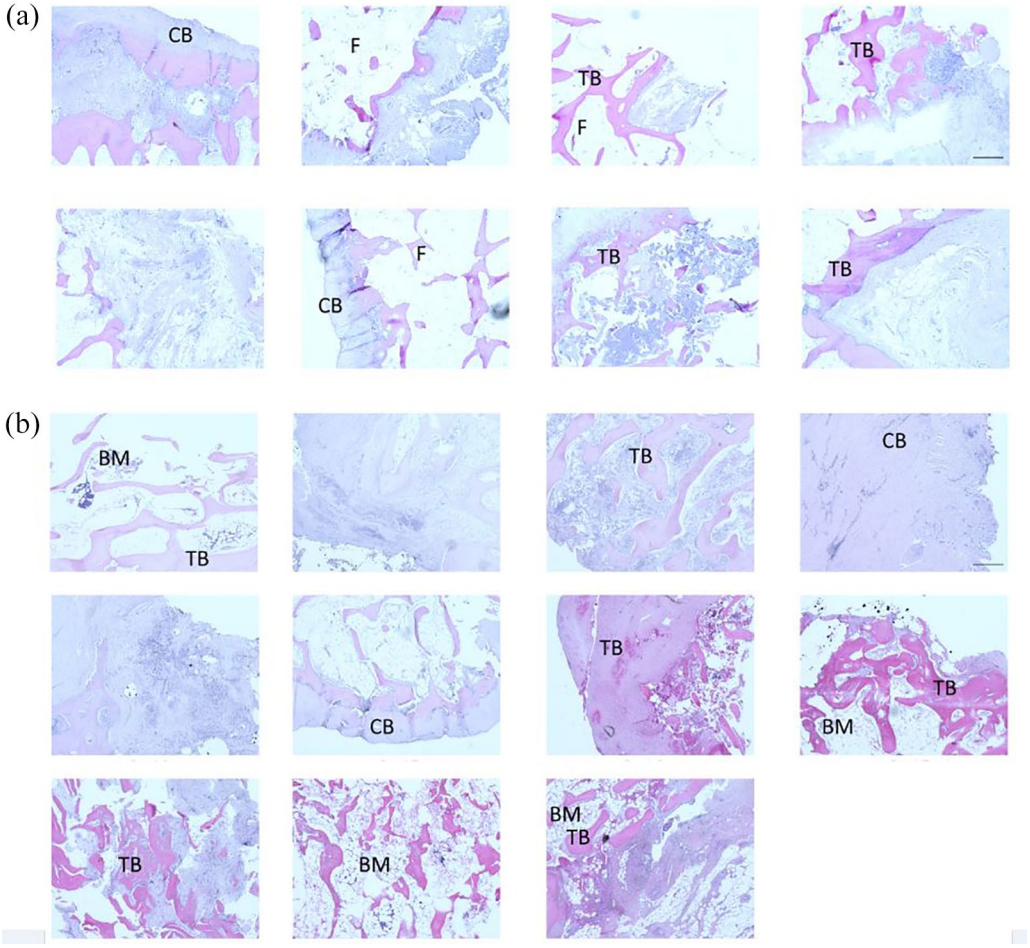
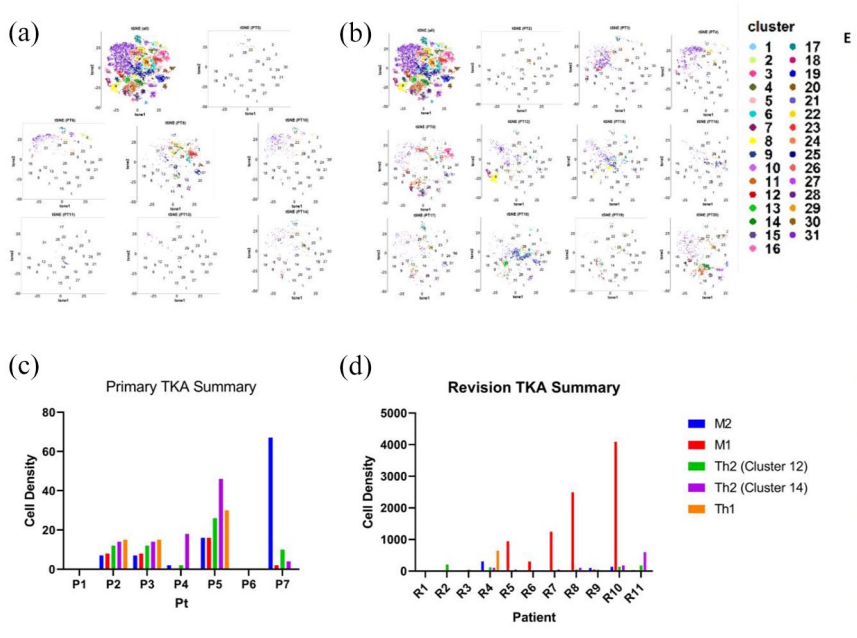


Figure 2. (a) H&E for primary TKA bone specimens and (b) H&E for revision TKA bone specimens. Specimens were cut and embedded in paraffin. H&E staining was performed. Representative tissue image was selected for each patient. Scale bar = 50 μM.
BM, bone marrow; CB, cortical bone; F, fat; TB, trabecular bone.

To evaluate whether LTT (or patch) testing adequately predicts patients who will have a T4HR, the LTT or patch testing was compared with the inflammatory cells found within the bone sample. LTT testing was performed mainly for concern for metal hypersensitivity and cutaneous reaction to jewelry. In addition, prior to revision TKA, the surgeon's working diagnosis for primary TKA failure due to metal hypersensitivity occurred in 45% (5/11) of cases. Among the 11 revision TKA cases, 1 patient had a patch test and 9 had an LTT result. Among revision patients who had a predominance of Th2 cells, four (44%) of nine patients had a negative LTT/ patch test. If only LTT results were considered, four (50%) of eight patients who had a negative LTT demonstrated a predominance of Th2 cells in bone specimens (Table 1). Five of nine patients had a positive LTT that was confirmed by Cytof staining that was performed on bone specimens (Table 1). This indicates that the current standard of care is not sensitive enough to capture patients who will have a T4HR to metal implants. However, Cyof staining provides additional information to allow for further understanding to this complex reaction. Because Cytof is able to quantify the relative prevalence of inflammatory cells within the joint space following primary TKA failure, further understanding of this mechanism is gained. To determine the relative presence of fibrosis, synovium was obtained from the same patients as secondary sample, and the presence of fibroblasts (indicated by α-SMA (alpha – smooth muscle actin)-containing clusters) was determined. Among primary TKA patients, there was significantly more fibrosis markers present (Figure 5).



E

Cluster	Cell type	Type of Immune Cell
1	Ki67+	Proliferating
2	PanCK+aSMA+	Endothelial
3	PanCK+aSMA+CCR4+CXVR3+	Endothelial
4	Ecad+	Fibroblast/Endothelial
5	CD31+Collagen+	Endothelial
6	Collagen+	
7	CD206+CD14+CD163+	Myeloid/ M2 macrophage
8	CD86+CD68+CD45+	Myeloid cell /M1
9	CD86+	Myeloid cell
10	CD80+	B cell, monocyte, dendritic cells
11	CD14+	Monocytes
12	CD3+CD4+CCR4+	T cells (Th2)
13	CD4+CCR6+CD45+	T regs
14	CD4+CCR4+	T cells (Th2)
15	CD4+CD3+CD45+CCR4+CXCR3+	T cells (Th1)
16	CD8+	T cell
17	CD20+	B cell
18	CD56+	NK cell
19	CD1c+	Dendritic Cell
20	CD15+CCR4+CCR6+GramB+	Myeloid cell
21	CXCR3+GramB+CD1c+	Dendritic Cell
22	CXCR3+	T cell
23	CXCR3+CCR4+	CD4 cell
24	Gata3+CCR6+	CD8 cell
25	CCR6+	T cell
26	CCR4+	T cell
27	PD1+	T cell / myeloid cell
28	Tbet+	CD4
29	PDL1+	Many types of cells
30	RORgt+	CD4
31	Surface-	

Figure 3. (a) T-SNE plot primary TKA specimens (P1–P7, from top left to bottom right; P8 did not have valid DNA staining, so Cytof was not possible), (b) T-SNE plot revision TKA specimens (R1–R11, from top left to bottom right), (c) summary of T-cell and macrophage response in primary TKA, (d) summary of T-cell and macrophage response in revision TKA, and (e) Cytof antibody staining used on bone specimens. (b) Demonstrates increased numbers of inflammatory cells among revision TKA cases compared with primary TKA. (d) Revision TKA demonstrates a 1000-fold higher number of macrophages compared with primary TKA.

Clinical observations

Non-metal alloy implants that were used in this patient population included titanium tibial and femoral components, or surface-coated/treated implants. Medical conditions that were considered immunologic in nature were arthritis, allergies, history of cancer, hypothyroidism, diabetes, gastroesophageal reflux disease (GERD), obesity, and hypertension. All the patients in this study were graded 0, +, ++, or +++ . There were no patients classified as '0' (Tables 2 and 3).

Of the eight patients who were primary control patients, five had an LTT (four positive and one negative). We used primary TKA as the control because following a successful primary TKA, it is not feasible to utilize successful primary TKA patients as a control until post-mortem to obtain bone specimens. The four patients who had positive LTTs had negative Cytof tests. The three patients who had no LTT had positive Cytof tests. The negative LTT testing patient received a standard (metal alloy) implant with no clinical issues. Interestingly, the LTT result did not agree with the Cytof result in all five of the tested patients: four false positives and one false negative. The three patients who had a stated history of metal allergy (but no LTT) had a positive Cytof result (Table 4). This demonstrates a discordance between LTT and Th1/Th2 ratios.

In the primary group, one patient underwent a postoperative MUA (manipulation under anesthesia) with a final successful result and one patient in addition to a postoperative MUA underwent an open lysis of adhesions with a final range of motion of 0–90°.

For the 11 patients in the revision group, all but one had LTT or patch test. Four of 10 patients who had pre-operative testing had a negative LTT/patch test (Table 1). Nine of 10 had a positive Cytof test. Of patients who were clinically suspected for metal hypersensitivity, all but one received a non-metal alloy implant. One patient had a poor result but had three previous revision procedures all with a non-metal alloy implant. There was concordance in the LTT testing in 5 of 10 patients (Table 1B).

Tables 2 and 3 provide information on the type of implants used in the primary TKA, as well as in the revision TKA. Differences in implants were due to surgeon preference rather than because of

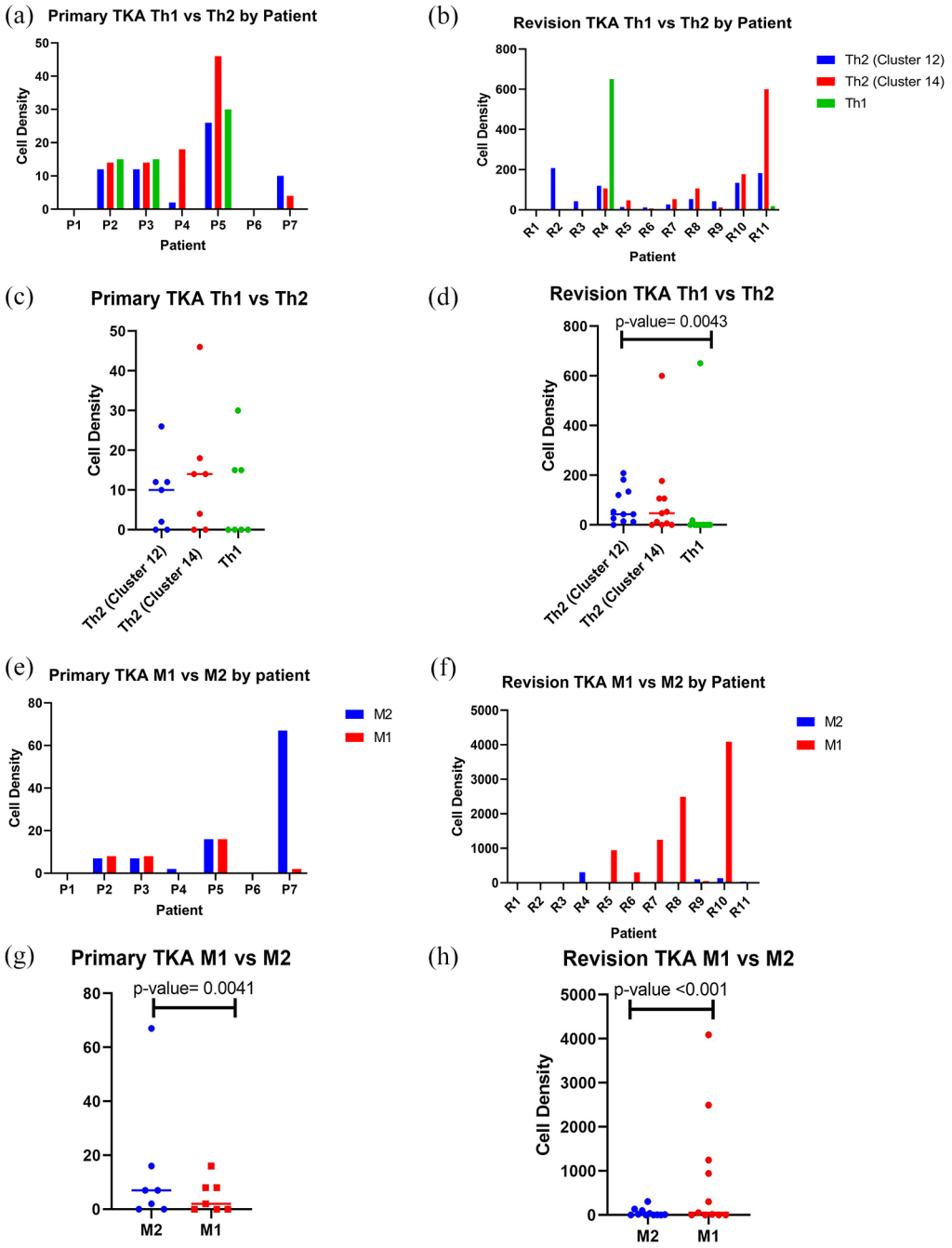


Figure 4. Th1 *versus* Th2 response and M1 *versus* M2 response: (a) primary TKA Th1 *versus* Th2 response by patient, (b) revision TKA Th1 *versus* Th2 response by patient, (c) difference in cell density number in primary TKA, (d) difference in cell density number in revision TKA, (e) primary TKA M1 *versus* M2 response by patient, (f) revision TKA M1 *versus* M2 response by patient, (g) difference in cell density number in primary TKA, and (h) difference in cell density number in revision TKA. (a) Primary TKA cases do not show a propensity for Th1 *versus* Th2, (b) revision TKA patients have a predominance of Th2, except for patient 9, (c) primary TKA cases do not show any difference in mean number of Th2 *versus* Th1 cells, (d) revision TKA cases have a significantly higher number of Th2 (cluster 12) cells compared with Th1 ($p = 0.0043$), (e) primary TKA cases do not show a propensity for M1 *versus* M2 macrophages, (f) revision TKA patients have a predominance of M1 macrophages, except for patient 9, (g) primary TKA shows an difference in mean number of M2 *versus* M1 macrophages ($p = 0.0041$), and (h) revision TKA cases have a significantly higher number of M2 macrophages compared with M1 ($p < 0.001$).

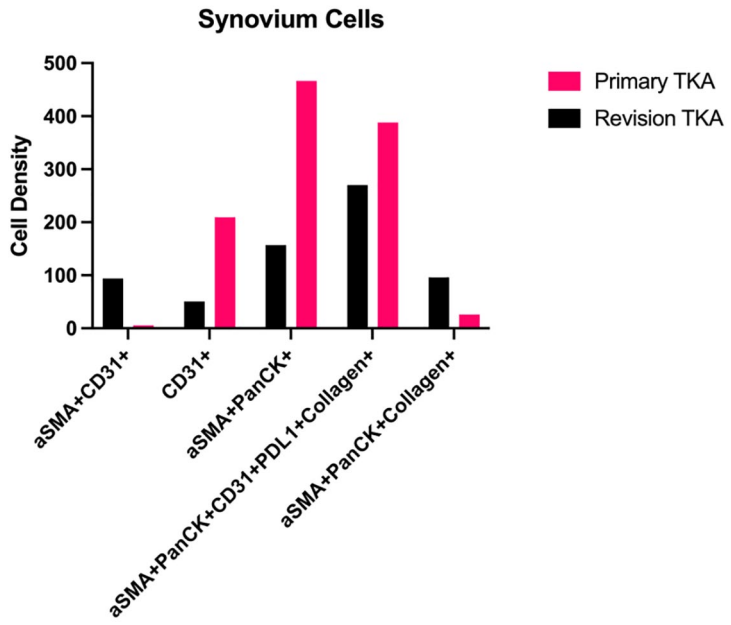


Figure 5. α-SMA-positive cells detected in the synovium of TKA cases.

correlation to lab or clinical results. Patients included in the study were from four different orthopedic surgeons, with no one surgeon contributing significantly more cases.

We utilized the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) case–control reporting guidelines in this study. (von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.)

Discussion

We were able to determine that among patients undergoing revision TKA due to no other likely cause of failure, specimens obtained from bone exhibited a presence of Th2 and M1 macrophages. This suggests that a T4HR occurred due to the presence of the implant, possibly due to metal exposure. In addition, among patients who revealed inflammatory cell populations that suggested a possible T4HR via Cytof staining of specimens, half of the patients had negative LTT test pre-operatively. This suggests that metal hypersensitivity within the knee joint is more complex than systemic T4HR, and the combination of multiple screening tests may be needed to more accurately predict and understand the reaction.

The influence of metal implants on postoperative outcomes is highly debated.[9–16] Although the population rates for cutaneous metal allergy are low,[25] metal hypersensitivity has been reported as a cause of implant failures due to a T4HR.[26] Evidence of a T4HR would be determined by the predominance of Th2 and M1 cells in the bone samples surrounding the implant. This study is the first study to use Cytof to evaluate inflammatory cells within cortical bone at the site of implant. Cytof was previously not performed in bone specimens due to the technical difficulty in processing the samples. In addition, this study utilizes an advanced technique that was able to definitively determine what inflammatory cells were present among patients who presented with a primary TKA failure that underwent a revision TKA. By utilizing this novel technique, we were able to determine that the relative prevalence of inflammatory cells present suggests that a T4HR reaction may have occurred in all but one patient (R4) among revision cases. However, Cytof also revealed that the inflammatory cells present following primary TKA failure suggest that the reaction and mechanism to metal implants are much more complex than previously thought. In R4, the absence of a T4HR was determined by a predominance of M2 macrophages, as well as Th1 cells ($p < 0.05$). Interestingly, this patient's LTT demonstrated mild to moderate reactivity to nickel and vanadium with a documented clinical concern for metal allergy. The disconnect between the LTT and bone specimen findings may be due to the fact that the inflammatory reaction within the bone is different than the systemic inflammatory reaction. It could also be due to an error in selecting a representative specimen, as Cytof examines only a small area. In conclusion, although we are unable to determine whether the failure of the primary TKA would not have occurred if a hypoallergic implant was utilized among patients who exhibited a T4HR, a predominance of inflammatory cells, specifically cells (Th2 and M1 macrophages), suggests the presence of a T4HR. In addition, the discordance between current test results and Cytof results suggests that it may be more conclusive to evaluate a global picture of multiple test results to better predict which patients would benefit from a non-metal alloy implant in primary TKA.

Table 1. (A) Revision total knee arthroplasty patients' LTT *versus* response within the bone and (B) LTT *versus* immune response found in bone in revision cases.

A.							
Pt #	**M1**	**M2**	**Th1**	**Th2 (cluster 12)**	**Th2 (cluster 14)**	**LTT testing**	**Difference between LTT and bone sample**
R1	0	0	0	0	0	Mild reactivity to nickel and iron	Yes (not detected bone)
R2	2.04	0	0	208.16	0	No LTT, negative patch test	Yes (not detected LTT)
R3	0	0	0	42.86	0	Mild reactivity to nickel	
R4	0	308.16	651.02	120.41	106.12	Mild to moderate reactivity to nickel, mild reactivity to vanadium	
R5	944.90	8.16	0	14.29	46.94	No LTT testing	N/A
R6	301.01	2.02	0	12.12	6.06	Mild zirconium reactivity, mild cobalt reactivity, highly reactive to nickel	
R7	1245.91	10.18	0	26.41	52.99	Negative LTT testing	Yes (not detected LTT)
R8	2491.82	20.37	0	52.81	105.99	Moderate reactivity to nickel	
R9	51.02	102.04	0	42.86	12.24	Negative LTT testing	Yes (not detected LTT)
R10	4089.75	134.61	0	134.20	177.30	Negative LTT testing	Yes (not detected LTT)
R11	16.33	36.73	18.37	181.63	600	Mild reactivity to nickel, vanadium, chromium	

(B)		
	LTT positive	**LTT negative**
Bone positive for T4HR	5	4
Bone negative for T4HR	1	0

LTT, lymphocyte transformation test; T4HR, type IV hypersensitivity reaction.

Table 2. Primary TKA patient characteristics.

Pt #	Sex	Age (years)	BMI	PMH	Implant used in TKA	Hypoallergenic implant used in primary TKA	Reason for LTT testing	LTT testing
P1	M	67	27.29	+++	Smith & Nephew Genesis PS Oxinium	Y	Patch test positive	++ Nickel ++ Cobalt
P2	F	73	21.64	+++	Smith & Nephew Genesis PS Oxinium	Y	Allergic to jewelry	++ Nickel
P3	F	65	31.20	++	Microprot Evolution NitrX CS	Y	Allergic to jewelry	+ Chromium + Aluminum
P4	F	74	24.62	+++	Zimmer Persona	N	Concern for metal allergy	No reactivity
P5	F	64	39.56	++	Microprot Evolution NitrX CS	Y	N/A	Cost prohibitive to patient
P6	M	68	27.21	++	Microprot Evolution NitrX CS	Y	N/A	Cost prohibitive to patient
P7	F	70	32.93	+++	Microprot Evolution NitrX CS	Y	Allergic to jewelry	Cost prohibitive to patient
P8	F	49	32.74	+	Smith & Nephew Genesis PS Oxinium	Y	Concern for metal allergy	+++ Nickel

+, 1–2 diseases related to immune response, ++ = 3–5 diseases related to immune response, +++ = 5 + diseases related to immune response; BMI, body mass index; LTT, lymphocyte transformation test; PMH, past medical history, TKA, total knee arthroplasty.

Table 3. Revision TKA patient characteristics.

Pt #	Sex	Age (years)	BMI	PMH	Primary implant that was replaced	ESR (Erythrocyte Sedimentation Rate) prior to revision TKA (mm/h)	CRP (C-Reactive Protein) prior to revision TKA (mg/dl)	Synovial fluid count prior to revision TKA	Synovasure prior to revision TKA	Implant used for revision TKA	Hypoallergic implant	Working diagnoses prior to revision	Reason for LTT testing	LTT testing
R1	F	68	35.57	++	Depuy Sigma	26	<0.3	N/A	N/A	S&N Legion Oxinium	Y	Stiffness	Concern for metal allergy	+ Nickel + Iron
R2	F	69	34.00	++	Rotating Platform	9	0.25	N/A	N/A	Zimmer RHK	N	Instability	Patch test performed prior	No LTT, negative patch test
R3	M	67	34.72	++	Depuy Attune PS	7	<0.3	N/A	N/A	S&N Legion Oxinium	Y	Instability, metal allergy	Concern for metal allergy	+ Nickel
R4	F	39	22.89	++	Zimmer NextGen PS Legacy	N/A	N/A	N/A	N/A	S&N Legion Pressfit Oxinium	Y	Instability, metal allergy	Concern for metal allergy	++ Nickel + Vanadium
R5	F	55	39.30	++	Wright Medical Evo MP	8	0.42	1289	Negative	Microport Evo	N	Loosening	N/A	No LTT testing
R6	M	57	28.56	+	Zimmer & Biomet Vanguard	9	0.76	3625	N/A	Zimmer & Biomet vanguard complete titanium	Y	Metal allergy, stiffness	Inconclusive patch test	+ Zirconium + Cobalt +++ Nickel
R7	M	47	32.96	+++	S&N Genesis II	N/A	0.5	248	Negative	Aesculap	Y	Instability	Concern for metal allergy	Negative LTT testing
R8	M	50	45.70	+++	Microport Evolution	26	0.1	N/A	N/A	S&N Legion Pressfit Oxinium	Y	Instability, metal allergy	Concern for metal allergy	++ Nickel
R9	M	72	22.36	+	CCK Implant	19	0.84	428	Negative	Zimmer RHK	N	Infection	Concern for metal allergy	Negative LTT testing
R10	F	68	32.13	+++	Conformis Total Knee	10	<.3	N/A	N/A	Zimmer Vanguard Tibia	N	Instability	Allergic to jewelry	Negative LTT testing
R11	F	81	26.86	+++	Zimmer & Biomet Vanguard	7	<.3	N/A	N/A	S&N Legion Pressfit Oxinium	Y	Instability, metal allergy	Concern for metal allergy	+ Nickel + Vanadium + Chromium

+, 1–2 diseases related to immune response; + +, 3–5 diseases related to immune response; + + +, 5 + diseases related to immune response.BMI, body mass index; LTT, lymphocyte transformation test; PMH, past medical history; TKA, total knee arthroplasty.

Table 4. (A) Clinical outcomes of revision TKA cases and (B) primary TKA cases.

(A)			
Pt #	**Cytof + for T4HR**	**Revision TKA–involved hypoallergenic implant**	**Clinically satisfactory result**
R1	N	Y	N
R2	Y	N	Y
R3	Y	Y	Y
R4	Y	Y	Y
R5	Y	N	Y
R6	Y	Y	Y
R7	Y	Y	Y
R8	Y	Y	N
R9	Y	N	Y
R10	Y	N	Y
R11	Y	Y	Y

(B)					
Pt #	**LTT +**	**Cytof + for T4HR**	**Primary TKA–involved hypoallergenic implant**	**Clinical satisfactory result**	**Any revision surgery with hypoallergenic implant?**
P1	Y	N	Y	Y	N
P2	Y	N	Y	Y	N
P3	Y	N	Y	Y	N
P4	Y	N	Y	N	N
P5	N	Y	N	Y	N
P6	N/A	Y	Y	Y	N
P7	N/A	Y	Y	Y	N
P8	N/A	Y	Y	Y	N

LTT, lymphocyte transformation test; T4HR, type IV hypersensitivity reaction; TKA, total knee arthroplasty.

In addition, among primary TKA, there was not a predominance of M1 or Th2 cells among the majority of patients. However, there was a predominance of Th2 cells in three patients (P1, P2, P3). Interestingly, these patients also had underlying medical conditions (i.e. type 2 diabetes, seasonal allergies, hypothyroidism, asthma), suggesting an over-active immune response. Moreover, all revision TKAs had metabolic syndrome, which is a pro-inflammatory state. This suggests that a patient's underlying medical conditions may assist in determining which patients are at a higher risk of a T4HR.

This study showed that there is a predominance of many inflammatory cells following a primary TKA (Figure 3). This suggests that the reaction that occurs between a metal implant and bone is more complex than simply a T4HR reaction. Although a T4HR occurs, there seems to be other

inflammatory reactions that occur simultaneously. Additional studies are needed to further delineate the specific inflammatory reactions that occur when bone interacts with foreign metal to develop a way to predict, prevent, or decrease this reaction.

In addition, when evaluating the relative prevalence of inflammatory cells present, the clinical picture and clinical laboratory results were compared. For patients who required a revision TKA, all patients had a normal C-Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR) value, and only two patients had elevated synovial fluid counts (Table 3). This demonstrates that infection was ruled out and primary TKA failure was due to some other cause.

Moreover, we also evaluated the fibrosis in the synovium to determine whether the fibrotic environment was different between revision TKA cases and primary TKA. The presence of fibroblasts (α-SMA positive) indicates the formation of a different fibrotic reaction toward the metal implant than the inflammatory reaction expected in osteoarthritis. This further suggests that the reaction to metal implants is complex and involves many different inflammatory-related cells and mechanisms.

Among revision TKA, we sought to determine whether the LTT and patch test adequately identified patients who displayed a T4HR during their revision case. Within our sample, we found a high discordance between LTT results and Cytof analysis. We found that the LTT and patch test did not capture four (44%) of nine patients; when examining only LTT results, four (50%) of eight patients had a negative result that did not match with Cytof analysis. However, Cytof staining is currently impossible to implement clinically. This suggests that the current standard of care for metal allergy testing (LTT) prior to TKA might be not accurate to describe a local inflammatory reaction. This may be because the hypersensitivity response that occurs systemically or cutaneously is different than the inflammatory response that occurs within the knee joint. The mechanism of antigen presentation within the joint space is unknown, and it is debated whether the response within the joint is the same as the systemic response. Moreover, LTT testing appears best suited for work-up of painful TKA with no discernible cause of failure. Prior to primary TKA, questioning patients about metal hypersensitivity (i.e. jewelry) appears to also be a good strategy to predict whether a non-metal alloy implant is warranted.

A more sensitive screening test or the use of multiple screening tests prior to TKA using different tissue as a probe could more accurately predict patients who need a non-metal alloy implant.[3] Non-metal alloy implants are often not used unless an LTT patch test is positive in a primary TKA due to the high costs associated with the implant. However, our data suggest that non-metal alloy implants may be warranted in a larger proportion of patients undergoing TKA than previously thought. This would avoid the costs associated with revision TKA cases and prevent the use of expensive hypoallergic implants in patients who do not display a T4HR. It may be that Cytof staining is too sensitive and will inappropriately capture patients who demonstrate a T4HR preoperatively, but then fail to display signs and symptoms of primary TKA failure clinically. Although it may be argued that Cytof staining has too high specificity, the debate remains whether the costs and patient morbidity associated with revision TKA if only LTT results are used are higher compared with potentially inappropriately utilizing non-metal alloy implants if different preoperative tests were to be implemented. Moreover, although we believe that Cytof staining is a strong technique to detect the inflammatory local infiltrate, it might be logistically challenging to readily implement this technique clinically.

This study has some limitations. The clinical work-up after a primary TKA failure occurred because it was believed by the surgeon that the cause of failure was metal hypersensitivity. Work-up included testing for infection, postoperative X-rays, and a clinical examination. Although it is unknown whether a well-functioning implant creates a mild reaction within the bone that would mimic a T4HR, it is not possible to obtain bone samples from a well-functioning TKA except post-mortem. However, our results from Cytof demonstrate a significantly higher prevalence of Th2 T-cells and M1 macrophages. In addition, it could be argued that there was a hypersensitivity reaction to the cement, causing the primary TKA failure, rather than the implant. However, among the revision TKA surgeries, when a non-metal alloy implant was used in the revision surgery, clinically satisfactory results were obtained, suggesting that the implant was the cause of primary failure rather than the

cement. In addition, all revision TKA patients have metabolic syndrome, which is a pro-inflammatory state. Although this may have contributed to some of the inflammatory cells within the specimens evaluated by Cytof, it is unlikely that the relative prevalence of inflammatory cells (Th1 *versus* Th2, M1 *versus* M2) was affected by this medical history. Finally, there were two patients who had MUA, which may have resulted in arthrofibrosis, skewing the inflammatory cells present detected by Cytof. However, as MUA was rare, it is unlikely that the overall findings of this study would have changed.

In conclusion, this study suggests that primary TKA failure could be due to a T4HR (possibly due to metal presence), demonstrated by the presence of Th2 and M1 inflammatory cells present within the joint space following implant removal. This study uses Cyof to examine inflammatory cell populations in bone. In addition, we are able to demonstrate that screening tests currently utilized prior to TKA may lack sensitivity and may miss a part of patients who exhibit a T4HR following primary TKA (44% in this study). This study should raise awareness that metal hypersensitivity to implants could exist, and metal hypersensitivity should be considered prior to primary TKA. If any concern does exist, hypoallergic implants should be considered. Finally, it appears that a patient's medical history of cutaneous metal hypersensitivity provides additional information when considered with results gained from LTT and patch testing results. Thus, LTT and patch testing results may have limited clinical use when determining whether a patient needs a non-metal alloy implant, and the use of multiple screening tests should be evaluated together to provide a more global picture of test results.

Acknowledgements

The authors thank Shu-Hsia Chen, PhD, and the Immunomonitoring Core at Houston Methodist Research Institute, Houston, TX. They thank Thomas Sullivan for his assistance in the coordination of this project and David Lionberger for contributing to the collection of patient samples.

Author contribution(s)

Ava Brozovich: Conceptualization; Data curation; Formal analysis; Investigation; Writing – original draft.

Terry Clyburn: Conceptualization; Methodology; Writing – review & editing.

Kevin Park: Conceptualization; Data curation; Methodology; Supervision.

Katharine D. Harper: Data curation; Investigation.

Thomas Sullivan: Methodology.

Stephen Incavo: Conceptualization; Supervision; Writing – original draft; Writing – review & editing.

Francesca Taraballi: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Resources; Writing – original draft; Writing – review & editing.

Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Internally funded.

ORCID iD

Francesca Taraballi https://orcid.org/0000-0002-4959-1169

References

1. Inacio MCS, Paxton EW, Graves SE, *et al.* Projected increase in total knee arthroplasty in the United States – an alternative projection model. *Osteoarthritis Cartilage* 2017; 25: 1797–1803.
2. Lavernia CJ, Guzman JF and Gachupin-Garcia A. Cost effectiveness and quality of life in knee arthroplasty. *Clin Orthop Relat Res* 1997; 345: 134–139.
3. Hallab N, Merritt K and Jacobs JJ. Metal sensitivity in patients with orthopaedic implants. *J Bone Joint Surg Am* 2001; 83: 428–436.
4. Patel A, Pavlou G, Mújica-Mota RE, *et al.* The epidemiology of revision total knee and hip arthroplasty in England and Wales: a comparative analysis with projections for the United States. A study using the National Joint Registry Dataset. *Bone Joint J* 2015; 97-B: 1076–1081.
5. Caicedo MS, Solver E, Coleman L, *et al.* Females with unexplained joint pain following total joint arthroplasty exhibit a higher rate and severity of

hypersensitivity to implant metals compared with males: implications of sex-based bioreactivity differences. *J Bone Joint Surg Am* 2017; 99: 621–628.

6. Scott CE, Howie CR, MacDonald D, *et al.* Predicting dissatisfaction following total knee replacement: a prospective study of 1217 patients. *J Bone Joint Surg Br* 2010; 92: 1253–1258.
7. Wylde V, Hewlett S, Learmonth ID, *et al.* Persistent pain after joint replacement: prevalence, sensory qualities, and postoperative determinants. *Pain* 2011; 152: 566–572.
8. Beswick AD, Wylde V, Gooberman-Hill R, *et al.* What proportion of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. *BMJ Open* 2012; 2: e000435.
9. Granchi D, Cenni E, Giunti A, *et al.* Metal hypersensitivity testing in patients undergoing joint replacement: a systematic review. *J Bone Joint Surg Br* 2012; 94: 1126–1134.
10. Eftekhary N, Shepard N, Wiznia D, *et al.* Metal hypersensitivity in total joint arthroplasty. *JBJS Rev* 2018; 6: e1.
11. Saccomanno MF, Sircana G, Masci G, *et al.* Allergy in total knee replacement surgery: is it a real problem? *World J Orthop* 2019; 10: 63–70.
12. Carulli C, Villano M, Bucciarelli G, *et al.* Painful knee arthroplasty: definition and overview. *Clin Cases Miner Bone Metab* 2011; 8: 23–25.
13. Innocenti M, Carulli C, Matassi F, *et al.* Total knee arthroplasty in patients with hypersensitivity to metals. *Int Orthop* 2014; 38: 329–333.
14. Innocenti M, Matassi F, Carulli C, *et al.* Oxidized zirconium femoral component for TKA: a follow-up note of a previous report at a minimum of 10 years. *Knee* 2014; 21: 858–861.
15. Carossino AM, Carulli C, Ciuffi S, *et al.* Hypersensitivity reactions to metal implants: laboratory options. *BMC Musculoskelet Disord* 2016; 17: 486.
16. Innocenti M, Vieri B, Melani T, *et al.* Metal hypersensitivity after knee arthroplasty: fact or fiction? *Acta Biomed* 2017; 88: 78–83.
17. Lavernia C, Lee DJ and Hernandez VH. The increasing financial burden of knee revision surgery in the United States. *Clin Orthop Relat Res* 2006; 446: 221–226.
18. Lachiewicz PF, Watters TS and Jacobs JJ. Metal hypersensitivity and total knee arthroplasty. *J Am Acad Orthop Surg* 2016; 24: 106–112.
19. Lazzarini R, Duarte I and Ferreira AL. Patch tests. *An Bras Dermatol* 2013; 88: 879–888.
20. Muthupalaniappen L and Jamil A. Prick, patch or blood test? A simple guide to allergy testing. *Malays Fam Physician* 2021; 16: 19–26.
21. Niki Y, Matsumoto H, Otani T, *et al.* Phenotypic characteristics of joint fluid cells from patients with continuous joint effusion after total knee arthroplasty. *Biomaterials* 2006; 27: 1558–1565.
22. Frisch N and Jacobs JJ. Hypersensitivity: 'doc, Am I allergic to my implant?' *Semin Arthroplasty* 2017; 28: 53–57.
23. Thomas P. Patch testing and hypersensitivity reactions to metallic implants: still many open questions. *Dermatitis* 2013; 24: 106–107.
24. Subrahmanyam PB and Maecker HT. CyTOF measurement of immunocompetence across major immune cell types. *Curr Protoc Cytom* 2017; 82: 9.54.1–9.54.12.
25. Schäfer T, Böhler E, Ruhdorfer S, *et al.* Epidemiology of contact allergy in adults. *Allergy* 2001; 56: 1192–1196.
26. Teo WZW and Schalock PC. Metal hypersensitivity reactions to orthopedic implants. *Dermatol Ther* 2017; 7: 53–64.