

Letter to the Editor

Development of a validated blood test for nickel sensitization

To the Editor:

Of the 1 million hip and knee replacements that are performed annually in the United States, 7.5% to 10% will fail and require revision (www.AAOS.org). As the number of implants is expected to exceed 2 million by 2015, the number of joint failures is also expected to rise. Immunologic sensitization to implant metals is one of the causes of implant failure,^{1,2} along with infection and biomechanical mismatch, and distinct from metallosis caused by metal wear particles.³ Metal sensitization has only recently been recognized as a cause of joint failure; the delay in recognition is in part due to the lack of convenient, standardized, and validated laboratory tests for sensitization. It is a delayed type IV hypersensitivity reaction, currently diagnosed by skin patch testing, that indicates that sensitized memory T cells have recognized the metal antigen-protein conjugate presented by dendritic cells in regional lymph nodes, proliferated, and then migrated to the source of the antigen. *In vitro* tests, such as the lymphocyte proliferation test (LPT), measure sensitized T-cell reactivity to the suspected antigen in culture. They are good surrogates for the skin reaction and are easier to administer at a distance without the need for multiple clinic visits. Although, in the United States, other metal LPTs are available to assess metal sensitization, none have been validated against the gold standard, patch testing. Recognizing the need for a diagnostic tool that is easier to implement, yet provides the same information, we have developed and validated a blood LPT for nickel sensitization with good sensitivity and excellent specificity that we present here.

Patients referred by local orthopedists for evaluation for possible nickel sensitization were enrolled sequentially. Controls were volunteers without a history of joint replacement. Following informed consent, demographic information and medical history were collected by questionnaire, and all subjects provided 30 mL of blood, which was collected in heparin tubes. PBMCs were separated from whole blood within 24 to 30 hours of collection by using ficoll-hypaque density gradient centrifugation.⁴ PBMCs from all controls and 31 patients were cultured with 8 concentrations of NiSO₄ (Fisher 73-100, Ft Lawn, NJ)—0.1, 0.5, 1, 2.5, 5, 10, 20, and 50 mcg/mL—and sampled at day 4 and day 6. We established optimal assay conditions on the basis of the peak stimulation index (PSI) in the 16 controls without a history of nickel allergy, and the first 31 consecutive patients, and then applied these conditions to the remaining 45 patients. The threshold for positive responses was based on the mean of the PSIs from the nonsensitized control population, plus 3 SDs. Because nickel is known to be nonspecifically stimulatory at higher concentrations,⁵ we required stimulation indices above this threshold in at least 2 culture conditions to be considered positive. Patch testing was performed according to NACDRG⁶ by using standard concentrations of 2.5% and 5% nickel sulfate hexahydrate (AllergEAZE by SmartPractice, Calgary, Alberta, Canada). Sites were scored and photographed at 48 hours, and 72 or 96 hours. We conducted summary statistics and sensitivity/specificity analysis by using JMP (SAS Institute, Inc, Cary, NC) and compared groups by using a 2-tailed paired *t* test, with significance set at *P* less than .05.

TABLE I. Demographics of patient and control populations

Variable	Patient	Control	<i>P</i> value
Age (y), mean (range)	58.6 (19-84)	41.5 (23-63)	<.0001
Sex, n (%)			
Male	28 (37)	2 (11)	
Female	48 (63)	17 (89)	.03
Prosthesis, n (%)			
Knee	42 (55)		
Hip	12 (16)		
Other	6 (8)		
Preoperative evaluation, n (%)	16 (21)		
Totals	76	19	Total = 95

Note. Results of the NiLPT dichotomized as positive (2 or more conditions >3 SD above the normal mean) or negative were compared with patch test results for nickel, and sensitivity, specificity, and positive and negative predictive values were derived. NPV, Negative predictive value; PPV, positive predictive value.

Patients were significantly older and included a higher percentage of men than did the control population (Table I). Most patients were referred for allergy evaluation for a failed joint replacement, most frequently the knee; 20% were referred for an initial preoperative evaluation. We found that 50 mcg/mL of NiSO₄ proved toxic, while the majority of PSI values occurred between 0.1 and 10 mcg/mL for both patients' and controls' cells (Fig 1). Three log dilutions of nickel sulfate—0.1 mcg/mL, 1 mcg/mL, and 10 mcg/mL—were ultimately chosen as optimal to detect positive responses. Under these conditions, the mean PSI for cells from controls without nickel allergy was 2.24 ± 1.17 (1.15-4.67). The threshold for positive was thus set at 5.7 (mean + 3 SD). Applying this threshold to patients' results, those with negative patch test results demonstrated a mean PSI of 3.19 (0.91-19.1), not significantly different from controls. In contrast, the mean nickel LPT (NiLPT) PSI was 21.5 (0.84-73.8) in patients with a positive nickel patch test result, significantly different from controls and from patients with a negative patch test result (*P* < .0001). By using these optimized conditions and threshold, we then analyzed the sensitivity and specificity of the NiLPT against patch testing (Table II). Overall, 19 of 76 patients (25%) had a positive patch test result to nickel, of whom 13 also had a positive NiLPT result, for a sensitivity of 68%. All but 1 patient were concordant for a negative patch test and NiLPT result, for a specificity of 98%.

The blood-based proliferation assay we propose has several advantages. It requires a single blood draw, eliminating multiple patient visits. Blood can be drawn remotely and shipped to the laboratory for testing. And it may provide objective results that are perhaps more reflective of the *in vivo* cellular interaction. Other studies have demonstrated proliferative responses to metals *in vitro*, but have not as rigorously established culture conditions, or set a positive threshold based on normal controls. Taking into account that nickel is known to be immunostimulatory at high concentrations,⁵ we set our positive threshold at 5.7, compared with other published cutoffs of 2 or 3, which may identify more false-positive reactions.

Although whether the patch test and the LPT measure the same immune responses has been debated, numerous examples indicate that immune responses in the target organ may be accurately measured in another site, for example, tuberculosis testing by

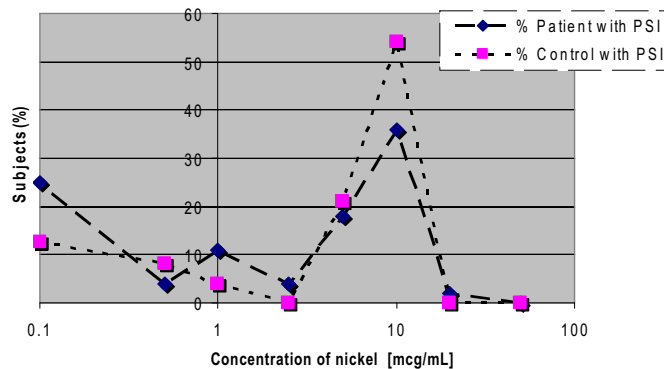


FIG 1. Distribution of PSI by nickel concentration. PBMCs were incubated with increasing concentrations of NiSO_4 , to which ^3H -thymidine was added at 4 or 6 days. Counts incorporated with each concentration of nickel were compared with background counts to determine a stimulation index. The percentage of patients (-♦-) and controls (-■-) with their highest stimulation index (PSI) for each concentration of nickel are shown on the y-axis.

TABLE II. Sensitivity and specificity of the NiLPT versus patch testing

NiLPT	Nickel patch test			Predictive value
	Positive	Negative	Total	
Abnormal	13	1	14	PPV = 93%
Normal	6	56	62	NPV = 90%
Totals	19	57	76	
Sensitivity = 68% Specificity = 98%				

purified protein derivative skin testing or by the QuantiFERON-TB Gold assay in blood. The TCR-V repertoire is the same in blood- and skin-derived T-cell clones specific for nickel,^{7,8} as shown for beryllium-specific T-cell clones in blood and lung.⁹ Thus, T-cell responses in blood and on the skin plausibly reflect the sensitization that occurred in the failed joint.

Comparison to patch test results greatly strengthens the validity of our test. We used the same nickel salts in both patch and *in vitro* testing, allowing us to make direct comparisons between the 2 methods. Our overall concordance between the 2 methods was 68%, but this may be affected by the exposure, as those without a current implant were more likely to be discordant between the patch test and the NiLPT (57% or 4 of 7), whereas only 19% (13 of 68) of those with an implant already in place were discordant. Viewed another way, for patients with a current joint replacement, and hence tissue and systemic exposure to nickel, the NiLPT performed with a sensitivity of 78% ($P < .0001$). In patients with a remote history of metal reactions, but without current systemic exposure to nickel, the sensitivity of the NiLPT was 60%, although specificity remained high ($P = .03$). It is possible that current, systemic exposure to nickel enhances the sensitivity of the NiLPT

by eliciting more circulating nickel-responsive T cells that also react to nickel *in vitro*. Metal sensitization may also be induced by the implant, and prospective studies are needed to understand the conditions favoring this development, and its implications for joint failure.

In summary, we have developed a blood test specific for nickel sensitization with a robust assay threshold based on nonsensitized controls, and clinically validated by patch testing. It is a useful addition to help further establish the role of metal sensitization in joint implant failure.

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REFERENCES

1. Thomas P, Braathen LR, Dorig M, Aubock J, Nestle F, Werfel T, et al. Increased metal allergy in patients with failed metal-on-metal hip arthroplasty and peri-implant T-lymphocytic inflammation. *Allergy* 2009;64:1157-65.
2. Hallab N, Merritt K, Jacobs JJ. Metal sensitivity in patients with orthopaedic implants. *J Bone Joint Surg Am* 2001;83-A:428-36.
3. Andrews RE, Shah KM, Wilkinson JM, Gartland A. Effects of cobalt and chromium ions at clinically equivalent concentrations after metal-on-metal hip replacement on human osteoblasts and osteoclasts: implications for skeletal health. *Bone* 2011;49:717-23.
4. Mroz MM, Kreiss K, Lezotte DC, Campbell PA, Newman LS. Reexamination of the blood lymphocyte transformation test in the diagnosis of chronic beryllium disease. *J Allergy Clin Immunol* 1991;88:54-60.
5. Cederbrant K, Anderson C, Andersson T, Marcusson-Stahl M, Hultman P. Cytokine production, lymphocyte proliferation and T-cell receptor Vbeta expression in primary peripheral blood mononuclear cell cultures from nickel-allergic individuals. *Int Arch Allergy Immunol* 2003;132:373-9.
6. Fransway AF, Zug KA, Belsito DV, Deleo VA, Fowler JF Jr, Maibach HI, et al. North American Contact Dermatitis Group patch test results for 2007-2008. *Dermatitis* 2013;(24):10-21.
7. Budinger L, Neuser N, Totzke U, Merk HF, Hertl M. Preferential usage of TCR-Vbeta17 by peripheral and cutaneous T cells in nickel-induced contact dermatitis. *J Immunol* 2001;167:6038-44.
8. Werfel T, Hentschel M, Kapp A, Renz H. Dichotomy of blood- and skin-derived IL-4-producing allergen-specific T cells and restricted V beta repertoire in nickel-mediated contact dermatitis. *J Immunol* 1997;158:2500-5.
9. Fontenot AP, Maier LA, Canavera SJ, Hendry-Hofer TB, Boguniewicz M, Barker EA, et al. Beryllium skin patch testing to analyze T cell stimulation and granulomatous inflammation in the lung. *J Immunol* 2002;168:3627-34.

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