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Delayed-type Hypersensitivity to Metals in Newly Diagnosed Patients with Nonischemic Dilated Cardiomyopathy

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Abstract

The causes of nonischemic dilated cardiomyopathy are classified as genetic or nongenetic, but environmental factors such as metal pollutants may interact with genetic susceptibility. The presence of metal particles has been detected in the myocardium, including in those patients with dilated cardiomyopathy. It is also known that hypersensitivity reactions can induce inflammation in tissue. The present study aimed to verify if metal-induced delayed-type hypersensitivity is present in patients with nonischemic dilated cardiomyopathy. The patient group consisted of 30 patients with newly diagnosed dilated cardiomyopathy; the control group comprised 41 healthy subjects. All patients and control subjects provided blood samples for lymphocyte transformation testing (MELISA®) to assess possible hypersensitivity to seven common metals. Specific exposure to metals was based on interview data. Results showed that exposure to cadmium and lead ($p=0.0002$), aluminum ($p=0.0006$), nickel ($p=0.0012$), and chromium ($p=0.0065$) was more often reported by patients than controls. The patients also had significantly more frequent hypersensitivity reactions to mercury (26.7% vs. 7.3%, $p=0.014624$), nickel (40% vs. 12.2%, $p=0.02341$), and silver (20% vs. 4.8%, $p=0.025468$) than the control group. Patients with dilated cardiomyopathy had greater exposure to certain metals compared with healthy controls. Hypersensitivity to metals was more frequent in patients with dilated cardiomyopathy, suggesting a possible association that warrants further investigation.

Keywords Dilated cardiomyopathy · Metal exposure · Delayed-type hypersensitivity · Lymphocyte transformation test · Inflammation

Introduction

Nonischemic dilated cardiomyopathy, which is a major cause of heart failure, is a clinical diagnosis characterized by dilatation and impaired contraction of the left or both

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ventricles, which is not due to abnormal loading or coronary artery disease [1]. The causes are either genetic or nongenetic, and interaction with environmental factors, e.g., metal pollutants, could play a certain role [2].

It is known that particles of metal pollutants can be found in the myocardium of the heart ventricles [3] and that their biological effects may result in myocardial damage [4, 5]. Some studies showed that patients with dilated cardiomyopathy had high concentrations of mercury and antimony in their myocardium [6–8]. In a large group of total hip arthroplasty patients, it was shown that individuals with a metal head prosthesis were subsequently more frequently diagnosed with dilated cardiomyopathy or heart failure [9]. These data suggest that metal exposure through medical devices could have a biological effect on the myocardium. On the other hand, a number of studies have indicated that immune reactions, particularly delayed-type hypersensitivity, may be involved in the development of myocardial damage.

The term delayed-type hypersensitivity refers to the type IV immunopathological response, in which specific T-lymphocytes are stimulated in susceptible individuals. It targets intracellular pathogens (i.e., viruses, *Borrelia, Chlamydia*) and tumor cells under physiological conditions. These immunopathological (inflammatory) pathways are genetically determined. Patients with hypersensitivity to dental alloys, containing metals such as mercury, have significantly more frequent HLA-B37, B47, and DR4 antigens in HLA genotyping [10]. In the general population, these hypersensitive reactions are more common in women [11, 12] and are most often induced by chronic exposure to low concentrations of an antigen [13, 14]. Autoimmune systemic diseases, such as rheumatoid arthritis and lupus erythematosus, have also been associated with hypersensitivity to metals that can cause inflammation in tissue, including the myocardium [15, 16]. During chronic inflammatory processes, the activated T-lymphocytes interact with other cellular elements of the immune system [17].

Delayed-type hypersensitivity can be assessed using a lymphocyte transformation test, which is based on the principle of allergen/antigen-specific mediated response of a memory lymphocyte following contact with the corresponding antigen. A positive lymphocyte transformation test response proves the presence of antigen-specific T cells in peripheral blood [18, 19].

The aim of the present study was to determine if newly diagnosed nonischemic dilated cardiomyopathy patients have more frequent hypersensitivity reactions to metals than healthy individuals.

Materials and Methods

The Ethics Committee at the Brno University Hospital approved the protocol of the study. All participants (patients and controls) signed informed consent before their participation.

Participants

The participants in the patient and the control groups were age- and sex-matched. The primary characteristics of both study groups are displayed in Table 1.

The patient group included 30 subjects (23 males) with a mean age of 51.0 ± 12.4 years presenting with new heart failure signs and symptoms and/or who had echocardiographically confirmed left ventricular systolic dysfunction with left ventricular ejection fraction (LVEF) < 50%. Patients with coronary artery disease, significant valvular disease, tachycardia-induced left ventricular dysfunction, cardiac stimulation, toxic myocardial damage caused by alcohol or chemotherapy, decompensated severe endocrine disorders, or known hypersensitivity to drugs were excluded. Patients with acute myocarditis, including specific forms (i.e., giant cell myocarditis or eosinophilic myocarditis), were also excluded, according to the previously reported protocols [20].

All subjects were tested within three months of their diagnosis, and all were clinically stable (functional class NYHA II or III) and following optimal medical treatment protocols for heart failure. Patients and controls were not taking immunosuppressive medication since this would interfere with the measurement of hypersensitivity reactions. Four patients showed concomitant hypothyroidism. This could not have had any influence on the manifestation of heart failure as three patients were well controlled on hormone replacement therapy, and one patient had a subclinical form of the disease not requiring medication. Within three months of the heart failure diagnosis, seven patients underwent implantation of a cardioverter-defibrillator (four were primary preventative indications).

Diagnostic endomyocardial biopsy was performed in 22 (73.3%) subjects, depending on the clinical manifestation and severity of the heart failure symptoms. This procedure was performed under echocardiographic and fluoroscopic guidance according to a standard operating protocol [21]. Tissue samples were histologically and immunohistochemically evaluated, and the presence of inflammation was assessed.

The control group included 41 healthy volunteers (28 males) with a mean age of 51.7 ± 13.6 years. These individuals were hospital personnel, their family members, or

acquaintances who were randomly requested to participate in the study. None of them presented with any signs or symptoms associated with cardiovascular dysfunction or impairment or had recently taken any medication. A certain number of control subjects showed fatigue and palpitation—this was later confirmed to be connected to their age and to anxiety induced by participation in a research study.

Table 1 Basic characteristics and comorbidities of patients and healthy control group

Characteristics[a]	Patients ($N=30$)	Control group ($N=41$)	p value
Sex (male)	23 (76.6%)	28 (68.3%)	0.5943
Age (years)	51.0 ± 12.4	51.7 ± 13.6	0.8650
BMI (kg/m^2)	26.9 ± 4.2	27.8 ± 3.6	0.6540
LVEF (%)	26.5 ± 8.2	62.9 ± 4.6	**< 0.00001**
LVEF < 40%	29 (96.7%)	0 (0%)	**< 0.0001**
NYHA class			
NYHA II	17 (56.7%)	0 (0%)	**< 0.0001**
NYHA III	13 (43.4%)	0 (0%)	**< 0.0001**
Heart failure symptoms duration (months)	2.2 ± 1.8	NA	
Dominating (heart failure) symptoms			
Cardiopulmonary resuscitation	2 (6.7%)	0 (0%)	0.0710
Ventricular fibrillation/tachycardia	3 (10.0%)	0 (0%)	0.0710
Peripheral edema	7 (23.3%)	0 (0%)	**0.0015**
Palpitations	10 (33.3%)	4 (9.7%)	**0.0177**
Chest pain	12 (40.0%)	0 (0%)	**< 0.0001**
Dyspnea	23 (76.6%)	0 (0%)	**< 0.0001**
Fatigue	27 (90.0%)	5 (12.1%)	**< 0.0001**
Laboratory findings			
Troponine T (pg/l)	20 ± 16	5 ± 3	**< 0.00001**
NTproBNP (ng/l)	1504 ± 1549	171 ± 98 (*)	**< 0.00001**
CRP (mg/l)	17 ± 35	2 ± 1	**0.0069**
Endomyocardial biopsy	22 (73.3%)	Not done	
Verified myocardial inflammation	8 (26.7%)	Not done	
PCR positive for cardiotropic virus	5 (16.6%)	Not done	
Comorbidities			
Hypertension	13 (43.4%)	0 (0%)	**< 0.0001**
Contact dermatitis/atopy	7 (23.3%)	3 (7.3%)	0.0839
Dyslipidemia	5 (16.6%)	2 (4.9%)	0.1246
Other allergies	5 (16.6%)	2 (4.9%)	0.1246
Thyreopathy	4 (13.4%)	0 (0%)	**0.0282**
Depression/anxiety	3 (10%)	0 (0%)	0.0710
Hyperuricemia	3 (10%)	0 (0%)	0.0710
Vertebralgia	3 (10%)	2 (4.9%)	0.6438
Atrial fibrillation	2 (6.7%)	0 (0%)	0.1751
Diabetes mellitus	2 (6.7%)	0 (0%)	0.1751
Bronchial asthma	2 (6.7%)	0 (0%)	0.1751

BMI body mass index, *CRP* C-reactive protein, *LVEF* left ventricular ejection fraction, *NTproBNP* N-terminal pro-B-type natriuretic peptide, *NYHA* New York Heart Association classification of dyspnea, *PCR* polymerase chain reaction (*) below 300 ng/L

[a]All study subjects were exposed to car exhaust, particulate matters (PM_{10}), smog, urban pollution, and food additives, all commonly present in urban life. None of the controls were occupationally exposed to the tested metals, or had undergone cardiac surgery or other surgical interventions

Delayed-type Hypersensitivity Testing by Lymphocyte Transformation Test

Seven metals for hypersensitivity testing were selected based on subjects' answers captured in a specially designed Questionnaire on Metal Environmental Burden. The interview data were also used to compare metal exposure between patients and control subjects.

MELISA, a modified in vitro lymphocyte transformation test, was performed according to the standardized methodology [18, 22, 23]. Lymphocytes were isolated from anticoagulated blood on Ficoll gradient, washed and resuspended in medium containing 20% pooled, heat-inactivated human serum. Partial depletion of monocytes was achieved by incubation of cells at 37 °C with 5% CO_2 in plastic flasks. Lymphocytes were cultured with previously defined metal solutions in two to three concentrations, for five days at 37 °C with 5% CO_2. Three negative controls (lymphocytes only in 10% medium) and one positive control (Pokeweed Mitogen) were included in each test. After 5 days, the cells were pulsed for four hours with 3^H-thymidine. Cells were harvested onto filter paper, the filter paper dried in a microwave oven, and the radioactivity was measured in a liquid scintillation counter.

A positive reaction was defined as a Stimulation Index (SI) ≥ 3, where SI = cpm (counts per minute) in a test well/average cpm in negative control wells. A SI between two and three was interpreted as "weakly positive," and SI < 2 was considered negative. Cells from the five-day cultures were additionally analyzed morphologically after staining with cytospin preparations. Only tests in which the radioactively counted positive results showed a presence of lymphoblasts were evaluated as positive. Laboratory personnel were blinded to the subject groups.

Statistics

Statistics was performed analyzing data as mean ± standard deviation. Stimulation indices of hypersensitivity reactivity were described by the mean ± standard deviation, median, minimal and maximal value, and by 25th and 75th percentile. To compare the statistical significance of the continuous variables between the patients and control individuals, a Wilcoxon test was used, once a nonparametric distribution was assessed via a Kolmogorov–Smirnov test and a Shapiro–Wilk test. Confounders were analyzed according to previously published reports [24]. Moreover, Kendall tau multivariate rank test was performed to evaluate the best correlations between patients and controls regarding the different metal-induced delayed-type hypersensitivity and possible interactions due to Type 1 errors and outliers. These were tested via an Iglewicz and Hoaglin's robust test for multiple outliers (two-sided test), having an outline criterion with a modified z score ≥ 3.5. The statistical significance of the categorical data was compared by Fisher's Exact Test. The level of significance was set at $\alpha = 0.05$ ($p < 0.05$) for all analyses.

Results

Cardiovascular Conditions

Patients with newly diagnosed nonischemic dilated cardiomyopathy had a much lower left ventricular ejection fraction (26.5 ± 8.2% and 62.9 ± 4.6%, respectively; $p < 0.00001$); 96.7% of patients had LVEF less than 40%. The most frequent symptoms of heart failure were fatigue (90.0%) and exertional dyspnea (76.6%). Some control subjects showed anxiety and fatigue without any cardiovascular signs and symptoms, diagnosed as age-related muscular fatigue. Patients also had significantly higher levels of some laboratory markers such as troponin T ($p < 0.00001$), CRP ($p < 0.00001$), and NTproBNP ($p = 0.0069$). Despite having dilated cardiomyopathy, the patient group had very few other comorbidities. The most common comorbidity was hypertension (43.4% and 0%, respectively; $p < 0.0001$). Of note, contact dermatitis/atopy and other allergies were found in 23.3% and 16.6% of the patients with heart failure but only in 7.3% and 4.9% of the healthy individuals, respectively ($p = 0.0839$ and $p = 0.1246$, respectively).

Delayed-type Hypersensitivity to Metals Evaluated by MELISA

A comparison of metal exposure in the study patients and the control subjects is shown in Table 2. The patients were significantly more often exposed to aluminum, cadmium, chromium, lead, and nickel. They were also significantly more likely to be smokers and significantly more often engaged in occupations with high exposure to metals.

The prevalence of the patient group's hypersensitivity reactions to mercury, nickel, and silver is statistically significant. Additionally, variation between patients and controls could be seen when looking at reactions to individual metals. Hypersensitivity (SI ≥ 3) to at least one tested metal was found in 17 patients and 11 controls (56.6% vs. 26.8%, $p = 0.0146$). A positive response to only one metal was detected in ten (33.3%) patients and eleven (26.8%) controls, reactivity to two and three metals in three (10%), and four (13.3%) patients but in no controls. Thirteen patients (43.3%) and thirty (73.2%) controls did not respond to any of the tested metal salts. The SI values of the hypersensitivity reactions to the metals tested are shown in Table 3. Significant differences were observed in three metals: silver

Table 2 Comparison of metal exposure in study patients and a healthy control group

Metal	Source	Patients ($N=30$)	Control group ($N=41$)	p
Silver (Ag)	Dental alloys	28 (93.3%)	41 (100%)	0.1751
Aluminum (Al)	Dental alloys, implanted devices, cosmetics, glass ionomer cement	21 (70%)	11 (26.6%)	**0.0006**
Gold (Au)	Dental alloys	6 (30%)	7 (17.1%)	0.7657
Cadmium (Cd)	Dental alloys	19 (63.3%)	8 (19.5%)	**0.0002**
Cobalt (Co)	Dental alloys	11 (36.6%)	13 (31.7%)	0.8002
Copper (Cu)	Dental alloys	28 (93.3%)	41 (100%)	0.1751
Chromium (Cr)	Cigarette smoke	24 (80%)	19 (46.3%)	**0.0065**
Mercury (Hg)	Dental alloys	28 (93.3%)	41 (100%)	0.1751
Nickel (Ni)	Dental alloys, food	25 (83.3%)	18 (43.9%)	**0.0012**
Lead (Pb)	Cigarette smoke	19 (63.3%)	8 (19.5%)	**0.0002**
Tin (Sn)	Dental alloys	28 (93.3%)	41 (100%)	0.1751
Titanium (Ti)	Dental alloys, implanted devices, cosmetics and toothpaste	30 (100%)	41 (100%)	0.9999
Occupational metal exposure		7 (23.3%)	2 (4.9%)	**0.0305**
Smoker/ex-smoker		19 (63.3%)	8 (19.5%)	**0.0002**

Metal exposure was assessed by studying the composition of the different dental alloys commonly used. Amalgam alloy: Ag, Cu, Hg, and Sn; metal-bound ceramics, dental crowns or bridges, stainless steel: Ni, Co, Cr, and Cd; Ti alloys in dental prostheses, cardiac pacemakers/defibrillators: Ti, Al; glass ionomer cement: Al; cigarette smoke: Al, Cd, Cr, Cu, Ni, and Pb; toothpastes, medicines, and cosmetics: Ti

(patients = 2.26 ± 0.99 SD and controls = 1.26 ± 0.55 SD, $p = 0.025468$), nickel (patients = 2.45 ± 1.43 SD and controls = 1.90 ± 0.86 SD, $p = 0.02341$), and inorganic mercury (patients = 3.68 ± 0.31 SD and controls 1.49 ± 0.53 SD, $p = 0.014624$). In both groups, the most frequent reactions were hypersensitivity to nickel, inorganic mercury, and silver. To better represent the correlation between dilated cardiomyopathy with delayed-type hypersensitivity to metals, a Kendall tau test was performed for each metal, with the exclusion of tin, as some data are biased (Table 4). Results show that outliers may distort the evaluation, which is why they are excluded from the Wilcoxon evaluation reported in Table 3. Although Kendall tau results would show an unexpected correlation for copper, this result is a consequence of statistical interactions, while only those correlations with silver, mercury, and nickel showed the best performance in p values. This may depend on sample distribution and stratification, as data in Table 4 were plotted without considering the bias effect of outliers. In this regard, interaction effects were observed between controls in the cobalt and silver groups. Outliers, due to a sample size effect, biased the distribution of copper groups in the Pearson evaluation (copper vs. chromium and copper vs. silver) and for mercury/nickel in the Spearman rank correlation. A meta-analysis on the Type 1 error distribution showed that this kind of error increased only with a nonparametric distribution, as expected, so assessing the quality of the Kendall tau evaluation.

Discussion

This pilot study compared the presence of delayed-type hypersensitivity to seven metals, frequently occurring in the environment (chromium, cobalt, copper, mercury, nickel, silver, and tin), in patients with newly diagnosed nonischemic dilated cardiomyopathy and healthy control subjects. A significant limitation of the present study is the relatively small sample size of recruited patients (although within the statistical limits of sample size) and, in particular, that metal exposure was only assessed through interview. A further limitation is the omission of other medical histories/risk factors/exposures in matching the patient and control groups. However, the present preliminary study outlines an association between metal hypersensitivity and patients with dilated cardiomyopathy.

Sources of metal exposure included, but were not limited to, tobacco smoke [25], metal alloys used in various implants and dental restoration materials [26–31], frequently used products [32], and occupations such as metalworking, casting, or welding. In genetically predisposed individuals, delayed-type hypersensitivity to these metals may be the cause of inflammation in tissue [10, 15, 33].

Patients with nonischemic dilated cardiomyopathy were significantly more frequently smokers and more often worked in the metals industry, and thus they were significantly more often exposed to aluminum, cadmium, chromium, lead, and nickel than the healthy controls. Despite

Table 3 Hypersensitivity reactivity to metals in study patients and healthy control group described as Stimulation Index (SI) values

Metal	Patients ($N=30$)		Control group ($N=41$)		Mean ± SD		Statistic test	p
					Patients	Controls		
	SI:median (25th; 75th percentile)	SI minimum–maximum	SI:median (25th; 75th percentile)	SI Minimum–maximum				
Silver	1.45 (1.07; 2.55)	0.75–13.00	1.10 (0.90; 1.62)	0.30–4.45	2.26 ± 0.99	1.26 ± 0.55	564.5	**0.025468**
Cobalt	1.15 (0.82; 1.37)	0.25–3.85	1.00 (0.80; 1.25)	0.25–2.90	1.18 ± 0.65	1.06 ± 0.46	461.5	0.52824
Chromium	1.05 (0.90; 1.55)	0.70–3.20	1.10 (0.85; 1.51)	0.50–2.55	1.28 ± 0.60	1.24 ± 0.55	424	0.96721
Copper	0.85 (0.70; 1.05)	0.50–2.45	0.90 (0.60; 1.06)	0.30–2.20	0.92 ± 0.40	0.93 ± 0.41	297.5	0.77784
Mercury	1.80 (1.23; 3.30)	0.90–27.50	1.40 (1.17; 1.62)	0.85–3.85	3.68 ± 0.31	1.49 ± 0.53	615.5	**0.014624**
Nickel*	2.12 (1.50; 4.02)	0.95–23.25	1.60 (1.07; 2.32)	0.50–4.10	2.45 ± 1.43	1.90 ± 0.86	704	**0.02341**
Tin	0.95 (0.67; 1.15)	0.30–1.90	1.00 (0.70; 1.30)	0.55–3.90	0.93 ± 0.40	1.21 ± 0.87	99	0.58057

*Four outliers excluded from the average

this, the frequent hypersensitivity to mercury, nickel, and silver suggests that delayed-type hypersensitivity is more often related to exposure to dental materials and everyday products than to occupational exposure. The variation in exposure might be of clinical relevance since smoking, for example, does not only increase mortality in patients with symptomatic atherosclerosis (ischemic heart disease, stroke, and peripheral artery disease) but also in patients who have idiopathic dilated cardiomyopathy without proven atherosclerosis. Moreover, the fatal effects of some metal toxins, for example, cobalt, have been described previously [29]. In general, patients with a newly diagnosed nonischemic dilated cardiomyopathy might represent cases of weaker (not fatal) toxicity to metal exposure. Nevertheless, in patients with dilated cardiomyopathy, identification of frequent exposure to metals using questionnaires or an interview should not be solely relied upon in clinical practice. It is recommended that concentrations of metals are measured in the blood or directly in the myocardium [3, 5–7].

In this study, both the patients and control subjects were most often positive to nickel (40% and 12.2%) in a delayed-type hypersensitivity test (MELISA). The clinical significance of these results in patients with cardiomyopathy is unclear, due to the high frequency of nickel allergy in both groups. In the general population, according to patch test results, the most common hypersensitivity is to nickel, with a prevalence of 17% to 31% in women and approximately 3% in males [11]. Various reasons may explain the difference in nickel allergy prevalence between women and men [34]. Women may more easily become sensitized to different allergens and have a stronger immune response than men. Women with pierced ears have higher levels of nickel allergy than those with unpierced [35]. In men, raised nickel sensitization is most commonly due to occupational exposure to nickel [34, 36].

More surprisingly, this study also detected delayed-type hypersensitivity to mercury and silver. As most of the enrolled subjects in this study had undergone dental treatment at some point, this could explain the immune responses to the metals, frequently used in dental alloys. Interestingly, SI ≥ 3.0 was mostly recorded in patients with dilated cardiomyopathy and not in healthy controls.

Patients with newly diagnosed nonischemic dilated cardiomyopathy had significantly more frequent hypersensitivity reactions to mercury, nickel, and silver than healthy subjects. According to the data obtained from the questionnaires, the main source of nickel in the patients was from dental nickel–cobalt–chromium restorations. Both inorganic mercury and silver are present in dental amalgam [37]. The number of subjects with at least one dental amalgam filling was comparable between the groups (93.3% and 100%, respectively). Why there is a significant difference in the occurrence of hypersensitivity to nickel,

Table 4 Kendall tau correlation matrix

Patients/controls	Pearson *r* (1)	Spearman rho (2)	Kendall *k* (3)	*p*
Mercury (Hg)	0.1169	0.6364	0.4628	p = 0.5394 **p < 0.0001** **p < 0.0001**
Copper (Cu)	0.0417	0.4141	0.2847	p = 0.8268 **p = 0.0229** **p = 0.0337**
Nickel (Ni)	0.4081	0.1559	0.1146	**p = 0.0252** p = 0.4108 p = 0.3806
Cobalt (Co)	0.103	0.1718	0.0969	p = 0.5881 p = 0.3639 p = 0.4626
Chromium (Cr)	− 0.0564	0.0334	0.0285	p = 0.7672 p = 0.8609 p = 0.8296
Silver (Ag)	− 0.1052	− 0.0916	− 0.0381	p = 0.5801 p = 0.6302 p = 0.7741
Interactions				
Between controls	$p > 0.05$ (n.s.)	Ctrl Co/Ctrl Ag	***p = 0.024*** ***p = 0.0407*** ***p = 0.0226***	Note (a): presence of outliers
Patients Hg/Cu	$p > 0.05$ (n.s.)	Patients Hg/Ni	$p = 0.945$ ***p = 0.029*** ***p = 0.0253***	Note (a): presence of outliers
Patients Hg/Co	$p > 0.05$ (n.s.)	Patients Hg/Cr	$p > 0.05$ (n.s.)	
Patients Hg/Ag	$p > 0.05$ (n.s.)	Patients Cu/Ni	$p > 0.05$ (n.s.)	
Patients Cu/Co	$p > 0.05$ (n.s.)	Patients Cu/Cr	***p < 0.0001*** $p = 0.0835$ $p = 0.0666$	Note (a): presence of outliers
Patients Cu/Ag	$p > 0.05$ (n.s.)	Patients Ni/Co	$p > 0.05$ (n.s.)	
Patients Ni/Cr	$p > 0.05$ (n.s.)	Patients Ni/Ag	$p > 0.05$ (n.s.)	
Patients Co/Cr	$p > 0.05$ (n.s.)	Patients Co/Ag	***p < 0.0001*** $p = 0.0711$ $p = 0.0626$	Note (a): presence of outliers
Patients Cr/Ag	$p > 0.05$ (n.s.)			
Meta-analysis and correlation test Number of significant by the total number of correlations				
Type 1 error	Pearson r	Spearman rho	Kendall tau	Notes
0.01	0.06	0.03	0.03	
0.02	0.06	0.05	0.05	
0.03	0.09	0.08	0.08	
0.04	0.09	0.09	0.09	
0.05	0.09	0.11	0.11	
0.06	0.09	0.12	0.11	
0.07	0.09	0.14	0.14	
0.08	0.09	0.15	0.14	
0.09	0.09	0.17	0.17	
0.1	0.09	0.2	0.2	

silver, and mercury between the two groups and whether there is the clinical relevance of this finding is unclear and would require more extensive investigation. Hypersensitivity to mercury has also been found more frequently in Takotsubo patients in comparison to healthy controls, as well as in patients with connective tissue disease [15, 38]. To clarify, in this study, only reactivity to inorganic mercury was tested, which is the type of mercury used in dentistry. Reactivity to other forms of mercury, such as methyl mercury present in fish, or phenylmercury, used as a preservative in eye drops and root fillings, was not tested.

In the body, metal ions bind with high affinity to thiol groups of structural proteins and enzymes [5]. Metal particles in tissues induce specific biological effects, including DNA damage, mitochondrial dysfunction, the breakdown of cellular metabolism, protein destruction, enzyme blockade, and oxidative stress [39, 40]. They have also been associated with autoimmunity [15, 33, 41] and may serve as a potential antigen for hypersensitive immunopathological reactions. As a result, certain pro-inflammatory cytokines are synthesized, and free radicals are produced. These biologically active substances are responsible for inducing and maintaining (chronic) inflammation in tissues [5, 13, 14, 28, 39]. Exogenous antioxidants [42] and particularly the endogenous methionine-homocysteine cycle [43] are the major protective (antioxidative) factors. Certain hormones, particularly female sex hormones, are also protective. Estrogens, in general, have a protective effect on the cardiovascular system [44], and progesterone has a strong immunosuppressive effect [45]. Cellular immunity primarily protects against all intracellular pathogens (viruses, including *Chlamydia and Borrelia*). Dysfunction of cellular immunity may interfere with other components of the immune system and with multiple chemical or biological factors [46]. These negative interactions may contribute to myocardial damage.

Limitations of the Study

The present study has several limitations. It was a monocentric, exploratory, and nonrandomized study, which included a small number of patients and healthy controls. However, the results of the study confirm that hypersensitivity reactions to metals are relatively common in these patients, and the highest SI values were more frequently found in patients than in healthy controls. The study subjects were exposed to other metals in addition to the seven that were selected for testing, based on a survey of metal exposure. Reactivity to other metals (aluminum, cadmium, lead, etc.) was not evaluated mainly due to financial constraints. Direct blood/myocardial concentrations of metals were not assessed.

Conclusion

The present study demonstrated that subjects with nonischemic dilated cardiomyopathy exhibit more frequent delayed-type hypersensitivity to mercury, nickel, and silver compared to healthy controls. Assessed correlation statistics showed that the higher hypersensitivity rates are closely related to dilated cardiomyopathy, despite the observation that the amount of metal exposure was similar for both patients and controls. Metals involved in these reactions are ubiquitous in daily life; they are present in tobacco smoke, in metal alloys used in dentistry and orthopedics, in food and medication.

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Compliance with Ethical Standards

Conflict of interest The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Ethical Approval All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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