Metal-induced inflammation triggers fibromyalgia in metal-allergic patients

Vera Stejskal [1], Karin Öckert [2], Geir Bjørklund [3]

1 Department of Immunology, University of Stockholm, Stockholm, Sweden
2 Gårdatandläkarna, Gothenburg, Sweden
3 Council for Nutritional and Environmental Medicine, Mo i Rana, Norway

Correspondence to: Prof. Vera Stejskal,
August Wahlströms väg 10, 18231 Danderyd, Stockholm, Sweden.
TEL: +46 8753 2322; FAX: +44 20 8711 5958; E-MAIL: vera@melisa.org

Submitted: 2013-09-05 Accepted: 2013-09-15 Published online: 2013-11-25

Key words: **fibromyalgia; delayed type hypersensitivity; inflammation; lymphocyte transformation test; MELISA; mercury; metals; nickel**

Neuroendocrinol Lett 2013; **34**(6):559–565 **PMID:** 24378456 NEL340613A10

Abstract

BACKGROUND: Fibromyalgia (FM) is a disease of unknown aetiology. Inflammation could be one of the mechanisms behind this disease.
OBJECTIVES: We studied the frequency and clinical relevance of metal allergy in FM patients.
METHODS: Fifteen female FM patients were included in the study. Metal allergy was measured by a lymphocyte transformation test, MELISA®. Ten healthy age-matched women were used as controls for *in vitro* studies. Reduction of metal exposure in the FM patients was achieved by replacement of dental metal restorations and by the avoidance of known sources of metal exposure. Objective health assessment was performed 5 years after treatment. Subjective health assessment was established by a questionnaire, completed 2, 5 and in some cases 10 years after the start of the study. Follow-up MELISA was also performed.
RESULTS: All FM patients tested positive to at least one of the metals tested. The most frequent reactions were to nickel, followed by inorganic mercury, cadmium and lead. Some healthy controls responded to inorganic mercury in vitro but most of the tests were negative. Objective examination 5 years later showed that half of the patients no longer fulfilled the FM diagnosis, 20% had improved and the remaining 30% still had FM. All patients reported subjective health improvement. This correlated with the normalisation of metal-specific responses *in vitro*.
CONCLUSION: Metal allergy is frequent in FM patients. The reduction of metal exposure resulted in improved health in the majority of metal-sensitized patients. This suggests that metal-induced inflammation might be an important risk factor in a subset of patients with FM.

Abbreviations:

FM	- fibromyalgia
CFS	- chronic fatigue syndrome
ME	- myalgic encephalopathy
SI	- stimulation index
MCS	- multiple chemical sensitivity
Au	- gold
EtHg	- ethyl mercury
Hg	- mercury (inorganic)
MeHg	- methyl mercury
Ni	- nickel
Pb	- lead
Pd	- palladium
PhHg	- phenyl mercury
Sn	- tin
Thim	- thimerosal
Ti	- titanium

ORIGINAL ARTICLE

INTRODUCTION

Fibromyalgia (FM) is a disease of unknown aetiology. It is characterised by widespread pain in 11 of 18 tender points experienced for at least three consecutive months (Wolfe *et al.* 1990). Patients with FM suffer from general fatigue, widespread musculoskeletal pain and stiffness, cognitive impairment, sleep disorders and other symptoms that affect their quality of life (Salaffi *et al.* 2009; Arranz *et al.* 2010). The disease also has a considerable overlap in non-musculoskeletal symptoms with allied conditions such as chronic fatigue syndrome/myalgic encephalopathy (CFS/ME), post-viral fatigue syndrome, migraine and tension headaches, affective disorders and irritable bowel syndrome (Clauw 1995; Sivri *et al.* 1996; Hamilton *et al.* 2005). Fibromyalgia often leads to working and social inability and no curative treatment is currently available. The prevalence of FM is 0.5% to 6% in the general population of the North America and Europe (Arranz *et al.* 2010; Branco *et al.* 2010; Lawrence *et al.* 2008; Wolfe *et al.* 2013). As FM is a frequently occurring condition, better knowledge is warranted to find an effective treatment.

It has been suggested that mercury from dental amalgam fillings may play a role in the aetiology of FM (Kötter *et al.* 1995). Other studies suggest a link between allergy to nickel and FM (Marcusson *et al.* 1999; Regland *et al.* 2001). Since signs of inflammation have been described in patients with FM (Kadetoff *et al.* 2012), metal-induced inflammation may be a risk factor. The present study aims to investigate if metals ubiquitous in our environment, such as nickel, and metals commonly used in dentistry might trigger inflammation in FM patients.

METHODS

Fifteen female patients with primary FM (mean age 47.6 years, range 34–66 years) provided informed consent to participate in this study. A specialist in rheumatology diagnosed the patients according to the American College of Rheumatology 1990 criteria for FM (Wolfe *et al.* 1990). The mean duration of illness at the time of study was 11 years (range 2–29 years). All patients had clinical metal allergy, such as eczema when wearing cheap metal earrings. Other allergies to food, pollen and drugs were also frequent and were reported by 80% of the patients. The evaluation of oral health, performed by one of our group (Karin Öckert), showed that all patients had amalgam fillings. All but three also had restorations containing gold, such as crowns and bridges. Most of the patients had root-filled teeth; some containing gold-plated metal posts (Table 1). Other known metal exposures were: living in a polluted area (near motorway, airport or crematorium), exposure to cigarette smoke, occupational exposure or contact with occupationally exposed family member. Further sources of metal exposure were thimerosal-containing vaccines and pills coated with titanium dioxide. All patients underwent amalgam replacement. In most patients, other metal restorations were removed as well. Maximal precaution was taken to minimize the metal release. Metal restorations were replaced with metal-free alternatives such as composites and non-metallic ceramics. In some patients, titanium-containing medication was replaced with a titanium-free alternative.

Five years after treatment, a rheumatologist evaluated the patients' health. Subjective health assessment was done by patients using a questionnaire; 2, 5 and in some cases 10 years after the treatment.

In vitro testing

The presence of metal allergy in FM patients was measured by an optimized lymphocyte transformation test, MELISA (Stejskal *et al.* 1994; 2006; Valentine-Thon & Schiwara 2003; Valentine-Thon *et al.* 2006).

This test uses the property of memory cells to be re-stimulated by a specific allergen *in vitro*. If memory cells are present in the blood, they start to divide and differentiate to so-called lymphoblasts. When allergens are low-molecular substances, allergen-specific memory cells are found in the blood of patients experiencing exposure-related clinical symptoms but not in the majority of healthy subjects (Stejskal *et al.* 1986; 1990; 1999; Stejskal & Forsbeck 1996; Tibbling *et al.* 1995). Lymphocytes isolated from peripheral blood were cultivated for 5 days with various concentrations of metal salts *in vitro*. Lymphocyte proliferation was measured by the uptake of radiolabeled thymidine and calculated as a Stimulation Index (SI): the quotient of counts per minute in metal-treated cultures and mean counts per minute from control cultures cultivated in the absence of metal salts. SI <3 was regarded as negative, SI ≥3 was taken as a positive response and SI ≥10 as a strongly positive response. In addition to objective radioisotope measurement, the number of lymphoblasts in cultures was morphologically evaluated.

RESULTS

Lymphocyte responses to metals in 15 patients with FM and in 10 healthy controls are shown in Table 2. All FM patients responded *in vitro* to one or more of the metals tested. The most frequent reaction was to nickel followed by inorganic mercury and phenyl mercury. One third of patients responded to palladium and tin and one fifth to gold. Only patients who had both amalgam and gold restorations responded to gold and palladium salts *in vitro;* the three patients exposed to amalgams only did not. Titanium was tested in the form of titanium dioxide and induced low positive responses in 40% of FM patients. Four patients responded to thimerosal, a mercury-containing preservative. None of the patients responded to copper, silver or platinum (data not shown).

Tab. 1. Metal exposure, positive lymphocyte responses and health outcome in fibromyalgia patients.

Patient code	Age	Duration of symptoms (years)	Reported metal exposure	Smoker	Dental exposure[1]	Lymphocyte responses to metals *in vitro*[2]	Health status 5 years after treatment
P5	50	21	Vaccines	Yes	16A, 3G, 3RF	Hg, PhHg**, Pd,** MeHg, EtHg, Thim, **Ni**	cured
P2	63	3	Orthodontics, environment	Yes	9A, 4G, 5RF	PhHg, **Au**, Pd, Pb, Ti, **Ni**	cured
P14	51	20	Vaccines	No	12A, 1G, 2RF	Sn, **Ni**	cured
P6	50	15	Not reported	Yes	16A, 1G, 1RF	**Hg**, PhHg, Au, Ti, EtHg, Thim, **Ni**	cured
P7	44	8	Not reported	Yes	12A, 4RF	Hg, Cd	cured
P15	39	19	Anti-D globulin injection	Yes	15A	Hg	cured
P13	66	2	Occupational	No	13A	**Hg, PhHg,** Pb**,** Cd, Ni	cured
P9	50	3	Orthodontics, environment	No	11A, 1G	Cd	possible FM
P10	42	21	Vaccines	Yes	15A, 3G, 2RFB	Pd, Cd, MeHg, Thim, **Ni**	possible FM
P12	34	10	Not reported	Yes	18A	Ti, Ni	possible FM
P1[3]	53	29	Husband works at car factory	No	1A, 6G, 2RFB	**Hg**, PhHg, Sn, **Au,** Pd**,** Pb**,** Ti**,** Ni	FM
P3[4]	49	10	Lived near chemical factory	Yes	7A, 3G, 1RF	Hg, Sn, Pb, **Ni**	FM
P8	40	5	Father welder, vaccines, environment	No	13A, 1G, 4RFB	Hg, PhHg, Au, Pd, Pb, Cd, Ti, MeHg, EtHg, Thim, **Ni**	FM
P11[5]	54	7	Anti-D globulin injection	Yes	4A, 6G, 2RF, Ti	Sn, Cd, Ni	FM
P4	53	7	Contact lenses	No	6A, 4G, 2RFB, 2Ti	**Hg**, **PhHg**, Sn, Pb, Cd, Ti, Ni	not available

1. Number of amalgam fillings (A), gold restorations (G), root fillings (RF), root fillings with gold-plated brass pins (RFB), titanium crown and screw (Ti)
2. Positive lymphocyte responses to metals are shown; strongly positive responses are in bold
3. Onset of FM following placement of root filling containing lead and phenyl mercury
4. Worsening of FM after placement of gold bridge
5. Worsening of FM after placement of titanium crown; patient refused to replace the crown

Three healthy controls responded to mercury with weak positive responses; one of the responders showed positive response to titanium dioxide as well.

Objective assessment performed by a rheumatologist 5 years after treatment, showed that half of the patients no longer fulfilled the diagnostic criteria of FM and thus were regarded as "cured". One fifth of the patients had less trigger points than the 11 necessary for the diagnosis of FM and were therefore labelled as "possible FM". The remaining one third of the patients still fulfilled the criteria for FM. Subjective health evaluations was done by patients 2, 5 and 10 years following treatment. All patients reported improvement of health. Follow-up MELISA, performed at the same time as the subjective evaluation, showed a marked decrease in lymphocyte reactivity to metals *in vitro* compared to the reactivity at the beginning of the study (Table 3).

DISCUSSION

In this small cohort of women with primary FM, metal allergy to nickel, mercury and other metals was frequent. Following reduced exposure to metals, most FM patients experienced long-term health improvement.

Similar findings in CFS patients have previously been reported (Stejskal *et al.* 1999; 2006; Sterzl *et al.* 1999). In those studies, as well as in the current study, the most frequent metal allergens were nickel and inorganic mercury. Two recent studies, one from France and one from Italy, reached the same conclusion. In the French study, 100 patients with FM and CFS/ME were tested for metal allergy by the MELISA test. Nickel and mercury were found to be the most frequent sensitizers (Tournesac P. Fibromyalgia: nickel and mercury hypersensitivity in general practice. Poster presented at: Controversies in Rheumatology and Autoimmunity; 2013 April 4–6; Budapest, Hungary.)

In the second study, Pigatto and his colleagues from Italy studied 41 patients diagnosed with multiple chemical sensitivity (MCS) (Pigatto *et al.* 2013). Metal allergy had been diagnosed with patch testing and MELISA testing *in vitro*. The authors reported that 91% of the patients (39 women and 2 men) were sensitized to at least one metal, indicating a very high presence of metal allergy in the Italian MCS cohort. This high frequency of metal allergy in MCS patients corroborates the data obtained in this study. Another Italian group (De Luca *et al.* 2010) compared serum cytokine profiles in 226 MCS patients with the corresponding cytokine levels in 218 healthy Italians. Significant up-regulation of pro-inflammatory cytokines such as interferon (IFN)-gamma was found in the MCS group compared

Tab. 2. Lymphocyte responses to metals in patients with fibromyalgia and healthy controls expressed as Stimulation Index.

Code[1]	Inorganic mercury	Phenyl mercury	Tin	Copper	Silver	Gold	Palladium	Platinum	Lead	Cadmium	Titanium dioxide	Methyl mercury	Ethyl mercury	Thimerosal	Nickel
P1	**15.2**[2]	3.6	**5.3**	0.9	2.1	***11.0***	**3.2**	2.0	**6.6**	1.7	**3.0**	1.3	ND	2.6	**4.5**
P2	2.6	3.3	1.4	1.0	1.1	***14.0***	**3.0**	2.5	**4.0**	2.3	**3.1**	1.6	1.4	1.3	***11***
P3	**6.1**	2.9	**5.4**	1.6	0.7	1.4	2.7	1.1	**3.6**	1.6	1.5	1.4	1.9	1.5	***12***
P4	***20***	***12.9***	**8.1**	1.0	1.0	2.5	1.5	1.1	**8.6**	**4.3**	**9.6**	2.2	2.8	1.9	**3.1**
P5	**6.1**	**6.2**	0.8	0.5	0.5	2.0	***13.2***	0.6	2.8	2.4	2.6	**5.1**	**6.1**	**6.3**	***18***
P6	***19***	4.3	0.8	1.3	1.0	**4.4**	2.3	1.6	1.6	2.9	**4.8**	1.3	**4.9**	**6.7**	***17***
P7	**9.4**	ND	2.0	ND	ND	ND	ND	ND	ND	**3.8**	ND	1.9	ND	ND	2.6
P8	**9.2**	**3.6**	0.8	1.7	2.1	**8.0**	4.0	2.3	**3.4**	**3.8**	**4.5**	**6.2**	6.0	**4.9**	***17***
P9	2.0	1.5	1.2	0.8	1.9	1.9	1.6	1.2	0.9	**5.9**	1.3	1.2	0.9	1.4	2.3
P10	1.4	1.0	1.0	2.5	0.9	1.4	5.6	1.2	0.9	**3.3**	1.5	**4.5**	0.5	**4.5**	***90***
P11	1.9	1.2	**3.6**	1.1	1.0	1.8	1.9	1.5	1.4	**4.0**	2.3	2.7	0.3	1.3	**7.4**
P12	1.6	1.2	1.2	1.0	1.0	1.2	1.7	1.0	1.0	2.7	**3.2**	2.9	1.6	2.1	***19***
P13	***10***	***11***	1.0	0.3	0.5	2.9	0.8	0.4	3.6	**3.6**	2.1	2.1	0.9	1.1	**3.6**
P14	1.7	1.4	**5.4**	0.4	0.7	1.0	1.9	1.0	0.8	1.3	1.0	1.9	1.1	1.5	**29**
P15	**3.6**	1.0	1.4	0.4	0.6	1.4	1.3	1.2	1.5	2.1	1.5	2.4	0.5	0.7	2.5
C1	1.6	2.2	1.4	0.8	0.9	0.7	1.0	0.9	1.4	1.5	1.4	1.4	1.1	0.3	0.9
C2	1.9	ND	0.1	0.5	0.7	2.2	ND	0.7	0.4	1.1	1.0	1.4	0.1	0.9	2.5
C3	0.5	0.3	1.2	0.7	0.6	0.8	1.3	0.8	0.8	1.0	0.9	1.1	0.9	0.9	0.9
C4	**4.0**	ND	1.3	0.6	0.9	0.8	0.8	0.7	1.1	1.5	2.1	ND	ND	0.7	1.3
C5	0.7	0.7	2.3	1.0	1.0	1.0	1.2	0.9	1.0	1.2	1.1	1.0	1.1	1.0	2.6
C6	**4.4**	**3.1**	1.4	0.9	0.7	1.0	1.1	0.9	1.7	1.8	1.5	1.2	1.0	0.8	2.6
C7	1.8	**3.9**	1.1	0.7	0.5	2.0	2.0	0.6	1.4	1.6	1.2	2.5	1.5	1.3	1.0
C8	1.7	2.4	1.2	0.7	1.3	1.7	1.6	0.5	1.5	1.1	1.7	1.4	1.2	0.9	2.0
C9	**4.4**	2.9	1.2	0.5	1.1	2.8	2.5	1.2	1.1	1.2	3.7	1.7	0.2	1.5	1.6
C10	0.7	0.8	0.5	ND	ND	0.5	0.8	0.5	0.8	1.2	0.6	ND	ND	0.3	ND

1. P1–P15: patients with FM, C1–C10: healthy controls, SI: Stimulation index
2. SI ≥3 are positive responses and are shown in **bold,** SI ≥10 indicates strongly positive responses are shown in ***bold cursive***, ND: Not done

to healthy subjects (De Luca *et al.* 2010). Other findings, such as changes in free radical/antioxidant homeostasis, and low levels of glutathione are all compatible with a metal-induced pathology in the MCS group.

Most patients suffering from nonspecific symptoms fulfilling the criteria of FM, CFS/ME and MCS, are women. It is well known that women suffer more frequently from nickel allergy than men. This may be due to exposure to nickel-containing earrings (Sterzl *et al.* 1999) as well as by widespread use of nickel-containing cosmetics (Liu *et al.* 2013). In men, occupational exposure is the most frequent factor behind sensitization to nickel (Pizzutelli 2011). Nickel occurs in soil, water, air and in the biosphere. It is present in most of the constituents of a normal diet and also in many metallic everyday items. In some countries, metal alloys with a high concentration of nickel are still used in dental crowns and bridges. Nickel can also be present as an impurity in amalgam and dental gold alloys (Forsell *et al.* 1997). Other sources of nickel exposure are cigarette smoke and piercing (Dotterud & Falk 1994; Schäfer *et al.* 2001).

Patch testing is the gold standard for diagnosis of delayed cell-mediated hypersensitivity. The prevalence of positive patch tests to nickel in the general population in Europe is about 20% (Nielsen & Menne 1992).

Marcusson *et al.* (1999) reported on the increased prevalence of nickel positive patch tests in Swedish patients with FM and CFS/ME. In a previous study from this group, 397 patients were referred for metal patch testing in Sweden. Over 50% of the patients had systemic symptoms such as fatigue and muscular pain, while local oral symptoms were less frequent (Marcusson 1996). This confirms the *in vitro* data showing increased lymphocyte responsiveness in this patient group (Stejskal *et al.* 1994; Sterzl *et al.* 1999).

Muris and Feilzer (2006) describe two patients with a known nickel allergy that experienced the disappearance of symptoms and general health improvement after removal of nickel-containing dental appliances (a bridge and orthodontic wire respectively). One of the patients, a 45-years old woman, suffered from profound fatigue, migraine and joint pain of the wrists, hands and fingers. Two months after the removal of a nickel-containing orthodontic wire, the patient's profound fatigue disappeared. Regland *et al.* (2001) investigated 204 women that fulfilled the criteria for both FM and CFS/ME. About half of the patients in the present study had a positive history of nickel-induced contact dermatitis.

The second most common allergen found was inorganic mercury. Dental amalgams commonly consist of 50% mercury, ~22–32% silver, ~14% tin, ~8% copper, and other trace metals (Ferracane 2001). Mercury released from dental amalgams makes the predominant contribution to human exposure to inorganic mercury and vapour in the general population (Clarkson *et al.* 1988). No exposure to mercury vapour can be considered totally harmless since mercury vapour has no toxic threshold (IPCS 1991).

All mercury salts tested have immunomodulatory potential (Shenker *et al.* 1992) as well as allergenic properties (Stejskal *et al.* 1996). One third of the FM patients tested positive to thimerosal and nearly half reacted to phenyl mercury in MELISA. Both thimerosal, also called Merthiolate™, and phenyl mercury are organic mercury compounds used as antiseptics and preservatives in eye drops and vaccines (Rietschel & Fowler 2001). Organic mercurials are strong allergens and induce local and systemic delayed type hypersensitivity (Tosti *et al.* 1989; Seidenari *et al.* 2005). In our study, three out of four patients responding to thimerosal *in vitro* responded to ethyl mercury as well.

Lymphocyte responses to methyl mercury were rarely detected in this study. The most common source of methyl mercury in humans is from polluted fish. Methyl mercury is also produced by conversion of metallic mercury through oral and gastrointestinal bacteria (Liang & Brooks 1995). It is important to note that memory lymphocytes induced by various mercury compounds do not cross-react (Santucci *et al.* 1998; Tosti *et al.* 1989). Therefore, to determine the presence of possible mercury allergy, both organic and inorganic mercury should be tested.

Tab. 3. Frequency of positive lymphocyte responses to metals in fibromyalgia patients.

Metal	Before treatment [1,2]	%	After treatment	%
Mercury	6/9	67%	0/9	0%
Phenyl mercury	2/4	50%	0/4	0%
Tin	3/3	100%	1/3	33%
Gold	3/5	60%	1/5	20%
Palladium	3/7	43%	1/7	14%
Lead	2/5	40%	1/5	20%
Cadmium	4/5	80%	0/5	0%
Titanium dioxide	4/6	67%	0/6	0%
Methyl mercury	2/7	29%	0/7	0%
Thimerosal	2/5	40%	0/5	0%
Nickel	8/11	73%	3/11	27%
Total	39/67	58%	7/67	10%

[1)] Number of positive tests/total tests
[2)] The treatment consisted of reduction of metal exposure by replacement of dental metals with ceramic or composite restorations as well as removal of other known sources of metal exposure

One third of the patients, but none of the controls, reacted to gold, palladium and tin. These metals are frequently used in dental restorations and the allergenic potential of gold and palladium has been discussed previously (Marcusson 1996).

Titanium is generally considered to be a safe option for metal allergic patients, although the risk of titanium allergy might be increased in patients already sensitized to other metals (Hallab *et al.* 2001). Around 40% of our FM patients tested positive to titanium dioxide. In addition to the titanium used in dentistry and orthopedic surgery, titanium is frequently used as a white pigment (E171) in pharmaceuticals, sunscreens, cosmetic products, toothpastes, foods and other items. As a consequence of a positive MELISA test to titanium dioxide, some FM patients limited their exposure to for example titanium-containing cosmetics and requested titanium-free medication from their physicians. Avoidance of titanium-containing cosmetics in a strongly titanium-positive patient, who was required to wear titanium-containing make up daily, resulted in resolution of her eczema and joint pain (Müller and Valentine-Thon 2006).

Fibromyalgia is often present as a co-morbidity with other inflammatory diseases. In a recent study, the frequency of metal allergy was determined in 41 patients suffering from systemic lupus erythematosus, rheumatoid arthritis and Sjögren's syndrome (Stejskal V and Reynolds T. Metal allergy – the missing link in autoimmune connective tissue disorders? Poster session presented at: Controversies in Rheumatology and Autoimmunity (2013 April 4–6; Budapest, Hungary).

The results showed that the majority of the patients had clinical metal allergy, and responded *in vitro* to nickel, mercury, gold and palladium. In one patient, the symptoms of rheumatoid arthritis resolved after removal of stainless steel chest wires containing nickel, molybdenum and chromium; metals that the patient reacted to in the MELISA test. These findings, as well as previously published data (Stejskal *et al.* 2006; Prochazkova *et al.* 2004), suggest that metal-induced allergy might be a risk factor not only in CFS/ME, MCS and FM but also in rheumatoid diseases.

In conclusion, dental and environmental metals can trigger chronic inflammation in metal-sensitized FM patients. Reduction of inflammation by removal of these specific triggers could present a new way to treat not only FM but also other chronic inflammatory diseases.

REFERENCES

1 Arranz LI, Canela MÁ, Rafecas M. (2010). Fibromyalgia and nutrition, what do we know? Rheumatol Int. **30**: 1417–1427.
2 Branco JC, Bannwarth B, Failde I, Abello Carbonell J, Blotman F, Spaeth M *et al.* (2010). Prevalence of fibromyalgia: a survey in five European countries. Semin Arthritis Rheum. **39**: 448–453.
3 Clarkson TW, Friberg L, Nordberg GF, Sager P. (1988). Biological monitoring of metals. Plenum Press, New York.
4 Clauw DJ. (1995). The pathogenesis of chronic pain and fatigue syndromes, with special reference to fibromyalgia. Med Hypotheses. **44**: 369–378.
5 De Luca C, Scordo MG, Cesareo E, Pastore S, Mariani S, Maiani G *et al.* (2010). Biological definition of multiple chemical sensitivity from redox state and cytokine profiling and not from polymorphisms of xenobiotic-metabolizing enzymes. Toxicol Appl Pharmacol. **248**: 285–292.
6 Dotterud LK, Falk ES. (1994). Metal allergy in north Norwegian schoolchildren and its relation with ear piercing and atopy. Contact Dermatitis. **31**: 308–313.
7 Ferracane JL. (2001). Materials in dentistry: principles and applications. Lippincott Williams & Wilkin, Philadelphia.
8 Forsell M, Marcusson JA, Carlmark B, Johansson O. (1997). Analysis of the metal content of in vivo-fixed dental alloys by means of a simple office procedure. Swed Dent J. **21**: 161–168.
9 Hallab M, Mikecz K, Vermes C, Skipor A, Jacobs J. (2001). Differential lymphocyte reactivity to serum-derived metal-protein complexes produced from cobalt-based and titanium-based implant alloy degradation. J Biomed Mater Res. **56**: 427–436
10 Hamilton WT, Gallagher AM, Thomas JM, White PD. (2005). The prognosis of different fatigue diagnostic labels: a longitudinal survey. Fam Pract. **22**: 383–388.
11 IPCS - International Programme on Chemical Safety. (1991). Environmental Health Criteria 118: Inorganic mercury. WHO, Geneva.
12 Kadetoff D, Lampa J, Westman M, Andersson M, Kosek E. (2012). Evidence of central inflammation in fibromyalgia-increased cerebrospinal fluid interleukin-8 levels. J Neuroimmunol. **242**(1–2): 33–38.
13 Kötter I, Dürk H, Saal JG, Kroiher A, Schweinsberg F. (1995). Mercury exposure from dental amalgam fillings in the etiology of primary fibromyalgia: a pilot study. J Rheumatol. **22**: 2194–2195.
14 Liang L, Brooks RJ. (1995). Mercury reactions in the human mouth with dental amalgams. Water Air Soil Pollut. **80**: 103–107.
15 Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA *et al.* (2008). Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum. **58**: 26–35.
16 Liu S, Hammond K, Rojas-Cheatham A. (2013). Concentrations and potential health risks of metals in lip products. Environ Health Perspect. **121**: 705–710.
17 Marcusson JA. (1996). Contact allergies to nickel sulfate, gold sodium thiosulfate and palladium chloride in patients claiming side-effects from dental alloy components. Contact Dermatitis. **34**: 320–323.
18 Marcusson JA, Lindh G, Evengård B. (1999). Chronic fatigue syndrome and nickel allergy. Contact Dermatitis. **40**: 269–272.
19 Müller K, Valentine-Thon E. (2006). Hypersensitivity to titanium: clinical and laboratory evidence. Neuro Endocrinol Lett. **27**(Suppl 1): 31–35.
20 Muris J, Feilzer AJ. 2006. Micro analysis of metals in dental restorations as part of a diagnostic approach in metal allergies. Neuro Endocrinol Lett. **27**(Suppl 1): 49–52.
21 Nielsen NH, Menné T. 1992. Allergic contact sensitization in an unselected Danish population. The Glostrup Allergy Study, Denmark. Acta Derm Venereol. **72**: 456–460.
22 Pigatto P, Minoia C, Ronchi A, Brambilla L, Ferrucci SM, Spadari F, *et al.* (2013). Allergological and toxicological aspects in a multiple chemical sensitivity cohort. Oxid Med Cell Longevity. Article ID 356235, 12 p.
23 Prochazkova J, Sterzl I, Kucerova H, Bartova J, Stejskal V. (2004). The beneficial effect of amalgam replacement on health in patients with autoimmunity. Neuro Endocrinol Lett. **25**: 211–218.
24 Regland B, Zachrisson O, Stejskal V, Gottfries C. (2001). Nickel allergy is found in a majority of women with chronic fatigue syndrome and muscle pain. J Chronic Fatigue Syndr. **8**: 57–65.
25 Rietschel RL, Fowler JF. (2001). Fisher's contact dermatitis. 5th ed. Lippincott Williams and Wilkins, Philadelphia.
26 Salaffi F, Sarzi-Puttini P, Girolimetti R, Atzeni F, Gasparini S, Grassi W. (2009). Health-related quality of life in fibromyalgia patients: a comparison with rheumatoid arthritis patients and the general population using the SF-36 health survey. Clin Exp Rheumatol. **27**(5 Suppl 56): S67–S74.
27 Santucci B, Cannistraci C, Cristaudo A, Camera E, Picardo M. (1998). Thimerosal sensitivities: the role of organomercury alkyl compounds. Contact Dermatitis. **38**: 325–328.
28 Schäfer T, Böhler E, Ruhdorfer S, Weigl L, Wessner D, Filipiak BJ *et al.* (2001). Epidemiology of contact allergy in adults. Allergy. **56**: 1192–1196.
29 Seidenari S, Giusti F, Pepe P, Mantovani L. (2005). Contact sensitization in 1094 children undergoing patch testing over a 7-year period. Pediatr Dermatol. **22**: 1–5.
30 Shenker BJ, Rooney C, Vitale L, Shapiro IM. (1992). Immunotoxic effects of mercuric compounds on human lymphocytes and monocytes. I. Suppression of T-cell activation. Immunopharmacol Immunotoxicol. **14**: 539–553.
31 Sivri A, Cindas A, Dinçer F, Sivri B. (1996). Bowel dysfunction and irritable bowel syndrome in fibromyalgia patients. Clin Rheumatol. **15**: 283–286.
32 Stejskal VD, Cederbrant K, Lindvall A, Forsbeck M. (1994). MELISA – an *in vitro* tool for the study of metal allergy. Toxicol In Vitro. **8**: 991–1000.
33 Stejskal VD, Danersund A, Lindvall A, Hudecek R, Nordman V, Yaqob A *et al.* (1999). Metal-specific lymphocytes: biomarkers of sensitivity in man. Neuro Endocrinol Lett. **20**: 289–298.
34 Stejskal VD, Forsbeck M, Cederbrant KE, Asteman O. (1996). Mercury-specific lymphocytes: an indication of mercury allergy in man. J Clin Immunol. **16**: 31–40.
35 Stejskal V, Forsbeck M, Nilsson R. (1990). Lymphocyte transformation test for diagnosis of isothiazolinone allergy in man. J Invest Dermatol. **94**: 798–802.
36 Stejskal V, Hudecek R, Stejskal J, Sterzl I. (2006). Diagnosis and treatment of metal-induced side-effects. Neuro Endocrinol Lett. **27**(Suppl 1): 7–16.
37 Stejskal V, Olin R, Forsbeck M, (1986). The lymphocyte transformation test for diagnosis of drug-induced occupational allergy. J Allergy Clin Immunol. **77**: 411–426.
38 Sterzl I, Procházková J, Hrdá P, Bártová J, Matucha P, Stejskal VD. (1999). Mercury and nickel allergy: risk factors in fatigue and autoimmunity. Neuro Endocrinol Lett. **20**: 221–228.

39 Tibbling L, Thuomas KÅ, Lenkel R, Stejskal V. (1995). Immunological and brain MRI changes in patients with suspected metal intoxication. Int J Occup Med Toxicol. **4**: 285–294.
40 Tosti A, Guerra L, Bardazzi F. (1989). Hyposensitizing therapy with standard antigenic extracts: an important source of thimerosal sensitization. Contact Dermatitis. **20**: 173–6.
41 Valentine-Thon E, Muller KE, Guzzi G, Kreisel S, Ohnsorge P, Sandkamp M. (2006). LTT-MELISA® is clinically relevant for detecting and monitoring metal sensitivity. Neuro Endocrinol Lett **27**(Suppl1): 17–24.
42 Valentine-Thon E, Schiwara HW. (2003). Validity of MELISA® for metal sensitivity. Neuro Endocrinol Lett. **24**: 57–64.
43 Wolfe F, Brähler E, Hinz A, Häuser W. (2013). Fibromyalgia prevalence, somatic symptom reporting, and the dimensionality of poly-symptomatic distress: results from a survey of the general population. Arthritis Care Res (Hoboken). **65**: 777–785.
44 Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL *et al.* (1990). The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum. **33**: 160–172.