ORIGINAL ARTICLE

# The beneficial effect of amalgam replacement on health in patients with autoimmunity

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**Abstract**

**BACKGROUND**: Patients with certain autoimmune and allergic diseases, such as systemic lupus, multiple sclerosis, autoimmune thyroiditis or atopic eczema, often show increased lymphocyte stimulation by low doses of inorganic mercury *in vitro*. The patients often report clinical metal hypersensitivity, especially to nickel.

**OBJECTIVE AND METHODS**: In this study we examined the health impact of amalgam replacement in mercury-allergic patients with autoimmunity. The suitability of MELISA®, an optimized lymphocyte stimulation test, for the selection of susceptible patients and monitoring of sensitization was also examined. Amalgam fillings were replaced with composites and ceramic materials. Follow-up health status and lymphocyte reactivity were assessed and evaluated half a year or later following amalgam removal.

**RESULTS**: Results of lymphocyte reactivity measured with MELISA® indicate that *in vitro* reactivity after the replacement of dental amalgam decreased significantly to inorganic mercury, silver, organic mercury and lead. Out of 35 patients, 25 patients (71%) showed improvement of health. The remaining patients exhibited either unchanged health (6 patients, 17%) or worsening of symptoms (4 patients, 11%). The highest rate of improvement was observed in patients with multiple sclerosis, the lowest rate was noted in patients with eczema. The initial mercury-specific lymphocyte reactivity was significantly higher in the responder group, than in the non-responders, whose health was not improved by amalgam removal. All patients with health improvement after amalgam replacement showed reduced proliferation to inorganic mercury in follow-up MELISA®. *In vitro* responses to phenylmercury and nickel did not differ between the groups.

**CONCLUSIONS**: Mercury-containing amalgam may be an important risk factor for patients with autoimmune diseases. MELISA® is a valuable tool for selection of patients for amalgam replacement and also for monitoring of metal allergies.

**Abbreviations**

| | |
|---|---|
| AT | autoimmune thyroiditis |
| AE | atopic eczema |
| CFS | chronic fatigue syndrome |
| Cpm | counts per minute |
| DNA | deoxyribonucleic acid |
| $HgCl_2$ | mercury chloride |
| HPA-axis | hypothalamus-pituitary-adrenal axis |
| LST | lymphocyte stimulation test |
| LTT | lymphocyte transformation test |
| MELISA® | Memory Lymphocyte Immuno Stimulation Assay |
| MS | multiple sclerosis |
| No. | number |
| P | value of probability for statistical significance |
| PWM | pokeweed mitogen |
| RPMI 1640 | Roswell Park Memorial Institute (culture medium) |
| SI | stimulation index |
| SLE | systemic lupus erythematosus |

## Introduction

Metals, such as mercury and gold, can induce autoimmunity in susceptible strains of experimental animals [1–4]. Mercury and lead may also accelerate systemic autoimmunity in lupus-prone mice [5]. In humans, certain metals may accelerate or worsen clinical autoimmune disease in susceptible populations [6–7]. High levels of autoantibodies were found in populations exposed to high doses of inorganic mercury [8–10]. El-Fawal et al. [8] studied workers in fluorescent light factories exposed to mercury vapor in a concentration of 0, 05 mg/m$^3$. The titers of autoantibodies to neurofilament proteins and to myelin basic protein were significantly increased in exposed workers as compared with non-exposed workers. Antibodies were predominantly of the IgG type, indicating secondary antigen challenge or antigen persistence. The strong allergenic potential of mercury compounds is well recognized by dermatologists since thimerosal, thiosalicylate salt of ethylmercury, is the number one allergen in children [11]. Many patients with autoimmune diseases have hypersensitivity to heavy metals in the anamnesis [12]. In addition to mercury, well-known allergens such as gold, beryllium and nickel are frequently used in dental alloys [13–16]. Dental amalgam has been implicated as a risk factor in multiple sclerosis [17], in Alzheimer's disease [18–19] and recently in infertility [20].

Corrosion products from dental alloys may serve as triggers of chronic inflammation in susceptible subjects. Metal-induced allergy in man is caused by the contact of the metal with the surface of memory lymphocytes. Upon re-exposure to the same or structurally similar epitope, memory cells become activated and start to produce lymphokines. The resulting inflammation can occur in the skin or elsewhere in the body where metal ions are located [7, 12]. The activation of memory cells can be measured outside the body, *in vitro,* by so called lymphocyte stimulation test (LTT, LST). Lymphocytes from patients suffering from metal hypersensitivity (so called delayed type allergy) often show increased proliferation after exposure to metal salts *in vitro* [7, 12, 21–25]. Increased lymphocyte responses to inorganic mercury are frequent in patients with lichenoid changes and positive patch test to mercury [23], in patients with autoimmune thyroiditis [12] and in patients with atopic eczema and psoriasis [26–27].

In this study the impact of amalgam replacement on lymphocyte stimulation and health was studied in a subgroup of patients with various autoimmune diseases who showed lymphocyte reactivity to mercury in an optimized lymphocyte proliferation test, MELISA®.

## Material and Methods

### *Patients*

From a cohort of 305 patients, referred to the Institute of Dental Research in Prague during the period 1996–2003, 35 patients diagnosed with autoimmune diseases enrolled in the study. The selection criteria were: 1) increased responsiveness to a low concentration of inorganic mercury in a modified lymphocyte stimulation test, MELISA®, and 2) the presence of amalgam fillings as the single restorative material. All patients fulfilled the diagnostic criteria for a given autoimmune disease such as the presence of specific autoantibodies in serum. The patient group consisted of 27 females and 8 males. The mean age of patients was 36 years (range 20–65 years).

At the beginning of the study, all patients completed a detailed questionnaire regarding current and past metal exposure and possible clinical metal hypersensitivity (Table 1).

Prior to enrolment in the study, patients underwent oral examination. The former dentist of the patient was consulted in some cases. With the patient's informed consent, amalgam was removed in two to four sessions under maximal protection, such as the use of rubber dam, Clean up® and fresh air [28] and replaced by composites and/or ceramic inlays. Follow-up examination, performed half a year or later, included re-testing of lymphocyte reactivity in MELISA® and detailed re-evaluation of patient's health. Objective findings such as changes in the medication protocol, results of laboratory parameters as well as subjective "well-being" were taken into account. Decreased medication, normalization of laboratory parameters and an increase in patients' well being were considered as signs of health improvement.

### *MELISA®*

Peripheral blood was collected into vacutainer tubes with polystyrene beads (Becton Dickinson, UK), defibrinated by shaking and diluted 1:1 in a modified culture medium RPMI 1640 with 10 mM Hepes (Gibco BRL) and gentamycin (Krka, Yugoslavia). Mononuclear cells were separated by centrifugation for 30 minutes at 600 g on a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden), followed by washing. Cells were resuspended in 20% autologous inactivated serum and incubated for 30 minutes at 37°C and in 5% $CO_2$ atmosphere in culture flasks (Costar, USA). This procedure results in partial depletion of monocytes

**Table 1:** Summarized data from questionnaires ( M - male; F - female; B - better; U - unchanged; W - worse)

| Diagnosis | Patient code | Age at diagnosis | Sex | Duration of illness (months) | Exposure to cigarette smoke | Clinical metal hypersensitivity | Number of removed fillings | Short term worsening of health after amalgam replacement | Long term health improvement | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Objective | Subjective |
| **Systemic** | 1 | 48 | F | 9 | no | yes | 3 | no | B | B |
| **Lupus** | 2 | 36 | F | 4 | no | yes | 7 | yes | B | B |
| **Erythematosus** | 3 | 65 | F | 8 | no | no | 4 | no | B | B |
| | 4 | 20 | M | 34 | yes | no | 3 | no | W | U |
| | 5 | 31 | F | 9 | no | yes | 12 | yes | B | B |
| | 6 | 20 | M | 19 | no | yes | 11 | yes | U | U |
| | 7 | 34 | F | 14 | no | yes | 7 | no | B | B |
| | 8 | 56 | F | 7 | no | no | 10 | yes | U | U |
| | 9 | 35 | F | 16 | no | yes | 9 | yes | U | B |
| | 10 | 30 | F | 36 | yes | no | 4 | no | U | U |
| | 11 | 48 | M | 8 | yes | no | 6 | no | B | B |
| | 12 | 49 | M | 11 | no | no | 9 | yes | B | B |
| | 13 | 28 | F | 11 | yes | yes | 3 | no | B | B |
| | 14 | 20 | F | 7 | no | yes | 3 | no | B | B |
| | 15 | 27 | M | 7 | yes | yes | 8 | no | B | B |
| **Autoimmune** | 16 | 35 | F | 32 | yes | yes | 4 | no | W | W |
| **thyroiditis** | 17 | 27 | F | 9 | yes | yes | 5 | no | U | B |
| | 18 | 48 | F | 19 | no | no | 3 | no | B | B |
| | 19 | 50 | F | 7 | no | no | 7 | yes | B | B |
| | 20 | 49 | F | 10 | yes | yes | 9 | yes | B | B |
| | 21 | 25 | F | 8 | yes | yes | 11 | yes | B | B |
| | 22 | 49 | M | 5 | yes | yes | 8 | yes | U | U |
| | 23 | 47 | F | 12 | yes | yes | 6 | no | B | B |
| **Multiple** | 24 | 37 | F | 12 | no | no | 6 | no | B | B |
| **sclerosis** | 25 | 38 | F | 15 | no | no | 4 | no | B | B |
| | 26 | 29 | M | 15 | no | yes | 9 | yes | B | B |
| | 27 | 50 | F | 18 | no | no | 16 | yes | B | B |
| | 28 | 25 | F | 5 | yes | yes | 4 | no | B | B |
| | 29 | 27 | M | 7 | no | yes | 13 | yes | B | B |
| **Atopic eczema** | 30 | 25 | F | 14 | no | no | 4 | no | B | B |
| | 31 | 37 | F | 19 | yes | yes | 5 | no | B | B |
| | 32 | 40 | F | 14 | yes | yes | 9 | no | W | W |
| | 33 | 29 | F | 16 | no | yes | 11 | yes | B | B |
| | 34 | 23 | F | 19 | yes | no | 3 | no | W | W |
| | 35 | 23 | F | 23 | no | no | 10 | no | B | B |

from lymphocyte suspension. After incubation, cells were resuspended in complete medium containing glutamine and 10% inactivated autologous serum in concentration 1x $10^6$ cells per ml. One ml of cell suspension was added to each well in 48-well tissue plates (Costar, USA) pre-coated with metal salts. Stock metal solutions were prepared by dilution of metal salts with sterile filtered tissue water (TW) and further diluted to working solutions at the time of plate preparation [22]. Three wells with 100 µl TW without metals were used as negative controls. Pokeweed mitogen (PWM, Sigma; in concentration 10 µg/ml) was used as positive control. After 5 days incubation at 37°C and 100% humidity, 600 µl of cell suspension from each well was transferred to a new plate and 111 kBq of methyl$^3$H thymidine (UVVVR, Prague) was added. After 4-hour incubation, samples were harvested using an automatic cell harvester (Inotech, Switzerland) and the radioactivity incorporated into DNA of cells was counted on a Microbeta counter (LKB/Wallac, Finland). Lymphocyte stimulation in metal-containing cultures was expressed as stimulation index, SI:

SI = cpm (counts per minute) in experimental cultures divided by mean cpm in control cultures.

SI equal or more than 2 indicates positive response. Since the level of SI is dependent on the control value (higher control values may result in lower stimulation index), lymphocyte stimulation was also expressed as delta cpm (Δ-cpm):

Δcpm = cpm in experimental cultures – cpm in control cultures.

Delta cpm is less sensitive to changes in control values compared to SI [unpublished]. Statistical analysis of data was performed using two-tailed Fisher's exact test.

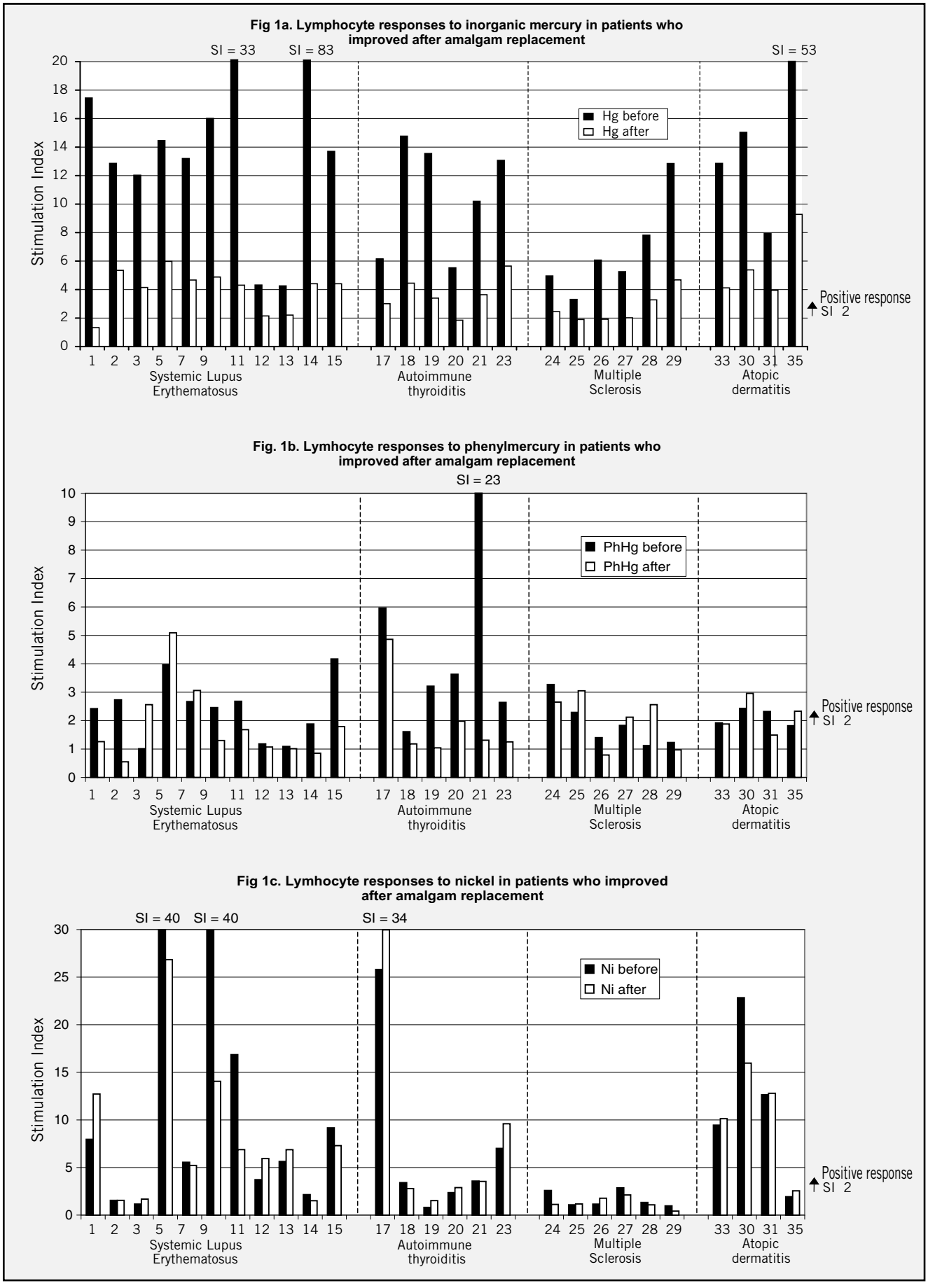


**Fig 1a.** Lymphocyte responses to inorganic mercury in patients who improved after amalgam replacement
**Fig 1b.** Lymphocyte responses to phenylmercury in patients who improved after amalgam replacement
**Fig 1c.** Lymphocyte responses to nickel in patients who improved after amalgam replacement

**Fig 2a. Lymphocyte responses to inorganic mercury in patients who did not improve after amalgam replacement**

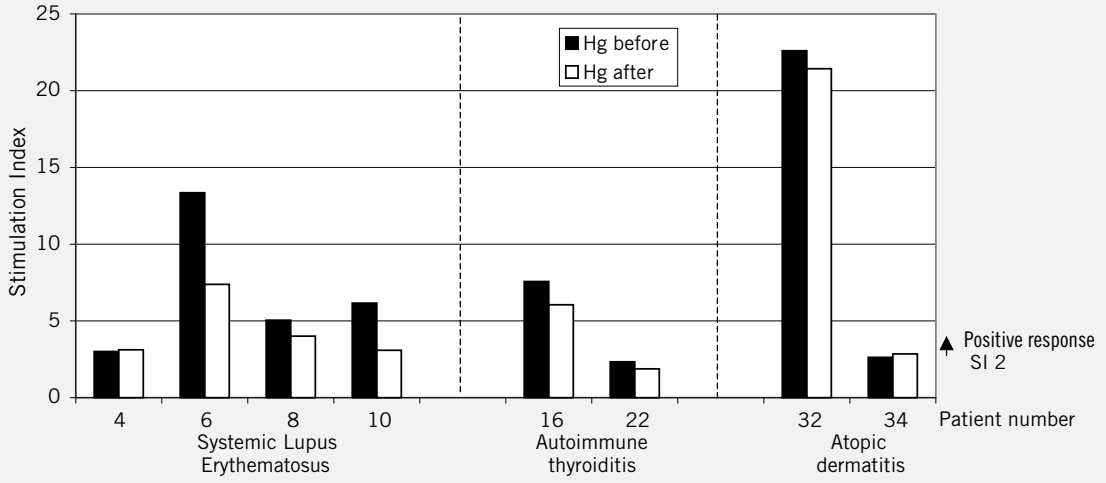


**Fig 2b. Lymphocyte responses to phenylmercury in patients who did not improve after amalgam replacement**

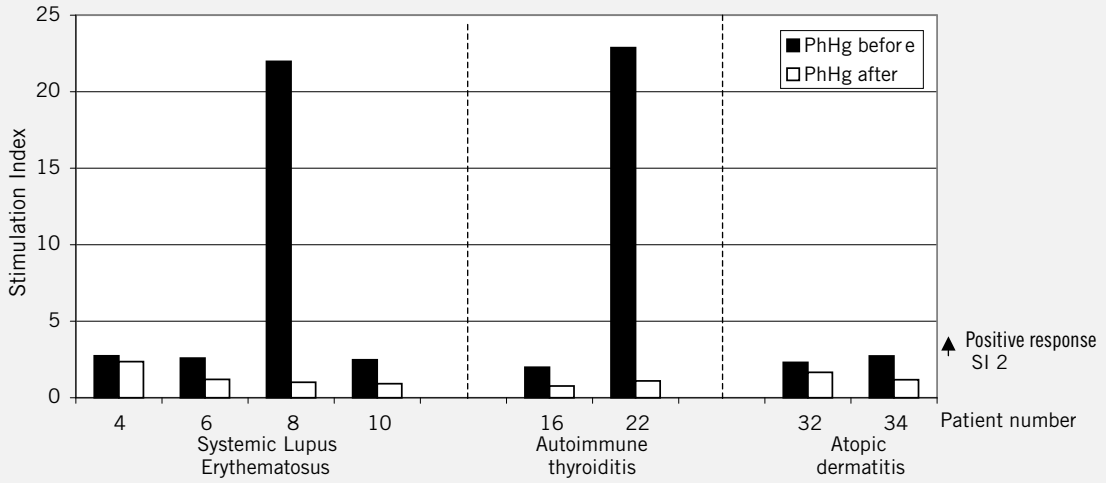


**Fig 2c. Lymphocyte responses to nickel in patients who did not improve after amalgam replacement**

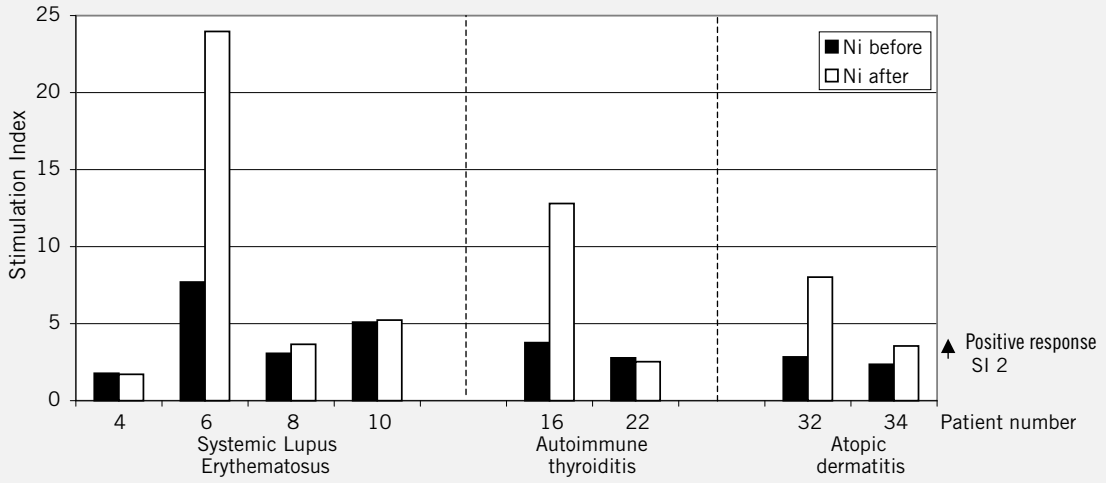


**Fig 2a.** Lymphocyte responses to inorganic mercury in patients who did not improve after amalgam replacement
**Fig 2b.** Lymphocyte responses to phenylmercury in patients who did not improve after amalgam replacement
**Fig 2c.** Lymphocyte responses to nickel in patients who did not improve after amalgam replacement

**Table 2:** Results of MELISA® (mean values of Stimulation Index) prior and after amalgam replacement and significance values (P)

| Metal | SI prior replacement | SI after replacement | P |
|---|---|---|---|
| Ag | 1,6 | 0,7 | 0,012 |
| Al | 2,3 | 1,8 | 0,600 |
| Au | 2,7 | 1,9 | 0,700 |
| Cd | 2,9 | 2,0 | 0,09 |
| Co | 1,3 | 0,7 | 0,023 |
| Cr | 1,1 | 0,8 | 0,14 |
| Cu | 0,9 | 0,8 | 0,68 |
| Fe | 2,0 | 1,2 | 0,19 |
| Inorganic Hg | 10,7 | 4,1 | 0,001 |
| Phenyl Hg | 4,1 | 1,7 | 0,001 |
| Mo | 3,6 | 2,8 | 0,61 |
| Ni | 7,1 | 6,2 | 0,75 |
| Pb | 3,0 | 1,2 | 0,018 |
| Pd | 1,5 | 2,1 | 0,289 |
| Pt | 0,9 | 1,2 | 0,321 |
| Sn | 2,2 | 1,6 | 0,305 |
| Ti | 1,7 | 1,4 | 0,614 |
| Positive control | 97 | 98 | 0,995 |
| Negative control (1) | 3713 | 1277 | 0,668 |

Foot note:
1) Value measured in counts per minute

## Results

The mean lymphocyte proliferation responses from 35 patients with autoimmunity before and after removal of metal fillings are shown in Table 2. Lymphocyte responses decreased significantly to the following metals: silver (P=0,027), inorganic mercury (P=0,009), phenylmercury (P= 0,031) and lead (P = 0,034). In total, 25 patients out of 35 studied (71%) showed health improvement after amalgam replacement, both by subjective reporting and by laboratory findings (Table 1). Two patients reported subjective improvement but no change in objective findings. The health of four patients was unaffected by amalgam replacement while the conditions of other three patients worsened. The patients who felt worse after amalgam replacement usually underwent more dental sessions due to a higher number of amalgam fillings.

The effect of amalgam removal on the individual responses of patients to inorganic mercury, phenylmercury and nickel is shown in Fig.1 and Fig.2. Fig.1 illustrates lymphocyte reactivity of patients whose health improved after amalgam replacement (responders) while Fig.2 shows the lymphocyte responses of patients with unchanged or worsening of health (non-responders). All but one patient (MS patient no.25) with improved health exhibited high levels of reactivity to inorganic mercury prior to amalgam replacement (Fig.1a and 2a). The mean SI value of the responder group was 15,4 compared to the mean SI value of 7,8 in a non-responder group. Lymphocyte responses to inorganic mercury were dramatically reduced in all patients with health improvement (Fig.1a).

One female SLE patient (no.14) reacted with SI of 83 at the start of the study (delta cpm 134 740) while follow up MELISA® showed SI of 4,4 (delta cpm 2189). The other patient with extremely high initial value to inorganic mercury was a female eczema patient (no.35) with initial SI of 53 (delta cpm122 936) and follow up SI values of 9,2 (delta cpm 1822). In the non-responder group only two patients exhibited strong proliferation to mercury prior amalgam replacement; one SLE patient (no.6) and another one with eczema (no.32). Interestingly, both patients retained strong mercury-specific reactivity even after amalgam removal (Fig.2a). The same was true for five other patients from the non-responder group. Most of lymphocyte responses were low and were not significantly altered by amalgam removal.

In contrast to inorganic mercury, the organic form of mercury, phenylmercury, did not induce strong lymphocyte responses in the majority of the patients (Fig.1b and Fig.2b). In the responder group, one of the few patients who exhibited strong reactivity to phenylmercury was a female patient with thyroiditis (no.21). Her reactivity diminished after amalgam replacement from SI 23 (delta cpm 799 973) to SI 1,3 (delta cpm 1772) and so did her reactivity to inorganic mercury. In the non-responder group, two patients, one female with SLE (no.8) and one male with AT (no.22) reacted strongly to phenylmercury prior to amalgam replacement but not after (Fig 2b). The responses of the remaining patients were low, in the range of SI 2 – SI 3.

When *in vitro* reactivity to the most common sensitizer, nickel, was compared between the responder and non-responder groups, 19 out of 27 patients (70%) alternatively 7 patients out of 8 (88%) reacted to nickel at the start of the study (Fig.1c and Fig.2c). While nickel-specific stimulation in responders usually remained unchanged or decreased after amalgam replacement, nickel-specific reactivity strongly increased in some patients from the non-responder group. In a female patient with SLE and strong reactivity to mercury, the reactivity to nickel at the beginning of the study was moderate (SI 7,7 and delta cpm 7 330). After amalgam replacement, the reactivity to nickel increased (SI 24, delta cpm 11 875). Another patient from the non-responder group, a woman with AT and health worsening following amalgam removal, showed increased sensitivity to nickel after replacement as well. Her SI prior replacement was 3,8 and after 13 (delta cpm739 and 15 585).

## Discussion

This study shows the beneficial effect of amalgam replacement on the health of autoimmune patients with lymphocyte reactivity to low concentrations of inorganic mercury. Many patients showed short-term aggravation of symptoms 1–2 days after amalgam replacement. Since patients were selected into the study

on the basis of lymphocyte reactivity to mercury, this reaction can be explained by systemic delayed type mercury hypersensitivity. To our knowledge this is the first time when a specific biomarker of mercury sensitivity was used for the selection of patients for amalgam replacement. It is important to realize that any risk factor maybe diluted if evaluated in heterogeneous population. As suggested by Weiss [29], analyses based on clinical markers of susceptibility (phenotypic markers) may be suitable for elucidation of causal pathways, identification of specific environmental risk factors and elimination of methodical flaws which exist in some studies concerning health risks of amalgam [30].

One of the important factors for a beneficial outcome of amalgam replacement seems to be an initially strong reactivity of lymphocytes to low concentrations of inorganic mercury (0, 5 $\mu$g of $HgCl_2$ per ml and $1x10^6$ lymphocytes). Low concentrations of mercury have been found to activate lymphocytes of patients with oral lichen adjacent to amalgam fillings. In addition to oral problems, patients also suffered from arthralgia, myalgia, eczema, diabetes and chronic malaise. Under these conditions, lymphocytes from healthy amalgam bearers and from amalgam-free subjects were not activated [23]. Inorganic mercury is the second most common sensitizer in patients with central nervous symptoms and suspected intoxication from dental amalgam [31], in patients with CFS syndrome [7] and in a group of patients with Hashimoto thyroiditis [12]. As mentioned previously, thimerosal, an organic mercury compound, ranks as the most frequent allergen in children and adolescent men, probably due to repeated exposure to thimerosal-containing vaccines in the past [11,32]. Positive patch test to inorganic mercury is also frequent in dermatological praxis [33–34].

One important observation in this study is a decrease of lymphocyte reactivity to inorganic mercury half a year after amalgam removal in patients with health improvement but not in patients with unchanged or worsened health. All patients with multiple sclerosis showed improved health and reduction of mercury-specific reactivity *in vitro*. All patients with thyroiditis who improved showed a decrease of mercury-induced activity to half or less of original SI value.

The non-responder group, whose health was unchanged or worsened after amalgam replacement, consisted of eight patients, four with systemic lupus, two with thyroiditis and the remaining two with atopic eczema. Only two patients from this group (25%) responded strongly to inorganic mercury *in vitro* prior to amalgam replacement as compared to 17 out of 27 (63%) of the patients in the responder group. In the majority of patients, lymphocyte responses to inorganic mercury were low and did not change significantly after amalgam replacement.

With regards to smoking habits, all patients with worsened health after amalgam replacement were smokers. As reported previously, nickel, mercury, cadmium and lead are present in cigarette smoke [35]. Some patients started smoking again after the initial health improvement and this could unfavorably affect their health. Patient no.32 had been a heavy smoker and the persisting reactivity to mercury which was not affected by amalgam removal could be possibly explained by continuous exposure to mercury from the cigarettes. Another source of nickel exposure, often not discussed, is that nickel may be present as an impurity in certain amalgams, so called copper amalgams. Nickel is the most common metal allergen [36–37] and its ubiquitous presence makes complete avoidance difficult. The beneficial effect of a nickel-free diet on the health of patients with chronic fatigue syndrome has been reported (38).

It has been postulated that chronic metal-driven inflammation may through cytokine release up regulate the hypothalamus-pituitary-adrenal axis (HPA-axis) resulting in unspecific multi-symptoms such as chronic fatigue, depression, sleep disturbances and other psychosomatic symptoms [39]. The reduction of mercury-specific responses after amalgam replacement has been published previously in patients with autoimmune thyroiditis [12], and in patients with chronic fatigue syndrome [7]. Similarly, Valentine-Thon and Schiwara demonstrated marked reduction of lymphocyte reactivity to titanium dioxide in patients with abnormal fatigue who used titanium-containing cosmetics on a daily basis [25]. Avoidance of exposure resulted in disappearance of fatigue and reduction of lymphocyte reactivity to titanium *in vitro*. Thus, the decrease of the exposure to metals in susceptible patients may result in down-regulation of inflammation and reduced symptomatology. In this respect, metal-induced inflammation resembles the inflammation caused by bacteria, viruses and other allergens. It is known that patients with metal sensitivity may experience influenza-like symptoms following the dental treatment.

In contrast to strong lymphocyte responses to inorganic mercury, only a few patients responded to phenylmercury, an organic form containing a benzene ring. Phenylmercury is used as a preservative in ointments, cosmetics and lotions. In the dental praxis, phenylmercury is present in root filling materials, such as N2 ("Nerve second"), together with arsenic and lead. In Sweden, N2 is banned since several years but is still illegally used by some dentists. Inorganic mercury and phenylmercury are structurally different and are therefore both used in patch testing. The same is true for thimerosal. The marked reduction of phenylmercury-specific responses in some patients suggests decreased exposure, possibly through avoidance of phenylmercury-containing pharmaceutical products.

No significant health risks of mercury in an unselected population were described in a report written on behalf of the European Union (40). However, such risks may become apparent when the effect of mercury exposure is studied in susceptible groups, such as children and patients with autoimmunity. This article will hopefully create an interest for larger studies on metal-susceptible populations in the future.

## Conclusion

This is the first time when the selection of patients for amalgam removal was based on initial sensitivity to inorganic mercury at lymphocyte level as measured by the MELISA® test. The results show that mercury-containing amalgam may be an important risk factor for patients with autoimmune diseases who are sensitized to mercury. Hence, the removal of amalgam fillings is a useful complementary treatment for such patients. Nickel sensitivity was found to be another risk factor that may negatively affect the chance of regaining health.

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REFERENCES

1 Weening JJ, Fleuren GJ, Hoedemaeker PJ. Demonstration of antinuclear antibodies in mercuric chloride – induced glomerulopathy in the rat. Lab Invest 1978; **39**:405–11.
2 Druet E, Sapin C, Gunther E, Feingold N, Druet P. Mercuric chloride-induced anti-glomerular basement membrane antibodies in the rat: genetic control. Eur J Immunol 1977; **7**:348–51.
3 Balasz T. Immunogenetically controlled autoimmune reactions induced by mercury, gold and d-penicillamine in laboratory animals: a review from the vantage point of premarketing safety studies. Toxicol Industrial Health 1987; **3**:331–336.
4 Bigazzi PE. Autoimmunity induced by metals. In: Chang LW, editor. Toxicology of metals. Boca Raton, FL: CRC Lewis Publishers; 1996. p. 835–52.
5 Pollard KM, Pearson DL, Hultman P, Deane TN, Lindh U, Kono DH. Xenobiotic acceleration of idiopathic systemic autoimmunity in lupus-prone mice. Environ. Health Perspect 2001; **109**:27–33.
6 Silbergeld E, Silva I, Nguyen P, Hoover D, Lustberg M, Via C. Interactions between mercury and experimental autoimmune disease [Abstract]. Autoimmunity Rev 2002; **1**:5.
7 Stejskal VDM, Danersund A, Lindvall A, Hudecek R, Nordman V, Yaqob A et al. Metal-specific lymphocytes: biomarkers of sensitivity in man. Neuroendocrinol Lett 1999;**20**:289–298
8 El-Fawal H, Waterman SJ, De Feo A, Shamy MY. Neuroimmunotoxicology: humoral assessment of neurotoxicity and autoimmune mechanisms. Environ Health Perspect 1999; **107**:767–75.
9 Silva I, Gorman A, Perisse A, Ventura AM, Santos ECO, Burek L et al. Mercury exposures and prevalence of serum autoantibodies ANA/ANOA in Amazon populations in Brazil [Abstract]. Autoimmunity Rev 2002; **1**:5.
10 Bartova J, Prochazkova J, Kratka Z, Benetkova K, Venclikova Z, Sterzl I. Dental amalgam as one of the risk factors in autoimmune diseases. Neuroendocrinol Lett 2003; **24**:65–7.
11 Wantke F, Hemmer W. Patch test reactions in children, adults and elderly. A comparative study in patients with suspected allergic contact dermatitis. Cont Derm 1996; **34**:316–19.
12 Sterzl I, Prochazkova J, Hrda P, Bartova J, Matucha P, Stejskal VDM. Mercury and nickel allergy: risk factors in fatigue and autoimmunity. Neuroendocrinol Lett 1999; **20**:221–8.
13 Bruze M, Edman B, Björkner B, Möller H. Clinical relevance of contact allergy to gold sodium thiosulfate. J Am Acad Dermatol 1994; **31**:579–583.
14 Ahlgren C, Ahnlide I, Björkner B, Bruze M, Moller H, Liedholm etal. Gold allergy and dental gold alloys [Abstract]. 5th Congress of the European Society of Contact Dermatitis, Amsterdam. 2000.
15 Svensson A, Möller H, Björkner B, Bruze M, Leden I, Theander J, et al. Rheumatoid arthritis, gold therapy, contact allergy and blood cytokines. BMC Dermatol 2002; **2**:2.
16 Newman LS. Significance of the blood beryllium lymphocyte proliferation test. Environ Health Perspect 1996; **104**(suppl 5):953–6.
17 Siblerud RL, Kienholz E. Evidence that mercury from silver dental fillings may be an etiologic factor in multiple sclerosis. Sci Total Environ 1994; **142**:191–205.
18 Ely JT. Mercury induced Alzheimer's disease: Accelerating incidence? Bull Environ Contam Toxicol 2001; **67**:800–6.
19 Pendergrass JC, Haley BE. Inhibition of brain tubulin-guanosine 5'-triphosphate interactions by mercury: similarity to observations in Alzheimer diseased brain. Metal Ions Biol Syst 1997; **34**:461–78.
20 Podzimek S, Prochazkova J, Pribylova L, Bartova J, Ulcova-Gallova Z, Mrklas L et al. Vliv tezkych kovu na imunitni reakci u pacientu s prokazanou neplodnosti. (Effect of heavy metals on immune reactions in patients with infertility) (In Czech with English abstract). CLC 2003; **142**:285–8.
21 Kapsenberg ML, Res P, Bos DJ, Schootemijer A, Teunissen MB, van Schooten W. Nickel-specific T lymphocyte clones derived from allergic nickel-contact dermatitis lesions in man: heterogeneity based on requirement of dendritic antigen-presenting cell subsets. Eur J Immunol 1987; **7**:861–5.
22 Stejskal VDM, Cederbrandt K, Lindvall A, Forsbeck M. MELISA–an in vitro tool for the study of metal allergy. Toxicol in vitro 1994; **5**: 991–1000.
23 Stejskal VDM, Forsbeck M, Cederbrandt K, Asteman O. Mercury-specific lymphocytes: an indication of mercury allergy in man. J Clin Immunol 1996; **16**:31–40.
24 Büdinger L, Hertl M. Immunologic mechanisms in hypersensitivity reactions to metal ions: an overview. Allergy 2000; **55**:108–15.
25 Valentine-Thon E, Schiwara HW. Validity of MELISA® for metal sensitivity testing. Neuroendocrinol Lett 2003; **24**:49–55.
26 Ionescu G. Schwermetallbelastung bei atopisher Dermatitis und Psoriasis-Diagnose und Therapie. (Heavy metal load in atopic dermatitis and psoriasis-Diagnosis and therapy) (In German with English abstract). Biol Med 1996; **2**:65–8.
27 Kohdera T, Koh N, Koh R. Antigen-specific lymphocyte stimulation test in patients with psoriasis vulgaris. [Abstract]. XVI International Congress of Allergology and Clinical Immunology, October 19–24, 1997, Cancoon, Mexico. 1997.
28 Hudecek R, Danersund A, Kinigalakis G, Lindvall A. Experience of medical and odontological treatment: removal of incompatible dental material (RID) in patients with intolerance to dental materials. In: Amalgam and health–new perspective on risks. Report from Conference of Swedish Council for planning and coordination of research, 14 November 1997, Stockholm, Sweden. 1997.
29 Weiss NS, Liff JM. Accounting for the multi-causal nature of disease in the design and analysis of epidemiological studies. Am J Epidemiology 1983; **117**:14–8.
30 Mutter J, Naumann MD, Sadaghiani C, Walach H, Drasch G. Amalgam studies: Disregarding basic principles of mercury toxicity. Int J Indust Hyg Environ Health. In press.
31 Tibbling L, Thuomas KA, Lenkei R, Stejskal VDM. Immunological and brain MRI changes in patients with suspected metal intoxication. Int J Occup Med Toxicol 1995; **4**:112–20.
32 Hansson H, Möller H. Patch test reactions to merthiolate in healthy young subjects. Br J Dermatol 1970; **83**:949–56.
33 Finne K, Göransson K, Winckler L. OLP and contact allergy to mercury. Int J Oral Surg 1982; **11**:236–9.
34 Lundström IMC. Allergy and corrosion of dental materials in patients with OLP. Int J Oral Surg 1984; **13**:16–24.
35 Blaha K, Kasparova L, Cabelkova Z, Cikert M. Koureni jako zdroj expozice niklu, rtuti a manganu. (Smoking as a source of nickel, mercury and manganese intake) (In Czech with English abstract). Czech. Hyg 1989; **24**:103–10.
36 Marzulli F, Maibach HI, editors. Dermatotoxicology. 4th ed. New York, Hemisphere Publishing Corporation; 1991.
37 Veinen NK, Menné T, Maibach HI. Systemically induced allergic contact dermatitis. In: Menné T, Maibach HI, editors. Exogenous Dermatoses: Environmental Dermatitis. CRC Press; 1991. p.267–82.
38 Regland B, Zachrisson O, Stejskal V, Gottfries CG. Nickel allergy is found in a majority of women with chronic fatigue syndrome and muscle pain – and may be triggered by cigarette smoke and dietary nickel intake. J Chronic Fatigue Syndrome 2001; **8**:57–65.
39 Stejskal J, Stejskal VDM. The role of metals in autoimmunity and the link to neuroendocrinology. Neuroendocrinol Lett 1999; **20**: 351–64.
40 Floyd P, Crane M, Tarkovski S, Bencko V. Risks to health and the environment related to the use of mercury products. Final report prepared for The European Commission, DG Enterprise, Risk & Policy Analysts Limited, Farthing Green House, Norfolk, UK, 2002.