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ORIGINAL ARTICLE

Continuing the quest for autoimmunity due to oral metal exposure

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Abstract

Aim: The role of metal exposure in the development of autoimmune disease (AID) is still controversial. Here, we studied the relationship between oral metal exposure, metal allergy and autoimmunity. *Methods*: A mixed population ($n = 78$) of non-allergic volunteers, metal-allergic patients and patients with oral problems putatively due to metal alloys was evaluated for oral Ni, Pd, Au and Hg exposure and skin hypersensitivity. Clinical autoimmune parameters were based on medical histories; additionally, serum levels of the four most common autoantibodies were measured. *Results*: Skin hypersensitivity, as seen mainly for Ni and/or Pd, was not positively associated with autoimmune parameters. In contrast, metal hypersensitive individuals showed an extremely low frequency of thyroid autoantibodies (3% vs 20% in non-hypersensitive controls). Next, the relation between metal exposure and autoimmunity was evaluated in individuals >35 years ($n = 58$), since from that age on metal exposure had plateaued and was not correlated with age. In this subgroup, oral Ni exposure was associated ($p < 0.01$) with self-reported AID, irrespective of autoantibody levels. These unexpected findings warrant further confirmation in a larger test group. Of note, oral Pd, Au or Hg contacts were not associated with any of the clinical or serological autoimmune phenomena tested. *Conclusion*: The results of this study support the view that development of metal contact allergies may prevent autoimmune activation, and, second, that oral exposure to Pd, Au or Hg does not facilitate the development of AID.

Keywords

Amalgam, autoimmunity, allergy, gold, nickel, oral metal exposure, palladium

History

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Introduction

Oral exposure to dental alloys is associated with both local and systemic adverse reactions [1]. The release of metal ions from such alloys has convincingly been demonstrated. Depending on their concentration, these metals can have toxic inflammatory, allergenic or even mutagenic effects [2]. Recently, distinct pathways for immune activation by orally applied transition metals were unraveled: nickel (Ni), cobalt (Co) and palladium (Pd) were shown to directly bind to the endotoxin receptor TLR4 and Au to TLR3, thereby on one-hand triggering maturation of antigen presenting cells and on the other hand initiating immune reactions via the production of pro-inflammatory mediators such as IL-8 [3–6]. For other metals such as mercury (Hg), the mechanism of activating dendritic cells is still under investigation. Whether the capacity of distinct transition metals to trigger the innate immune system might facilitate the development of metal-specific allergy or immune responses to simultaneously presented (auto) antigens, remains an intriguing question. Here, we focus on the putative association between oral exposure to dental alloys and the presence of autoimmunity.

Both genetic and environmental factors play a role in the pathogenesis of AID. Metal exposure could be considered as one of the latter, but robust pathogenesis studies have mainly been performed in animal models. In rodents, exposure to high concentrations of heavy metals such as mercury, silver and gold resulted in proliferation of B and T cells, in increased levels of IgG1 and IgE and more specifically in anti-nucleolar antibody production [7–10]. More recently, also Ni was suggested to induce autoantibodies as well as clinical signs of cutaneous sclerosis in rodents [11]. In humans, much controversy still exists about the role of metal exposure in the development of autoimmunity. The majority of reports derive from the group of Stejskal et al. [12–14] relating *in vitro* metal responsiveness to systemic diseases with unknown etiology; Hg and Ni exposure were thus considered as potential risk factors for fatigue and auto-immunity [13]. A pathogenic role of amalgam exposure could, however, not be confirmed in a meta-analysis on multiple sclerosis [15]. Still, some reports provide indirect support for metal-induced autoimmune disease (AID), e.g. the

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removal of amalgam fillings would reduce the levels of thyroid antibodies [16] and urinary Pd levels were found to be associated with thyroid disease [17].

This study focuses on clinical and serological parameters for AID in relation to oral metal exposure in non-allergic and metal allergic individuals, as well as in patients with oral lesions attributed to dental restorations. Metal exposure, anamnesis and immune responsiveness to metals were evaluated extensively in these individuals in previous reports [18–20]. Hence, this study now tested, in conjunction to the clinical anamnestic data, objective and early parameters for autoimmunity, such as autoantibodies associated with connective tissue disease, rheumatoid arthritis and thyroid autoimmunity. Based on the literature, it was chosen to focus on Ni, Pd, Au and Hg exposure, thereby addressing the following questions: (a) Are autoimmune manifestations more frequent in oral metal-exposed individuals? (b) Are autoimmune manifestations more frequent in metal hypersensitive individuals? and (c) Do metals differ in these respects?

Materials and methods

Patients

The investigated population ($n = 78$) consisted of 26 volunteers without a history of metal allergy, recruited from the university campus, 26 metal allergic patients attending the Dermatology Department at VUmc with a positive history of metal allergy, and 26 patients with oral lesions possibly due to their dental restorations, referred to the adverse reaction unit of ACTA. From all the participants, a medical history was taken, oral examination was carried out, the number and composition of all the dental alloys were determined and skin test responsiveness to metals was evaluated [18–20]. Skin tests at the time of inclusion showed a positive reaction to at least one metal salt in 4/26 non-allergic, in 22/26 metal allergic [18] and in 8/26 patients with oral complaints [20]. The study was approved by the medical ethical committee of VUmc and ACTA. All the participants gave written informed consent.

Oral metal exposure

Dental alloys were categorized into palladium (Pd), nickel (Ni), mercury (Hg) and gold (Au)-based alloys as evaluated by Scanning Electron Microscopy-Energy-Dispersive X-ray spectroscopy analysis. Au-based alloys consisted of at least 45 atom percentage (At%) Au, and not more than 20 At% of Pd. The Pd-based alloys consisted of 30–90 At% Pd and are alloyed mainly with Au (<45 At%), copper (Cu) (15–30 At%) or silver (Ag) (15–55 At%) [20]. Further scoring at the moment of evaluation was based on numbers of Au- or Pd-based crowns and Hg (amalgam) fillings, and on the absence or presence of Ni-containing devices, specified as gingival or non-gingival oral Ni contacts. Thus, oral Ni exposure was found in 18, Pd in 35, Au in 37 and Hg in 53 of the 78 individuals.

Autoantibody assessment

Serum samples from all the 78 individuals were collected before skin testing and stored at −20 °C until analysis. All the serological assays were performed in a certified diagnostic laboratory (based on EN/ISO 15189) according to standard operating procedures and included positive and negative controls.

Anti-nuclear antibodies (ANA) were tested on Hep-2000 cells (Sacramento, CA; 1:40 diluted, resulting in a relatively sensitive ANA evaluation; titers exceeding 1:80 are considered clinically relevant), by indirect immunofluorescence according to the manufacturer's guidelines. The intensity of nuclear fluorescence (−, ±, +, ++, +++), as well as the staining pattern (h: homogeneous, n: nucleolar, s: speckled) were scored independently by two observers. Positive ANA's were additionally specified for reactivity to the antigens dsDNA, SS-A, SS-B, U1RNP, CenpB, Sm, Jo-1 and Scl70, using a Phadia 250 of Thermo Fisher Scientific , Freiburg, Germany [21]. The three sera with strong positive ANA staining (1 + ++s and 2 + ++ns) were additionally tested in a systemic sclerosis blot (Euroimmun, Lübeck, Germany), including fibrillarin as antigen.

Antibodies to cyclic citrullinated peptides (CCPs) were measured with a commercial EliA CCP (Thermo Fisher Scientific , Freiburg, Germany) according to the manufacturer's instructions. Levels > 10 U/ml were considered clinically relevant.

Rheumatoid factors (IgM-RF) were determined by a routinely used enzyme-linked immunosorbent assay (ELISA), using heat-aggregated rabbit IgG as antigen. Results were expressed as IU/ml and levels > 5 IU/ml were considered clinically relevant.

Thyroid peroxidase (TPO) antibodies [22] were measured with the IMMULITE 1000 (Siemens, Los Angeles, CA) according to the manufacturer's instructions. Results were expressed in IU/ml; levels > 50 IU/ml were considered clinically relevant.

Clinical AI-related parameters

Medical histories were taken by one of the clinicians [18–20]. Clinical potentially AI-related parameters (joint/muscle pain, dry mouth and pre-existing AID, such as thyroid dysfunction, psoriasis, T1DM, connective tissue disease or MS) were based on this anamnesis.

Data analysis

Categorical data were analyzed by using Fischer's Exact test. For non-parametric (semi) quantitative data, the Rank correlation test (Spearman's rank correlation) was used to evaluate potential correlations and the Wilcoxon test for comparison of groups. All statistics were performed by the MedCalc software (Mariakerke, Belgium). $p \leq 0.05$ was considered statistically significant.

Results

In 38 (49%) of the 78 individuals, one or more of the clinical AI-related parameters (joint/muscle pain, dry mouth or presence of AID) were reported. Additionally, as a more objective parameter for AI, serum levels of the most common autoantibodies were assessed, i.e. anti-nuclear antibodies (ANA) associated with connective tissue disease and

Table 1. Frequency of clinical and serological autoimmune parameters in relation to metal hypersensitivity.

	Metal skin test negative ($n=44$)	p^b Value	Metal skin test positive[a] ($n=34$)
Characteristics			
Age, median (95% CI)	48 (40–57)		47 (43–52)
Gender (female/male)	29/15	0.005	32/2
AID-related parameters			
AID[c]	9 (20%)		5 (15%)
AID types	2×CTD, Ps, T1DM, 5×TD		2×CTD, MS, 2×TD
Joint and/or muscle pain	11 (25%)		12 (35%)
Dry mouth	12 (27%)		13 (38%)
ANA ≥ 1:80	3 (7%)		3 (9%)
ANA patterns[d]	h, n/s, s		h, 2×n/s
Rheumatoid factors ≥ 5 IU/ml	0 (0%)		0 (0%)
Anti-citrullinated peptides ≥ 10 U/ml	0 (0%)		0 (0%)
Anti-TPO ≥ 50 IU/ml	9 (20%)	0.04	1 (3%)

[a]Positive skin test reaction to at least one of a panel metal salts at the moment of inclusion [18,19].
[b]p Values ≤ 0.05 (Fisher's Exact test) are given for the comparison between metal skin test positive vs negative individuals.
[c]Self-reported AI diseases; CTD, connective tissue disease; MS, multiple sclerosis; Ps, psoriasis; TD, thyroid dysfunction; T1DM, type 1 diabetes mellitus.
[d]ANA patterns recorded: h, homogeneous; n, nucleolar; s, speckeled.

anti-thyroid peroxidase (TPO) associated with thyroid autoimmunity. Autoantibodies to rheumatoid factors (RFs) and anti-citrullinated peptides (anti-CCP) were not detectable in clinically relevant concentrations in any of the individuals. Of note, autoantibodies do not always reflect overt AID, but may still be considered as early, additional signs of autoimmunity. Although some individuals with positive clinical AI parameters did show high levels of antibodies, there was no overall correlation between the presence of autoantibodies and the evaluated clinical symptoms. So, for analytical purposes, clinical and serological AI symptoms were summarized per individual. In total, 43/78 individuals showed 1 or more and 24/78 individuals showed 2 or more positive AI-related parameters (data not shown).

To investigate whether metal hypersensitivity would be associated with autoimmunity, the 78 individuals were divided according to metal skin test reactivity. Thirty-four individuals showed a positive reaction (≥ +) to at least one out of a panel of metal salts: 25 to Ni, 26 to Pd (20 to both Ni and Pd), 0 to Au and 3 to Hg salts [18]. Table 1 summarizes the characteristics, as well as the AI-related parameters in skin test positive and negative individuals. Age was comparable in the two groups, but females were overrepresented in the group with metal hypersensitivity. None of the single or combined AI parameters were increased in the hypersensitive group. On the contrary, TPO antibodies were relatively rare in the metal hypersensitive group (3% vs 20% in the non-hypersensitive individuals; Fischer's exact test $p<0.05$) and were present in significantly lower titers (Figure 1). Of the 20 patients with strong (++ or +++) Ni or Pd hypersensitivity, none showed clinically relevant anti-TPO levels (Figure 1b and c).

Subsequently, data were examined from a metal exposure point of view. Hence, all the 78 individuals were re-ordered according to oral metal exposure via dental devices, crowns and fillings. The number of Pd- and Au-based crowns, as well as the number of Hg fillings increased significantly with age (Figure 2), but oral metal exposure was not related to gender (data not shown). Therefore, to avoid a bias when evaluating AI-related parameters, which are known to be more frequent in elderly people, and indeed also in our group (Spearman's $r=0.36$; $p<0.01$) (data not shown), we evaluated only patients >35 years ($n=58$) (Figure 3), since from that age on metal exposure plateaued and was not significantly correlated with age. Table 2 shows that oral Pd, Au or Hg exposure (≥1 Pd- or Au-based crowns or amalgam fillings) were not significantly associated with the presence of any of the AI-related parameters, though exposure to Pd tended to be associated with a dry mouth (Fischer's Exact test $p=0.1$). Also, when evaluating, more quantitatively, the number of crowns or fillings in relation to the number of positive AI-related parameters present in an individual, no significant correlations were found. In contrast, oral Ni exposure was significantly associated with the presence of AID (three thyroid dysfunction, 1 T1DM, 1MS, 1RA; Fischer's Exact test $p=0.003$). Interestingly, this association became even stronger (Fischer's Exact test $p=0.0004$) when only the eight individuals with gingival Ni contacts were considered: six of these had reported to have an AID (data not shown). Oral Ni exposure was, however, not associated with any of the other clinical or serological AI-related parameters. Of note, the strongly positive ANAs ($n=3$), all from individuals with Hg exposure, were not positive for a set of systemic sclerosis-associated antibodies, including anti-fibrillarin.

Discussion

In the present study, we investigated the potential association between oral exposure to metals, metal hypersensitivity and the development of AID. We focused on oral Ni, Pd, Au and Hg exposure in a mixed population of non-allergic volunteers, metal allergic individuals and patients with oral complaints putatively due to orally applied dental alloys. Although the evaluated AI-related symptoms and autoantibodies do not provide conclusive evidence for AID, they provided useful parameters for this study.

As expected, metal hypersensitivity was in the majority of cases (88%) caused by Ni and/or Pd, whereas no skin hypersensitivity to Au was recorded and Hg hypersensitivity was detectable only in three individuals. Although we did find

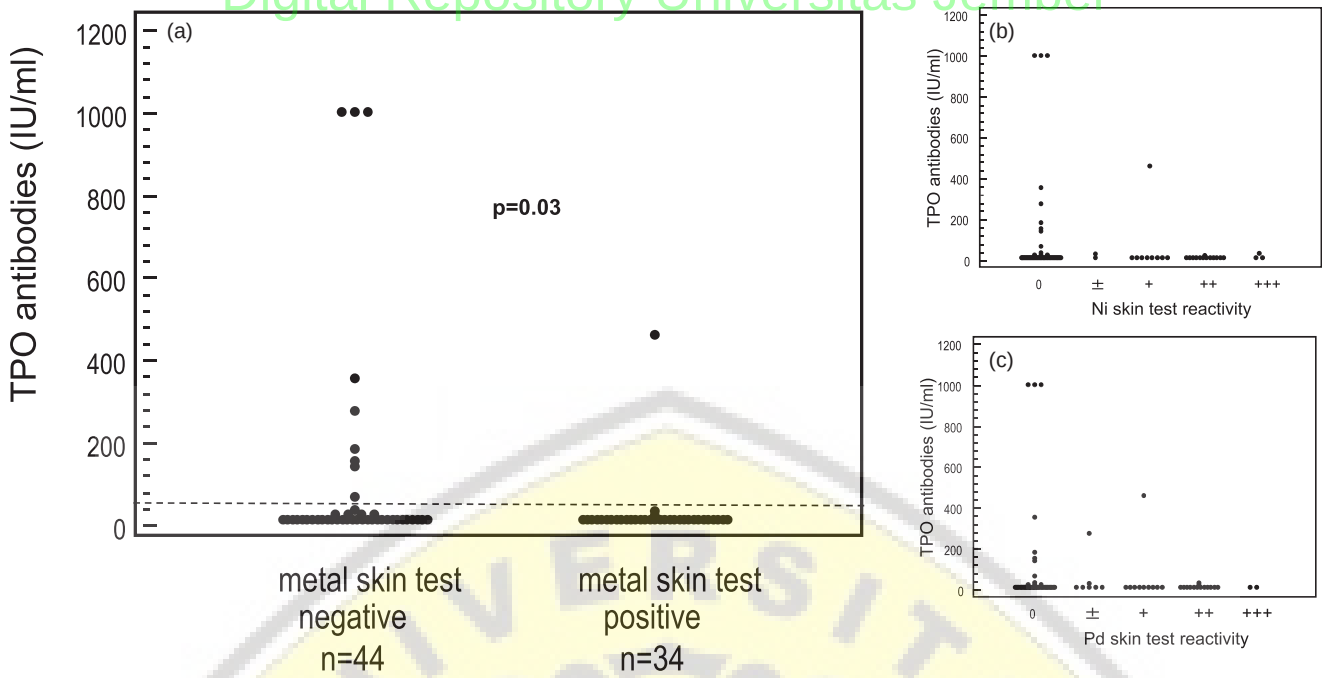
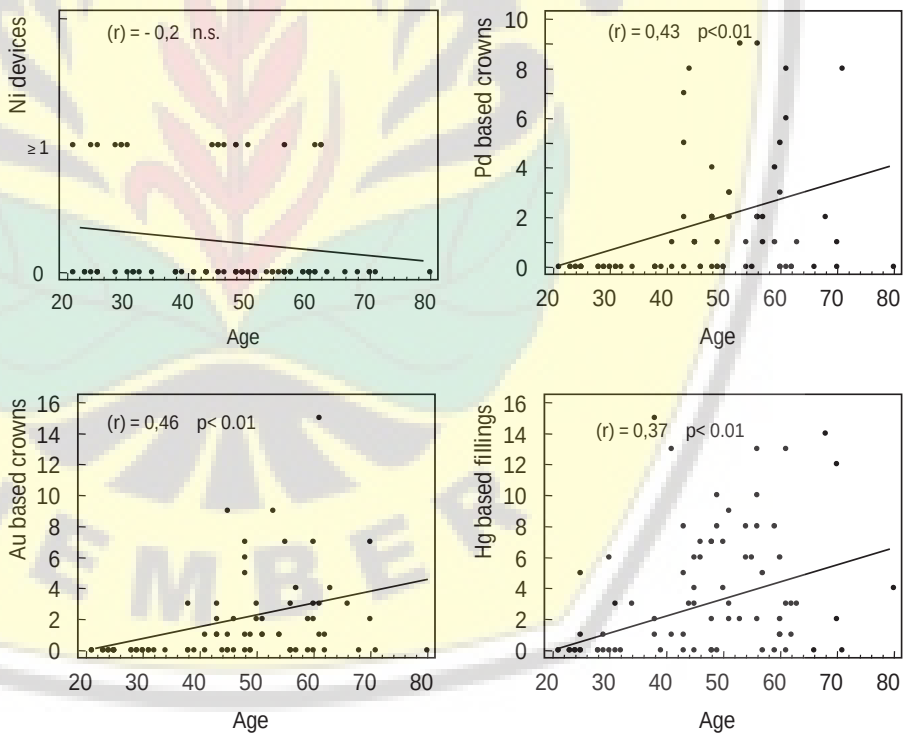


Figure 1. TPO antibodies in relation to metal hypersensitivity. TPO antibody levels (IU/ml) are given per patient ($n = 78$) in relation to metal skin test reactivity in general (i.e. to any metal out of a panel with Ni, Cr, Pd, Co, Au and Hg salts), as well as to Ni and Pd skin test reactivity separately. p Values < 0.05 (Wilcoxon test) are given. The dotted line represents the cut off for positivity of the TPO antibodies.

Figure 2. Oral metal exposure in relation to age. Metal exposure, i.e. the presence of Ni devices, the number of Pd- and Au-based crowns as well as the number of Hg-based amalgam fillings is shown per patient ($n = 78$) in relation to age. Spearman's rank correlation coefficients and p values are given if $p < 0.05$. n.s. indicates 'not significant'.



an association of Pd hypersensitivity with oral lesions, such as non-plaque-related gingivitis previously [20], no significant associations were found between Pd or Ni hypersensitivity and any of the single or combined autoimmune parameters. Interestingly, however, metal hypersensitive subjects displayed significantly lower levels of TPO antibodies, even though women – being more prone to thyroid autoimmunity than men – were overrepresented in the metal hypersensitive group. One might speculate that in hypersensitive individuals, strict avoidance of (skin) contact with the metal might prevent the development of TPO antibodies. Actually, very few studies reported on a positive or negative association between Ni or Pd allergy and AID. To our knowledge, the only human case control study in this context was performed by Jin et al. [23] showing higher levels of autoantibodies to metallothionein and hsp70 in metal allergic patients as compared to controls. Indirect evidence for an association between metal allergy and AID was also given by Hybenova et al. [24] who

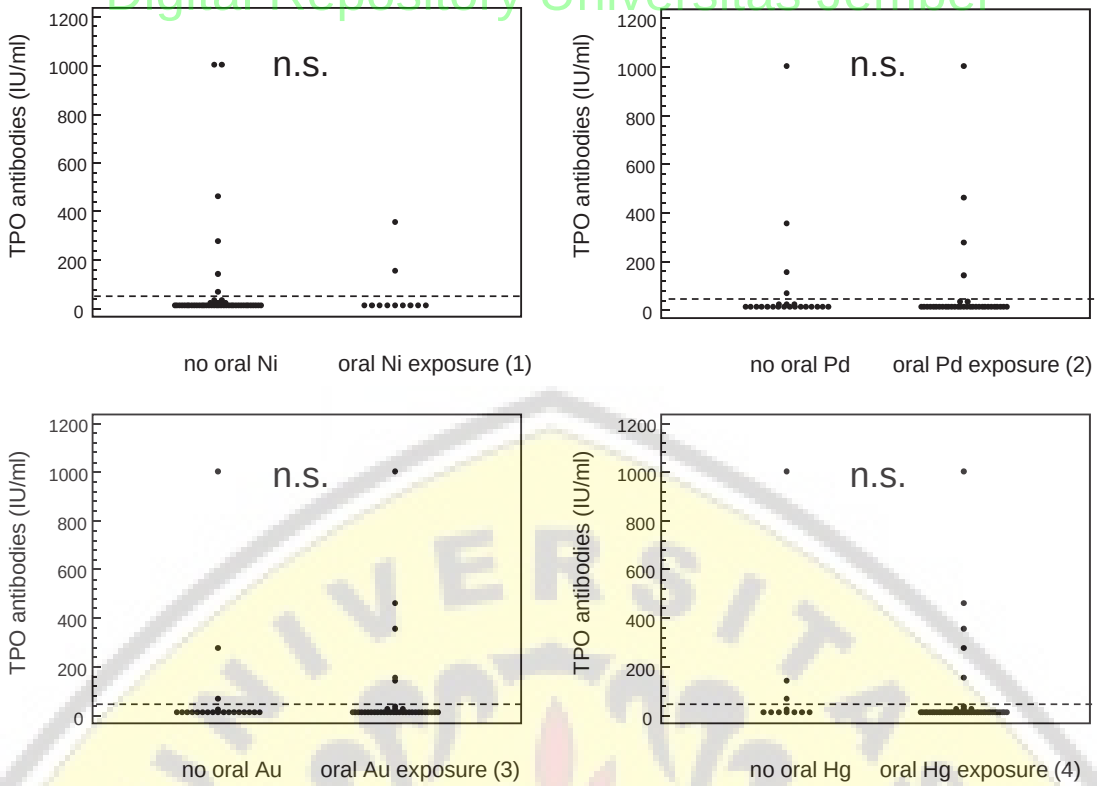


Figure 3. TPO antibodies in relation to oral metal exposure in individuals >35 years. TPO antibody levels (IU/ml) are given in 58 patients, aged > 35 years, in relation to oral Ni, Pd, Au or Hg exposure. p Values < 0.05 (Wilcoxon test) are given. n.s. indicates 'not significant'. The dotted line represents the cut off for positivity of the TPO antibodies. (1) Due to either gingival ($n = 8$) or non-gingival ($n = 3$) contacts. (2) Due to the presence of ≥1 Pd-based crowns. (3) Due to the presence of ≥1 Au-based crowns. (4) Due to the presence of ≥1 amalgam fillings. For numbers of metal-exposed individuals, see Table 2.

reported increased lymphocyte responsiveness to Ni and Hg in various AIDs, including thyroid AID. Data from experimental studies, showing increased levels of ANA in Ni chloride-treated rats, were less conclusive in this respect since exposure to Ni rather than a state of hypersensitivity was evaluated in that study [11]. Although sample sizes were relatively small, no increased ANA levels or other AI-related symptoms were found in metal hypersensitive individuals, arguing against a relation with metal allergy.

Still, in the absence of allergy, metal exposure *per se* could provide an important environmental factor in the development of autoimmune responses. We therefore evaluated the positivity of AI-related parameters in relation to oral metal exposure. Since Pd, Au and Hg exposure increased significantly with age, just like AI phenomena, we evaluated here the 58 individuals > 35 years, where exposure had plateaued and was no longer correlated to age. Although anamnesis-based AID was relatively infrequent (11/58 individuals), clinical symptoms often associated with AID, as well as the presence of serum autoantibodies were more frequent. Thus, taken these together, AI-related parameters scored positive in a substantial proportion of the test population (35/58 showed at least 1 and 22/58 at least 2 AI-related parameters).

Exposure to mercury in relation to AID has been studied extensively in the literature, both in experimental animals [8,11,25,26] and humans [27]. In men, long-term exposure to inorganic mercury was evaluated in Brazilian gold miners and was found to be associated with increased levels of ANA and systemic inflammatory cytokines [28] but no clinical AID symptoms were evaluated in that study. We did not find increased ANA levels upon exposure to amalgam, the most frequent source of Hg exposure in men, although the strongest ANA responses in our study were indeed found in amalgam-exposed individuals. Of note, none of these were directed to scleroderma-associated ANA antigens in a blot system. Neither did we find an association between Hg exposure and any of the other AI-related parameters. Although quite some reports in the literature show beneficial effects of the removal of amalgam fillings, including remission of lichenoid lesions [29] and reduction of anti-thyroid and anti-nuclear autoantibodies [30], no consensus for a pathogenic role of Hg in AID was reached [31–34]. Also, a meta-analysis on the relation between amalgam exposure and the most prominent AID in this context, multiple sclerosis, did not show a significant association [15].

Like mercury, gold was shown to induce ANA directed to the nucleolar protein fibrillarin in mice [35]. Despite the fact that about half of the individuals in our study were exposed to Au, no association with ANA was observed. Although most studies refer to the use of Au salts, Au ions can also be released from gold foils and taken up by macrophages [36]. Both salts and foils were shown to display distinct immunomodulatory effects on human dendritic cells and macrophage cell lines [6]. In addition, *in vivo* exposure to Au-based crowns was shown to be associated with oral lichenoid lesions in the present population [20]. Despite these

Table 2. Frequency of clinical and serological autoimmune parameters in relation to oral metal exposure in 58 individuals > 35 years.

	Oral Ni exposure[a]		Oral Pd exposure[b]		Oral Au exposure[c]		Oral Hg exposure[d]	
	− $n=47$	+ $n=11$	− $n=23$	+ $n=35$	− $n=21$	+ $n=37$	− $n=11$	+ $n=47$
Characteristics								
Age median (95% CI)	53 (38–80)	48 (44–62)	50 (38–80)	53 (41–71)	49 (38–80)	51 (38–70)	49 (39–71)	51 (38–80)
Gender (female/male)	36/11	8/3	16/7	28/7	15/6	29/8	10/1	34/13
AID-related parameters								
AI disease[e]	5 (11%)	6 (55%) $p=0.003$[f]	5 (22%)	6 (17%)	3 (14%)	8 (22%)	1 (9%)	10 (21%)
AID type	3×CTD 2×TD	CTD MS T1DM 3×TD	CTD T1DM 3xTD	3×CTD MS 2xTD	CTD 2×TD	3×CTD MS T1DM 3×TD	TD	4xCTD MS T1DM 4xTD
Joint/muscle pain	15 (32%)	6 (55%)	8 (35%)	13 (37%)	5 (24%)	16 (43%)	5 (45%)	16 (34%)
Dry mouth	18 (38%)	5 (45%)	6 (26%)	17 (49%) $p=0.1$[f]	9 (43%)	4 (38%)	5 (45%)	8 (38%)
ANA ≥ 1:80	3 (6%)	2 (18%)	3 (13%)	2 (6%)	2 (10%)	3 (8%)	2 (18%)	3 (6%)
ANA patterns[g]	h, n/s, s	h, n/s	h, 2xn/s	h, s	h, s	h, 2×n/s	n/s, s	2×h, n/s
Anti-TPO ≥ 50 IU/ml	6 (13%)	2 (18%)	4 (17%)	4 (11%)	3 (14%)	5 (14%)	3 (27%)	5 (11%)

[a]Due to either gingival ($n=8$) or non-gingival ($n=3$) contacts.
[b]Due to the presence of ≥1 Pd-based crowns.
[c]Due to the presence of ≥1 Au-based crowns.
[d]Due to the presence of ≥1 amalgam fillings.
[e]Self-reported AI diseases; CTD, connective tissue disease; MS, multiple sclerosis; Ps, psoriasis; T1DM, type 1 diabetes mellitus; TD, thyroid dysfunction.
[f]p Values ≤ 0.1 (Fisher's Exact test) are given for the comparison between exposed and non-exposed individuals per metal. Values $p<0.05$ are given in bold.
[g]ANA patterns recorded: h, homogeneous; n, nucleolar; s, speckled.

well-established effects of Au exposure, in our present study, we did not find support for a relation between Au-based dental alloy exposure and AI phenomena.

Few studies reported on the risk of Ni and Pd exposure for the development of AID. One study, in 367 German students, found an association between urinary Pd levels and various AID, including T1DM and thyroid disease [17]. For Ni, only in experimental studies, using Brown Norway rats a clear association was found between nickel chloride administration and both clinical and serological (ANA) signs of scleroderma. We did, however, not find an association between either Ni or Pd exposure with ANA levels. Also, ANA specificity did not reveal reactivity with any of the known scleroderma-associated antigens, such as fibrillarin. But, with respect to the clinical AI parameters, we observed that Pd exposure tended to be associated with at least one of these, a dry mouth. Pd alloys, especially when alloyed with Ag or Cu, were shown to release metal ions and to be associated with adverse reactions [20,37,38]. Most remarkably, we did find a strong correlation between oral Ni exposure and the presence of clinical AID (thyroid dysfunction $n=3$, T1DM $n=1$, MS $n=1$, RA $n=1$). This association became even stronger when only gingival Ni exposure was evaluated. Interestingly, both Ni and Pd have recently been shown to display immunomodulatory effects on human dendritic cells, via TLR4 triggering, Ni providing the strongest signal [3–5]. Further studies on the relation between this type of immune-stimulating agents and the development of AID are therefore warranted.

This study has some limitations. First, the number of patients is relatively small, dictated by the extensive monitoring of clinical pathological, allergological and autoimmune data ($n=78$), so findings need to be confirmed in a larger group. Second, the focus in this study was on oral exposure. Environmental exposure, although expectedly low [39], or systemic exposure resulting from implants elsewhere in the body were not taken into account. Finally, oral exposure to Au, Hg and Pd appeared to increase with age, just like manifestations of AID. Potential, weak association could thus be masked. Future studies on extended groups should allow for statistical elimination of confounding factors such as age.

In conclusion, the results of this study support the view that oral exposure to Ni, but not to Pd, Au or Hg, may facilitate the development of AID. Therefore, further investigations into a possible role of transition metals, in particular Ni, in the development of AID are warranted.

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Declaration of interest

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References

1. Koch, P., and F. A. Bahmer. 1999. Oral lesions and symptoms related to metals used in dental restorations: a clinical, allergological, and histologic study. *J. Am. Acad. Dermatol.* 41: 422–430.
2. Cortizo, M. C., M. F. De Mele, and A. M. Cortizo. 2004. Metallic dental material biocompatibility in osteoblastlike cells: correlation with metal ion release. *Biol. Trace Elem. Res.* 100: 151–168.
3. Schmidt, M., B. Raghavan, V. Muller, et al. 2010. Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel. *Nat. Immunol.* 11: 814–819.
4. Raghavan, B., S. F. Martin, P. R. Esser, et al. 2012. Metal allergens nickel and cobalt facilitate TLR4 homodimerization independently of MD2. *EMBO Rep.* 13: 1109–1115.
5. Rachmawati, D., H. J. Bontkes, M. I. Verstege, et al. 2013. Transition metal sensing by Toll-like receptor-4: next to nickel, cobalt and palladium are potent human dendritic cell stimulators. *Contact Dermatitis*. 68: 331–338.
6. Rachmawati, D., I. W. A. Alsalem, R. J. Scheper, et al. 2015. Innate stimulatory capacity of high molecular weight transition metals Au (gold) and Hg (mercury). *Toxicol. In Vitro* 29: 363–369..
7. Bagenstose, L. M., P. Salgame, and M. Monestier. 1999. Cytokine regulation of a rodent model of mercuric chloride-induced autoimmunity. *Environ. Health Perspect*. 107: 807–810.
8. Bagenstose, L. M., P. Salgame, and M. Monestier. 1999. Murine mercury-induced autoimmunity: a model of chemically related autoimmunity in humans. *Immunol. Res.* 20: 67–78.
9. Rowley, B., and M. Monestier. 2005. Mechanisms of heavy metal-induced autoimmunity. *Mol. Immunol.* 42: 833–838.
10. Schiraldi, M., and M. Monestier. 2009. How can a chemical element elicit complex immunopathology? Lessons from mercury-induced autoimmunity. *Trends Immunol.* 30: 502–509.
11. Al-Mogairen, S. M. 2010. Induction of autoimmunity in brown Norway rats by oral and parenteral administration of nickel chloride. *Lupus*. 19: 262–267.
12. Stejskal, V., and J. Stejskal. 2006. Toxic metals as a key factor in disease. Forword. *Neuro. Endocrinol. Lett.* 27: 3–4.
13. Sterzl, I., J. Prochazkova, P. Hrda, et al. 1999. Mercury and nickel allergy: risk factors in fatigue and autoimmunity. *Neuro. Endocrinol. Lett.* 20: 221–228.
14. Stejskal, J., and V. D. Stejskal. 1999. The role of metals in autoimmunity and the link to neuroendocrinology. *Neuro. Endocrinol. Lett.* 20: 351–364.
15. Aminzadeh, K. K., and M. Etminan. 2007. Dental amalgam and multiple sclerosis: a systematic review and meta-analysis. *J. Public Health Dent.* 67: 64–66.
16. Sterzl, I., J. Prochazkova, P. Hrda, et al. 2006. Removal of dental amalgam decreases anti-TPO and anti-Tg autoantibodies in patients with autoimmune thyroiditis. *Neuro. Endocrinol. Lett.* 27: 25–30.
17. Helm, D. 2002. Association between palladium urinary concentrations and diseases of the thyroid and the immune system. *Sci. Total Environ.* 299: 247–249.
18. Muris, J., C. J. Kleverlaan, T. Rustemeyer, et al. 2012. Sodium tetrachloropalladate for diagnosing palladium sensitization. *Contact Dermatitis.* 67: 94–100.
19. Muris, J., A. J. Feilzer, C. J. Kleverlaan, et al. 2012. Palladium-induced Th2 cytokine responses reflect skin test reactivity. *Allergy* 67: 1605–1608.
20. Muris, J., R. J. Scheper, C. J. Kleverlaan, et al. 2014. Palladium-based dental alloys are associated with oral disease and palladium-induced immune responses. *Contact Dermatitis.* 71: 82–91.
21. Damoiseaux, J. G., and J. W. Tervaert. 2006. From ANA to ENA: how to proceed? *Autoimmun. Rev.* 5: 10–17.
22. Sinclair, D. 2006. Clinical and laboratory aspects of thyroid autoantibodies. *Ann. Clin. Biochem.* 43: 173–183.
23. Jin, G. B., H. Nakayama, M. Shmyhlo, et al. 2003. High positive frequency of antibodies to metallothionein and heat shock protein 70 in sera of patients with metal allergy. *Clin. Exp. Immunol.* 131: 275–279.
24. Hybenova, M., P. Hrda, J. Prochazkova, et al. 2010. The role of environmental factors in autoimmune thyroiditis. *Neuro. Endocrinol. Lett.* 31: 283–289.
25. Kubicka-Muranyi, M., J. Kremer, N. Rottmann, et al. 1996. Murine systemic autoimmune disease induced by mercuric chloride: T

helper cells reacting to self proteins. *Int. Arch. Allergy Immunol.* 109: 11–20.
26. Nielsen, J. B., and P. Hultman. 2002. Mercury-induced autoimmunity in mice. *Environ. Health Perspect.* 110: 877–881.
27. Stejskal, V., R. Hudecek, J. Stejskal, and I. Sterzl. 2006. Diagnosis and treatment of metal-induced side-effects. *Neuro. Endocrinol. Lett.* 27: 7–16.
28. Gardner, R. M., J. F. Nyland, I. A. Silva, et al. 2010. Mercury exposure, serum antinuclear/antinucleolar antibodies, and serum cytokine levels in mining populations in Amazonian Brazil: a cross-sectional study. *Environ. Res.* 110: 345–354.
29. Issa, Y., P. A. Brunton, A. M. Glenny, and A. J. Duxbury. 2004. Healing of oral lichenoid lesions after replacing amalgam restorations: a systematic review. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 98: 553–565.
30. Pigatto, P. D., and G. Guzzi. 2010. Linking mercury amalgam to autoimmunity. *Trends Immunol.* 31: 48–49.
31. Bates, M. N., J. Fawcett, N. Garrett, et al. 2004. Health effects of dental amalgam exposure: a retrospective cohort study. *Int. J. Epidemiol.* 33: 894–902.
32. Miller, F. W., K. M. Pollard, C. G. Parks, et al. 2012. Criteria for environmentally associated autoimmune diseases. *J. Autoimmun.* 39: 253–258.
33. Ong, J., E. Erdei, R. L. Rubin, et al. 2014. Mercury, autoimmunity, and environmental factors on cheyenne river sioux tribal lands. *Autoimmune Dis.* 2014: 325–461.
34. Pollard, K. M., and D. H. Kono. 2013. Requirements for innate immune pathways in environmentally induced autoimmunity. *BMC Med.* 11: 100.
35. Havarinasab, S., U. Johansson, K. M. Pollard, and P. Hultman. 2007. Gold causes genetically determined autoimmune and immunostimulatory responses in mice. *Clin. Exp. Immunol.* 150: 179–188.
36. Seifert, O., A. Matussek, F. Sjogren, et al. 2012. Gene expression profiling of macrophages: implications for an immunosuppressive effect of dissolucytotic gold ions. *J. Inflamm. (Lond.)* 9: 43(1–8).
37. Berzins, D. W., I. Kawashima, R. Graves, and N. K. Sarkar. 2008. Heat treatment effects on electrochemical corrosion parameters of high-Pd alloys. *J. Mater. Sci. Mater. Med.* 19: 335–341.
38. Syverud, M., J. E. Dahl, H. Hero, and E. Morisbak. 2001. Corrosion and biocompatibility testing of palladium alloy castings. *Dent. Mater.* 17: 7–13.
39. Kielhorn, J., C. Melber, D. Keller, and I. Mangelsdorf. 2002. Palladium – a review of exposure and effects to human health. *Int. J. Hyg. Environ. Health.* 205: 417–432.