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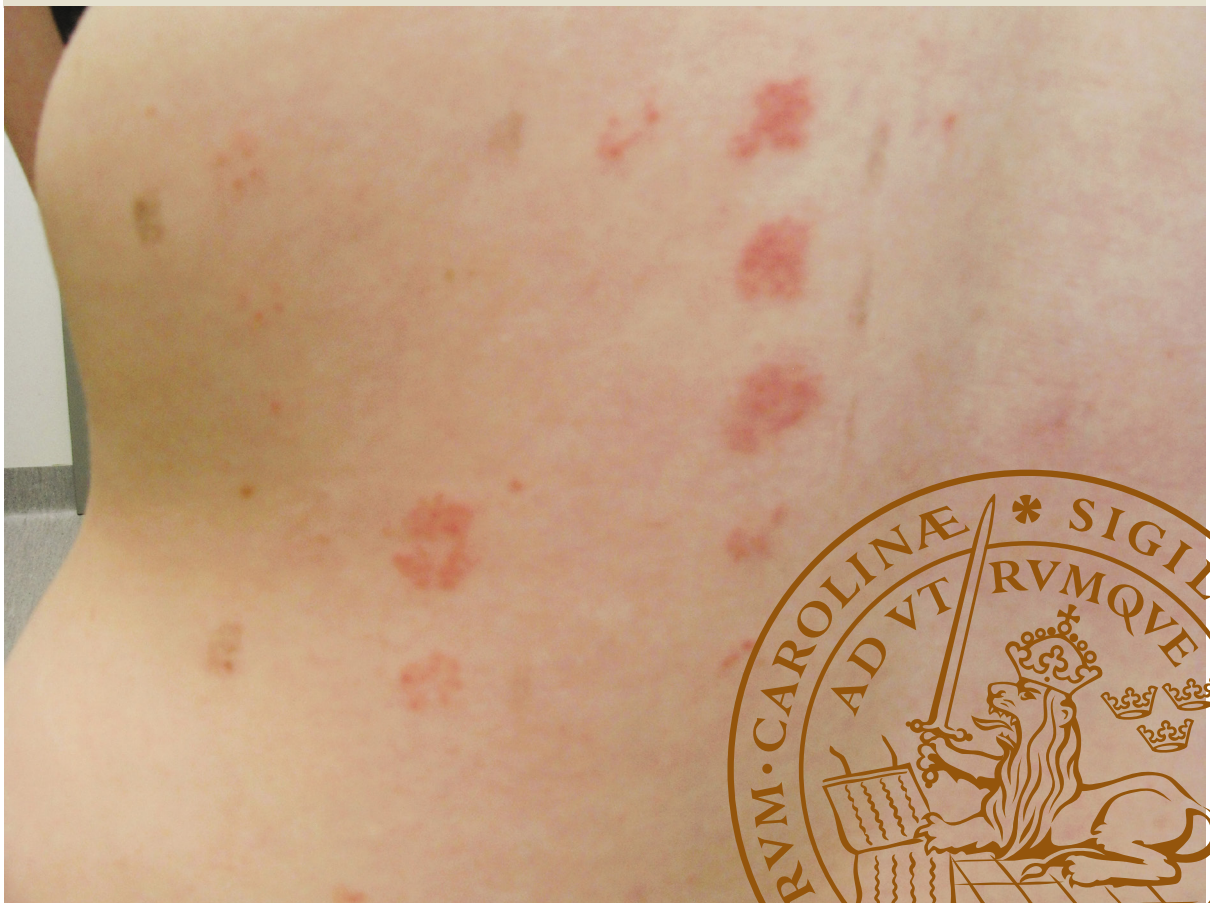

Patch testing with palladium and aluminium

Epidemiological and experimental studies

Lisbeth Rosholm Comstedt

LUND UNIVERSITY

DOCTORAL DISSERTATION

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The aim of this thesis was to investigate the prevalence and significance of contact allergy to palladium from a Swedish perspective. Our initial findings made it necessary to also explore the importance of the metal aluminium used in test chamber systems and the effect of aluminium chloride (Al-Cl) in patch test preparations.

A retrospective study (study I), with 18,306 patch test results obtained from 1995-2016, showed that the prevalence of contact allergy to palladium is following that of nickel. After the introduction of the EU Nickel Directive in 2001, there was a significant decrease in contact allergy to sodium tetrachloropalladate (Na-PdCl), palladium chloride (Pd-Cl), and nickel sulphate (Ni) among younger females, age six to 30 years. Regression analysis revealed that women with contact allergy to Ni were approximately 36 times more likely to have contact allergy to Pd-Cl compared to females with no allergy to Ni.
The prevalence of isolated palladium (Pd) allergy in the whole study population (men and women) was 1.4% and remained stable from 1995 to 2016.

In study II, Na-PdCl showed less variability in patch test results, compared to Pd-Cl. When re-testing the same 15 participants with known contact allergy to Ni, Na-PdCl, and Pd-Cl, a seasonal variation was seen. In wintertime, there were significantly higher summarized test scores compared to in late summertime for the three metal salts Pd-Cl, Na-PdCl, and Ni.

A retrospective study (study III) showed that the use of Finn Chambers in patch testing patients with contact allergy to aluminium could be a risk for false-positive patch test reactions to Na-PdCl and Pd-Cl. No such risk was seen in patients patch tested with Finn Chamber Aqua.

In study IV, the use of Al-Cl in test preparations with Ni seemed to increase the sensitivity for detecting Ni allergy. When adding 30.0% Al-Cl to Ni 15.0% aqua, the sensitivity increased to 91% from 50.0% in Ni 5.0% in petrolatum. This increase in sensitivity was only seen when adding Al-Cl to Ni and was not seen when adding Al-Cl to methylisothiazolinone and to fragrance mix I.

Key words Prevalence of contact allergy to metals, palladium chloride, sodium tetrachloropalladate, patch test systems, adjuvants in patch testing, patch test variability, isolated palladium allergy, Finn Chamber,

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‘The modern aim is to learn more and more about less and less’

-Professor Geddes, 1916

Table of Contents

Abbreviations

Al-Cl:	Aluminium chloride hexahydrate
Al-lac:	Aluminium lactate
Aq:	Aqua
Co:	Cobalt chloride
FM I:	Fragrance mix I
IL:	Interleukin
K-Cr:	Potassium dichromate
LPT:	Lymphocyte proliferation test
LTT:	Lymphocyte transformation test
MEC:	Minimal elicitation concentration
MI:	Methylisothiazolinone
MP:	*Myroxylon pereirae*
Na-PdCl:	Sodium tetrachloropalladate
Ni:	Nickel sulphate hexahydrate
Ni-Cl:	Nickel chloride
Pet:	Petrolatum
Pd-Cl:	Palladium chloride
SLS:	Sodium lauryl sulphate
STS:	Summarized test score
Tixocortol:	Tixocortol-21-pivalate

Definitions

Contact allergy to aluminium

Defined as contact allergy to Al-Cl or Al-lac or both.

Contact allergy to palladium

Defined as contact allergy to Pd-Cl or Na-PdCl or both.

Co-reactivity in patch test reactivity

In this thesis, co-reactivity is defined as present if the mean MEC to two different test salts from four different test occasions, shows a positive correlation (for further details, see the Materials and Methods section).

Co-variation in patch test reactivity

In this thesis, co-variation is defined as present if the change in MEC to a certain test salt, between two test occasions, shows positive/negative correlation to the change in MEC in another test salt and between the same two test occasions (for further details, see the Materials and Methods section).

Isolated cobalt allergy

Positive patch test reaction to Co without a concomitant positive patch test reaction to Ni.

Isolated palladium allergy

Positive patch test reaction to Pd-Cl and/or Na-PdCl without a concomitant positive patch test reaction to Ni.

Positive patch test reaction

Only 1+, 2 +, and 3+ reactions are considered positive allergic reactions (1).

Preface

Heading towards this thesis

When this project was initiated, palladium chloride (Pd-Cl) had been used for patch testing in the extended baselines series in Malmö for more than 20 years. Monica Hindsén began to study the cross-sensitization between nickel and Pd-Cl. She and my colleague, Cecilia Tillman, also carried out a study investigating clinical symptoms from earrings, made of palladium, in patients with contact allergy to Pd-Cl. Monica got me into the project in 2013.

Nine years have passed since I started my education to become a Doctor of Philosophy in Medical Science. A change of main supervisor, a cancer disease, and a pandemic were my biggest challenges. They forced me to change priorities, look at other options, and also offered me new possibilities.

In the beginning, this thesis was planned to be all about contact allergy to palladium. Studies I and II are part of this original plan. Because of lack of clinical problems due to palladium allergy, the funding opportunities were zero and, as the Covid-19 pandemic was knocking at the door, I was forced to look at other relevant possibilities. A lecture from Yolanda Hedberg, about aluminium release from Finn Chambers, gave my supervisors and me the idea of a retrospective study (study III) that I could perform at home, a study that came to play an important part in this thesis. A cooperation with a laboratory in Lyon were discussed for study IV, but the Covid-19 pandemic was closing Europe down, and I was forced to look at projects I could do at our own laboratory in Malmö. When my colleague, Ingrid Siemund, had new results concerning the use of aluminium when added to common nickel preparations, I chose to continue with aluminium in patch test material and the ideas were settled for study IV.

This thesis therefore ended up becoming much more about *patch testing* than about *contact allergy*. Palladium is still the metal in focus but factors influencing the patch test results became the thread joining together my studies. Aluminium, as such a factor, plays a main role in my last two studies.

Abstract

The aim of this thesis was to investigate the prevalence and significance of contact allergy to palladium from a Swedish perspective. Our initial findings made it necessary to also explore the importance of the metal aluminium used in test chamber systems and the effect of aluminium chloride (Al-Cl) in patch test preparations.

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List of papers

Paper I

Rosholm Comstedt L, Dahlin J, Bruze M, Åkesson A, Hindsén M, Pontén A, Isaksson M, Svedman C. 'Prevalence of contact allergy to metals: nickel, palladium and cobalt in Southern Sweden from 1995-2016.' *Contact Dermatitis*. 2020;82(4):218-26. The paper has been reprinted with permission from the publisher.

Paper II

Rosholm Comstedt L, Engfeldt M, Svedman C, Åkesson A, Hindsén M, Bruze M. 'Variation, and co-variation in patch test reactivity to palladium and nickel salts.' *Eur J Dermatol*. 2018 Oct 1;28(5):668-676. Reproduced with permission from Springer Nature.

Paper III

Rosholm Comstedt L, Dahlin J, Bruze M, Hedberg Y, Matura M, Svedman C. 'Patch testing with aluminium Finn Chambers could give false positive reactions in patients with contact allergy to aluminium.' *Contact Dermatitis*, 2021;85:407-414.

Paper IV

Rosholm Comstedt L, Siemund I, Dahlin J, Bruze M, Svedman C. 'Effects of aluminium chloride hexahydrate added to common patch test substances.' Submitted October 2022

Introduction

Contact allergy to metals

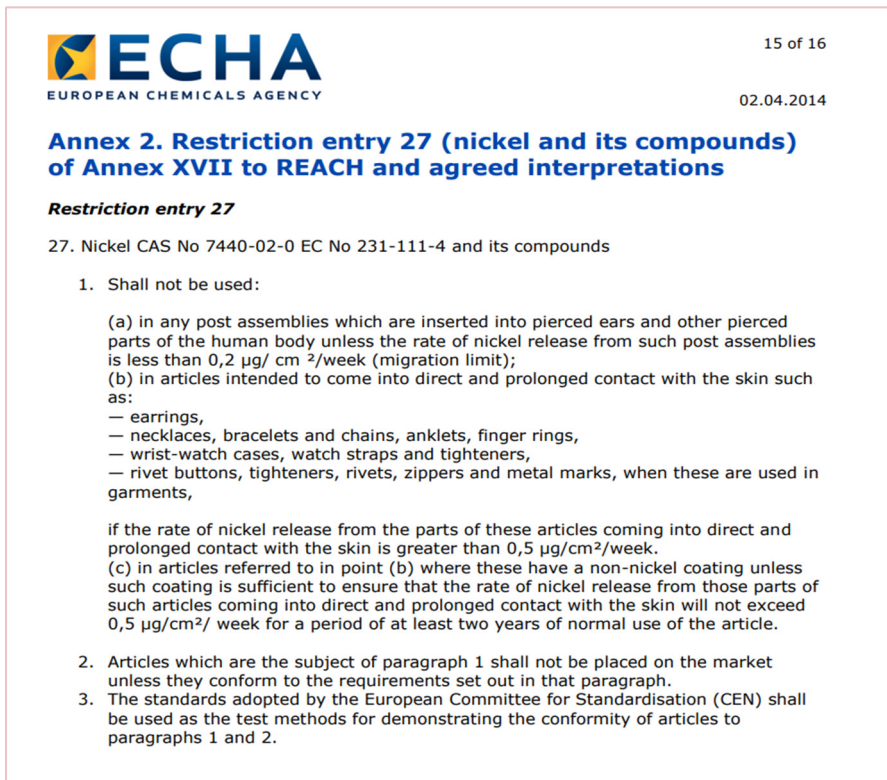
In general

Today, contact allergy in patients undergoing patch testing has an overall prevalence of 20.1% in Europa and North America. The prevalence is significantly higher among women (27.9%) than among men (13.2%) (2). Metals belong to the most common allergens. Metals are found in the earth´s crust and some occur naturally in our drinking water and food and are essential nutrients for life. Industrialization made the demand for, and consumption of, metals grow, which led to an increased cutaneous exposure not only occupationally but also in consumer products. During the 20th century, the prevalence of contact allergy to metals increased (3). Contact allergy to nickel sulphate hexahydrate (Ni), cobalt chloride (Co), and potassium dichromate (K-Cr), the three most common metal allergens, had prevalence rates in Europe 2019/2020 of around 19.8%, 6.2%, and 4.4% respectively among consecutive patients (4). Contact allergy to nickel and cobalt is more common in women. This has been explained by a higher exposure to nickel and cobalt in earrings, and other prolonged contact with jewellery (3).

For sensitization to take place to a metal, the skin or mucosa needs to be in contact with metal ions, which means the metal must be in an ionised form, which can happen from corrosion of a metal or alloy. Differences in metal properties and their compositions in different alloys determine how easily they corrode. In contact with the skin or mucosa, the metal surface is affected by sweat or saliva which can evoke corrosion and release of metal ions (3).The type of alloy, type of contact, and the local environment on the skin are decisive factors as to whether a person becomes sensitized to a metal. The corrosion process in the oral mucosa is different from the corrosion at the skin, primarily because of the constantly wet environment and lower pH. In the oral cavity, saliva works as an oxidizer of dental alloys. Electrons can be extracted from a metal in an alloy, which results in a positively charged metal surface and the release of positively charged metal ions into the saliva. The corrosion process therefore happens faster in the oral cavity than when metals are exposed to the skin (3).

The EU Nickel Directive.

Due to the increased prevalence of especially contact allergy to Ni in the late 20th century, Denmark and Sweden were the first nations in Europe to enact legislation about nickel. In 1990, the first legislation regarding nickel content in earrings and piercing posts came into force in Sweden by the National Board of Health and Welfare (5). A similar legislation was made in Denmark as well (3). Based on these national initiatives, The European Commission published a draft for a European directive in 1993, which was adopted as the Nickel Directive in 1994. It came into force in 2001 (3, 6). In 2004, the directive was amended by changing the limits of nickel contained in post assemblies to a maximum nickel release/week (7). Today the directive is a restriction under the REACH regulation. It is found in annex XVII, entry 27, under the European Chemicals Agency (8). Figure 1 shows the three parts of the nickel restriction.



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Annex 2. Restriction entry 27 (nickel and its compounds) of Annex XVII to REACH and agreed interpretations

Restriction entry 27

27. Nickel CAS No 7440-02-0 EC No 231-111-4 and its compounds

1. Shall not be used:

   (a) in any post assemblies which are inserted into pierced ears and other pierced parts of the human body unless the rate of nickel release from such post assemblies is less than 0,2 µg/ cm ²/week (migration limit);
   (b) in articles intended to come into direct and prolonged contact with the skin such as:
   — earrings,
   — necklaces, bracelets and chains, anklets, finger rings,
   — wrist-watch cases, watch straps and tighteners,
   — rivet buttons, tighteners, rivets, zippers and metal marks, when these are used in garments,

   if the rate of nickel release from the parts of these articles coming into direct and prolonged contact with the skin is greater than 0,5 µg/cm²/week.
   (c) in articles referred to in point (b) where these have a non-nickel coating unless such coating is sufficient to ensure that the rate of nickel release from those parts of such articles coming into direct and prolonged contact with the skin will not exceed 0,5 µg/cm²/ week for a period of at least two years of normal use of the article.

2. Articles which are the subject of paragraph 1 shall not be placed on the market unless they conform to the requirements set out in that paragraph.
3. The standards adopted by the European Committee for Standardisation (CEN) shall be used as the test methods for demonstrating the conformity of articles to paragraphs 1 and 2.

Figure 1. Screen dump from the European Chemical Agency, www.echa.europa.eu (8). The piercing posts or post assembly is the part of a product designed for insertion into the wound caused by piercing, together with parts that hold the piece in and against the wound. Prolonged contact with the skin is defined as contact with the skin by nickel and of potentially more than: 10 minutes on three or more occasions within two weeks or 30 minutes on one or more occasions within two weeks (8).

The delayed hypersensitivity reaction

The immune response behind contact allergy is called a delayed hypersensitivity reaction or type IV reaction. This reaction contains two phases: the sensitization and the elicitation phases. The sensitization phase can take 10-14 days and is the phase when an individual gets sensitized to an allergen. The elicitation phase is shorter and might only take 24-72 hours and is the phase when an individual comes into contact with an allergen, which he/she has already been sensitized to. Both phases start with the activation of the innate immune system. This activation is similar in the two phases (9). The sensitization phase starts with a chemical so small, < 500 kilodalton, that it can penetrate stratum corneum. The chemical needs to be able to bind to proteins, to activate the immune system. When a chemical is able to do this, it is called a hapten. Some chemicals need to be activated to become haptens. This is called haptenization. Different chemicals undergo haptenization in different ways. A pro-hapten is a molecule that requires activation by the host metabolism to make it able to bind to proteins. Pre-haptens are chemicals that are activated by oxidative processes, which is the case with some fragrances and *p*-phenylenediamine in haircolouring (10). When haptens bind to proteins they are defined allergens. Allergens react to cellular products, such as extracellular matrix components, which stimulate the release of damage-associate molecular patterns (DAMP). DAMP serves as an innate immune response trigger by activating toll-like receptors on the dendritic cells, which is essential for the development of the delayed hypersensitivity reaction (11).

The metals nickel, palladium, and cobalt are able to activate the innate immune system in a more direct manner by binding directly to specific histidine residues on toll-like receptor 4 (TLR4), located at the dendritic cells in stratum corneum (3, 12).

When the dendritic cells are activated, they capture the allergen, binding it to MHC I and II receptors and transporting it to the regional lymph nodes, where they present it to the T-cells. This presentation activates the adapted immune system (11, 12). The role of dendritic cells is essential in delayed hypersensitivity reaction because the cells serve as a link between the innate and adapted immune system.

In the adapted immune response, naïve T-cells, when presented to the allergens, are activated and a subset are triggered to become memory T-cells which will be able to recognize the allergen later in life and start an elicitation reaction. The CD4+ T-cells, Th1, Th2 and Th17, produce different cytokines, such as tumour-necrosis factor alfa (TNFα), interferon gamma (INFγ), inter-leukin (IL) 4, IL5, IL13, IL17 and IL22 (3, 9). These cytokines promote further migration of T-cells to the site of allergen exposure in the skin and killing of haptenized cells, which leads to inflammation in the skin and

development of allergic contact dermatitis. Upon re-exposure to an allergen, the elicitation phase starts, and the innate immune system is again activated but the allergen presentation results in activation of T-memory cells, which recognize the allergen, and promote the inflammatory response. It is in the elicitation phase that cross-sensitization can occur. When exposure is to an allergen that has the same chemical structure, the T-memory cells recognize it as a former allergen and start the inflammatory response, even though an earlier exposure to this specific allergen has never occurred (9). When first sensitized, the amount of allergen to stimulate an elicitation of the immune response does not have to be as high as the amount in the sensitization phase (13).

Patch testing

A patch test is an, *in vivo*, provocation test. It was developed more than 100 years ago and is still today the gold standard to diagnose contact allergy. The purpose of patch testing is to elicit a delayed hypersensitivity reaction in the skin to an allergen the patient is allergic to and decide whether this found allergy can explain the patient's symptoms. The standardized procedure of patch testing has been developed and optimized based on clinical studies and many years of experience (1, 9, 14-23).

Test preparations, chamber, occlusion time, and reading

Allergens are applied on the patient´s skin as a test substance dispersed in a vehicle. Petrolatum (pet) is the most used vehicle because it is able to disperse most test substances. Formaldehyde and methylisothiazolinone (MI) cannot be distributed in pet, and aqua (aq) is used instead in these preparations. Pet has several advantages. It is non-irritating, stable, cheap and able to protect test substances from degradation before contact with the skin. However, the allergen might be unevenly distributed, which can decrease the reproducibility of patch testing. Aq, as a vehicle, does also have the advantage of being non-irritating and cheap, and if a substances dissolves, it gets evenly distributed. However, many allergens do not dissolve in water. Liquid test preparations compared to pet preparations are easier to dose in an exact manner, but the test preparations must be applied immediately before skin testing, so that it does not evaporate. In practical, clinical settings where testing a series of substances in many patients takes place, pet is therefore the preferred vehicle. In research studies or when testing the patient´s own products, liquid, such as water, acetone, or ethanol are used (9).

In 1984, Friedmann et al showed that there is a dose-response relationship between an allergen and the immune reaction (24). This is explained by the immune system's ability to enhance an elicitation reaction to an allergen dependent on the amount of Langerhans cells coming into contact with the allergen (25). The challenge is, for every test chamber system, to determine an optimal dose of a test preparation that can elicit an allergic response in those previously sensitized, without causing skin irritation or sensitization in patients not already allergic. Today, the dose of common test preparations has been standardized according to type of test chamber system (16, 20, 22, 23, 26, 27). Besides Finn Chambers, True Test, and IQ Chambers are commonly used in Sweden and other European countries. True Test and IQ chambers are plastic chambers. Their quality and accuracy have been compared with Finn Chambers and the chambers have been proven equal (28-30) .

Test chambers with test preparations are applied on the patient´s back for 48 hours. This occlusion time is chosen as a compromise. Because many allergens are tested simultaneously, it is most practical if the patient can take off all the chambers at the same time. Some allergens penetrate the skin faster than others, but 48 hours is a balanced exposure time that has proven effective (9). With a high suspicion of a strong allergy, the specific allergen can be tested on the upper arm alone, so that the patient is able to take this test preparation off earlier if needed.

The occlusion time and the time before the patch test reaction is read were initially presumably decided based on practical reasons and by trial and error. However, since some allergens are slow reactors, it has been shown that in order to catch as many positive reactions as possible two patch test readings are recommended (14). Clinical studies have shown that up to 30% of positive patch test results would be missed if the test results were only read once (9, 31-33). Doses, occlusion time, and patch test reading times are related, and a change in one of them can influence the patch test results (9).

In 1981, the scoring of patch test results, as we know it today, was defined (1). This scoring is still recommended by the International Contact Dermatitis Research Group and is used in the Department of Occupational and Environmental Dermatology in Malmö with the small modification that the clinical findings should cover the whole test area. See Table 1.

Table 1: Description of patch test reactions according to Malmö classification based on the critieria from the International Contact Dermatitis Research Group.

Patch test reaction	Recorded	Morphology
Negative	-	Normal skin
Weak positive	+	Erythema and infiltration covering the whole test area
Strong positive	++	Erythema, infiltration, and papules covering the whole test area.
Very strong positive	+++	Erythema, infiltration, and vesicles covering the whole test area.
Irritative	IR	Sharp-edged margins, wrinkling, pustular reactions.
Doubtful	?/(+)	Erythema/ infiltration/ papules, but not fulfilling all the above- mentioned criteria for a positive reaction.

The limits of this scoring system are the inter-individual differences between doctors. Education is needed to read a patch test, and calibration by morphology protocols can make the clinical judgement more consistent among doctors (9).

When a test preparation elicits a positive reaction, the final step in patch testing is to interpret whether the found allergy has any relevance to the patient´s symptoms. Clinical relevance is defined as existing exposure to the allergen and dermatitis or other symptoms; for example, symptoms from mucosa in the oral cavity, that can be explained by the allergy (9). It is essential to explore the patient´s personal and occupational exposures to the found allergen. The clinical relevance of a contact allergy should be considered with respect to all positive patch test reactions the patient might have, as the different reactions can be of current and/or past relevance. In cases when one substance is known to cross-react with another, a previous exposure and sensitization to this cross-reacting substance is not necessary for developing a positive reaction (14).

Patch test series

Baseline series are standard screening series that every patient under investigation for contact allergy, is tested with. In Europe, the European Society of Contact Dermatitis regularly recommends what should be in the European baseline series. These recommendations are based on population exposure and the current prevalence of contact allergy (26). This series is meant to be complemented with allergens of local importance (14). The Swedish Society of Occupational and Environmental Dermatology, and the Swedish Contact Dermatitis Group, have, based on the European baseline series, recommended a Swedish baseline series. As new allergens appear, and others disappear, this series is evaluated twice yearly, and it is changed according to the European baseline series and environmental exposure in the Swedish population. In Malmö, besides the Swedish baseline series, all patients are also tested with an extended baseline series, which is based on local exposure to allergens in the

population in southern Sweden as well as to the ongoing quality and research project at the clinic. Today, the extended baseline series contains 48 substances and is revised yearly. In addition to these baseline series, specific test series are used; they categorize according to occupation and/or environmental exposure.

Factors, not involved in the standard procedure, that can affect the diagnostic outcome of a patch test

Irritative contact dermatitis, atopy, and the role of seasons

Irritative contact dermatitis is defined as dermatitis due to exposure to an irritating substance that enhances an inflammatory reaction through the innate immune system, thus causing dermatitis, but where no T-cell mediated response towards the substance is created. Substances that cause irritant dermatitis can be water, acids, alkalis, oils, and organic solvents. Irritative contact dermatitis is a risk factor for increased susceptibility to developing contact allergies and the ability to develop irritative contact dermatitis shows large variation between individuals (9). The hypothesis that atopic dermatitis is a risk factor for developing contact allergies has been confirmed in some studies and rejected in others (34). However, a study of atopy and skin irritability has shown that persons with active atopic dermatitis show more susceptibility to irritant contact dermatitis compared to persons with a history of atopic dermatitis but with no active lesions and persons with no history of atopic dermatitis at all (9, 35). Seasonal variation, as found in temperate climate zones, can change the skin´s susceptibility to developing irritant reactions, as dry and cold weather with low humidity make the skin more irritant (36). For Ni and Co, a significant increase in prevalence of weak positive reactions has also been reported during wintertime but, during that season, the prevalence of strong and very strong reactions remained stable (36, 37). A seasonal variation in the form of increased positive reactivity during wintertime, in general among patients suspected to have contact allergy, has been reported from our clinic, and from Israel (38-40). The latter study excluded weak positive and doubtful reactions, and found an increase in strong and very strong patch test reactions during wintertime (38). This is explained by the fact that UVB exposure suppresses the Langerhans cells in stratum corneum (41).

Gender

Women are more often diagnosed with contact allergies with the misinterpretation that women have more sensitive skin than men. However, studies regarding skin thickness and trans-epidermal water loss have concluded that the skin in women and men has equal properties (9). Hence, the problem seems to be the grade of exposure to irritants

and allergens that might be different among gender. For women, small studies have shown that the hormonal variation in the menstrual cycle can influence patch test reactivity. Premenstrual patch test results to Ni seem to have lower minimal elicitation reaction (MEC) as well as summarized test score (STS) compared to patch test reactivity in the same individuals in the follicular phase of the cycle. The hypothesis is that oestradiol has an inhibitory effect on the cells in the delayed hypersensitivity reaction (42).

Medication

Immunomodulatory medicine might affect the elicitation response to an allergen with the risk of false-negative reactions. This is especially present with oral corticosteroids >20mg/day (9). When it comes to other immunosuppressive medications, the overall recommendation is to patch test before the patient starts treatment; however, if this is not possible, patients in general seem to benefit from patch testing despite receiving immune-suppressive medications. Positive patch test results, especially 2+ and 3+ reactions can appear for several allergens in patients treated with Methotrexate, TNF-alfa inhibitors and Ustekinumab in psoriasis patients, and Dupilumab and Cyclosporine in dermatitis patient. When treated with Dupilumab, it has been shown that approximately 10.0% of positive patch test results are lost (43).

Diagnostic *in vitro* tests

As described above, there are a lot of steps involved in the procedure of patch testing. Despite standardization and experience, these steps can vary from clinic to clinic, from technician to technician, and from doctor to doctor. Even though a patient is patch tested by the same technician at the same clinic and read by the same doctor, patch test results can vary in the same individual over time (33, 44) and simultaneously at the same patch test time, when tested with the same substance on each side of the back (21). A high clinical suspicion of contact allergy but with a doubtful or negative result might therefore lead to repeated patch testing with a higher concentration or, in the case of an irritant reaction, repeated patch testing with dilution of the test substance. Alternative *in vitro* methods to diagnose contact allergy are desirable. The lymphocyte proliferation (LPT) test was introduced already in the 1960s (45). This test shows the T-cell´s ability to proliferate in response to allergens *in vitro* and, when it was supplemented with analysis of the cytokine production, it was named lymphocyte transformation test (LTT) (3). The cytokine production can be measured by different techniques such as ELISA, ElisaSpot, or FlouroSpot assays (3). The LPT and LTT have

been used in experimental settings to explore the T-cell response to different metals in allergic individuals (3, 46-49). Different cytokine productions have been found for nickel, palladium, and gold in patients with contact allergy to the respective metals (48-50). Comparing LTT with patch test results has also mainly been done in patients with contact allergy to metals. One study investigated the correlation between patch test reactivity to Ni and the cytokine response from T-cells in 15 females with contact allergy to Ni. They were patch tested three times and before every test occasion, blood samples were taken for LTT. T-cells were stimulated with nickel chloride (Ni-Cl) and the mean cytokine response was found to have a positive correlation with the mean patch test reactivity in the patients (51). The LTT in patients with contact allergy to palladium has showed similar results with a positive correlation between patch test reactivity and the cytokine response created in LTT, when T-cells have been stimulated with sodium tetrachloropalladate (Na-PdCl) (52). According to the sensitivity and specificity for detecting nickel allergy by LTT, different studies show variable results, possibly because of differences in patient population and exact method of LTT. In one study, the sensitivity for detecting nickel allergy was found to be 88% and the specificity 96% (53), while in a second study regarding patients with implant failure, the LTT had a sensitivity of 68%, but with a specificity of 98% in detecting contact allergy to nickel (54). Patch testing was used as the gold standard in both studies. The LTT has also been investigated in patients with contact allergy to gold. When performing LTT and patch testing in 77 patients with contact allergy to gold thiosulfate, LTT had a sensitivity of 54.5% among the patients that had a positive patch test reaction to gold. The specificity was, however 92.9% and the test was unfortunately unable to distinguish between patients with positive patch test reactions and patients with irritative patch test reactions to gold (50). The LTT still needs standardization and investigations into reliability as well as cost-benefit analysis by comparing the method to patch testing (3). The method is today not part of clinical practice as a method for diagnosing contact allergy.

Palladium

Facts about palladium

Palladium is a hard, silver coloured, noble metal. In the periodic table it is in group X, with the atom number 46 and the atom mass 106.42. It belongs to the platinum group of metals together with rhodium, ruthenium, iridium, osmium, and platinum. The platinum group of metals is found in the earth´s crust in low concentrations <1 µg/kg (55). Palladium has strong catalytic properties. The two main demands for palladium

are in electrical equipment and in automobile catalysts. In electronics it exists mixed with silver or other metals in conductive paste, or in alloys in contact- and swishing systems. In catalysts in cars, palladium reduces levels of nitrogen oxides, carbon monoxide, and hydrocarbons. Because of the increased market for electronics and automobiles, the demand for palladium has increased enormously during the past 30 years (56, 57). For many years, the price of palladium was lower than the price of gold, and so the metal became a substitute for gold both in dental alloys and in jewellery. In Sweden, palladium-silver alloys were introduced in 1974 and gold-palladium alloys in 1977 in dental materials. However, due to the increasing prices of these alloys, chrome-cobalt alloys eventually became more common in Sweden. Today, palladium is only used to a very small extent in dental materials in Sweden (58, 59). In the Netherlands and Japan the use of palladium in dental materials is still common (60). When it comes to jewelleries, palladium has gained much popularity during the past 20 years in Sweden, as a substitute for white gold and platinum in wedding rings. Though the price has now exceeded that for gold, there is still an increased demand for palladium due to the electronic communication industry.

The environmental levels of palladium in water, soil, and air are increasing, due to the increased use in automobiles, but the levels are still very low, under the detection limit in many places in the world, even in places near the mining and ore-processing facilities. The intake from food is very small, as palladium is only found in very low amounts (0.3-0.9 microgram/kg) in milk, poultry, and vegetables. The highest amount of 3 microgram/kg is found in nuts (57).

Occupational exposure to palladium is found in mining, recycling of palladium, the electronic industry, the manufacturing of catalysts in automobile, and in dental personnel as well as in jewellers. The exposure in the general population comes from dental material and jewellery (56).

Patch testing with palladium

In the 1980s and 1990s, contact allergy due to palladium in dental alloys was reported (61-65), and patch testing with palladium chloride (Pd-Cl) in aq or pet in 0.1%, 1.0% and 2.0% started in the US and in Europe (66, 67). In the late 1980s, Pd-Cl 2.0% in pet was introduced at the Department of Occupational and Environmental Dermatology in Malmö as part of the extended base line series. In 2009, Na-PdCl 3.0% pet was found to have a better penetration through the skin compared to Pd-Cl. In clinical studies it was shown to find nearly twice as many allergic patients than Pd-Cl 1.0% (60, 68-70). Therefore, it was inserted in the extended baseline series in Malmö, together with PdCl 2.0%.

Figure 2. The two palladium salts in petrolatum, prepared for patch testing in Finn Chambers**.**
Photo: Lisbeth Rosholm Comstedt

Prevalence of palladium allergy in Europe.

Several departments from different countries in Europe have patch tested with Pd-Cl during the 1990s and early 2000s due to the increased use of palladium in dental alloys. A future problem with increased prevalence of palladium allergy was predicted (65). With the introduction of sodium tetrachloropalladate (Na-PdCl) in 2009, differences in frequency rates among different European countries became noticeable. In a multicentre study in Europe, investigating the most optimal concentration of Na-PdCl in 2011-2012, there were large differences in prevalence of palladium allergy among departments (60). Amsterdam and Basel found a prevalence above 30%. In Amsterdam, the prevalence equalled that for Ni, and in Basel, it was higher than the prevalence to Ni allergy. Barcelona, Bari, and Coimbra had a prevalence of contact allergy to palladium of 19-26%. Odense had 11% and Malmö had the lowest at 10%. These large differences among countries in Europe were interpreted as differences in the use of palladium in dental alloys (60).

Clinical symptoms due to contact allergy to palladium

Several authors have described a high prevalence of positive patch test reaction to Pd-Cl without any clinical relevance (64, 67, 71-74). However, symptoms due to contact allergy to palladium have been reported, mainly from the oral cavity due to palladium-containing alloys. The oral symptoms reported have been, swelling, pain, gingivitis, stomatitis, and xerostomia (61, 62, 70, 75). Skin symptoms due to dental alloys with palladium have been described in two case reports from Japan. One patient developed dermatitis over the mandibles and forehead due to palladium- containing dental alloys (76), and one patient developed more widespread dermatitis due to systemic contact allergy to palladium from dental alloys (77). In both cases, the patient´s skin symptoms disappeared the dental alloys being replaced with another dental material. Case reports about contact dermatitis from palladium jewelleries are even more sparse. The very first report about palladium allergy actually described contact dermatitis due to a wedding ring of platinum and palladium (78). In 2001 came a case report from Finland about contact dermatitis due to spectacle frames decorated with palladium (79). Granulomatous reactions in the skin due to earrings made of palladium have been described (80-83). To the knowledge of the author of this thesis, there is no published case report from Sweden about clinical symptoms due to contact allergy to palladium.

Due to the paradox of a high prevalence of palladium allergy and no reports on contact dermatitis from palladium in our population (or even indications that reactions found were interpreted as possibly relevant) a study of exposure to pure palladium metal in a clinical setting was performed at the Department of Occupational and Environmental Dermatology in Malmö 2013. Tillman et al carried out this study with a usage test with palladium-coated earrings, in which 40 females with known sensitization to Ni and Pd-Cl and/or Na-PdCl wore palladium-coated earrings for nine weeks. None of the participants developed contact dermatitis or other skin symptoms that could be related to the earrings (84).

Cross-reaction with nickel

When patch testing with Pd-Cl became common, a high prevalence of positive reactions was noticed with concomitant reactions to Ni. On the other hand, many patients still had positive patch reactions to Ni without reactions to Pd-Cl. The significance of the positive reactions to Pd-Cl remain unknown (64, 65, 67). As nickel and palladium belong to the same group in the periodic table, group X, cross-reaction between the metals was suggested (64, 67). An often mentioned study that confirmed cross-reactivity between the metals is from Wahlberg and Boman (85). In their study, guinea pigs were sensitized with Pd-Cl. Upon subsequent stimulation with Ni, the animals developed elicitation reactions. A study in humans was done in Malmö in

2005. Twenty-seven females, known to be sensitized to both Ni and Pd-Cl, were patch tested with dilution series containing Ni and Pd-Cl, and one month later were given an oral capsule containing Ni or placebo. Flare-up reactions at previously Ni and Pd-Cl-positive patch tested areas were seen only in the group of patients receiving oral nickel and none in the group receiving placebo ($p = 0.012$ for flare-up reactions at Ni-positive patch test sites and $p = 0.006$ for palladium-positive patch test sites compared to the placebo group). The stronger the patch test reaction to Ni and Pd-Cl, the more flare-up reactions were noticed at both Ni and Pd-Cl- positive patch test sites, after oral challenge with Ni (86). A significant correlation between positive patch test reactions to Ni and Na-PdCl (*rho* = 0.78, $p < 0.0001$) and Pd-Cl (*rho* = 0.61, $p < 0.0001$) has been described by Muris et al (69).

Cross-reactivity has also been investigated at the T-cell level. T-cells from Ni-allergic patients can become activated upon stimulation with palladium (46, 47). Some T-cell clones can react to palladium and others to copper, whereas some clones do not react at all, which perhaps might reflect the incomplete or complexed pattern in cross-reactions in patch test reactivity among patients with contact allergy to Ni (46, 47). All the above-mentioned studies confirmed that cross-reactions between Ni and Pd-Cl exist.

Aluminium

Exposure of aluminium

Aluminium is a common metal, found in many different consumer products in our everyday life. Aluminium forms bonds with oxygen to become aluminium-oxide, which has a surface stable to corrosion. Contact with elemental aluminium does therefore not elicit a contact allergic reaction, as the Al^{3+}-ions are tightly adhered. Once released, Al^{3+} is, however, found very reactive and it forms salts in several ways, giving Al^{3+} different properties in many consumer products (87). In sunscreen, aluminium oxide can be found working as a physical filter (88), and in toothpaste, aluminium oxide is a polishing agent (89). Aluminium chloride (Al-Cl) and aluminium chlorohydrate are used in antiperspirants where they block sweat excretion. In high concentration, aluminium chloride has astringent properties used in local anti-coagulants. Aluminium hydroxide is used in antacids as an acid neutralization agent. Aluminium hydroxide, aluminium phosphate, and aluminium potassium sulphate are used in vaccines as adjuvants (90). Taking into consideration the extensive use of aluminium, it is considered a weak allergen.

Patch testing with aluminium

Since the end of the 1970s, Finn Chambers have been used as the standard test chamber at the Department of Occupational and Environmental Dermatology in Malmö. Before the introduction of the Finn Chamber, the Al-test was used, developed by Sigfrid Fregert. This chamber was made of aluminium and, to avoid direct contact between the test substance and the aluminium, there was a thin layer of polythene covering the aluminium surface (personal communication with Magnus Bruze). The Finn Chamber was developed by Pirilä to optimize the adhesion ability to the skin. The border of the chamber was turned towards the skin to enclose the test substances more tightly. Softer and more porous tape, like Scanpor (Norgesplaster, Oslo) was sufficient to keep the chambers in place (91). The Finn Chamber was smaller than the Al-test and gave the opportunity to test with many more allergens at a time. The Finn Chamber is also made of aluminium, and it has been seen not only as light and cheap, but also as resistant to corrosion, and non-allergenic. However, there are some, though few, case reports describing contact allergy to Finn Chambers in patients with strong contact allergy to Al-Cl (20, 92, 93). When it comes to the corrosion stabile properties, it was already noticed in 1980 that mercury salt in aqueous solution was able to corrode the aluminium surface and react with the aluminium in Finn Chambers, which gave rise to irritant reactions and the risk of false-positive reading (94). Later, it was concluded that when mercury was tested in pet preparations the risks of irritant reactions were minimized, as pet protected the skin against irritation (95). In 1985, chemical studies with cobalt and nickel salts showed reactions between aluminium and cobalt and nickel. Once again it was confirmed that pet as a vehicle in test preparations will reduce problems with the interaction between aluminium and metal salts (96). The aluminium release from Finn Chambers has also proven able to induce a false-negative patch test reaction. In a case report about contact allergy to ethyl cyanoacrylate glue, a patient was patch tested with ethyl cyanoacrylate glue in acetone using a Finn Chamber. Hence, the aluminium promoted a catalytic reaction leading to a high polymerization of cyanoacrylate which decreased the number of molecules in the chamber, with the result that no positive patch test appeared. The same test preparation evoked a positive reaction when tested with a van der Bend chamber (plastic chamber) and in a Finn Chamber with pet as the vehicle (97). In 2020, Hedberg et al published new chemical results about aluminium release from Finn Chambers. They showed that when a Finn Chamber with the test preparation Na-PdCl 3.0% pet and cain mix II 10.0% pet was exposed to artificial sweat, there was a significant aluminium release from the chamber surface, corresponding to a skin dose of Al-Cl of 0.03-0.5% in a plastic chamber. They also noticed that chambers with *Myroxylon pereirae* (MP) 25.0% pet, and Pd-Cl 2.0% pet showed increased Al-release, though for these two preparations the increase was not statistically significant. Many patch test preparations from the European baseline series

showed decreased release of aluminium in the study, compared to an empty Finn Chamber only exposed to artificial sweat. This was explained as above: due to the protection of pet in the preparations. However, when exposed to the preparations of Na-PdCl, Pd-Cl, cain mix II, and MP, the petrolatum was found insufficient to protect from corrosion on the surface of the aluminium-oxide due to formation of Cl^- ions and acid in the preparation (98).

Today an upgraded version of the Finn Chamber exists – Finn Chamber Aqua. In this chamber, the aluminium surface is coated with a pre-fixed filter paper. It was developed to avoid loose filter paper when patch testing with liquids. Finn Chamber Aqua is resistant to moisture and can remain in place even if the patient takes a shower (99). Because of the paper-coated surface, the preparation is not in direct contact with the aluminium-oxide surface. Due to a change in working habits the Department of Occupational and Environmental Dermatology in Malmö changed the test chamber system to Finn Chamber Aqua in 2018.

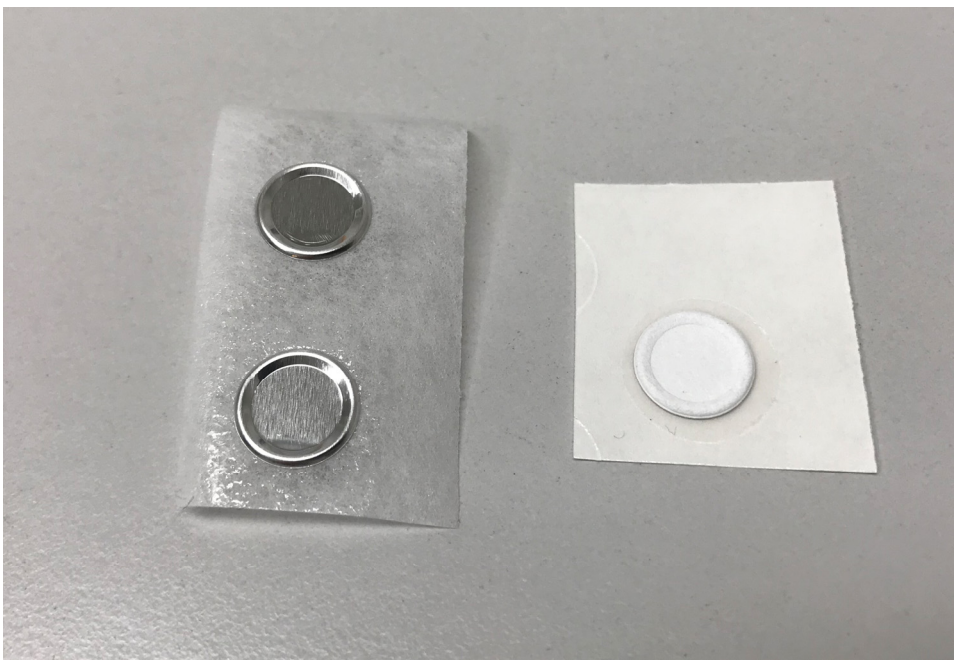


Figure 3 . Finn Chambers. To the left, Finn Chamber used as test chamber in Malmö from late 1970s until 2018. To the right, Finn Chamber Aqua. Paper coated aluminium Finn Chamber used in Malmö since 2018. Photo by Lisbeth Rosholm Comstedt

Since February 2010, Al-Cl has been part of the extended baseline series in Malmö. Aluminium lactate (Al-lac) was in the extended baseline series from February 2010 until 31st December 2017. The most optimal dose of Al-Cl to test with is 10.0% w/v pet (20, 100, 101). In a study with 21 participants with known allergy to aluminium, Siemund et al found that Al-Cl 10.0%, as well as Al-lac 7.7%, gave rise to more positive reactions than higher or lower concentrations of these test salts (20). This phenomenon has been explained based on different theories. One explanation is that Al-Cl in high concentrations has an astringent effect on human tissue, leading to impaired penetration through the epidermis in high doses. Another explanation could be that aluminium has a modulatory effect on the immune system; in vaccines and in allergen immunotherapy, Al^{3+}, works as an adjuvant early in the immune response. It activates the innate immune system to stimulation of the adapted immune system. In vaccines, T-helper cells are stimulated which leads to development of antibodies against the injected inactive virus (102). In allergen immunotherapy, the stimulation of the adapted immune system enhances and activates T-regulatory cells, which in turn leads to increased tolerance and less symptoms to the injected allergen (103). Could Al^{3+} play a role in the immune system in delayed hypersensitivity as well, hence up-regulating the patch test reactions or down-regulating them? The latter could explain weaker reactions at the higher concentrations. Aluminium's effect in patch test reactions has not been investigated.

Rationale

When this project was initiated in 2013, studies from other European countries reported high prevalence of contact allergy to Pd-Cl 1.0% pet and Na-PdCl 3.0% pet with, however, few relevant symptoms and mainly from dental alloys. In Malmö, a high rate of positive patch test results to Pd-Cl and Na-PdCl together with concomitant reactions to Ni was commonly noticed without any clinical relevance found. Questions arose as to whether the patients had cross-reactions to Ni. Could we confirm cross-reactivity between the metals by investigating co-reactivity and co-variability over time? Was it possible to distinguish the cytokine response, when T-cells were stimulated with Ni-Cl or Pd-Cl, so that we could distinguish between cross-reaction to Ni and true palladium allergy? How did the EU Nickel Directive in 2001 affect the prevalence of contact allergy to palladium, and was it following that for nickel?

The prevalence of isolated contact allergy to palladium in the patients at the Department of Occupational and Environmental Dermatology in Malmö was unknown. In other European countries isolated palladium allergy was and still is associated with dental alloys. In southern Sweden, palladium is seldom used in dental alloys, which makes our population different.

The results from study I and study II changed my focus to factors affecting patch test results. As the standard test chamber in patch testing, Finn Chamber, is made of aluminium, and laboratory investigation in 2020 showed that Pd-Cl and Na-PdCl induced corrosion in the Al-surface in the chamber, the question arose as to whether this was clinically relevant in our patients. At the Department of Occupational and Environmental Dermatology in Malmö, we had unique data because we had tested with Finn Chamber, Al-Cl and Al-lac, and both palladium salts for several years. The role of Al-Cl in patch test reactivity in general came into focus. Could it be down– or up regulating the patch test reactions?

Aims

The general aim of the thesis was to improve our knowledge of patch testing with palladium and the interpretation of the results. Our initial findings made it necessary to also explore the importance of the metal aluminium used in test chamber systems and the effect of aluminium chloride in patch test preparations.

Specific aims:

- To determine the prevalence of contact allergy to palladium in southern Sweden with a focus on gender, cross-reactions, the EU Nickel Directive, and the introduction of the test salt, Na-PdCl in the extended baseline series.
- To investigate the reproducibility of patch test results to Na-PdCl and Pd-Cl and explore factors influencing these results. The reactivity of and variation in contact allergy to Ni, Na-PdCl, and Pd-Cl in the same individuals was studied, and factors such as atopy, hormonal and seasonal changes, levels of nickel and palladium in the blood, and the degree of cytokine response at the T-cell level were analysed.
- To study the diagnostic outcome of palladium allergy when patch testing with aluminium Finn Chambers in patients with contact allergy to aluminium.
- To explore the effect of mixing Al-Cl with common patch test preparations such as Ni, fragrance mix I (FM I), and MI.

Materials and Methods

Study designs and participants

Study I

A retrospective study was performed with patch test results from all consecutive patients tested from January 1995 to December 2016 at the Department of Occupational and Environmental Dermatology, Malmö, Sweden. The study population was extracted from the database Daluk, from January 1995 to December 2016. The data extraction was done by a consultant in 2017 and was supposed to only contain consecutive patients tested with the Swedish baseline series and the extended baseline series. Because of an upgraded database, we are today able to extract data ourselves, and during the writing process of this thesis it was noticed that the patients tested with the dental series were included in the data from 2017.
The prevalence of contact allergy to Na-PdCl, Pd-Cl, Ni, and Co among men and women was analysed according to age group and test year.

Study II

This was an experimental, prospective study with 15 females with a known contact allergy to Ni and Pd-Cl and/or Na-PdCl. The participants were patch tested four times at 12-week intervals between September 2014 and May 2015. The patch tests contained dilution series of Na-PdCl, Pd-Cl, and Ni; see Table 2. At each patch test occasion, history about atopic eczema and the use of hormonal contraceptive as well as the number of days since their last menstruation, was obtained and a blood sample was taken before the application of the test chambers. Blood samples for measuring cytokine activity from peripheral mononuclear cells were sent to Mabtech in Stockholm for analysis. Blood samples for measuring the concentration of nickel and palladium in the blood were taken on test occasion 2-4. These tests were transported to the Department of Occupational and Environmental Medicine in Lund for further analysis.

Study III

A retrospective study was performed with consecutive dermatitis patients, tested at the Department of Occupational and Environmental Dermatology in Malmö from January 2010 to December 2019. Patients that had been tested with Finn Chamber or Finn Chamber Aqua and the test preparations Na-PdCl, Pd-Cl, Ni, *Myroxylon pereirae*, cain mix II, FM I, tixocortol-21-pivalate (tixocortol), and potassium dichromate (K-Cr) were included. In total 5,446 patients were enrolled. The positive patch test results for the above-mentioned test preparations and concomitant reactions to Al-Cl and Al-lac were investigated.

Study IV

This was an experimental study, divided into three parts (part 1, 2, 3). Part 1 was a pilot study and performed on consecutive patients referred to the Department of Occupational and Environmental Dermatology in Malmö. One hundred and twenty patients were enrolled and, besides the Swedish baseline series and the extended baseline series, the patients were patch tested with five extra patches containing Ni 15.0% in aqua; four of the patches also contained different concentrations of Al-Cl; see Table 3. After this first study, part 2 and part 3 were done slightly different. In part 2, participants were those already known to have contact allergy to MI but no allergy to Al-Cl, and in part 3 the participants were already known to have contact allergy to FM I but no allergy to Al-Cl. The participants were tested with the preparations shown in Table 3. Some of the participants reacted to the control patches with Al-Cl alone and were excluded. Twenty participants ended up being enrolled in part 2, and 19 participants in part 3. Participants without MI and FM I allergy were enrolled as controls in parts 2 and 3. The controls were previously patients, tested up to six months before the study and known to be negative to MI or FM I. Seven controls were tested with the MI preparations and 10 controls with the FM I preparations.

Patch test material

Table 2. Suppliers, vehicles, and concentrations of patch test substances and material used in studies I, II, III, and IV at the Department of Occupational and Environmental Dermatology in Malmö.

		Vehicles and concentrations			
Patch test substance	**Purchased from**	**Study I**	**Study II**	**Study III**	**Study IV**
Nickel sulphate hexahydrate	Acros Organics, Geel, Belgium (study I-II). Chemo-technique Diagnostics, Vellinge, Sweden (study III) Sigma Aldrich, Steinheim, Germany (study IV)	5.0% w/w pet	Dilution series in pet 5.0 - 0.00027% w/w	5.0% w/w pet	5.0% w/w pet and 15.0 % w/v aq
Sodium tetrachloropalladate	Acros Organics, Geel, Belgium (study I). Sigma Aldrich, Steinheim, Germany (study II- III)	3.0% pet w/w	Dilution series in pet 3.0 - 0.00093% w/w	3.0% w/w pet	
Palladium chloride	ICN Biomedicals, Eschwege, Germany (study I) Chemotechnique Diagnostics, Vellinge, Sweden (Study II, III)	2.0% pet w/w	Dilution series in pet 3.37 0.000181% w/w	2.0% w/w pet	
Cobalt chloride	Chemo-technique Diagnostics, Vellinge, Sweden	0.5% pet w/w			
Aluminium chloride hexahydrate	MPBiio-medicials, inc. Eschwege, Germany (study III) Sigma Aldrich, Steinheim, Germany (study IV)			10.0% w/w pet	Dilution series in aqua 2.0 -30.0% w/v and in 50/50 aq/ethanol v/v
Aluminium lactate	Sigma Aldrich, Steinheim, Germany			12.0% w/w Pet	
Myroxylon pereirae	Chemo-technique Diagnostics, Vellinge, Sweden			25.0% w/w Pet	
Cain mix II	Chemo-technique Diagnostics, Vellinge, Sweden			10.0% w/w pet	
Fragrance mix I	Chemo-technique Diagnostics, Vellinge, Sweden			8.0% w/w pet	
Potassium dichromate	Chemo-technique Diagnostics, Vellinge, Sweden			0.5% w/w pet	
Tixocortol-21-pivalate	Chemo-technique Diagnostics, Vellinge, Sweden			0.1% w/w pet	
Methylisothiazolinone	Sigma Aldrich, Steinheim, Germany			0.2% w/v aq	

		Vehicles and concentrations			
Patch test substance	**Purchased from**	**Study I**	**Study II**	**Study III**	**Study IV**
Frangrance mix components	International Flavors and Fragrances, New York, New York, USA (Amylcinnamal, Geraniol, Isoeugenol) Bedoukian Researc Inc. Connecticut, USA (Cinnamyl alcohol, Cinnamal) Firmenich Inc, Plainsboro, NJ, USA (Augenol, Hydroxycitronellal) Robertet, Grasse, France (Oak moss)				8*1.25% w/v aq/ethanol
Petrolatum	APL Stockholm				
Ethanol (alc)	Histolab, Askim Sweden (Study IV)				
Finn Chamber and Finn Chamber Aqua	Epitest Ltd Oy, Tuusula, Finland or Smart Practice, Phoenix, Arizona, USA. Study (I-III)				
IQ Ultimate Chambers	Chemo-technique Diagnostics, Vellinge, Sweden (study IV)				

Table 3: Substances and concentrations in the different preparations with Al-Cl used in study IV.

Part 1	**Nickel sulphate hexahydrate (%)**	**Aluminium chloride hexahydrate aq(% w/v)**
Ni-Al30	15.0 w/v aq	30.0
Ni-Al20	15.0 w/v aq	20.0
Ni-Al10	15.0 w/v aq	10.0
Ni-Al2	15.0 w/v aq	2.0
Ni-aq	15.0 w/v aq	0.0
Ni-pet	5.0 w/w pet	0.0
Part 2	**Methylisothiazolinone (% w/v aq)**	**Aluminium chloride hexahydrate (% w/v aq)**
MI-Al30	0.2	30.0
MI-Al20	0.2	20.0
MI-Al10	0.2	10.0
MI-Al5	0.2	5.0
MI-aq	0.2	0.0
Control-1	0.0	0.0
Control-2	0.0	5.0
Control-3	0.0	10.0
Control-4	0.0	20.0
Control-5	0.0	30.0
Part 3	**Fragrance mix I (% w/v aq/ethanol)**	**Aluminium chloride hexahydrate (% w/v aq /ethanol)**
FMI-Al30	10.0	30.0
FMI-Al20	10.0	20.0
FMI-Al10	10.0	10.0
FMI-Al5	10.0	5.0
FMI-aq/alc	10.0	0.0
Control-1	0.0	0.0
Control-2	0.0	5.0
Control-3	0.0	10.0
Control-4	0.0	20.0
Control-5	0.0	30.0

Patch testing, patch test readings, and calculating patch test results

Patch tests were performed according to the International Contact Dermatitis Research Group´s recommendations (1, 14). Patches were always removed from the patient´s back after two days and readings were carried out by a dermatologist on day 3/4 and day 7. At the Department of Occupational and Environmental Dermatology in Malmö, all patch test results with basic characteristics of the patients are accessible from our database Daluk back to 1995. From 2019, the results have been filed in a new database: Ekta.

In studies II and IV, the readings were modified according to the International Contact Dermatitis Research Group´s criteria with two classifications added to the three usual positive gradings. A doubtful reaction, with redness and infiltration not covering the whole test area, was graded as (+). A + reaction with a few papules was graded as +(+), and a ++ reaction with a few vesicles was graded as ++(+) (44).

To enable statistical calculations of the patch test results, irritant reactions were classified as negative reactions and doubtful reactions were classified as negative reactions in study I and study III. In studies II and IV, the test results were given the following numeric values: negative = 0; (+) = 0.5; + = 1; +(+) = 1.5; ++ = 2; ++(+) = 2.5; +++ = 3.

Test reactivity

Test reactivity was calculated in two ways in study II (44): (i) the lowest concentration eliciting at least a + reaction was registered as the minimal elicitation concentration (MEC), and (ii) the results for all reactions in a dilution series were given the above-mentioned values and were summed and registered as a summarized test score (STS) (44). This method was used in both studies II and IV.

In study II, by definition, all participants had contact allergy to Ni and Pd-Cl and/or Na-PdCl; therefore, when a negative reaction was noted to all dilutions, the MEC was defined as the concentration that would have been tested if the dilution series had an additional higher concentration. The positive reactions were not always continuous. When the same number or more of positive reactions followed the number of negative and/or doubtful reactions, the lowest positive reaction was registered as the MEC. In all other situations, the concentration above the first negative or doubtful reaction was registered as the MEC.

Co-reactivity was used in study II. The mean MEC to one test salt was calculated as the mean from the four test occasions for every participant. The mean MEC to one test salt

was then correlated with the mean MEC to another test salt. If there was a positive correlation, the test salts showed co-reactivity (51). Example is given in Figure 4.

Co-variation in reactivity was used in study II. The change in MEC to one test salt between two test occasions was found, and correlation analysis was done with the change in MEC to one of the other test salts between the same two test occasions. Even though two test salts did not show co-reactivity, they could still co-variate. For example, if the participant´s reactivity went up or down to the same extent for two test salts during the same time, and the changes in MEC between the two test salts showed correlation to one another, they co-variate (51). Example is giving in Figure 5.

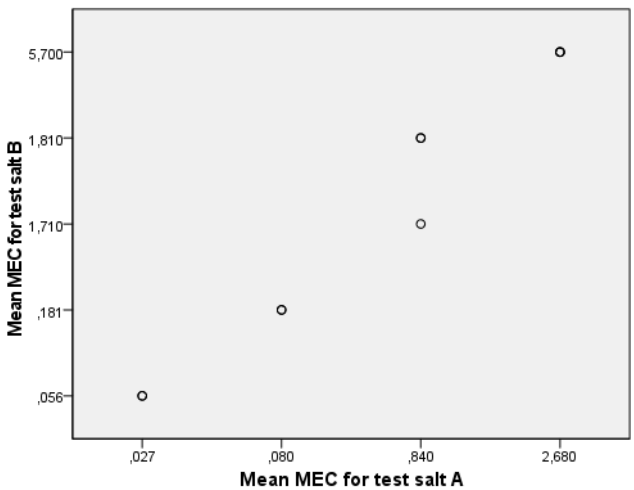


Figure 4. X-axis shows the mean minimal elicitation reaction (MEC) from all test occasions to the test salt A. Y-axis shows the mean MEC from all test occasions to the test salt B. Each dot represents one participant (in this example, five participants). The reactivity of test salt A and B co-reacted if the Spearman´s correlation coefficient, *rho,* was positive and $p < 0.05$.

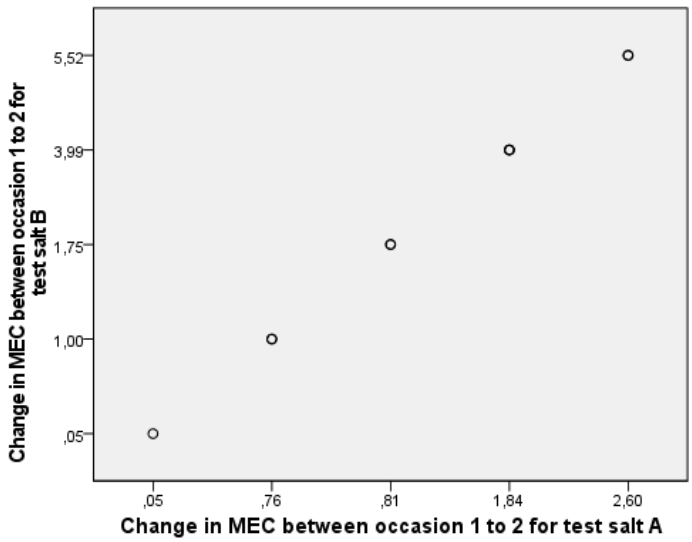


Figure 5. X-axis shows the change in minimal elicitation reaction (MEC) between test occasion 1 and 2 for test salt A. Y-axis shows the change in MEC between test occasion 1 and 2 for test salt B. Each dot represents one participant. The test salts were said to co-variate if Spearman´s correlation, coefficient, *rho,* was positive/negative as long as $p < 0.05$.

FluoroSpot assay

In FluoroSpot assay, cytokines released by T-memory cells, when exposed to a specific allergen, will be immediately captured by specific antibodies which are attached to a membrane in the cell culture plates. A captured cytokine is exposed to a fluorophore-labelled anti-tag detection antibody. In this way, a fluorescent spot will be formed when a memory cell secretes cytokines. By using different fluorophore-labelled anti-tags, multiple cytokines can be studied simultaneously in one well. The T-cells are also stimulated with a polyactivator, phytohaemagglutinin (PHA), which serves as a positive control, as PHA induces the production of many cytokines.

FluoroSpot assay, as described above, was carried out by Mabtech AB, Stockholm, in study II. A pilot study was performed using peripheral blood mononuclear cells (PBMC) isolated from a nickel-allergic person and PBMC from two palladium-allergic persons. A three colour FluoroSpot was optimized to be used for Pd-Cl-reactive PBMC. From this pilot study, it was found that 500,000 cells/well were needed and that the optimal concentration was 50 uM for Ni-Cl and 75 uM for Pd-Cl and that the best response was observed when detecting INFγ, IL13, and IL-22 in a triple FluoroSpot.

The cell culture was also investigated unstimulated, as negative control, and stimulated with PHA as a positive control.

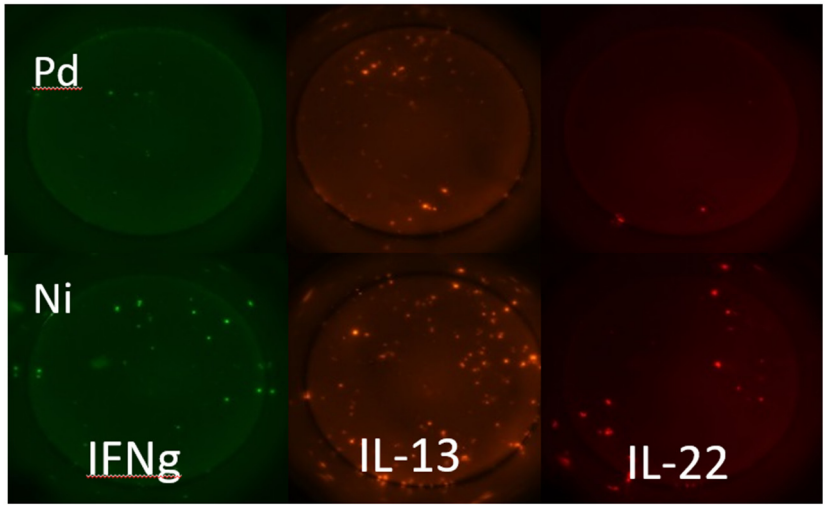


Figure 6. From the FluoroSpot pilot study. Peripheral mononuclear cells from a palladium-allergic individual. The cells were exposed to 75 uM palladium chloride and 50 uM nickel chloride. Interferon-gamma (IFN-γ), interleukin-13(IL-13) and interleukin-22 (IL"") were detected using FlouroSpot. Photo from Mabtech AB.

Blood concentrations of nickel and palladium

In study II, blood samples were taken from the participants at test occasions 2, 3, and 4. These samples were primarily meant to be investigated in another study by another PhD student. The idea of collecting the blood samples from the participants in study II came after the study had started. Due to this, blood samples were not collected from test occasion 1. The blood samples were analysed by the Department of Occupational and Environmental Medicine, Lund University for determination of nickel and palladium concentrations. Prior to analysis of metals, the blood samples were diluted 20-fold with an alkaline solution (104). The concentrations of metals were determined by inductively coupled plasma mass spectrometry (ICP-MS; iCAP Q, Thermo Fisher Scientfic, Bremen, GmbH Germany) equipped with collision cell with kinetic energy discrimination and helium as the collision gas. The limit of detection was 0.04 µg/L for nickel and 0.02 µg/L for palladium. The analytical accuracy was checked against two different reference materials. For nickel, Seronorm Trace Elements Whole Blood L-1 (lot 1103128;SERO AS, Billingsted, Norway) was used, and for palladium outdated blood from blood donors, spiked with 0,10 µg/L palladium was used.

Statistics

SPSS version 22-27 has been used for the statistical analyses. In studies I and II, the statistical analyses were performed with help and support from a statistics consultant (Anna Åkesson, Clinical Studies Sweden – Forum South, Skåne University Hospital). All analyses were considered statistically significant if $p < 0.05$.

Study I

Chi-square test (linear-by-linear association) was used to show trends of positive patch test results to Pd-Cl, Na-PdCl, Ni, and Co across test years, and between Na-PdCl and Ni.

Binary logistic regression models were performed to ascertain the effect on age and test year on the likelihood that women showed positive reactions to Pd-Cl, Na-PdCl Ni, and Co. The regression analyses were done both for Ni and Pd-Cl as the dependent variables and age group, test year and Co-allergy/Ni-allergy, respectively, as independent variables. All results were expressed as odds ratios (OR) with 95% confidence intervals (CI).

McNemar´s test was conducted to calculate the sensitivity for Na-PdCl and PdCl in the study population (unpublished).

Mann-Whitney U test was performed to look at the difference in the distribution of the positive reactions, +,++,+++ to Ni and Pd-Cl in the years 1995-1999 and 2012-2016 (unpublished).

Fisher´s exact test was used to determine the distribution of gender among the isolated patch test reactions to Pd-Cl, Na-PdCl, Ni, and Co.

Study II

Friedman´s test, with pairwise comparison, was used to calculate any significant variation for MEC and STS between the four test occasions for all three test salts. Mann-Whitney U test was used to compare reactivity between groups of atopic/non-atopic and groups with/without hormonal contraceptive. The Spearman´s rank-order correlation coefficient, *rho*, was used to measure the association between patch test reactivity and days of menstrual cycle on the different test occasions and to investigate correlation between co-reactivity and co-variation between the different test salts. It was also used to measure the association between patch test reactivity and levels of nickel in the blood.

Study III

Fisher´s exact test, two-sided, was used to calculate whether the difference in prevalence of MP, cain mix II, Na-PdCl, Pd-Cl, isolated palladium allergy, FM I, Ni, K-Cr, and tixocortol between patients with and without contact allergy to Al was statistically significant. The test was also used to calculate a difference in concomitant reactions in patients with a strong Al allergy compared to patients with a weak allergy to Al.

Study IV

McNemar´s test was conducted in part 1, to calculate the sensitivity of detecting Ni-allergy with the Ni-Al preparations. Mann-Whitney U test was used to compare the grade of patch test reactions to the Al-preparations in the groups of patients positive/negative to Ni 5.0% pet. In parts 2 and 3, Fisher´s exact test, two-sided, was used to compare any differences between allergic participants and controls. Wilcoxon signed rank test was then used to calculate pairwise comparison in STS for the different preparations with, Ni, FM I, and MI.

Ethics

When consecutive patients under investigation for contact allergy are patch tested in Malmö, they are informed that data may be used for comparisons on a group level and approval is mandatory if the patients´ data are stored in our database. Ethical approval to analyse results from the database in studies I and III were obtained from the Regional Ethical Review Board, Lund Sweden. Informed consent was obtained from all participants in studies II and IV and ethical approval for these studies was also obtained from the Regional Ethical Review Board, Lund, Sweden.

Results

Prevalence of contact allergy to palladium in southern Sweden.

The population in study I was extracted from the database Daluk, from January 1995 to December 2016. The 18,306 consecutive patients under investigation for contact allergy were patch tested at the Department of Occupational and Environmental Dermatology in Malmö. Of them, 11,722 (64.0%) were women, and 6,541 (36.0%) were men. Mean age for women was 44.3 years and 44.1 years for men. In the population, 9.8% had a positive patch test reaction to one or both palladium salts and 89.5% of all positive palladium reactions were found in women.

For the years 2009-2016 (when Na-PdCl was included in the extended baseline series), 6,943 patients (both men and women) were patch tested and 789 (11.4%) found positive to one or both palladium salts. Also, 89% of them were found by Na-PdCl and 74% were found by to Pd-Cl, McNemars test, $p< 0.001$ (unpublished).

Prevalence of contact allergy to Pd-Cl in women

Figure 7 shows the prevalence of contact allergy to Pd-Cl among all women and specifically for the youngest group of women, age 6-30 years, from 1995-1999 to 2012-2016. For all women $p = 0.05$, and for women, age 6-30 years, $p < 0.001$. The prevalence among middle-aged and older women was stable during the time period and is not shown in the figure. For more details, see Table 1 in paper I.

Binary logistic regressions analysis with Pd-Cl as the dependent variable showed that the prevalence of positive patch test results to Pd-Cl was dependent on age and the occurrence of contact allergy to Ni. The likelihood that a female patient had a positive patch test reaction to Pd-Cl was 36 times higher if she had a positive reaction to Ni. See Table 4.

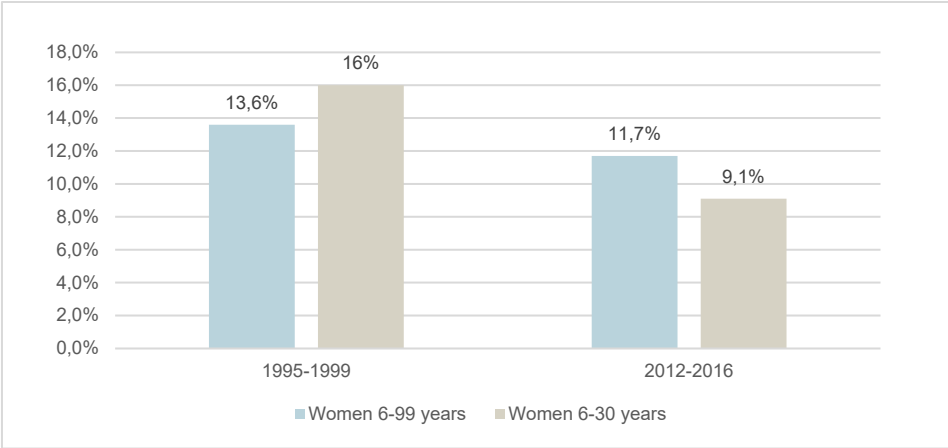


Figure 7. The prevalence of positive patch reactions to palladium chloride in women in the years 1995-1999 and 2012-2016.

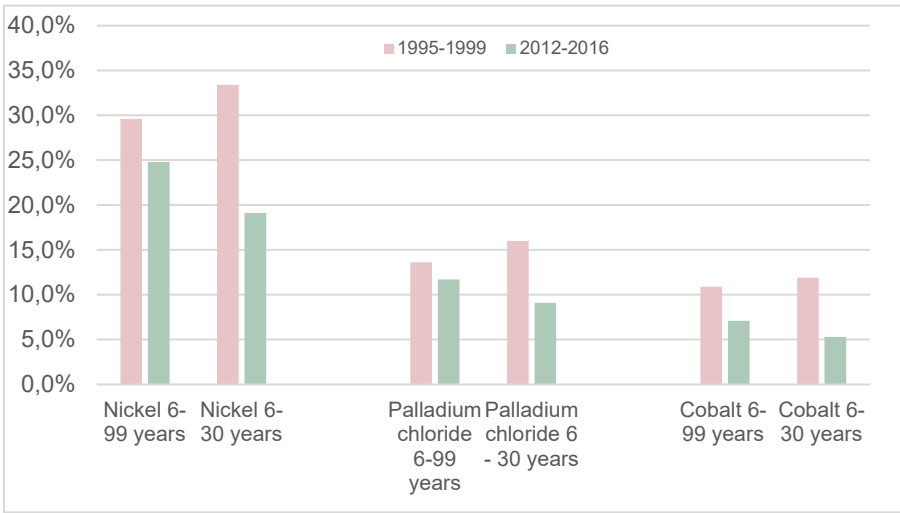
Table 4. Prevalence of positive patch test results to palladium chloride among 11,723 female patients tested from 1995-2016, -the results are stratified by age group and test year.

	Palladium chloride allergy % Positive/total number tested	Unadjusted OR (95% CI)	Adjusted OR(95% Ci)
Age groups			
6-30 years	11.7 (344/2936)	0.85 (0.75-0.97)	0.98 (0.84-1.15)
31-60 years	13.5 (901/6680)	1.0 (reference)	1.0 (reference)
61-99 years	8.1 (171/2107)	0.57 (0.48-0.67)	0.81 (0.66-0.98)
Test year			
1995-1999	13.6 (333/2454)	1.0 (reference)	1.0 (reference)
2000-2011	11.7 (713/6117)	0.84 (0.73-0.97)	0.77 (0.65-0.90)
2012-2016	11.7 (370/3152)	0.84 (0.72-0.99)	1.03 (0.85-1.24)
Nickel allergy			
Negative	141/8354	1.0 (reference)	1.0 (reference)
Positive	1275/3369	35.47 (29.61-42.48)	35.7 (29.78-42.80)

Prevalence of contact allergy to Pd-Cl compared to Co and Ni in women

In the whole study population in study I, 20.5% was shown to have contact allergy to Ni; of these, 89.8% were females. The prevalence of contact allergy to Ni decreased significantly among women from 29.6% in 1995-1999 to 24.8% in 2012-2016, $p < 0.001$. The largest decrease was seen in the youngest group; females 6-30 years. From 33.4% in 1995-1999, to 19.1% in 2012-2016, $p < 0.001$. Of the study population, 7.5% had a positive patch test reactions to Co; of these, 80.3% were females. Contact allergy to Co also decreased significantly among women $p < 0.0001$, and the largest

differences were found among the youngest females: from 11.9% in 1995 to 5.3 % in 2012-2016, $p < 0.001$. Figure 8 shows the prevalence of positive patch test results to Pd-Cl, Ni and Co among women. The prevalence of isolated Co allergy among women was 2.3% during the whole time period and is not shown in the diagram. For further details, see Table 1, in paper I.



Figur 8 The prevalence of contact allergy to nickel sulphate hexahydrate, palladium chloride and cobalt chloride among women, patch tested 1995-1999 and 2012-2016 in Malmö.

Grade of allergy to Ni and Pd-Cl(unpublished)

As the prevalence of contact allergy to Ni and Pd-Cl was decreasing among young females from 1995 to 2016, the patch test reactivity to Ni and Pd-Cl in 1995-1999 were compared with the reactivity in 2012-2016. Figure 9a shows that there was no difference in the strength of contact allergy to Ni in 1995-1999 compared to Ni allergy in 2012-2016. $p = 0.9$. Figure 9b shows the strength of allergy to Pd-Cl, and again no difference was found between 1995-1999 and 2012-2016, $p = 0.5$.

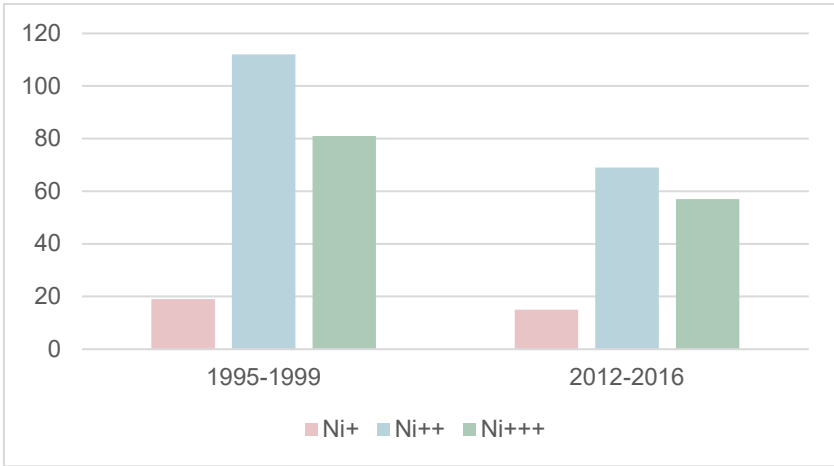


Figure 9a. The number of +, ++, +++ reactions to nickel sulphate hexahydrate (Ni) in 1995-1999 and in 2012-2016 among young females, age 6-30 years.

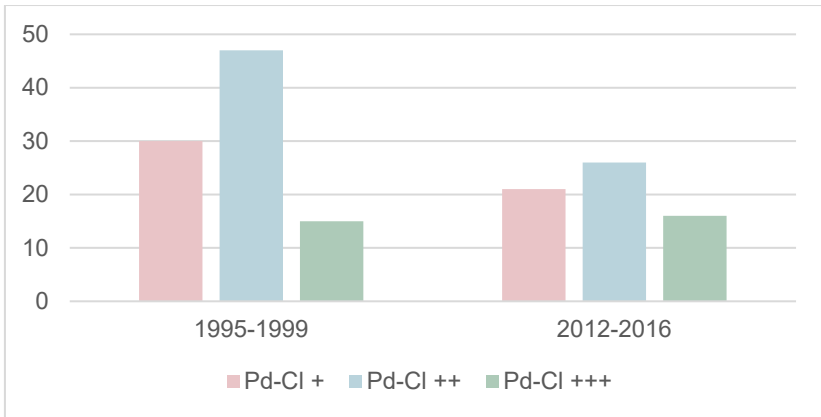


Figure 9b: The number of +, ++, +++ reactions to palladium chloride (Pd-Cl) in 1995-1999 and in 2012-2016 among young females, age 6-30 years.

Prevalence of positive patch test reactions to Pd-Cl and Ni in men

The prevalence of positive patch test reactions to Pd-Cl among men was stable for all age groups during the study period. For young men, 6-30 years old, the prevalence in 1995-1999 was 2.3% and in 2012-2016 it had decreased to 1.5%, though the difference was not statistically significant. The prevalence of Ni allergy among younger men, age 6-30 years, decreased from 5.9% in 1995-1999 to 2.1% in 2012-2016, p = .027. For the older age groups the prevalence of contact allergy to Ni remained stable.

Prevalence of contact allergy to Pd-Cl and/or Na-PdCl

Regarding positive patch test reactions to Pd-Cl and/or Na-PdCl in women, an increase in prevalence among the oldest female group was found, $p < 0.001$, with the greatest difference seen from 2000-2011 to 2012-2016. For women, age 31-61 years a slighter, but still significant, increase in the same time period was also seen, $p = 0.012$. Though Na-PdCl is more sensitive, the decrease in prevalence among the youngest group remained, $p = 0.006$. See Figure 10 and for further details, see Table 1 in paper I.

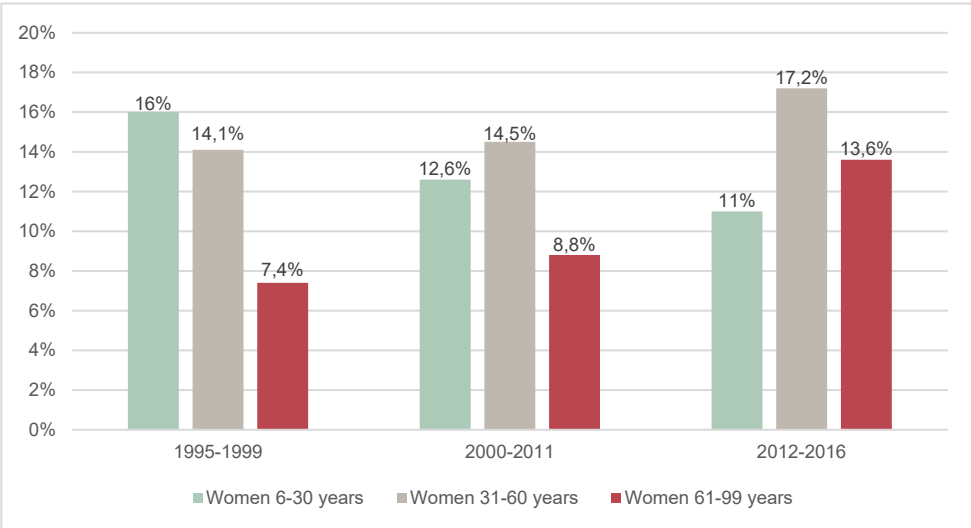


Figure 10. The prevalence of positive patch reactions to palladium chloride and/ or sodium tetra chloropalladate among women in different age groups from 1995-2016.

Test reactivity for Ni and Na-PdCl over time (unpublished)

The positive patch test reactivity to Ni and Na-PdCl on reading day 3/4 was analysed from 2009 (when Na-PdCl was inserted in the extended baseline series) to 2016 in all patients, men and women. There was a significant increase of stronger reactions to Na-PdCl in patients who also had strong reactions to Ni, $p < .001$. The results are illustrated in Figure 11.

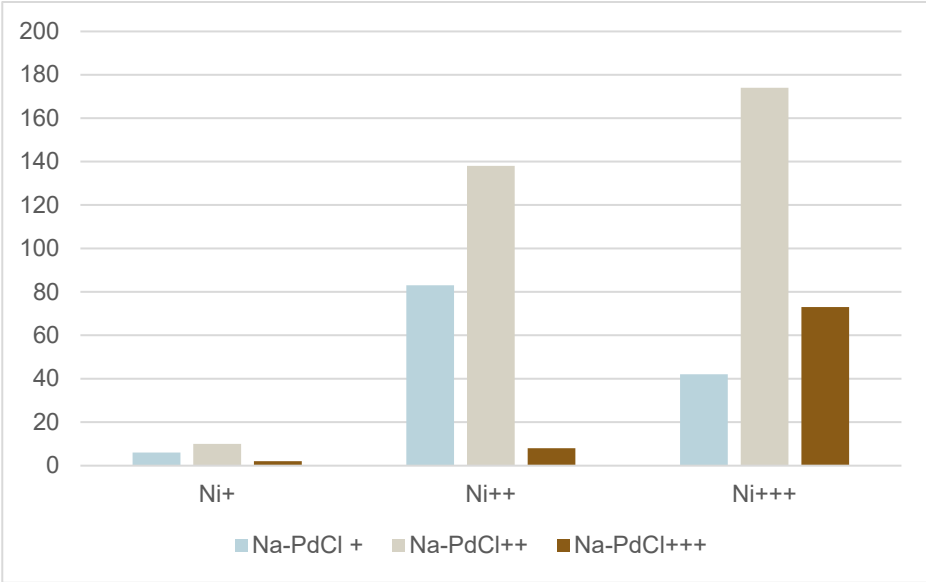


Figure 11. The number of concomitant positive reactions to nickel sulphate hexahydrate (Ni) and sodium tetra chloropalladate(Na-PdCl) in the years 2009-2016 in all patients. The concomitant reactions of Na-PdCl is distributed according to the grade of patch test reaction to Ni.

The reproducibility of patch test results for nickel and palladium.

Patch test reactivity to Ni, Pd-Cl and Na-PdCl at four test occasions, from September to May.

In study II, the participants mean age were 36.3 years, range 22-46, and they were all in their fertile age and none were known to be pregnant. Table 5 shows the MEC and STS for Ni, Pd-Cl, and Na-PdCl in the 15 female participants.

Ni

The largest individual difference in MEC for Ni between two test occasions was 32 times (participant 2). One participant (nr 15) was negative to Ni on one test occasion. The MEC was significantly different at the different test occasions, $x^2(3) = 13.629$, $p = 0.003$. Pairwise comparison revealed significant differences between test occasions 1 and 3, Bonferroni adjusted $p = 0.01$, but not between the other test occasions. The difference between test occasions 1 and 3 were noted as an increase in patch test reactivity during wintertime, as shown in Figure 12.

Pd-Cl

The largest individual difference in MEC was 100 times between test occasion 1 and 2 (participant nr 12). None of the participants had the same MEC on two test occasions. Three participants were negative to Pd-Cl on one test occasion (nr 3,13,15). MEC was significantly different at the different test occasions $x^2(3) = 9.410$, $p = 0.024$. Though the mean MEC for Pd-Cl was lower at test occasion 3 (February) than at test occasion 1 (September), pairwise comparison revealed no significant differences between the test occasions. See Figure 12.

Na-PdCl

The largest individual difference in MEC was 32 times between test occasions 1 and 2 in the same participant (nr 12) that had the largest difference in MEC for Pd-Cl. MEC was the same for Na-PdCl during the whole study period in two participants (nr 3 and 5). No participants were negative to Na-PdCl at any test occasion. MEC was therefore not significantly different between the test occasions, $x^2(3) = 6.469$, $p = 0.091$, but again we saw a numerically lower MEC at test occasion 3 compared to test occasion 1. See Figure 12.

Participant	Year for pre-study test		Pre-study reactivity to Ni[a]	Pre-study reactivity to PdCl[b]	Pre-study reactivity to NaPdCl[c]	Variable	Test 1 Ni	Test 2 Ni	Test 3 Ni	Test 4 Ni	Mean	Test 1 PdCl	Test 2 PdCl	Test 3 PdCl	Test 4 PdCl	Mean	Test 1 NaPdCl	Test 2 NaPdCl	Test 3 NaPdCl	Test 4 NaPdCl	Mean
1	2005		+++	+++	No test	STS MEC%	8.5 0.27	14 0.084	12.5 0.027	13.5 0.084	12.13 0.12	2 5.7	3 0.56	6 0.56	6.5 0.56	4.38 1.85	3.5 0.93	7 0.093	7 0.093	6 0.3	5.88 0.35
2	2014		+++	+++	++	STS MEC%	7.5 2.68	8 0.27	15 0.084	9.5 0.27	10.0 0.83	5 0.56	5 0.56	11.5 0.056	6.5 0.56	7.0 0.43	6.5 0.93	7 0.093	8.5 0.3	8 0.3	7.5 0.41
3	2014		++	+	+	STS MEC%	5.5 0.84	8 0.84	10.5 0.084	8.5 0.27	8.13 0.51	2 1.81	0 5.7	7 0.181	2.5 5.7	2.88 3.35	5 0.3	5 0.3	6 0.3	4.5 0.3	5.13 0.30
4	2012		+++	++	+	STS MEC%	7.5 0.84	12 0.27	7 2.68	11 0.27	9.38 1.02	7.5 0.181	12.5 0.056	10.5 0.056	17 0.018	11.88 0.08	9.5 0.3	8.5 0.3	11 0.093	13.5 0.03	10.63 0.18
5	2013		+++	++	++	STS MEC%	6 0.27	8 0.27	13 0.084	9 0.084	9.0 0.18	2 1.81	6 0.56	8 0.56	6 0.181	5.5 0.78	6 0.3	6 0.3	10 0.3	5 0.3	6.75 0.30
6	2013		++	+	++	STS MEC%	12.5 0.27	9 0.27	14 0.084	11.5 0.27	11.75 0.22	4.5 1.81	7 0.181	8.5 0.56	4 1.81	6.0 1.09	5 0.93	4 0.93	7 0.093	4.5 0.93	5.13 0.72
7	2001		+++	+++	No test	STS MEC%	11.5 0.27	11.5 0.27	11 0.084	11 0.084	11.25 0.18	7 0.56	7.5 0.181	7.5 0.181	7.5 0.056	7.38 0.24	6.5 0.3	6 0.3	7.5 0.093	6 0.3	6.5 0.25
8	2014		+++	++	++	STS MEC%	7.5 0.84	11.5 0.084	9 0.27	7.5 0.27	8.88 0.37	3.5 1.81	3 1.81	4 1.81	6 0.56	4.13 1.5	6 0.3	4 0.93	4 0.93	3 0.93	4.25 0.77
9	2012		++	++	++	STS MEC%	5 0.84	4 2.68	7.5 0.27	5.5 2.68	5.50 1.62	5 0.56	5 0.56	7.5 0.181	6 0.56	5.88 0.47	5.5 0.3	3 0.93	5.5 0.3	6 0.3	5.0 0.46
10	2007		+++	+++	No test	STS MEC%	17 0.027	17 0.027	17.5 0.027	18 0.027	17.38 0.03	12.5 0.056	14.5 0.018	9 0.181	15 0.018	12.75 0.07	10.5 0.093	12 0.03	10.5 0.093	15 0.0093	12.0 0.06
11	2008		++	+	No test	STS MEC%	6.5 0.84	13 0.084	10.5 0.27	11.5 0.27	10.38 0,37	2.5 0.56	8 0.056	5 0.56	4.5 1.8	5.0 0.74	2.5 0.93	5 0.3	6 0.3	4 0.93	4.38 0.62
12	2008		+++	++	No test	STS MEC%	9.5 0.27	13.5 0.084	13.5 0.084	13 0.027	12.38 0.12	4.5 1.81	14.5 0.018	17.5 0.018	16.5 0.018	13.25 0.47	6 0.3	12.5 0.0093	16 0.0093	14.5 0.0093	12.25 0.08
13	2005		+++	+	No test	STS MEC%	10 0.27	14 0.027	15 0.027	14.5 0.027	13.38 0.09	0 5.7	4 1.81	7.5 0.181	4 1.81	3.88 2.38	4 0.93	4.5 0.93	8.5 0.3	8 0.3	6.25 0.62
14	2007		++	++	No test	STS MEC%	10.5 0.084	12 0.084	11.5 0.084	8 0.27	10.50 0.13	6 0.56	5 0.56	4.5 0.56	5 1.81	5.13 0.87	6 0.3	4.5 0.93	5.5 0.3	5 0.93	5.25 0.62
15	2014		+	(+)	+	STS MEC%	1.5 5	0 8.5	2 2.68	4.5 0.27	2.0 4.11	4.5 1.81	0 5.7	5.5 0.56	3.5 1.81	3.38 2.47	5.5 0.3	4 0.93	6 0.3	5 0.3	5.13 0.46

Table 5: The patch test reactivity for nickel sulphate (Ni), palladium chloride (PdCl) and sodium tetra chloropalladate (NapdCl). [a] Patch test reaction to nickel sulphate hexahydrate, 5% in petrolatum [b] Patch test reaction to palladium chloride 2 % in petrolatum. [c] Patch test reaction to sodium tetrachloropalladate, 3% in petrolatum. Included in the extended baseline series since 2009.

When calculating the patch test reactivity based on STS, we found that STS for Pd-Cl, Na-PdCl, and Ni was significantly different at the different time points during the test period: Pd-Cl; $x^2(3) = 13.55$, $p = 0.04$, Na-PdCl; $x^2(3) = 12.52$, $p = 0.006$. Ni; $x^2(3) = 10.41$, $p = 0.015$. Pairwise comparisons revealed statistical differences (Bonferroni adjusted) between the STS for Pd-Cl ($p = 0.007$), Na-PdCl ($p=0.043$) and Ni ($p = 0.014$) between test occasions 1 and 3, see Figure 12, and for Na-PdCl also between test occasions 2 and 3 ($p = 0.009$).

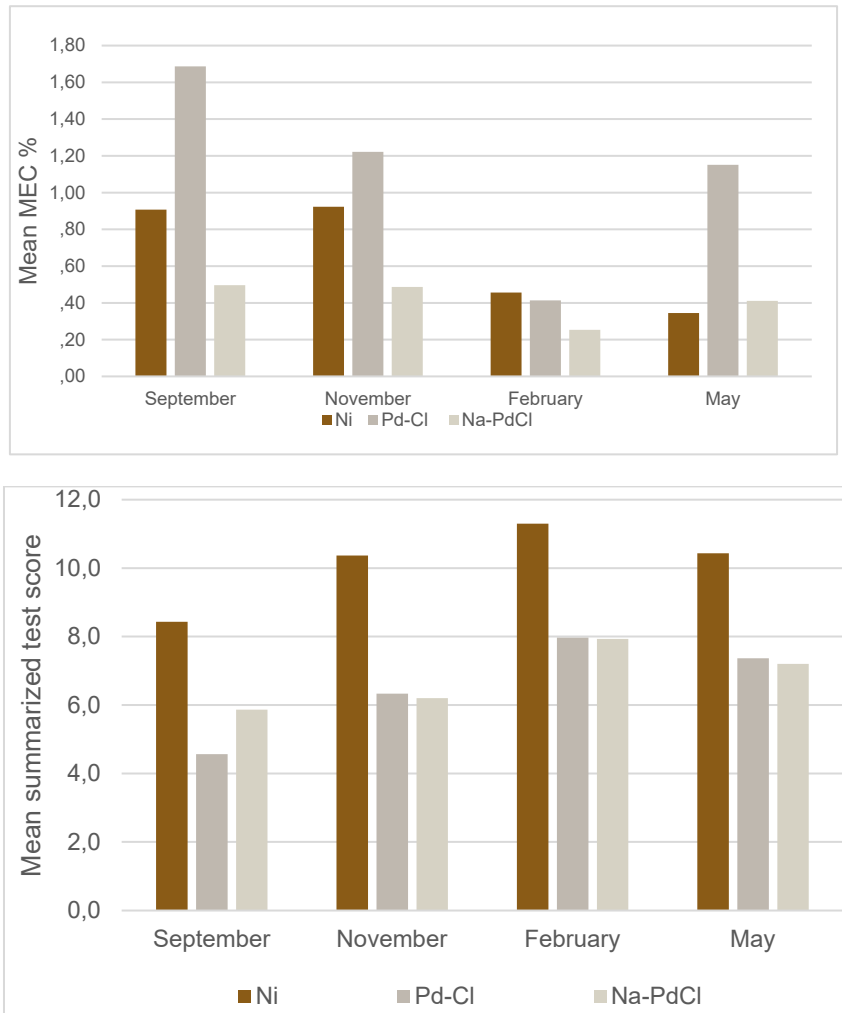


Figure 12. Shows the mean patch test reactivity to the three metal salts, for the four test occasions. The patch test reactivity is shown as mean minimal eliciation concentration(MEC) and as mean summarized test score. Ni:nickel sulphate hexahydrate, Pd-Cl: palladium chloride, Na-PdCl: sodium tetra chloropalladate

Concomitant reactivity between nickel and the two palladium salts

There was a positive and significant correlation between Pd-Cl and Na-PdCl, Spearman *rho* = 0.53, p =0.04. No correlations in patch test reactivity were found between Ni and the two palladium salts. For further details, see Figure 2, paper II.

Co-variation for nickel and the two palladium salts

The only significant correlations in the variation for MEC were seen between test occasions 1 and 2 for Ni and Na-PdCl, Spearman *rho* = 0.53, p = 0.04 and Pd-Cl and Na-PdCl, Spearman *rho* = 0.53, p =0.04 and between test occasions 2 and 3 for Ni and Pd-Cl, Spearman *rho* = 0.54, p = 0.04. The variation between the patch test reactivity for the different salts between the other test occasions was not significant; for further details, see Figure 3, paper II.

The influence of atopy and hormonal changes in the variability in patch test reactions

There were no differences in MEC for any of the test salts between the two groups, atopics and non-atopics, or between the participants on/not on hormonal contraceptive, p >3 in all tests.

No correlation was found between MEC and the number of days in the menstrual cycle for any of the test salts on any of the test occasions, p > 0.07 in all tests.

Cytokine release (unpublished)

Overall, the response to Ni-Cl was stronger than to Pd-Cl. All samples that responded to Pd-Cl at a test occasion had a stronger response to Ni-Cl at the same occasion. In some cases, a response to Ni-Cl but not to Pd-Cl was found. Figure 13 shows the cytokine response in two test occasions from the same patient.

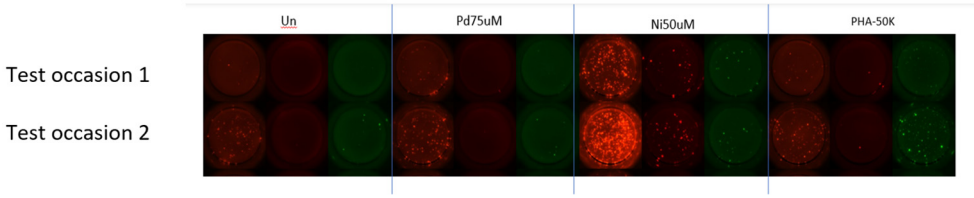


Figure 13. A picture of flourospot assay from a participant from two different test occasions. The response to nickel chloride were stronger than the response to palladium chloride. Un: unstimulated: negative control. Pd75uM: palladium stimulated, Ni50uM: Nickel stimulated. PHA-50K: polyactivator, positive control. INFγ: Interferon-gamma, IL-13: Interleukin 13, IL-22: Interleukin 22. Photo från Mabtech AB.

Unfortunately, the PHA response in the blood samples, (the positive control) varied too much between the samples and in some cases the PHA was very low. A correlation analysis of the variation in cytokine release and the variation in patch test reactivity for the four test occasions could therefore not be performed.

Concentration of nickel and palladium in blood samples(unpublished)

No palladium was detectible in the blood from any of the participants.

Table 5 shows the concentration of nickel in the blood from the participants. The mean concentration increased during the study period; thus, no correlation between MEC and the concentration of nickel in the blood at each test occasion could be found. Spearman *rho* = 0.16, $p = 0.95$.

Table 6. The concentration of nickel in the 15 female participants at the three last test occasions.

	Nickel concentration in blood samples µg/L		
Participant	**November**	**Februari**	**May**
1	0.33	0.42	0.77
2	0.50	0.77	0.89
3	0.32	0.40	0.70
4	0.38	0.61	0.74
5	0.37	0.55	0.65
6	0.39	0.33	0.61
7	0.44	0.46	0.71
8	0.48	0.42	0.40
9	0.33	0.40	0.73
10	0.26	0.30	0.77
11	0.43	0.57	0.82
12	0.37	0.39	0.77
13	0.55	0.38	0.84
14	0.45	0.41	0.64
15	0.50	0.45	0.75
Sum	6.1	6.86	10.79
Mean	0.41	0.46	0.72

Palladium allergy without nickel allergy

Data on isolated palladium allergy were extracted from the material in study I. In 249 patients (both men and women) out of 18,306, 1.4%, had a positive reaction to Pd-Cl and/ or Na-PdCl without a positive reaction to Ni. Women were overrepresented; 184 women (1.6%) and 65 men (1.0%), Fisher´s exact, $p < 0.005$. Na-PdCl has been tested since 2009. Looking at isolated patch test reactions to Pd-Cl alone in women, the prevalence was stable during the study period, $p = 0.29$. For further details, see Table 1 in paper I.

Isolated palladium allergy and age in women study I (Unpublished results)

The prevalence of isolated palladium allergy (Pd-Cl and/or Na-PdCl) in women is shown distributed over test year and age group in Table 7. This table has not been published before, as Table 1 in paper 1 only includes isolated contact allergy to Pd-Cl and does not concern Na-PdCl. There is an increase in 2012-2016, mainly seen in middle-aged women and slightly in the elderly, and this could be associated with the introduction of Na-PdCl in the extended baseline series.

Table 7. The prevalence of isolated palladium allergy, both Na-PdCl and Pd-Cl included, in 11,723 females suspected to have contact allergy at the Department of Occupational and Environmental Dermatology, Malmö. The results are stratified by test year and age group.

Isolated palladium allergy Pd-Cl and/or Na-PdCl	**Test years, % (positive/total tested)**			
	1995-1999	**2000-2011**	**2012-2016**	**P-value**
All age groups	1.1% (27/2454)	1.5% (91/6117)	2.1% (66/3152	0.002
6-30	1.3% (8/637)	1.1% (17/1561)	1.2% (9/738)	0.96
31-60	0.9% (13/1438)	1.5% (53/3524)	2.0% (35/1717)	0.009
61-99	1.6% (6/379)	2% (21/1031)	3.2%(22/697)	0.08

The patch reactivity to Na-PdCl on reading day 3/4 in women was investigated and divided into three groups, 1+, 2+ and 3+ reactions. The percentage of isolated palladium allergy was the same in the three groups and no tendency towards weaker or stronger reactions among women with isolated palladium allergy could be found. See Figure 14, $p = .43$.

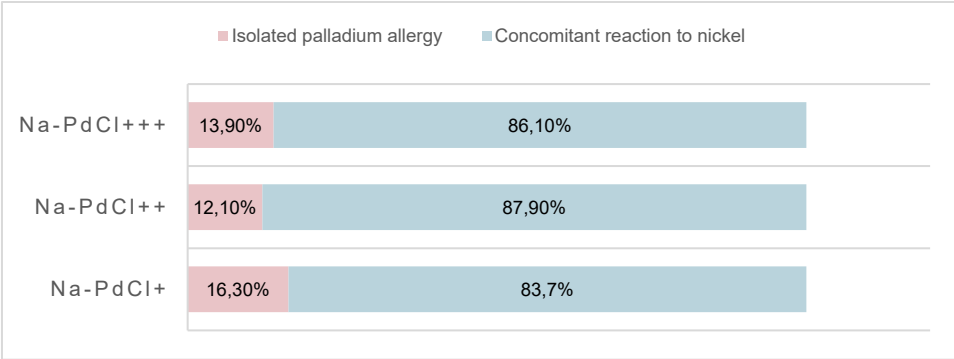


Figure 14: The percentage of isolated palladium allergy to sodium tetrachloropalladate (Na-PdCl) and percentage of concomitant allergy to nickel sulphate hexahydrate among females, data from study I. The percentages are distributed according to grade of patch test reactivity to Na-PdCl on reading day 3/4.

The diagnostic outcome of patch testing with palladium when using Finn Chamber in patients with contact allergy to aluminium.

During 2010-2017, 5,446 consecutive patients were tested with Finn Chamber and the following test preparations: Al-Cl, Al-lac, MP, cain mix II, Na-PdCl, Pd-Cl, FM I, Ni, K-Cr, and tixocortol. Of these, 3,636 were female (66.8%) and 1,810 were male (33.2%) with a mean age 44.4 years. Patients with a contact allergy to Al-Cl and/or Al-lac numbered 48 (0.9%). The age distribution of the 48 patients with contact allergy to Al-Cl and/or Al-lac is shown in Figure 15.

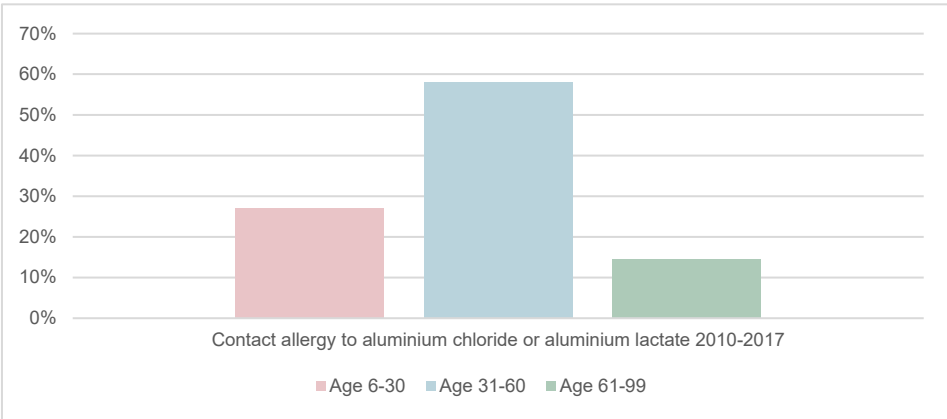


Figure 15. Age disbribution of patients with contact allergy to aluminium in the time period 2010-2017.

Positive patch test reactions to test preparations, known to release Al-ions *in vitro*, were overrepresented among the 48 patients with contact allergy to aluminium when the patients had been tested with Finn Chamber, Fisher´s exact, two-sided, $p = 0.009$ for MP, $p = 0.005$ for cain mix II, $p = 0.04$ for Na-PdCl and $p = 0.005$ for Pd-Cl. When tested with test preparations known *not* to release Al-ions, no statistical significance could be found; $p = 0.56$ for Ni, $p = 0.37$ for FM I, $p = 0.38$ for tixocortol and $p = 0.1$ for K-Cr. When tested with Finn Chamber Aqua, no difference between patients with or without aluminium allergy could be found; $p = 0.15$ for MP, $p > 0.9$ for Na-PdCl and Pd-Cl. No patients had concomitant reactions to Al-Cl and cain mix II, as well as no patients had contact allergy to Al-Cl together with isolated contact allergy to palladium. For further details, see Table 2 in paper III.

Gender and concomitant reactions to aluminium and palladium (unpublished)

Of the 48 patients with contact allergy to aluminium, 31 were female (64.6%) and 17 (35.4%) were male, We know from study I that more females had contact allergy to palladium; however, in study III, there was no difference in gender regarding concomitant reactions to aluminium and palladium (7/31 vs 3/17): Fisher´s exact, two-sided $p > 0.99$ or having contact allergy to aluminium and at the same time isolated palladium allergy: (3/31 vs 2/17), Fisher´s exact, two-sided $p > 0.99$.

Aluminium and Finn Chamber Aqua

From January 2018 to December 2019, 1,450 consecutive dermatitis patients were patch tested with Finn Chamber Aqua, the Swedish baseline series, and the extended baseline series at the Department of Occupational and Environmental Dermatology in Malmö. In this time period there was no overrepresentation of contact allergy to aluminium among patients with contact allergy to MP; $p = 0.15$, Na-PdCl $p > 0.99$, Pd-Cl, $p > 0.99$. No patients with aluminium allergy had positive reactions to cain mix II or had isolated palladium allergy. Figure 16 shows the distribution of positive patch test reactions to the two palladium salts including isolated palladium allergy, distributed according to contact allergy to Al-Cl, and test chamber; for further details, see Table 2 in paper III. Notable is that the prevalence of contact allergy to Pd-Cl and Na-PdCl as well as isolated palladium allergy increased from 2010-2017 to 2018-2019.

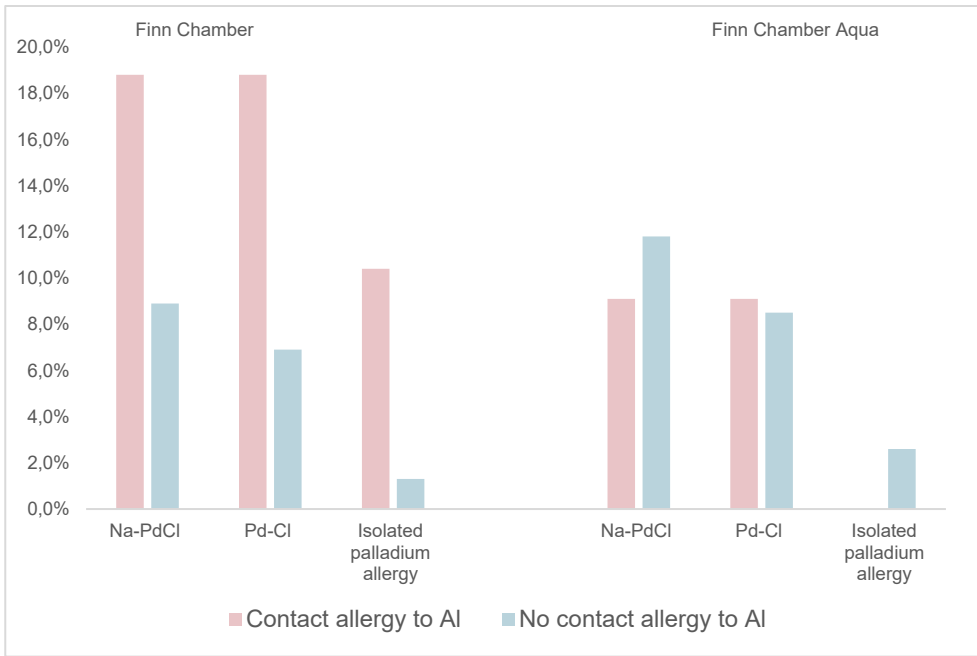


Figure 16. The prevalence of contact allergy to palladium chloride and sodium tetrachloropalladate distributed according to contact allergy to aluminium and test chamber.

Twenty patients out of 48 had a strong allergy (2+ or 3+) to aluminium and the remaining 28 patients had a weak allergy (1+) to aluminium. Among the patients with strong aluminium allergy, there were 19 concomitant reactions to MP and/or cain mix II and/or Na-PdCl, and/or Pd-Cl out of 80 possible reactions, see Figure 17, and among patients with weak aluminium allergy there were 11 concomitant reactions out of 112 possible reactions to MP and/or cain mix II and/or Na-PdCl, and/or Pd-Cl; see Figure 17. (19/80 vs 11/112 Fisher´s, two-sided, $p = 0.015$)

Figure 18 illustrates that the increased number of concomitant reactions in the group with strong Al allergy was due to an increased number of patients reacting to one or more of the corrosion-triggering preparations. For further details about the concomitant reactions to MP, cain mix II, and the palladium salts among patients with aluminium allergy, see Table 3 in paper III.

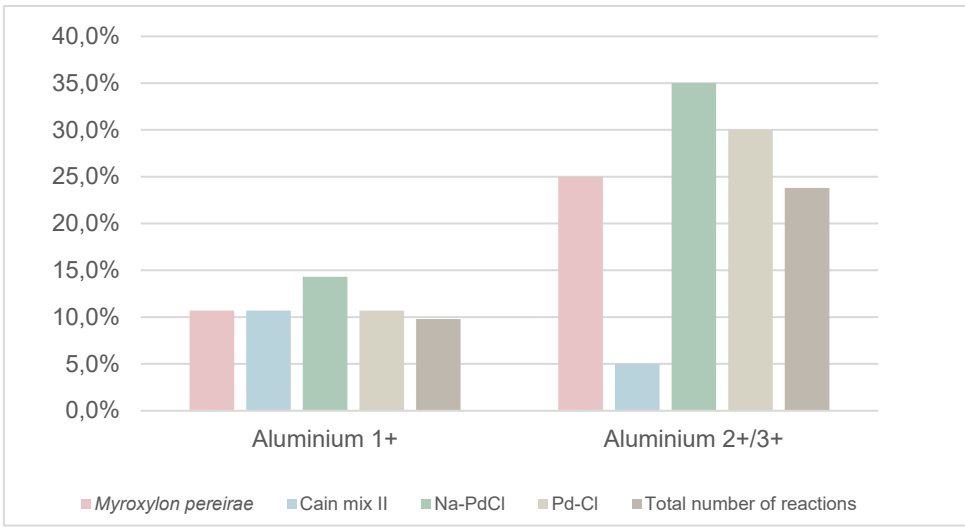


Figure 17. Percentage of patients with contact allergy to aluminium and concomitant reactions to *Myroxylon pereirae*, caine mix II, sodium tetrachloropalladate and palladium chloride. Each column represents the percentage of reactions to the specific test substance out of the number of possible reactions. For example, among the patients with a 2+/3+ allergy to Al, there were five reactions to *Myroxylon pereirae* out of 20 possible reactions (25%).

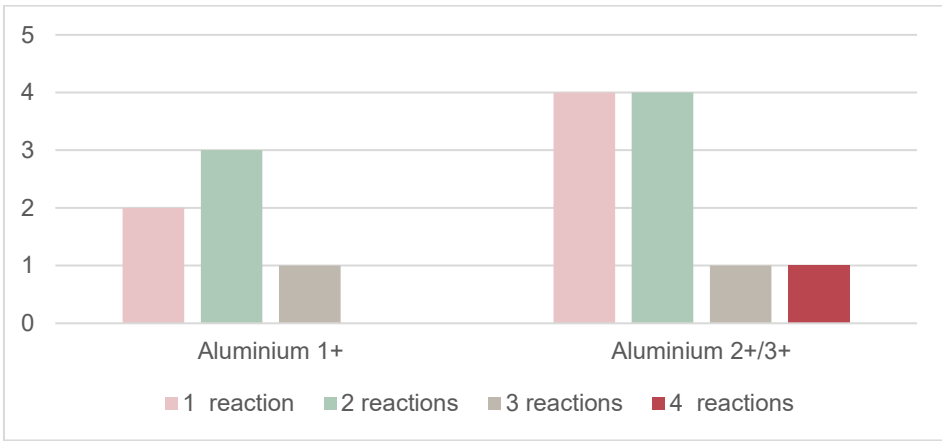


Figure 18. The number of patients with concomitant reactions to any of the four substances causing aluminium-ion release.

Aluminium used as additive in common patch test preparations

Aluminium added to nickel

Thirty-two out of 120 consecutive patients were positive to one or more of the Ni-preparations without being positive to Al-Cl 10.0% pet. Figure 19 shows the number of patients that had positive reactions to the different preparations containing Ni and Al-Cl. Test preparations Ni-Al30, Ni-Al20, Ni-Al10, and Ni-Al2 were compared to the preparations without Al-Cl, Ni-aq, and Ni-pet. Of the positive reactions, 91 % with Ni-Al30 and 88 % with Ni-Al20, compared to 47 % with Ni-aq and 50 % with Ni-pet; this was significantly higher sensitivity, $p < 0.001$.

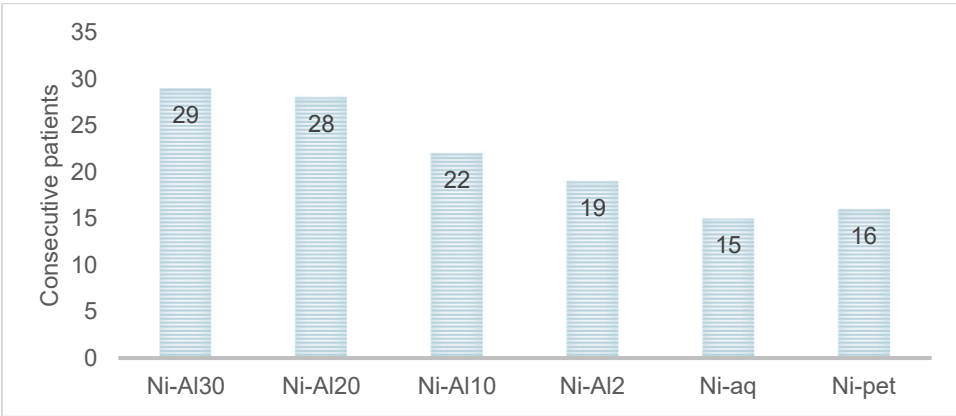


Figure 19. The number of patients that had positive patch test reactions to the different test preparations with nickel sulphate hexahydrate.
Ni-Al30: nickel sulphate hexahydrate 15.0% + aluminium chloride 30.0%, aqua.
Ni-Al20: nickel sulphate hexahydrate 15.0% + aluminium chloride 20.0%, aqua.
Ni-Al10: nickel sulphate hexahydrate 15.0% + aluminium chloride 10.0%, aqua.
Ni-Al2: nickel sulphate hexahydrate 15.0% + aluminium chloride 2.0%, aqua.
Ni-aq: nickel sulphate hexahydrate 15.0%, aqua.
Ni-pet: nickel sulphate hexahydrate 5.0%, petrolatum.

Dividing the 32 patients into two groups, according to their positivity to Ni-pet, revealed that 16 patients were positive, and 16 patients were negative. The group negative to Ni-pet had in general weaker patch test reactions to the preparations with Al-Cl, see Figure 20. Mann-Whitney U test, $p < 0.001$ for all preparations.

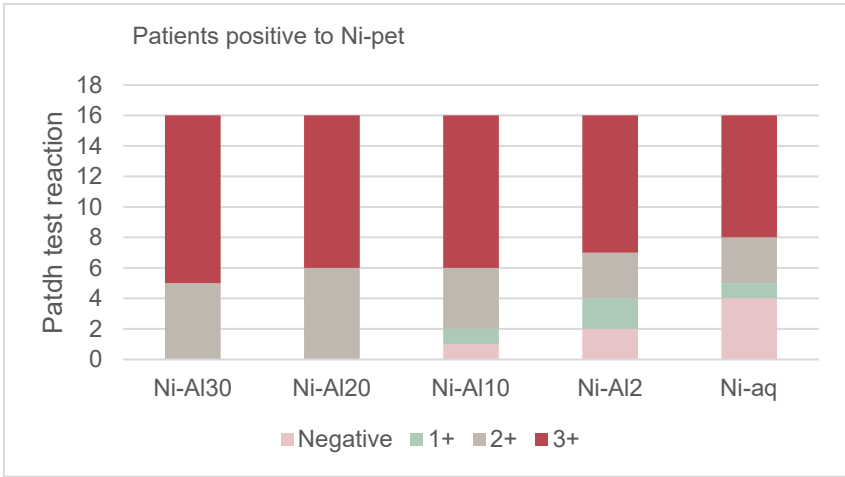


Figure 20a Number of reactions to the different mixes with nickel sulphate hexahydrate and aluminium chloride hexahydrat among the 16 patients with *positive* reactions to nickel sulphate hexahydrate 5.0% in petrolatum

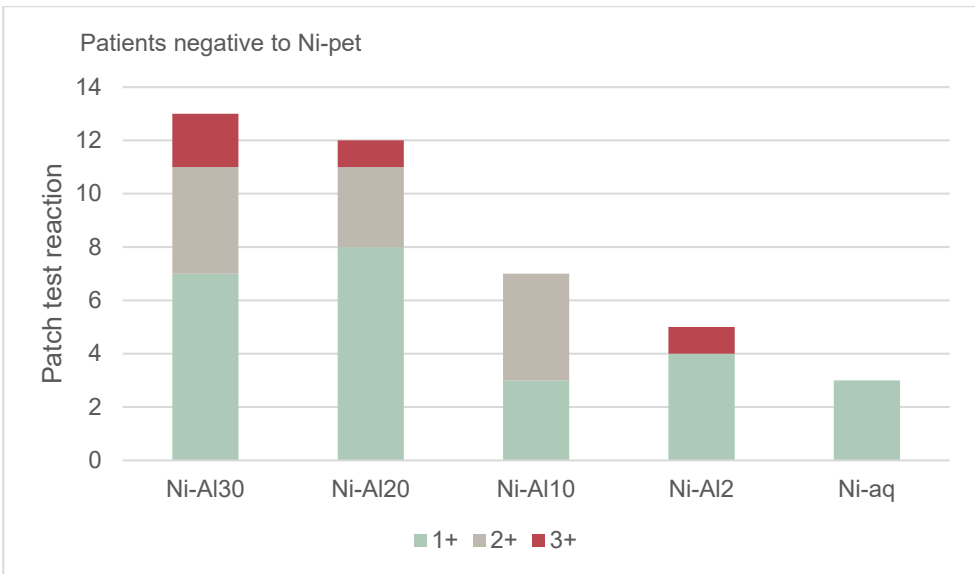


Figure 20b. The number of reactions to the different mixes with nickel sulphate hexahydrate and aluminium chloride hexahydrat among the 16 patients with *negative* reactions to nickel sulphate hexahydrate 5.0%, petrolatum

For the 16 patients positive to Ni-pet, STS was calculated for all preparations. STS for Ni-Al30 and Ni-Al20 was significantly higher than STS for Ni-Aq, shown in Figure 21; (Wilcoxon signed-rank test, $p = 0.03$ for Ni-Al30 versus Ni-aq and $p = 0.04$ for Ni Al20 versus Ni-aq).

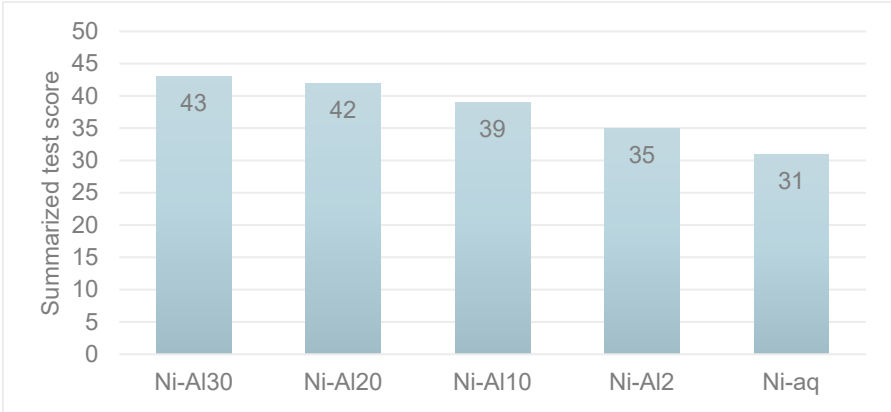


Figure 21. Summarized test score for the different Ni-Al preparations among the 16 patients all positive to Ni 5.0% petrolatum.

Aluminium mixed with methylisothiazolinone and Fragrance mix I.

Figure 22 shows the STS för the MI-preparations. MI-aq had significantly higher STS than MI-Al20 and MI-Al10, Wilcoxon signed rank test, $p = 0.02$ for both preparations. No differecet was found between MI-Al30 and/or MI-Al5 and MI-aq. Five of the allergic participants had irritant reactions, redness and without infiltration, to Al-Cl 30%, at day 3, but none of these participants had stronger reactions to any of the MI-AlCl reactions compared to MI-aq. Two other participants had irritant reactions to Al-Cl20. One of them had a slightly stronger positive reaction to MI-Al30, ++(+) compared to ++ to MI-aq.

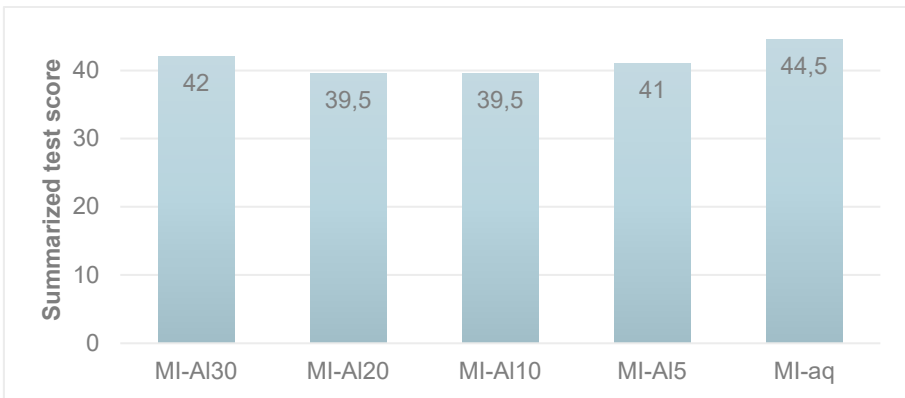


Figure 22. Summerised test score for the different MI-aluminium preparations.
MI-Al30: methylisothiazolinone 0.2% + aluminium chloride hexahydrate 30.0%, aqua.
MI-Al20: methylisothiazolinone 0.2% + aluminium chloride hexahydrate 20.0%, aqua.
MI-Al10: methylisothiazolinone 0.2% + aluminium chloride hexahydrate 10.0%, aqua.
MI-Al5: methylisothiazolinone 0.2% + aluminium chloride hexahydrate 5.0%, aqua.
MI-aq: methylisothiazolinone 0.2%, aqua.

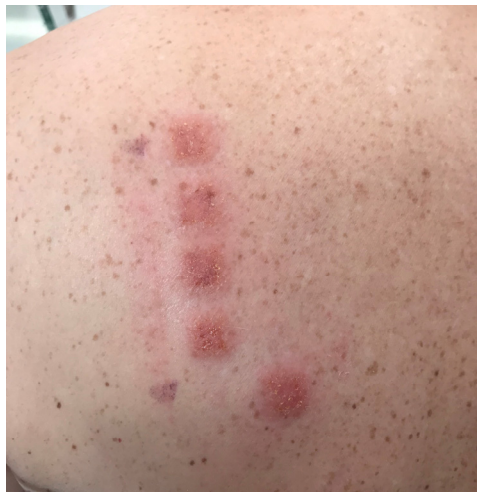


Figure 23. Patch test reactions to mixes of methylisothiazolinone and aluminium chloride hexahydrate. Participant with contact allergy to MI. +++ reactions was seen to all five MI-preparations, no matter the concentration of Al-Cl and negative reactions were seen to all five patches containing Al-Cl without MI. Photo by Lisbeth Rosholm Comstedt

Figure 25 shows the STS for the different preparations with FM I. No differences were found in STS. Among the participants allergic to FM I in part 3, one had an irritant reaction to the control patch nr 5, containing Al-Cl 30.0%, but did not have stronger patch test reactions to FMI-Al30 or FMI-Al20 than to FM1-aq/alc.

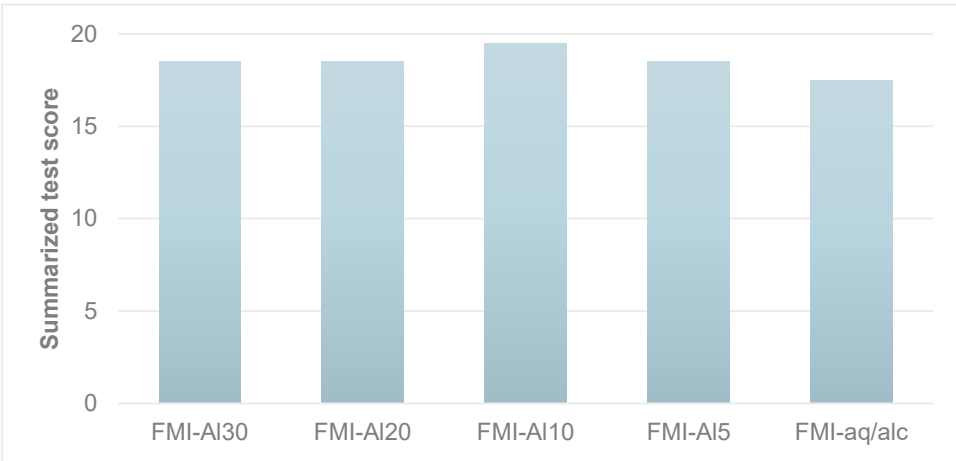


Figure 24. Summerized test score for the different FM I preparations.
FMI-Al30: fragrance mix I 10.0% + aluminium chloride hexahydrate 30.0%, aqua/ethanol.
FMI-Al20: fragrance mix I 10.0% + aluminium cloride hexahydrate 20.0%, aqua/ethanol
FMI-Al10: fragrance mix I 10.0% + aluminium cloride hexahydrate 10.0%, aqua/ethanol.
FMI-Al5: fragrance mix I 10.0% + aluminium cloride hexahydrate 5.0%, aqua/ethanol.
FM1-aq/alc: fragrance mix I 10.0% aqua/ethanol.

Discussion

This thesis aimed to improve our knowledge of patch testing with Pd-Cl and Na-PdCl and explore factors influencing the patch test results to these salts. The results of studies I and II changed the focus from palladium to factors affecting the patch test results in our patients and patch test results in general. Aluminium was investigated as an important factor in contact allergy to palladium and thereafter the effect of adding Al-Cl to patch test preparations was further investigated.

The prevalence of contact allergy to palladium with focus on factors such as legal frameworks, cross-sensitivity, and gender.

Study I is, to the author´s knowledge, the first study to investigate the prevalence of contact allergy to Pd-Cl and Na-PdCl over time and its association with contact allergy to Ni and the impact of the EU Nickel Directive´s introduction in 2001. When analysing the prevalence of contact allergy to Pd-Cl and Na-PdCl among the consecutive patients tested in Malmö 1995-2016, we found a decrease among the youngest females, age 6-30 years, despite the increased demand for palladium in electronics and jewelleries, and the introduction of a more sensitive test salt for detecting palladium allergy during the same time period. Among the middle-aged and elder female patients, 31-99 years, an increase in the years 2012-2016 was noticed, but when looking at patch test results to Pd-Cl, not considering results to Na-PdCl, the prevalence followed that of Ni. For the two salts, Ni and Pd-Cl, there was a decreased prevalence among young females, and a stable prevalence for middle-aged and elder patients in the whole study period. For the whole study population, the likelihood of having contact allergy to Pd-Cl was 36 times higher if contact allergy to Ni was present. Looking at the grade of patch test reactions in 1995-1999 and 2012-2016, see Figure 9, showed that despite fewer persons reacting to Ni and Pd-Cl, the strength of allergy remains the same for the individuals who do get sensitized.

Since palladium is not a commonly used dental material in Sweden (58, 59), the increased prevalence of palladium allergy when looking at both Na-PdCl and/or Pd-Cl is interpreted as due to the use of Na-PdCl in the extended baseline series since 2009. The sensitivity of Na-PdCl and Pd-Cl was 89% and 74% respectively. The prevalence of palladium allergy before 2009 might have been higher if Na-PdCl had been used as

a test salt earlier, thus the prevalence in middle-aged women might had been stable in later years. No significant results could be obtained among males when it came to prevalence of palladium allergy, probably because of the much lower number of allergic male patients.

Studies of the prevalence of contact allergy to Pd-Cl 2.0% from other European countries are sparse, as many have been using Pd-Cl 1.0% in patch testing (71, 72, 105-107). The overall prevalence of positive patch test results to Pd-Cl 2.0% from a recent Spanish study, in the time period 2004-2018, was found to be 8.6%, with the percentage of females being 87% (108). An American study showed similar results, in the time period 1997-2008; the overall prevalence of contact allergy to Pd-Cl 2.0% was 12%, and females were overrepresented, with 90% in this group (109).

When looking at the prevalence of contact allergy to the more sensitive palladium salt, Na-PdCl 3.0%, there seems to be a greater difference between countries in the EU. Numbers from Basel and Amsterdam showed a prevalence of more than 30%, Barcelona, Bari and Coimbra had a prevalence of 20-26%, and Odense and Malmö had approximately 10% (60).These numbers are interpreted as being due to the differences in countries´ use of palladium in dental alloys (60).

The decrease in prevalence to Ni allergy among females and men, age 6-30 years in the time period 1995-2016, is interpreted as a result of the EU Nickel Directive that came into force in 2001. This decrease in younger women has been noticed and described in other countries in the EU as well (110-113). The EU Nickel Directive has not only had a positive effect on Ni allergy, as it was meant to, but also to contact allergy to Pd-Cl, Na-PdCl, and Co among young patients in southern Sweden. As the directive does not limit the amount of palladium or cobalt in consumer products, the reason the legislation has influenced the prevalence of palladium can be interpreted as due to the cross-reactivity between palladium and nickel. The immunological relationship between nickel and cobalt is different, as it has been shown that nickel works as an adjuvant for cobalt sensitization (114). With less exposure to nickel means less exposure to cobalt, as the metals co-exist, and a smaller amount of 'adjuvants' to initiate an allergic reaction to cobalt.

Co-reactivity and co-variability in patch test results to palladium and nickel

Na-PdCl was introduced in 2009. Because of better skin penetration, this test preparation was able to increase the sensitivity to detect contact allergy to palladium. In the material in study II, Na-PdCl showed the smallest variability compared to both Ni and Pd-Cl, as none of the participants had any negative reactions to Na-PdCl at any of the four test occasions, see Table 5. This finding further supports the use of this salt for detection of contact allergy to palladium.

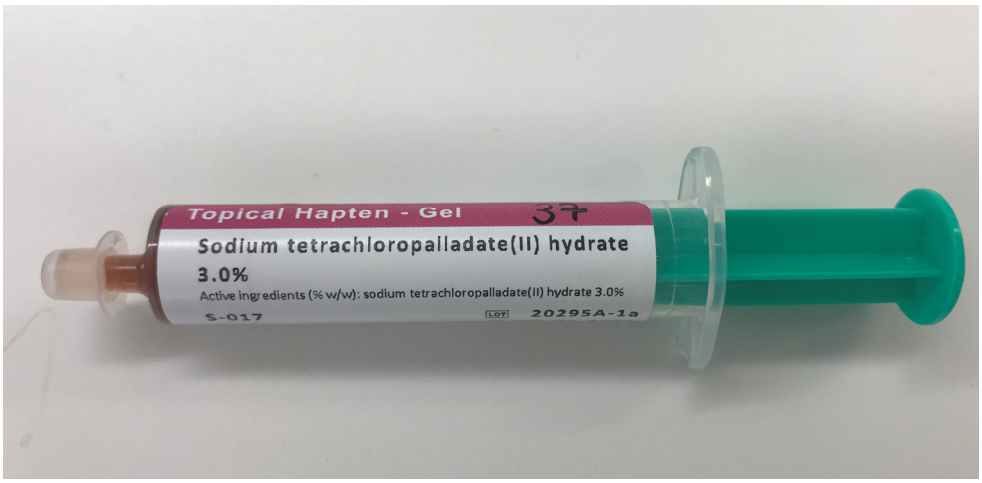


Figure 25. Sodium tetrachloropalladate, Na-PdCl, the recommended test salt for detecting palladium allergy. Photo by Lisbeth Rosholm Comstedt

In study II, the co-reactivity and co-variability for Ni, Pd-Cl, and Na-PdCl was investigated. Unfortunately, only 15 females participated out of 20 participants planned. The participation was time-consuming, with four test occasions with three visits each time, and the females were younger and could not participate due to other obligations. The study therefore became small and significant results about atopy and hormonal changes were difficult to obtain. However, despite the small population we did find a seasonal variation in patch test reactivity for all three metal salts. Ni, Pd-Cl, and Na-PdCl had increased patch test reactivity measured in STS, at the test occasion in February compared to the STS in September. This can be due to false negative or weaker reactions because of increased UV exposure during summertime and/or due to increased trans-water epidermal loss, because of low humidity in the Swedish winter (February). Different clinics get different results when evaluating seasonal variation in patch test results. In this study, the same participants were patch tested repeatedly in different seasons, which makes the seasonal variation very clear. Regarding Ni, a seasonal variation was also seen in a study by Hindsén et al (44) and seasonal variation among patients patch tested in Malmö has earlier been described (39, 40). The variation in Ni-patch test reactivity was overall smaller than the variation found in the study of Hindsén et al. However, that study was carried out using aq as the vehicle, which should be easier to dose (9); the routine in dosing pet in the laboratory in Malmö might have been amended since 2007, when studies about pipetting pet in Finn Chambers were done (16), and in study II, it was the same experienced person who prepared all tests.

There was a co-variation in patch test reactivity between test occasions 1 and 2 between the test salts Ni and Na-PdCl and between Pd-Cl and Na- PdCl. Between test occasions 2 and 3, a co-variability was seen between Ni and Pd-Cl. If the study population had been bigger, this co-variation might had been more obvious and seen between all test occasions. The initial goal was to be able to correlate change in cytokine response with the changes in patch test reactivity. As the laboratory (MabTech) for the cytokine analysis was not local, this turned out to be difficult to pursue. There was a big variation in the cytokine response to the polyactivator, PHA, which served as a positive control. Noticeable, however, was that the general response from the polyclonal cells was stronger when stimulated by Ni-Cl than when stimulated by Pd-Cl. This is in line with the unpublished results from study I, Figure 11, where the patch test reactivity to Ni was more often stronger than the reactivity to Na-PdCl in individuals with concomitant reactions. These results suggest primary sensitization to nickel in the participants, with cross-reaction to palladium. A recently published study has now investigated the cytokine response in three groups of patients with contact allergy to Ni and/or Pd-Cl. T-cells from the participants were stimulated with Pd-Cl and Ni. Group 1: participants with concomitant patch test reactions to both Ni and Pd-Cl, Group 2: participants with isolated Pd-Cl allergy, and group 3: participants with no contact allergy to Ni or Pd-Cl. Ni-stimulation of group 1 gave a 10-fold higher IL-5 response than upon stimulation with Pd-Cl. No IL-5 production was seen when stimulating group 2 or group 3 with Pd-Cl. According to this study, it seems, it is possible to differentiate between true palladium allergy and cross-reaction to Ni, when measuring the IL-5 response by LTT (115).

Normally, humans do not have detectable levels of palladium in the blood (55), and none of our 15 participants had measurable levels of palladium in their blood. Intake of nickel is normally through food, such as nuts, cacao, soyabeans and lentils (116). The concentration of nickel in the blood in the participants in study II was normal during the whole study period (117), and no correlation between patch test reactivity and nickel concentrations could be found. Earlier studies about gold and nickel concentrations in blood have shown that the gold concentration is positively correlated to patch test reactivity to gold thiosulfate, whereas nickel concentration in blood is not correlated to patch test reactivity to Ni (118).

Palladium allergy without nickel allergy

In the data from study I, the prevalence of isolated palladium allergy among women and men was 1.4%. More females than males had isolated palladium allergy, although the difference between gender was smaller in this group than the difference between gender in the whole study population.

Different hypotheses regarding the strength of the palladium allergy, whether it is isolated or not, have been suggested. Because of a 'true' allergy to palladium, one might think that more persons with 3+ reactions to Na-PdCl have isolated palladium allergy. Weak reactions to Na-PdCl are also a hypothesis, suggesting that the patients are actually allergic to Ni, but as the patch test reactivity to Ni varies, the patch test to Ni is negative at the test occasion and only a weak reaction for Na-PdCl is seen. This was the case in study II, participant number 15, test occasion 2; see Table 4. However, the percentage of isolated palladium allergy was the same in the different groups of reaction to Na-PdCl in study I. See Figure 14.

The isolated palladium allergy was mainly found in the middle-age and oldest group of patients, 31-99 years, in our data. Because of the introduction of Na-PdCl in 2009, we saw an increase in positive patch test results after 2009. The results collected for study III gave us the opportunity to continue to study the prevalence of isolated palladium from 2016-2019. The cohort in this study was slightly different, with more exclusion criteria. An increase in isolated palladium allergy and to palladium in general was seen up to 2019. The prevalence of palladium allergy from 2017-2019, from study III, has not been compared to the prevalence of contact allergy to Ni at the same time, as study III had other aims. The yearly prevalence of positive patch test reactions to palladium and other allergens is variable, and a trend should be investigated over several years. We changed test-chamber system in 2018, which could be the greatest reason for this sudden increase (119). No other studies have compared the prevalence of isolated palladium allergy over time up to 2016. Cristaudo et al, described isolated allergy to Pd-Cl 1% from 1996-1997 to 2007-2008 and found an increase from 0.1% to 1.8% among 5,350 patients and concluded that this increase was due to increased use of dental alloys (73). Regarding Pd-Cl 2.0%, our overall prevalence of isolated palladium allergy is in line with results from other countries. In the newly published Spanish study (108) there was a prevalence of isolated Pd-Cl 2.0% allergy at 0.7%. with only approximately 70% females, compared to 87% females among patients positive to both Ni and Pd-Cl. A tendency towards older patients was seen in the group with isolated Pd-Cl allergy. In an American study, the prevalence of isolated Pd-Cl was 1% (109). In results from a multicentre study in Europe (60), the prevalence of isolated palladium allergy was found as high as 4.2%. The Netherlands and Switzerland are presented as the countries with the highest prevalence. In this multicentre study, no difference was found among gender but, as in our study as well as in the Spanish study, there was a tendency of increasing age in the group with isolated palladium allergy. The reason for those differences in prevalence among countries seems to be interpreted as the differences in exposure to palladium in dental alloys. In Sweden, palladium was more common in dental alloys in the 1970s, but during the 1980s and 1990s cobalt/chrome

alloy became more and more common (59). The oldest patients in our study can have been exposed in the 1970s, and it can explain the difference in gender become smaller, but there is still a difference in gender that we cannot explain.

The use of aluminium in patch test material when testing for palladium allergy

When testing with Finn Chamber, 2010-2017, there was an overrepresentation of patients with positive patch test results to Na-PdCl, Pd-Cl, and to MP and cain mix II among patients with contact allergy to aluminium. The stronger the aluminium allergy was in the patients, the more concomitant reactions to the four aluminium-ion-releasing preparations were found. An overrepresentation of patients with isolated palladium allergy was also seen among patients with contact allergy to aluminium.

No overrepresentation of contact allergies to these four patch test preparations could be found in 2018-2019 when Finn Chamber Aqua was used, and no isolated palladium allergy was seen at all among the 1,450 patients with aluminium allergy, despite an increase in isolated palladium allergy among consecutive patients 2018-2019. Among patients with concomitant reactions to Na-PdCl, Pd-Cl, Al-Cl, and/or Al-lac there was no difference between gender (unpublished). This supports the suggestion that the reason for the increase in prevalence of palladium allergy among patients with aluminium allergy seems to be due to corrosion in the Finn Chamber, which is independent of gender. Contact allergy to aluminium was more frequent among the younger patient group.

The results in study III indicate that, when testing for palladium allergy, it is important to choose the right chamber. Among younger patients, who are more likely to have contact allergy to Al-Cl, a plastic chamber or Finn Chamber Aqua should be used. If Finn Chamber is used and the patients have positive test reactions to both Na-PdCl/Pd-Cl, and Al-Cl, re-testing for confirmation of the indicated palladium allergy is recommended. In the older patients, testing for palladium can be most relevant in dental series and here again, like in the younger patients, it is important to use a plastic chamber or Finn Chamber Aqua, even though detectable contact allergy to Al-Cl in the age group is less common.

Figure 26. Preparing patch test with Na-PdCl in IQ plastic chamber. Photo by Kajsa Davidson Källberg

Aluminium in patch test preparations

Developing test preparation for optimizing the sensitivity and specificity in patch testing is important. The differences in prevalence of palladium allergy seen between Na-PdCl and Pd-Cl shows the importance of using the most optimal test preparation to detect contact allergy. Na-PdCl was introduced because it had a higher penetration through the skin (68). Part I in study IV was carried out as a pilot study, to investigate any possible role Al-Cl might have on patch test reactions with Ni. Surprisingly, there was a very high increase in sensitivity for the test preparation with the highest concentration of Al-Cl. Adding 30.0% and 20.0% Al-Cl to Ni 15.0% in aq increased

the sensitivity from 50% to approximately 90%. As aluminium in other salts, aluminium potassium sulphate, aluminium hydroxide and aluminium phosphate, are used as an adjuvant in activating the immune system in vaccines, the results indicated that an adjuvant effect of aluminium was perhaps seen in patch testing. It became interesting to investigate if Al-Cl could have the same increasing effect in patch test reactivity in other test preparations. Al-Cl is the aluminium salt used in leave-on products, such as antiperspirants. Here it appears together with other allergens, such as fragrances and preservatives. To investigate the effect of fragrances and preservatives in sensitized individuals thus had an interesting clinical perspective. Part 2 and part 3 in study IV were therefore carried out to investigate Al-Cl added to MI and FM I. The preparations MI-Al and FM I-Al did not change the patch test reactivity; in fact, a small decrease was seen for MI-Al10 and MI-Al20 compared to MI-aq. Hence, the increased sensitivity was only seen when adding Al-Cl to Ni.

A lot can happen when mixing different substances in test preparations. The allergens can have a synergistic or additive effect on one another (120, 121) or a substance in the preparation can have an irritative effect on the skin, which could increase the hypersensitivity reaction to the allergen (122-125). As none of the patients, with Ni allergy, had contact allergy to Al-Cl, nor had irritative reactions to Al-Cl 10.0%, we assumed that this was not the reason. However, in part 1, the consecutive patients tested with mixes of Ni and Al-Cl were not tested with Al-Cl 20.0% or 30.0% in aq alone and irritative reactions to Al-Cl aq have not been evaluated. In part II and part III, the participants were tested with Al-Cl 20.0% and 30.0% in aqua and some participants had irritative reactions, but this irritative effect was not seen to enhance the patch test reactions to MI or FM I. Metal can build complexes (126) which might change the properties of the allergen, such as penetration through stratum corneum and the binding affinity to proteins. We investigated the penetration of Ni by the acid wipe method (127), and found a small but significant ($p = 0.04$) difference in the amount of Ni than could be wiped off after 48 hours occlusion to the skin, in the preparation with Al-Cl 20.0%. This technique was also performed for MI, where the opposite was found. For further details, see Supplement in paper IV. Al-Cl seems to inhibit penetration when added to MI in the concentration 20.0%, ($p = 0.04$). Exactly what is happening in the skin when Al-Cl is added to Ni, we do not know. The increased effect could be due to a combination of complex binding and increased penetration through the skin.

Ni has a different immunological pathway in enhancing a hypersensitivity reaction compared to FM I and MI. To develop adjuvants in patch testing might need further understanding of the immunohistochemistry actions of specific allergens, as different adjuvants might be needed to enhance the elicitation reaction to different allergens.

Conclusions

In southern Sweden, the EU Nickel Directive seems to have not only affected the prevalence of contact allergy to Ni, but also to Pd-Cl, Na-PdCl and to Co, as the number of young patients diagnosed with these contact allergies is decreasing.

Women are still having the highest prevalence of contact allergy to Ni, Pd-Cl, Na-PdCl, and Co. Among young female patients, age 6-30 years, the prevalence of Ni-allergy was 19.1%, palladiumallergi was 11.0%, and Co-allergy was 5.3% in 2012-2016, whereas for young men, 6-30 years, it was 2.1% for Ni and 1.5% for palladium in 2012-2016.

When testing women, age 31-99 years, we saw an increased prevalence in palladium allergy after 2009, which is interpreted as an effect of the introduction of the more sensitive test salt Na-PdCl in 2009.

When testing for contact allergy over time to Na-PdCl and Pd-Cl, Na-PdCl showed less variability compared to Pd-Cl.

Seasonal variation due to increased reactivity in wintertime was the most obvious explanation for the variation of patch test reactivity to Ni, Pd-Cl, and Na-PdCl.

Isolated palladium allergy has a prevalence of 1.4% among consecutive patients and is mainly found in the middle-aged and elderly. The male:female ratio was smaller at 1.6%:1.0% compared to the ratio in total prevalence of palladium allergy, where the male:female ratio was 10.5%:89.5%.

Patch testing with Na-PdCl or Pd-Cl in Finn Chambers could give rise to false positive reactions in patients with contact allergy to Al-Cl.

Adding Al-Cl in concentration of 20.0% and 30.0% to Ni preparation can increase the sensitivity of a patch test from 50% to 90%. This phenomenon is not seen when adding Al-Cl to FM I and MI. More investigations are needed to understand and develop methods to enhance weak allergies, without increasing the amount of allergens in test preparations.

Clinical implications

Patch testing with a palladium salt in a consecutive dermatitis patient with no specific suspicion of metal allergy from jewelleries or dental material does not seem to be relevant in southern Sweden. As contact allergy to Ni cross-reacts with that for Na-PdCl and Pd-Cl, a positive patch test reaction to one or both of the palladium salts seems not to provide the clinicians with further information if the patient simultaneously has a positive reaction to Ni. Furthermore, the palladium test preparations take up space on the back of the patient, which can be used for other relevant test preparations. Testing with palladium might only give the nickel allergic patient extra itching eczema lesions on the back as well as the concern about another allergy. It is better to inform the patients about possible cross-reaction patterns to nickel, and that avoiding palladium in dental alloys, if that becomes an issue, can be of value.

The interpretations of patch test results in relation to the patient´s environmental and occupational exposure is essential in determining which test substances are important in patch testing. A high prevalence of positive reactions does not have to be an indicator of a huge problem with an allergen among dermatitis patients or in the general population.

Reports and articles from other countries state that clinical symptoms from palladium allergy is most often related to dental alloys. With symptoms from oral mucosa and suspicion of contact allergy to dental alloys, patch testing with palladium as the salt Na-PdCl 3.0% in pet, is indicated as part of a dental series. One should be observant of which chamber to use, as Finn Chamber in a patient with contact allergy to Al-Cl could give false positive reactions. A plastic chamber or Finn Chamber Aqua is recommended.

With high suspicion of a palladium allergy and a doubtful reaction, especially in summertime, one could consider testing again, as the patch test reactivity to Na-PdCl shows seasonal variation. The patch test reading on day 7 is important when it comes to Na-PdCl. Looking at our latest patch test result from 2018-2021, 11.9% of consecutive patients are positive to Na-PdCl only on day 7.

Al-Cl 20.0-30.0% added to Ni 15.0% in aq gives a high sensitivity in a patch test for nickel allergy and seems able to detect what we normally would interpret as very weak nickel allergy. However, this method needs more investigation before it can be recommended in patch testing.

Lapptestning med palladium och aluminium (Swedish popular science summary)

Lapptestning är standardmetoden för påvisning av kontaktallergi. Metoden har använts i över 100 år och utvecklas fortfarande. På 80-talet började flera kliniker i USA och Europa, inklusive Yrkes-och miljödermatologisk avdelning i Malmö, att lapptesta med palladiumklorid for att kunna påvisa palladiumallergi.

Palladium är en metall som började användas mycket i metallegeringar i tandmaterial på 70-talet. Även i industrin, i elektronik och i katalysatorer i bilar, ökade användningen av palladium. Vid lapptestning kunde man sedan konstatera att kontaktallergi för palladium var relativt vanligt, och framför allt var det mycket vanligt att samtidigt ha båda palladium- och nickelallergi. Nickelallergi var då känt för att ge många problem med eksem, särskilt hos kvinnor. Förklaringen var håltagning i öronen, användning av oäkta örhängen och andra smycken samt andra föremål som var tänkt att ha en långvarig kontakt med huden. 2001 infördes ett europeiskt nickeldirektiv – en lagstiftning kring hur höga halter nickel avseende föremål, som var tänkta att vara i långvarig kontakt med huden, fick innehålla och utsöndra. Direktivet var tänkt att begränsa uppkomsten av nickelallergi, men också att minska exponeringen för de som redan var allergiska, så att de fick mindre besvär av sin allergi. Palladiumallergi gav inte lika många symptom från huden, däremot rapporterades det från utlandet att symptomgivande palladiumallergi oftast var relaterad till metallegeringar i munhålan. I Malmö fanns det dock många patienter där lapptestning tydde på kontaktallergi för palladium, utan att de hade palladiumlegeringar i munhålan. På 90-talet började man förstå, att palladiumallergin, troligen i många fall, representerade en så kallad korsreaktion till nickel. Nickel och palladium är nämligen två metaller som kemiskt liknar varandra. När en person med nickelallergi lapptestas med palladium känner immunförsvaret igen palladiummetallen som nickel, och reagerar. Resultatet blir ett positiv test, trots att patienten kanske aldrig har varit exponerad för palladium.

2009 visade den holländska tandläkaren Joris Muris att lapptestning med en annan palladiumsalt än palladiumklorid, nämligen natriumtetrakloropalladat (Na-PdCl), kunde hitta flera individer med palladiumallergi. Man noterade då att förekomsten av

palladiumallergi ökade, särskilt i Nederländerna och Schweiz där palladium i tandmaterial är mycket vanligare än i Sverige.

Under de senaste 20 åren har användningen av palladium ökat i Sverige, som en ersättning för vitguld och platina i smycken, men det är fortfarande inte vanligt i tandmaterial.

I avhandlingens första studie undersöktes förekomsten av kontaktallergi mot palladiumklorid och Na-PdCl bland patienter som utreddes för kontaktallergi på Yrkes- och miljödermatologisk avdelning i Malmö. Nästan 10% av alla de patienter som testades i Malmö från 1995–2016 hade palladiumallergi och cirka 90% av dessa var kvinnor. Det visade sig att på 90-talet var förekomsten bland yngre kvinnor, 6–30 år, cirka 16% medan förekomsten av palladiumallergi var 9% när samma åldersgrupp testades 2012–2016. Liknande nedgång i förekomst noterades även hos de som testades positiv för nickel. På 90-talet var förekomsten av nickelallergi 33% bland yngre kvinnor, 6–30 år, medan förekomsten av nickelallergi var cirka 20% när samma åldersgrupp testades 2012–2016. Denna nedgång tolkas som en direkt effekt av det europeiska nickeldirektivet. I hela materialet av patienter, var sannolikheten för att ha palladiumallergi 36 gånger högre om man även var allergisk mot nickel, vilket tyder på ett mycket starkt samband, d.v.s. korsreaktion, mellan nickel och palladium. När palladiumallergi utan samtidig nickelallergi studerades, var förekomsten mycket lägre, endast 1,4% och även densamma 1995 och 2016. Fortfarande noterades en liten högre andel kvinnor med denna form av palladiumallergi, 1,6% respektive 1% för männen.

I andra studien undersöktes reproducerbarheten (ett tests förmåga att visa samma resultat varje gång) av lapptestreaktionerna till palladium och nickel. 15 kvinnor, alla kända för att vara allergiska mot båda nickel och palladium, lapptestades med nickel och palladium i fyra omgångar med 12 veckors intervall. Av resultaten kunde man se, att det nya testsaltet Na-PdCl hade högre reproducerbarhet än palladiumklorid. Det fanns en variation i lapptestresultaten som kunde förklaras med en årstidsvariation, eftersom alla tre metallsalter (nickel, palladiumklorid och Na-PdCl) uppvisade kraftigast reaktioner vintertid. Laboratorieundersökningar av blodprov från dessa 15 kvinnor, visade att immunförsvaret reagerade kraftigare när det stimulerades med nickel och svagare eller inte alls, när det stimulerades med palladium. Detta tolkades som att kvinnorna i studien hade utvecklat en allergi mot nickel och att immunförsvaret reagerade med en korsreaktion till palladium.

Vid lapptestning läggs testämnen i en beredning med vaselin i små testkammare som sedan tejpas fast på huden. Olika testkammarsystem är tillgängliga och på Yrkes-och miljödermatologisk avdelning i Malmö, har Finn Chamber-systemet används sedan 70-talet. I avhandlingens tredje studie undersöktes om användningen av detta

testkammarsystem kan ha haft någon betydelse för risken att få diagnostiserad palladiumallergi. Finn Chamber är gjort av aluminium. Ytan består av aluminiumoxid som anses mycket hållbart och som sällan interagerar med andra ämnen. Dock vet man, från när Finn Chamber utvecklades och började användas i lapptestning, att vissa ämnen såsom nickel, cobalt och kvicksilver kan skapa korrosion i aluminiumytan vilket kan leda till utsöndring av aluminiumjoner i testberedningen. Vaselinet som används tillsammans med testämnena vid lapptestning har dock en tillräcklig skyddande effekt. Kammarna har därför ansetts mycket säkra och används vid lapptestning både i Malmö och vid många andra kliniker runt om i Europa. Nyare laboratorieanalyser har visat att de två palladiumsalterna, palladiumklorid och Na-PdCl skapar ökad korrosion, faktiskt så mycket att en patient med samtidig aluminiumallergi skulle kunna reagerar på aluminiumjonerna som frisätts. Det visade sig att bland patienter med aluminiumallergi var förekomsten av palladiumallergi nästan 19%, jämfört med cirka 9% hos patienter utan aluminiumallergi. Förekomsten av isolerad palladiumallergi, d.v.s. utan samtidig nickelallergi, bland patienter med aluminiumallergi var över 10%, jämfört med förekomsten på cirka 1,4% för patienter utan aluminiumallergi. Det finns därför bra underlag för att misstänka att en del av palladiumreaktionerna hos patienter med aluminiumallergi är falsk positiva, och snarare är en reaktion på de aluminiumjoner som utsöndrats från kammaren.

I avhandlingens sista studie undersöktas effekten av aluminiumklorid som tillsats till tre testberedningar som vanligtvis används vid lapptestning. När aluminiumklorid tillsattes till nickelberedningen i koncentrationerna 20–30% identifierades dubbelt så många individer med nickelallergi. De extra allergiska individerna som hittades med denna nickel-aluminiumberedning hade alltså en mycket svag nickelallergi som inte upptäcktes med den vanliga standardtestberedningen med nickel. Man kan fråga sig om det är relevant att diagnosticera en så svag allergi, men det kan finns situationer när patienter har en svag men ändå symptomgivande allergi där koncentrationerna i de vanliga testberedningarna inte räcker till för påvisning av allergi. Resultaten väckte funderingar kring effekten av aluminiumklorid i hygienartiklar. Aluminiumklorid finns i antiperspiranta deodoranter, som i sig kan innehålla andra allergener som parfym och konserveringsmedel. Det blev därför intressant att undersöka om aluminiumklorid som tillsats till parfymberedningen, Fragrance mix I, som används vid lapptestning och testberedningen med konserveringsmedlet methyliso-thiazolinone (MI) kunde ge kraftigare reaktioner. Cirka 20 individer med allergi mot Fragrance mix I respektive MI lapptestades med beredningar innehållande Fragrance mix I samt aluminiumklorid respektive MI samt aluminiumklorid. I försöket med Fragrance mix 1 noterades ingen skillnad med eller utan aluminiumklorid. I försöket med MI visade det sig bättre att testa utan aluminiumklorid än med.

I avhandlingen konkluderas det bland annat att palladiumallergi i södra Sverige framför allt är relaterat till nickelallergi och att det europeiska direktivet kring nickel har haft effekt på förekomsten av båda nickel- och palladiumallergi. Förekomsten av palladiumallergi utan samtidig nickelallergi är mycket lägre i befolkningen, och har inte påverkats av det europeiska nickel-direktivet. Om palladiumallergi misstänks bör lapptestning utföras med testsaltet Na-PdCl, då detta ger det mest tillförlitliga resultatet. Viktigt är dock att använda ett testkammarsystem med en yta som inte kan interagerar med palladiumsaltet. Användningen av aluminiumklorid som tillsats i testberedningar med nickel är än så länge experimentell.

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