Patch testing with metals with focus on gold

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Patch testing with metals

with focus on gold

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Abstract

In this thesis the aims were to improve our basic knowledge of gold as a contact allergen and our understanding of the patch testing technique. The thesis is based on four studies:

An experimental study where dilution series were patch tested simultaneously.

A provocation study where volunteers, none had any known allergy to gold or nickel, were exposed to gold and stainless steel discs.

An experimental double blind study where patients with lichen sclerosus and age and sexcorrelated controls were patch tested. A retrospective study with patients tested with gold and nickel between 1995 and 2014.

The aims were:

To investigate the reproducibility of the patch test technique with regard to where the allergen is patch tested and the reactivity.

To investigate if gold is released on the skin when the object is in prolonged contact in an occlusive environment, and to find if a systemic uptake can be found from this exposure.

To investigate whether contact allergy and especially contact allergy with regard to metals, is more frequent in patients with genital lichen sclerosus et atrophicus.

To retrospectively investigate the dermatitis patients patch tested in Malmö with regard to the metals gold and nickel.

The findings were as follows: In study I the reproducibility in patch testing did not differ on the back, however as the reactivity decreased so did reproducibility.

In study II it was shown that gold can be released to the skin when in prolonged contact, and the release increases with time. Study III aimed at finding clinical relevance of contact allergy, especially to gold, in patients with lichen sclerosus. No association was found. Study IV aimed at improving our knowledge on contact allergy to gold in general and possible associations with basic characteristica of the patients such as atopy, sex and age. In the study there was an association between gold-allergy, age, sex, atopy and facial dermatitis.

Key words: Contact allergy, reproducibility, metals, gold, patch test, systemic uptake, dental materials, genital lichen sclerosus.

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To my beloved family

Contents

Thesis at a glance

Paper I

The reactivity of the back revisited

Objective

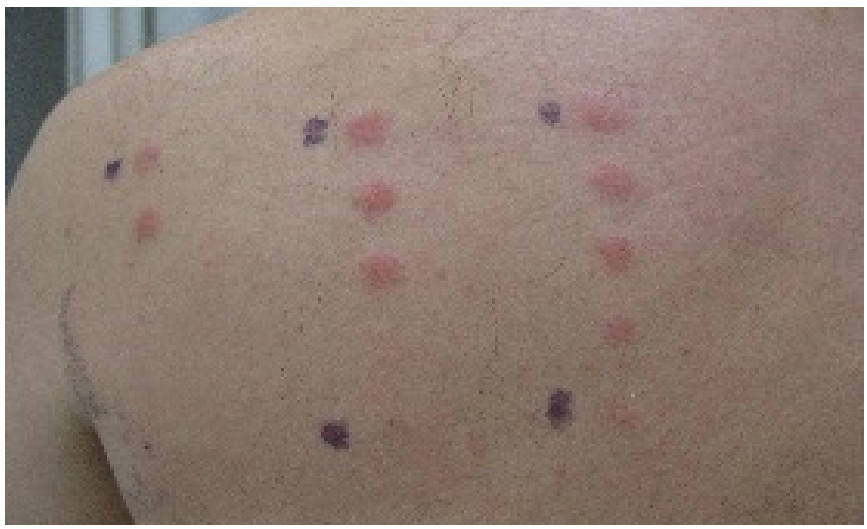
To study the reproducibility of patch testing at different locations on the upper back and the reproducibility over time with regard to reactivity pattern.

Method

31 subjects with contact allergy to gold or nickel were patch tested with serial dilutions in triplicate applications at different locations on the upper back.

Main findings/Conclusion

No differences in reactivity were found whether the patch test was applied to the left side or the right side of the back, or to the medial part or the lateral part of the back.



Paper II

How much metal is released to the skin during prolonged occlusion of gold objects?

Objective

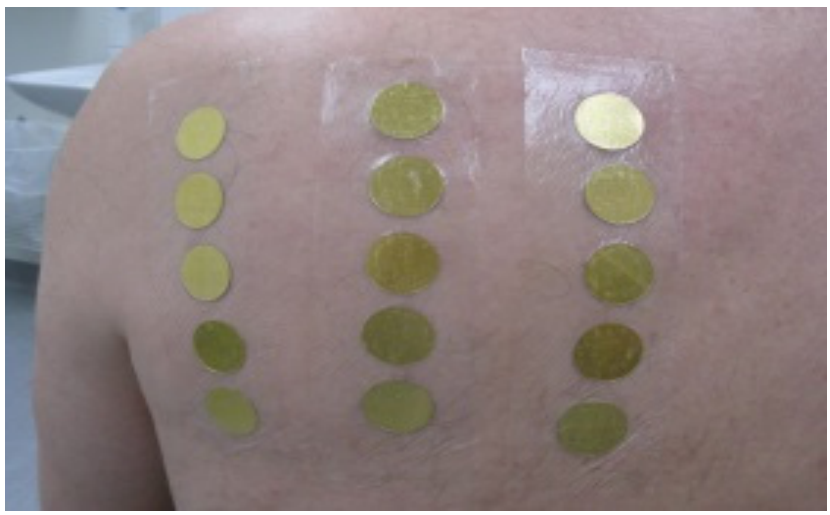
To study metal release from gold objects worn in close contact with the skin, to determine whether the amount increases over time, and to assess whether a measurable systemic uptake is possible.

Method

14 individuals were provoked with gold and stainless steel discs, occluded on the skin for different length of time. The skin was cleansed using the acid wipe technique and blood samples were drawn to investigate systemic uptake after exposure.

Main findings/Conclusion

With the acid wipe sampling technique used in 9 individuals, we found release of gold in 7 of the 9 subjects and release of nickel in all 9. We were able to detect a higher amount of metals on the skin when the provocation time was prolonged.



Paper III

Contact allergy and vulvar lichen sclerosus et athrophicus

Objective

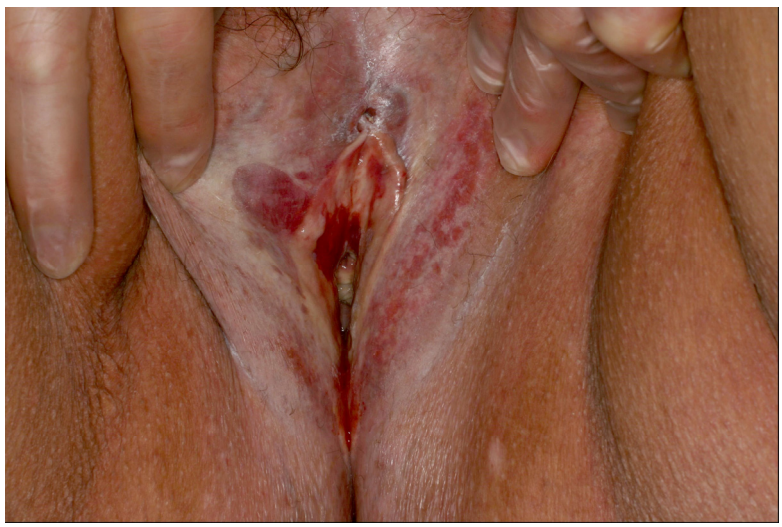
To study a possible association between genital lichen sclerosus and contact allergy to gold.

Method

41 women with genital lichen sclerosus (GLS) and 40 controls were tested with a modified baseline series and filled in a questionnaire about exposure to metals.

Main findings/Conclusion

No increase in the rate of contact allergy to gold was found in the GLS patients. The GLS group had positive patch test reactions to several more substances than the controls. Patch testing of patients with GLS with the baseline series and their own products is still a recommendation if the disease deteriorates or if treatment does not have the expected effect.



Paper IV

What can be learnt from patch testing with gold? A retrospective analysis of consecutive data from 1995 to 2014.

Objective

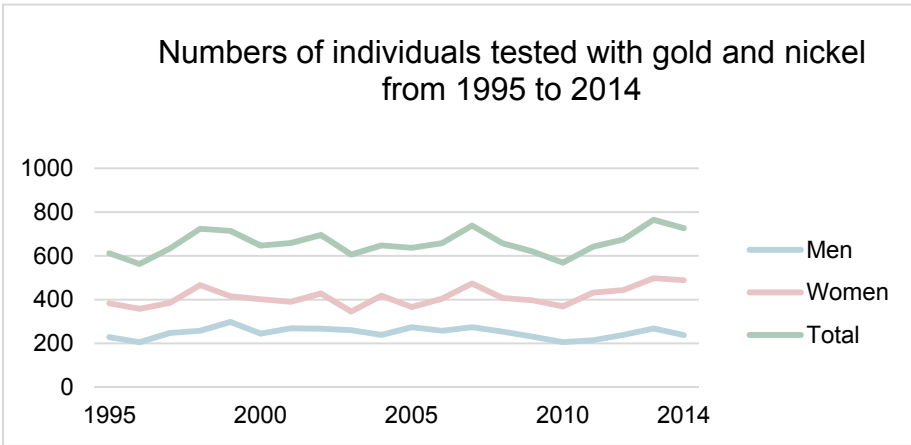
To study the rates of contact allergy to gold and nickel in dermatitis patients, retrospective analysis was performed on patch test data collected over 20 years.

Method

13,106 dermatitis patients were patch tested to gold with the extended baseline series. Patch test data were analysed regarding patient age, sex and location of dermatitis, and compared to atopy and contact allergy to nickel.

Main findings/Conclusion

In the material, both gold and nickel were frequent contact allergens, and more commonly in women. There was a trend of a slight decline in frequency of allergy to each metal. There was a correlation between gold allergy and age in women, atopy, and facial dermatitis. Nickel allergy was more common in younger female patients, and showed a correlation to atopy and hand dermatitis.



List of publications

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. **The reactivity of the back revisited**
Björk A-K, Bruze M, Engfeldt M, Nielsen Ch, Svedman C.
Contact Dermatitis 2016 Sep 4. doi: 10.1111/cod.12657. [Epub ahead of print]
PMID: 27593358

II. **How much metal is released to the skin during prolonged occlusion of gold objects?**
Björk A-K, Bruze M, Engfeldt M, Persson L, Lundh T, Svedman C.
Submitted for publication in the Contact Dermatitis

III. **Contact allergy and vulvar lichen sclerosus et atrophicus**
Björk A-K, Svedman C, Asplund H, Lingärde S, Hindsén M, Hradil E, Bruze M.
Immunome Res 2014: 10:2

IV. **What can be learnt from patch testing with gold? A retrospective analysis of consecutive data from 1995 to 2014**
Björk A-K, Bruze M, Edman B, Engfeldt M, Björk J, Lövkvist H, Isaksson M, Pontén A, Svedman C.
In manuscript

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Abbreviations

AAS	Atomic absorption spectrometry
ACD	Allergic contact dermatitis
aq	aqua
Au	Gold (from Latin: Aurum)
D	Day
DALUK	Database of Contact Allergy (the Swedish abbreviation for Databas för Lagring av Uppgifter om Kontaktallergi)
GLP	Genital lichen planus
GLS	Genital lichen sclerosus
GSTS	Gold sodium thiosulfate
ICDRG	International Contact Dermatitis Research Group
ICP-MS	Inductively coupled plasma mass spectrometry
MEC	Minimal eliciting concentration
Ni	Nickel
OLP	Oral lichen planus
pet	petrolatum
STS	Summarised test score
w/m	women/men
w/v	weight/volume
w/w	weight/weight

1 Introduction

There are different types of allergic reactions, the most common classification system being that of Coombs and Gell [1]. The allergic reactions are thus divided into four different groups, depending on the mechanism involved.

Table 1.
The four main types of allergic reactions, described by Coombs and Gell.

	Antibody-mediated reactions			**Cell-mediated reactions**
	Type I	Type II	Type III	Type IV
Mechanism	Immediate, IgE-mediated	Humoral, Cytotoxic	Immune complex-mediated	Delayed, T-cell-mediated
Symptoms	Allergic rhinitis, Bronchial asthma	Drug-induced, Cytopenia	Vasculitis, Allergic alveolitis	Allergic contact dermatitis

Patients who suffer from both type-I and type-IV reactions (Table 1) can be diagnosed in the clinic using the skin itself in two different provocation tests [2]. For type-I reactions, the immediate type of reaction mediated by rapid release of IgE [3] and giving rise to the typical reactions of allergic rhinitis and/or conjunctivitis and asthma, the prick test can be used. In this test, the allergen is put on the skin and the skin is then pricked. If there is an allergy to the substance, the skin will react with an urtica. The type-IV reaction is antibody- independent and relies on phagocytic and cytotoxic T-cells, as well as CD4+ cells. The onset is usually later than for type-I reactions, so symptoms do not occur until 24–48 hours after exposure. The skin is almost exclusively the organ that gives rise to symptoms. This reaction can be diagnosed using epicutaneous testing (patch testing). In the clinic it is often more difficult to suspect a type-IV reaction than to suspect a type-I reaction, due to the delay after exposure. It is also important to remember that the typical clinical symptoms of contact allergy, the eczematous reaction, are not the only possible clinical manifestation. Some allergens give rise to lichenoid or pustular reactions. Also, the route of administration of the allergen may influence the clinical picture. A systemic allergic contact dermatitis may occur if an individual with contact allergy is exposed to the allergen in question, or a chemically related substance—orally, percutaneously, or by inhalation [4]. The most specific set of clinical symptoms found in connection with systemic allergic contact dermatitis is probably the baboon syndrome [5]. The clinical picture can also be that of flare-up reactions on previous patch tested areas, or flare-up at previous sights of allergic contact dermatitis where the patient was previously exposed to the allergen. Systemic flu-like symptoms and fever can also arise [6-9].

The method used to establish contact allergy, patch testing, was introduced by Jadassohn more than a hundred years ago. It was then described in detail by Bloch in 1929 [10]. Patch testing is still the most accurate method for establishing contact allergy [11, 12]. It is a provocation test, i.e. the patient will experience an allergic reaction in the form of an eczema in miniature if positive on testing, which is a possible drawback of the technique. The patient has to come once for testing and twice for reading of the patch test. Today, the protocol for patch test reading has been standardised [11-15], but it is well known that the readings is subjective [12, 16, 17]. *In vitro* techniques have been tried, but considering the fact that patch testing can be used for most types of allergens—and the fact that the *in vitro* techniques may not perform as well in individual cases— makes patch testing the recommended method. However, for metals such as gold and nickel, the *in vitro* techniques using the lymphocyte transformation test give satisfying results at the group level [16-18]. The *in vitro* techniques have some advantages in that there is interference with the patient's immune response, and in that they are objective. The result, however, depends on the number of lymphocytes tested, the solubility of the metal, and the expertise of the laboratory [16].

The patch testing technique has many advantages: thousands of allergens are available for patch testing, and it is the size of the back that is the limit to how many allergens can be patch tested simultaneously. From the patient's point of view, the provocation of eczema at the site of application of the allergen is often found to be educative.

What we patch test with and how we perform the patch test— i.e. that the allergen is defined, that the vehicle is correct, and that the dose is standardised—is the first step to accurately diagnosing contact allergy[19]. Constant work is required to improve our knowledge of how to patch test correctly. The baseline series and the patch test technique need to be updated regularly [11, 18, 20, 21].

In our environment, some metals can be found in abundance and others can be very rare. When used in man-made objects, a metal is often present as an alloy. In this thesis, we address some basic aspects on how patch testing with metals should be performed and developed. In order to give rise to allergic contact dermatitis, metal has to be released from an object. These factors and how the metal is used will, of course, influence the prevalence of contact allergy. Metals can be contact allergens that may cause systemic contact dermatitis and reactions other than the typical eczematous reaction. Gold is associated with lichenoid reactions in the mucosa, and systemic allergic contact dermatitis with distant flare and systemic symptoms [8, 22].

In the work included in this thesis, we wanted to focus on gold as an allergen. In study I, we investigated the reproducibility of the patch test technique when testing with gold. In studies II and IV, we investigated how metals are released, which individuals acquire contact allergy to gold, and how the frequency of contact allergy has changed over the last few decades. The thesis also includes a retrospective study, presented in paper III, which was started because gold has been known to give rise to lichenoid reactions and because from questionnaires, contact allergy to gold has been associated with genital symptoms. For this reason, the aim was to study a large population with genital

symptoms such as lichen ruber planus and lichen sclerosus and to determine not only whether there was a possible effect of metal allergy but also whether this group had more allergies in general.

A retrospective study with patients with lichen sclerosus was therefore begun.

1.1 Lichen sclerosus

Lichen sclerosus et atrophicus is a chronic inflammatory disorder that can involve the skin, the nails, and/or the mucosa of the anogenital area. The genital lesions can be very difficult to differentiate from lichen ruber planus, an inflammatory disorder that can affect the skin, the anogenital mucosa, and the oral mucosa [23-25]. The two diseases can co-exist and overlap [26]. However, in lichen ruber planus, oesophageal lesions can occur with symptoms of dysphagia and pain [27]. The aetiology is still not fully understood, but hormonal mechanisms have been suggested and it is not uncommonly associated with autoimmune diseases [28, 29]. On the buccal mucosa, lichenoid reactions can occur adjacent to amalgam and gold restorations [22]. Several of the allergens found in the dental series can cause systemic reactions and localised oral lichenoid reactions [22, 30]. These patients often have positive patch test reactions to relevant allergens, and they are more likely to improve when they avoid the allergen or when the allergen is removed [31].

In a retrospective study involving patients with pruritus vulvae, it was found that of the 16 patients with lichen sclerosus, 7 (44%) had positive reactions when tested with the European baseline series, including selected preservatives, perfumes, local anaesthetics and medicaments [31]. Our knowledge of whether contact allergy may influence genital lesions either by systemic effects or through localised contact is, however, limited. A contact allergy can cause mucosal and skin diseases and could theoretically lead to deterioration of a pre-existing skin disease. We need to know more about the possible effects of contact allergy.

1.2 Contact allergy and allergic contact dermatitis

Contact allergy is known as a "type-IV allergy", meaning delayed hypersensitivity. The clinical manifestation of contact allergy is allergic contact dermatitis (ACD). An ACD will be elicited after exposure to the allergen at a concentration exceeding the individual's threshold [32]. Approximately 4,000 substances are known to cause contact allergy [33].

When the allergen has been identified, the individual often has the opportunity to avoid the substance and chemically related substances and can avoid developing ACD. Most

contact allergens are small, and to have any effect they must be able to penetrate the skin barrier. To penetrate the skin, the compound must be relatively lipophilic (log Po/w > 1) [34] and must also have a low molecular weight, usually below 500 [35]. In the skin, the contact allergen must react with proteins to form antigens. Since these molecules are too small to act as antigens themselves, contact sensitisers are generally referred to as haptens (incomplete antigens).

Contact allergy and ACD have two phases. The immunological memory is established in the first phase, the sensitisation phase, which requires at least 4 days to several weeks for the individual to become sensitised. The allergens penetrate the epidermis, react with protein, and form antigens. These antigens are taken up by antigen-presenting cells called Langerhans cells and transported to the regional lymph nodes where they are presented to uncommitted T-cells, which become activated. The activated T-cells release cytokines, which leads to proliferation and differentiation of the T-cells into hapten-specific memory T-cells. These memory cells are released into the blood circulation. The second phase, the elicitation phase, begins when the individual is re-exposed to the sensitiser. Langerhans cells present the antigen to these allergen-specific T-cells, which become activated and induce inflammatory events in the exposed skin area. This will result in an eczematous reaction usually within 1–4 days of exposure to the allergen [32]. However, for some substances the elicitation phase can be longer, sometimes more than 2–3 weeks [36-39]. If an individual who is sensitised to an allergen avoids exposure to the allergen, no ACD will occur. However, regarding the likelihood of ubiquitous substances acting as allergens, this would be impossible. The exposure to the allergen and the reactivity of the individual will decide whether the individual will have clinical symptoms of allergy. For some substances—gold, for example—the elicitation phase can be up to 2–3 weeks [36].

Contact allergy to metals is common. When acting as a contact allergen, the metal is in an ionised form; it must be protein-reactive to become immunogenic and evoke an immune response. In the skin, the metal undergoes coordinate covalent bonding with cellular and matrix proteins that usually contain key cysteine and histidine residues, which creates epitopes that can be recognised by T-cells. To become fully immunogenic, the free metal ion and/or metal-containing complex should also provide innate immune danger signals to the antigen-presenting cells, leading to cytokine production, and dendritic cell maturation, and mobilization to the draining lymph node [40]. In the lymph node the metal-containing complex is presented to the T-cells, as seen with organic haptens.

1.2.1 Contact allergy to gold and nickel

Allergy to nickel is the most frequent metal allergy found in the general population and in dermatitis patients, and the allergen is an integral part of the baseline series [41, 42]. Contact allergy to gold is also very common, but the allergen is usually not included (or

recommended to be included) in the baseline series since it can be difficult to assess the clinical relevance of a positive reaction [43] (Figure 1).

Nickel is a ubiquitous substance found not only in the environment—in water, in soil and in the air—but also in our bodies. Exposure can occur on and through the skin, in the airways, and in the gastrointestinal tract. Particulate nickel has been found in atmospheric aerosols and the concentration has been measured to be 0.8 ng/m^3 in remote areas, but to be as high as 180 ng/m^3 in urban areas—and even as high as 3.3 mg/m^3 around nickel smelters. Values of 40 mg nickel/kg have been found in house dust [44].

Gold is also abundant at low concentrations in the environment, mostly in metallic form, but it can also be found as gold telluride [45, 46]. For both metals, there is wide human exposure in products that are normally used in close contact with the skin—jewellery, for example—and even in implants and dental restorations [30, 47]. With nickel, exposure in Denmark is controlled by the Danish nickel regulation from 1990 and in the EU it is regulated by the EU nickel directive from 1994, the aim being to limit skin contact with objects from which nickel release can be detected [48-50]. In the last few decades, since the use of the metal and also habits have changed, new groups with allergic contact dermatitis caused by nickel release have been found. Nickel was previously common in jewellery and suspenders, but it has recently been found, for example, in smartphones [51].

Regard gold, there have been no legislative measures. Most individuals are exposed to gold through contact with jewellery. It has been used as implant material for stents and as dental restorative material, even though its use today appears to be on the decrease [52-60].

Gold as such has also been used in the treatment of arthritis since the 1960s [61, 62], but this is very uncommon nowadays. Gold has also been ingested in order to improve health. It has been used as a treatment for smallpox and measles (in China), and in Japan it has been ingested for its general beneficiary effect on health [63]. The allergen is often found to have no clinical relevance, so patch testing in the baseline series is not normally performed [43]. However, for aimed testing the allergen has been found to have clear clinical relevance—especially with regard to different implants, such as dental implants and stents [52-58]. Gold has been in the baseline series at the Department of Occupational and Environmental Dermatology in Malmö for more than 20 years, as there has been a special interest in the allergen [64].

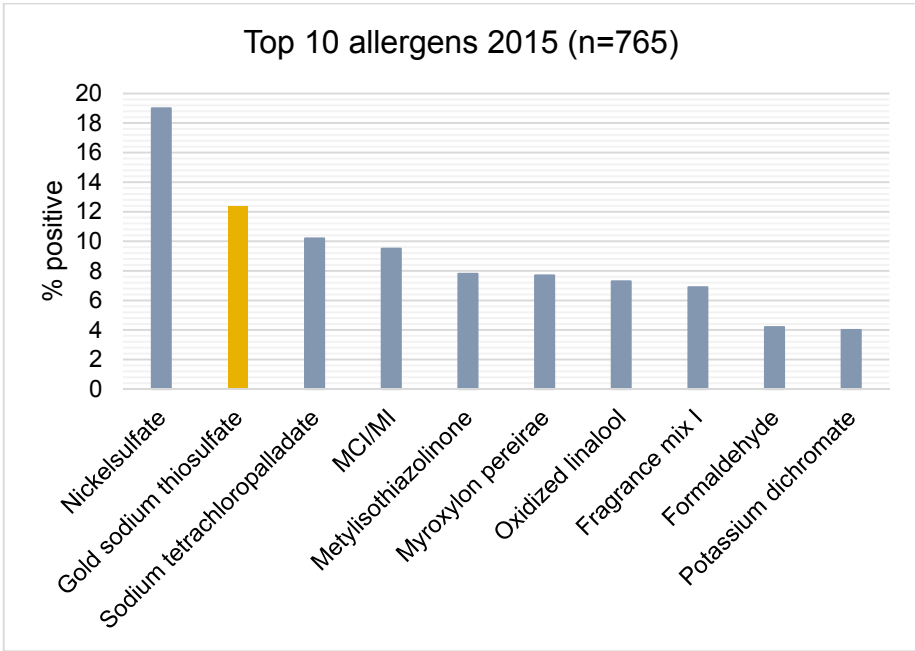

Figure 1

1.3 Exposure assessment

A metal has to be ionised to sensitise or to elicit an allergic contact dermatitis reaction. To prove metal release and quantify the release from an object, it is of utmost importance to prove relevance i.e. that the object has actually caused the reaction or caused deterioration.

1.3.1 Metal release *in vitro*

In the nickel directive, the method for investigation of metal release is to immerse the object in artificial sweat for a week followed by quantification of the release [49, 65]. In daily clinical practice, a spot test is used to prove nickel release. Regarding the immersion medium, different types have been used based on the nature of the metal and the environment in which the object is found.

It should be emphasised that it is not only the place where the object is found (e.g. the skin, blood vessels, or oral cavity) that may influence release. Local factors also affect the release, such as those in the mouth (chewing, characteristics of saliva, pH, temperature etc.) [47]. With regard to gold, cysteine solutions have been used to prove metal release *in vitro* [66].

The metals can be quantified by atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) [67]. In the work for this thesis, AAS was used for detection of ion release from metal objects.

1.3.2 Metal release *in vivo*

Another way of defining exposure to the metal is to investigate what is found on and in the skin, and in the blood. Monitoring of the amount of metals deposited on the skin can, for example, be accomplished by different wipe techniques or by tape stripping. For nickel, the presence of nickel ions on the skin has been shown by using a wipe technique based on the dimethylglyoxime spot test. Lidén and colleagues showed that the technique could be used to accurately assess skin exposure to possible contact allergens, and that it had a higher degree of recovery than tape stripping, a technique that has also been used to analyse penetration [68-70].

1.3.3 Detection

In paper II, AAS was used for the detection of gold and nickel released from metal objects and deposited on the skin whereas ICP-MS was used to monitor the amount of gold and nickel found in blood.

AAS

This analytical technique, which is used in many fields of chemistry such as for clinical analysis, for environmental analysis, and in industry, measures the concentration of metals. AAS can detect only one element at a time. The technique makes use of the fact that all atoms can absorb light and that the wavelengths at which light is absorbed are specific for each element. Thus, if a sample containing both nickel and gold is exposed to light at the characteristic wavelength for gold, then only gold atoms will absorb this light. The amount of light absorbed is proportional to the number of gold atoms. In AAS, the sample is atomised at high temperatures, i.e. converted into ground state free atoms. The beam of light with a specific wavelength is passed through the sample in this vaporised form. Thus, AAS requires the following three components: a light source, an atomiser to produce gaseous atoms, and a means of measuring the specific light absorbed. The light source is most often a hollow cathode lamp consisting of a tungsten anode and a cathode made of the element to be determined. Atomisation is accomplished with either of two systems: by sucking a solution of the sample into a flame or by placing a drop of the sample into a graphite tube which is then heated electrically. Finally, quantification is made possible by use of a light-sensitive detector.

ICP-MS

Exposure to metals not only occurs through the skin, but also through systemic intake. For this reason, in many studies efforts have been made to define the amount of metal

in blood. ICP-MS can detect several elements in a sample. It is a sensitive method that can be used to determine concentrations of various metals and non-metals, with atomic masses ranging from 7 to 250 atomic mass units. In the inductively coupled plasma (plasma is argon gas, ionised), ions are produced and then passed to a mass spectrometer that separates and detects them. ICP-MS is used to detect metals in blood.

1.4 Patch testing

When performing patch testing, a patient with suspected ACD is exposed to a suspected allergen on intact skin under controlled conditions. The patch test method is performed by applying appropriate concentrations of the suspected allergen in an appropriate vehicle in a test chamber mounted on adhesive tape fixed to the skin for 48 hours (Figure 2). It has been shown that paired readings of patch test reactions are the most accurate on day (D) 3/4 and D7 [14, 16, 71-74]. If an eczematous reaction occurs at the test site, a contact allergy is indicated. Over the years, the patch test technique has undergone standardisations and developments regarding to the substances used, the concentrations, the doses, the vehicles used, and the scoring [13, 20, 21].

It is recommended that reactions should be scored according to the International Contact Dermatitis Research Group (ICDRG) criteria as follows: (+), doubtful reaction; +, weak positive reaction; ++, strong positive reaction; +++, extreme positive reaction; [13, 75] (Figure 3).

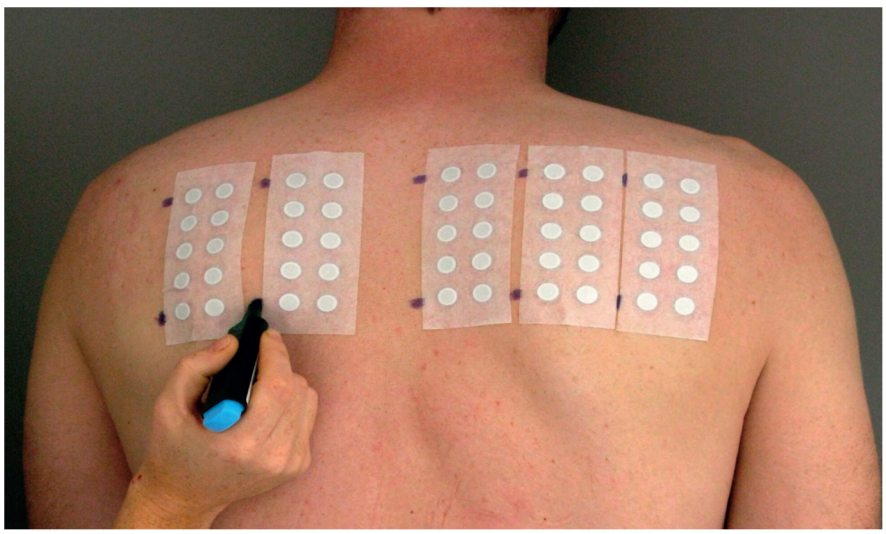

Figure 2.
Patch test applied to the back of a patient.

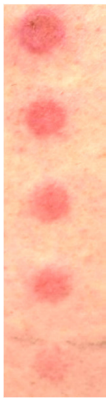	**+++**	**Extreme positive reaction; intense erythema, infiltration and coalescing vesicles.**
	+++	
	++	Strong positive reaction; erythema, infiltration, papules and vesicles.
	+	Weak positive reaction; erythema, infiltration and possibly papules.
	? +	Doubtful reaction; faint erythema only.
	-	Negative reaction.
	IR	Irritant reaction of different types.

Figure 3.
Recording of patch test reactions according to the ICDRG. Dilution series illustrating the fact that if the dose is too low, a doubtful reaction will occur even if the patient is allergic.

Doubtful reactions are reactions that do not fulfill the criteria of the ICDRG, i.e. there is only erythema and not infiltration covering the whole area—or there is only infiltration and not erythema covering the whole patch test area. A doubtful reaction is by definition not an allergic reaction but it may be an allergic reaction not fulfilling the criteria—for example, if the dose is not adequate at patch testing (i.e. the reaction may be false-negative and may be proven positive at retest with a higher concentration).

The easiest way to exemplify this is to scrutinise a serial dilution (Figure 3) in a contact allergic individual. As the dose is reduced, the reactivity decreases.

False-positive reactions are positive reactions defined as reactions caused by irritation, with a morphology indistinguishable from a contact allergic reaction. The general principle is to patch test with the highest concentration of the allergen not yielding active sensitisation or provoking irritation. Testing with serial dilutions of the test preparation and/or patch testing of controls may exclude the possibility that a reaction is false-positive. If the reaction is truly allergic, it is usually possible to decrease the concentration 100 times, giving a moderate patch test reaction without losing the possibility of eliciting a positive reaction [20].

False-negative reactions are negative reactions defined as a failure to elicit a positive patch test reaction although the individual tested has a contact allergy. Insufficient dose, too low a concentration, unstable substance, systemic treatment with corticosteroids during patch testing, improper vehicle or test chamber, and reading that is performed too early can all cause false-negative reactions [13, 73, 76, 77].

Late patch test reactions are positive reactions that appear at the site of a previously negative patch test, later than D7. Some allergens are known to cause late reactions. A

well- known example is when patch testing for corticosteroids, where the reason for the late- appearing reaction is probably the anti-inflammatory effect of the steroid, not that the reaction appears late as such. Another well-known example of late reactions is with gold. A low degree of reactivity in the patient and a low test concentration of—and/or slow penetration of—the allergen are possible causes of late reactions. A late patch test reaction can also indicate an active sensitisation caused by the patch test [37, 43].

Active sensitisation is an adverse effect of patch testing. With a negative patch test reaction followed by a positive reaction after 10–20 days or on D3/4 when re-testing, an active sensitisation is a possible explanation [16]. Some individuals may react to lower concentrations of an allergen, later than D7 [36, 37]. Patch testing with serial dilutions of the allergen in question should be performed when patch test sensitisation is suspected [20].

1.4.1 Patch testing with gold

Gold was initially considered to be an inert metal. A variety of gold salts, which did not give rise to contact allergy [78-81], were used for patch testing in addition to the metal as such [82]. In the 1960s, Kligman found that gold chloride was a strong sensitiser in the human maximisation test [83]. However, the solution with gold chloride is a strong irritant, and in 1987 Fowler recommended gold sodium thiosulfate dihydrate (GSTS) as a reliable, non-irritant preparation for patch testing with gold [84, 85].

This patch test substance, 2.0% (w/w) in petrolatum, was reported at the Jadassohn Centenary Congress in London in 1996 to be a good screening preparation for identification of contact allergy to gold [86]. In Malmö, GSTS has been patch tested within the extended baseline series in consecutive dermatitis patients since 1991, first at 0.5 % and after a few years at 2.0% [64].

Positive patch test reactions may appear late, and readings should therefore be performed on D3 or D4 and on D7 [14, 36]. The prevalence of contact allergy with a tested material is often high [87, 88] and differs between populations due to differences in exposure, but it has previously been found to be around 10% in dermatitis patients tested consecutively [89].

There are very few data on general populations, but in these, frequencies of 5–10% have been reported [90]. If the patch test result is doubtful and there is a high clinical suspicion of an allergic contact dermatitis, the patient can be re-tested with 5% GSTS in petrolatum.

1.4.2 Patch testing with nickel

The prevalence of contact allergy to nickel is high in the general population [91]. It is estimated that up to 17% of women and 3% of men are allergic to nickel. In dermatitis patients, the prevalence of metal allergy is even higher [40, 90]. The reason for nickel allergy being so common is not necessarily that it is a very strong allergen, but that exposure is difficult to avoid. The first patch tests with nickel were performed in 1925 [92]. Today, the salt nickel sulfate hexahydrate is used for patch testing all over the world, and is included in most baseline series. The test concentration is usually 5% pet. (w/w). If the reaction is doubtful and there is a high clinical suspicion of an allergic contact dermatitis, nickel sulfate hexahydrate can be tested in aqueous solution at 15% and 30% [93, 94]. Even intracutanous patch testing can be performed. It is known that the reactivity can vary with hormonal changes and diet [95].

2 Aims

In this thesis, the overall aim was to improve our basic knowledge of gold as a contact allergen and our understanding of patch testing as a technique. The specific aims of the studies included in this thesis were as follows:

- To investigate the reproducibility of the patch test technique with regard to where the allergen is patch tested and how strong the reactivity is.
- To investigate whether gold is actually released in detectable amounts onto the skin when the object is in prolonged contact in an occlusive environment, and to determine whether any systemic uptake can be detected from this exposure.
- To determine whether contact allergy, especially to metals, is more frequent in patients with genital lichen sclerosus et atrophicus and whether the disease may be a sign of a possible systemic effect of the metal as an allergen.
- To retrospectively investigate dermatitis patients who were patch tested in Malmö between 1995 and 2014, regarding contact allergy to gold and nickel.

3 Materials and methods

Detailed descriptions of the subjects and methods are given in the individual papers. This section aims to gives an overview.

Papers I and II were experimental studies whereas papers III and IV were retrospective studies.

3.1 Chemicals

The main chemicals used in the studies are listed in Table 2.

Table 2.
The main chemicals used, with manufacturers/suppliers.

Chemicals	Study	Manufacturer
Gold sodium thiosulfate dihydrate (GSTS)	I, III	Chemotechnique Diagnostics AB, Vellinge, Sweden
Hydrochloric acid, HCl (30%)	II	Merck, Darmstadt, Germany
Cysteine	II	ICN Biomedical, Aurora, OH, USA
Magnesium nitrate	II	PerkinElmer,Waltham, MA, USA
Nickel sulfate hexahydrate	I, III	Acros Organics, Geel, Belgium
Nitric acid, HNO_3 (0.2%)	II	Merck, Darmstadt, Germany
Palladium nitrate	II	PerkinElmer,Waltham, MA, USA
Atomic Spectroscopy Standard solution 1,000 µg/ml Au	II	PerkinElmer,Waltham, MA, USA
Nickel Standard solution, 1,000 µg/ ml Ni	II	Merck, Darmstadt, Germany

3.2 Subjects

In study I, 31 individuals, 12 with allergy to nickel and 19 with allergy to gold, were recruited from the patient data system (DALUK) and included in the study on the basis of previously found contact allergy.

In study II, 14 individuals were included. None had any known allergy to gold or nickel.

In study III, the study individuals (n = 41) all had genital lichen sclerosus whereas the controls (n = 40) had volunteered to participate and were previous patients of the

dermatology unit due to skin tumours. They had no connection with DALUK, but were actively recruited from the tumour clinic.

In the Department of Occupational and Environmental Dermatology, data from all patients investigated are stored in the DALUK database [96].

In study IV, we wanted to analyse the time period in which contact allergy to gold had been studied in a standardised manner by patch testing.

In this retrospective study, we analysed data from all 13, 106 consecutive patients who had been referred to the Department of Occupational and Environmental Dermatology in Malmö for suspected allergic contact dermatitis between the years 1995 and 2014. Table 3 gives information on the individuals included in these studies.

Table 3.
Demographic data on all individuals in the four studies

Study	No. of study subjects	No. of women	No. of men	Mean age of study subjects, years	Age range, years
I	31	25	6	54.6	19–73
II	14	13	1	44.2	20–69
III	81	81	0	62.5	26–86
IV	13,106	8,191	4,915	44.7	10–94

3.3 Patch testing technique and patch test preparations

In studies I, III, and IV all the patients were patch tested with 8 mm Ø Finn Chambers® (Epitest Ltd. Oy, Tuusula. Finland or SmartPractice, Phoenix, AZ, USA) mounted on Scanpor® tape (Norgeplaster A/S, Oslo, Norway). The patch test preparations that were tested are described in detail in sections 3.3.1., 3.3.2., and 3.3.3.and the manufacturers/suppliers are listed in Table 2. For aqueous solutions, a filter paper was mounted on the Finn chamber and 15 µl of test solution was applied to the chambers with a micropipette [72]. For preparations in petrolatum, 20 mg was applied to each patch test unit [21]. The patch test material was carefully marked, and extra tape was used to secure the patch test material to ensure that the material was equally occluded to the subject's back on both sides. To achieve standardisation, the personnel regularly calibrate to ensure that the amount of petrolatum preparations used is as close 20 mg as possible. Unless otherwise stated, the patch tests were removed from the back after 48 hours by the subjects themselves, and a reading was carried out by a dermatologist on D3/4 and on D7 according to the ICDRG guidelines [13].

3.3.1 Study I

All subjects were patch tested with their known allergen in serial dilutions. In this study, the dilution series of both allergens in aqua were tested and the highest concentrations were higher than those in the commercially available patch test preparations. GSTS was patch tested in water at 6.3, 2.0, 0.63, 0.2, 0.063, and 0.02% (w/v), and nickel sulfate hexahydrate was patch tested in water at 16.0, 5.0, 1.6, 0.5, and 0.16% (w/v) [72, 97]. If the subject had a previous 3+ reaction to gold, the serial dilution started at 2.0% and ended at 0.02% and if the individual had a previous 2+ reaction, the dilution series started at 6.3% and 0.063% was the last dilution step. Each subject was patch tested with 3 identical dilution series, and with the metal they had not previously reacted to in 2.0% w/w GSTS in petrolatum if nickel-allergic, and in 5.0 % w/w nickel sulfate hexahydrate in petrolatum if gold-allergic. Each serial dilution was applied separately and each subject was patch tested with 3 serial dilutions with the highest concentration on the proximal part of the back. 22 patients were patch tested on the left side and 9 were patch tested on the right side [98-101].

3.3.2 Study III

All the women were patch tested with a patch test series based on the Swedish baseline series and a modified dental series. Readings were performed by experienced dermatologists on D3 and D7 according to the ICDRG guidelines. Before the dermatologist met the participant, the nurse put a cloth over the participant's head and shoulders so that the reading of tests could be performed in a blind manner.

The dermatologist had no knowledge of the answers to the questionnaire, and was unaware of whether the study participant was a control or a patient with GLS. No-one was informed about the test results until both readings were finished.

3.3.3 Study IV

The individuals were all patch tested with the Swedish baseline series and also with the extended baseline series.

In Malmö, the baseline series includes the substances found in the Swedish baseline series but additional substances are always used in patch tests, such as GSTS, which has been consecutively patch tested in Malmö since 1991. Other allergens have been in the baseline series for a shorter length of time. The series is evaluated twice a year and changes regarding substances, patch test vehicles, and doses can be implemented. However, with regard to the patch test substances evaluated in the study, the Swedish baseline series has not been altered during this time.

3.4 Provocation with metal discs and analysis of metal release

In study II, provocations with metal discs were performed. The skin on the participants' backs was provoked with metal discs for defined periods of time. Metal release from the discs was investigated *in vitro* and *in vivo*. The skin was inspected before and after provocation.

3.4.1 Metal discs

Gold discs, 24 carat with a diameter of 20 mm and a thickness of 0.2 mm were obtained from KarAna Ädelmetall, Helsingborg, Sweden. Nickel discs with a diameter of 20 mm and a thickness of 0.2 mm (stainless steel, AISI 304/ EN 1.4307/ SS2333, AISI/SAE standard, a stainless steel alloy 18/8 with a nickel content of 8.3%) were obtained from MTA, Medical Technical Department, Skåne University Hospital SUS, Malmö, Sweden.

3.4.2 Metal release *in vitro*

The release of gold and nickel from the discs was investigated by placing one gold disc and one nickel disc separately in plastic test tubes (50 ml; Sarstedt AG & Co, Nümbrecht, Germany). To each tube was added 6.0 ml of 0.1 cysteine with the pH set to 8 [66] by addition of hydrochloric acid 30 % Suprapur. One gold disc and one stainless steel disc were put separately into identical plastic tubes in the same manner, and 6.0 ml of artificial sweat with a pH of 6.4 was added to the tubes. The discs were soaked in the solutions for one week and then analysed. A cysteine solution at pH 8 was chosen since this, in a prior study, has been found to be optimal for release of gold [66]. Both metals were immersed in cysteine and in artificial sweat for the same predefined time and the solutions then analysed for metal content [65]. The AAS technique (as described below) was used for detection and quantification of the samples.

3.4.3 Provocation with metal discs

The gold discs were divided and mounted on four strips (Tegaderm™ Film; 3M Health Care, St. Paul, MN, USA). Three strips had 5 discs on each and the fourth strip was smaller and contained only one disc. The five stainless steel discs were mounted on one strip in the same manner, Figure 4. Before and after the provocation, blood samples were drawn to analyse systemic uptake of gold/nickel. An acid wipe technique [68] for analysis of metal on skin was performed. An overview of which investigations were performed on each volunteer is shown in Table 4.

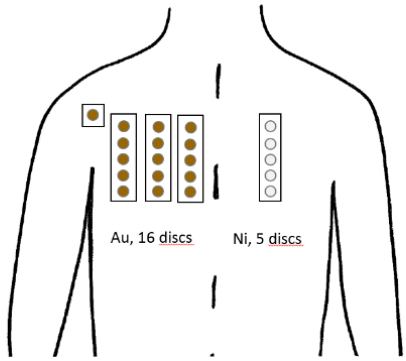

Figure 4.
Schematic overview of how provocation discs were placed. 16 gold (Au) discs were mounted on three strips with five each, one disc was mounted alone, and 5 nickel (Ni) discs were mounted on one strip. For the volunteers who removed gold discs on several occasions (nos. 1-9), the strips were removed in a randomized order.

Table 4.
Overview of which investigations were performed on each volunteer.

Volunteers and investigations	**Hours**			
	0	48	96	168
Volunteers 1–5				
Provocation with Au discs		x	x	x
Provocation with Ni discs				x
Blood sample for analysis	x	x	x	x
Acid wipe of skin	x	x	x	x
Volunteers 6–9				
Provocation with Au discs		x	x	x
Provocation with Ni discs				x
Blood sample for analysis	-	-	-	-
Acid wipe of skin	x	x	x	x
Volunteers 10-14				
Provocation with Au discs				x
Provocation with Ni discs				x
Blood sample for analysis	x	-	-	x
Acid wipe of skin	-	-	-	-

3.4.4 Metal release measured *in vivo*, the acid wipe technique

An acid wipe sampling technique [68] was used to sample metals from the skin before and after exposure to metal discs. For each metal disc, the skin was wiped off with one wipe (Mediplast, Malmö Sweden) moistened with 1.0 ml 0.2% HNO_3 in aqua and one dry wipe, Figure 5. The samples were stored and frozen in clean plastic tubes until analysis. To extract the metal for analysis, the technique differed slightly for gold and nickel (for details, see paper II): for nickel, 0.2% HNO_3 was used, and for gold, two

different concentrations of aqua regia were tried and 10.0% (HNO_3:HCl at a ratio of 1:3) proved superior. Samples were analysed using the AAS method described below. For the gold standard, curves were prepared by diluting a standard gold solution of 1,000 µg/ml Au in the same aqua regia concentration as above, to the following concentrations: 10, 20, and 40 ppm. For nickel, standard curves were prepared by diluting a nickel standard solution of 1,000 µg/ml Ni in 0.2% HNO_3 in aqua to the following concentrations: 10, 25, and 50 ppm. Triplicate injections were done of each sample and the result was calculated as the mean of the three.

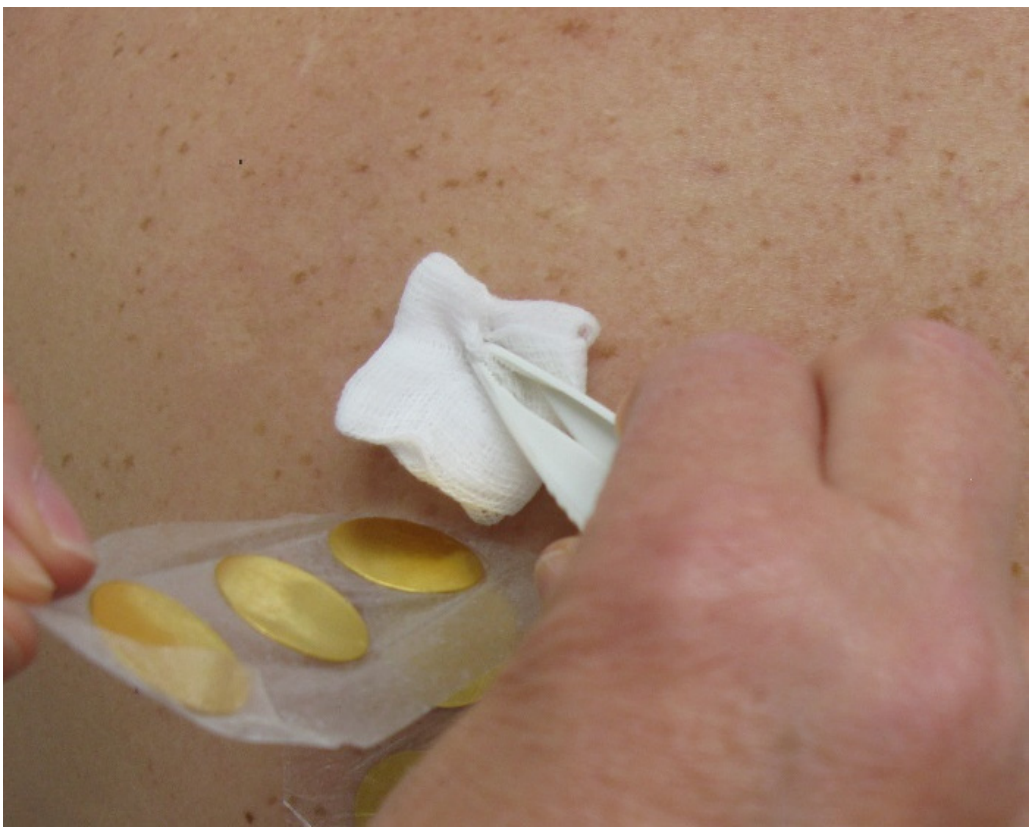

Figure 5.
For each metal disc the skin was wiped off with one wipe moistened with 1 ml 0.2% HNO_3 in aqua and one dry wipe.

3.4.5 The AAS technique used for determination of gold and nickel from metal discs and skin

Analysis was performed using AAS with a detection limit of > 0.003 µg/ml Au and > 0.001 µg/ml Ni. The spectrometer used was an AAnalyst 800 (PerkinElmer, Waltham, MA, USA) equipped with graphite furnace and nickel and gold hollow cathode lamps. Absorption of gold was measured at 242.8 nm and the spectral bandwidth was 0.7 mm,

while absorption of nickel was measured at 232 nm with a spectral bandwidth of 0.2 mm. Sample analysis was performed using Zeeman background correction. 20 µl of each sample was injected; for gold, the samples were injected together with a matrix modifier consisting of 0.005 mg palladium nitrate (PerkinElmer) and 0.003 mg magnesium nitrate (PerkinElmer). Triplicate injections were analysed for all samples. The coefficient of variation of 10 injections of the same sample was used to determine the repeatability. This was 16.7% and 6.3% for gold and nickel, respectively.

3.5 Analysis of gold and nickel in blood

Au and Ni were determined by inductively coupled plasma mass spectrometry (ICP-MS; iCAP Q, Thermo Fisher Scientific, Bremen, Germany) equipped with collision cell and helium as collision gas. The samples were diluted 20 times with an alkaline solution according to Bárány et al. [102]. The detection limit, calculated as 3 times the standard deviation (SD) of the blank, was 0.010 µg/l for Au and 0.12 µg/l for Ni. All the samples analysed were prepared in duplicate and the imprecision of the method (calculated as the coefficients of variation in measurements from duplicate preparations) was 8.8% for Au and 11% for Ni. To ensure the analytical accuracy, quality control samples were analysed along with the samples collected. For Ni, Seronorm Trace Elements Whole Blood L-1, Lot 0903106 (SERO AS, Billingstad, Norway) was used. Because no certified reference samples for Au in blood are available, outdated blood from blood donors spiked with 0.10 µg/l Au was used. The results obtained (mean ± SD) for Au were 0.11 ± 0.004 µg/l vs. the recommended 0.10 µg/l, and for Ni they were 1.2 ± 0.11 µg/l vs. the recommended 0.70–1.7 µg/l.

3.6 Questionnaires

3.6.1 Study III

In study III, a questionnaire, Table 5, was used that had been adopted from previous studies in which exposure to gold and contact allergy was evaluated [48]. The study subjects answered the questionnaire before taking part in the study, and the results were analysed after the study was terminated.

Table 5.
The questionnaire used in study III.

Questions			
	Yes	No	Don´t know/ No answer
Have you had itch, flush, or swelling after skin contact with gold jewellery such as earrings or finger rings.			
Have you had itch, flush, or swelling after skin contact with jewellery or other objects in metal (not gold)?			
Have you ever had your ears pierced?			
Have you ever had pierced skin or mucosa?			
Do you have or have you had dental material in gold?			
Do you have any symptoms from the oral mucosa?			
Do you have any genital symptoms?			
Do you smoke?			
Are you a snuff user?			
Do you regularly use chewing gum?			
Do you work or have you been working with material that contains gold, for example as a jeweller?			
Do you work or have been working with nickel or material containing nickel?			
Do you take or have you taken drugs containing gold?			

3.6.2 Study IV

Questions to the patients about their history regarding atopy, atopic dermatitis, and localisation of present dermatitis are asked and the answers collected before patch test results are available, which reduces the risk of bias. The aim of the questions is to get information on whether the patient has atopy, i.e. if the patient has rhinoconjunctivitis/asthma or atopic dermatitis, or if the patient has had any history of this. Does the patient have a dermatitis at the time of investigation, and where then is the dermatitis located? The sites are divided into: face/neck, trunk/arms/legs, hands/feet, or general spread. If the dermatitis is located on the hands, a differentiation is made between several anatomical parts of the hands. The answers are stored in the computer system, and they were retrieved for this retrospective study.

3.7 Ethics

In studies I, II, and III, the subjects were informed about the nature of the test and possible adverse reactions. Informed written consent was obtained from all the subjects, and the studies were approved by the Regional Ethical Review Board, Lund, Sweden.

Study IV was a retrospective one based on all patch tested individuals between 1995 and 2014. The patients are always informed (before the patch test) that the test data will be stored and that the data may be retrieved to be used for research. Identification of individuals is not possible. Every patient has the opportunity of objecting to this. An advertisement was published in the local newspaper to ensure that any patient who did not want his/her data to be used could make contact and have the data removed. No-one declined participation. The study was approved by the Regional Ethical Review Board, Lund.

3.8 Statistical calculations

3.8.1 Study I

To enable statistical calculations concerning the possible significance of anatomical localisation for the patch test result, the test reactions were transformed to numerical values:

− = 0, (+) = 0.5, + = 1, + (+) = 1.5, ++ = 2, ++ (+) = 2.5, +++ = 3 [95]. The reactivity could thus be measured in two ways, either as a summarised test score, STS, where the scores for all reactions in one patch test series were summed, or as minimal eliciting concentration (MEC) [95], defined as the lowest concentration to elicit at least a + reaction.

The positive reactions were not always continuous. When the number of negative and/or doubtful reactions was followed by the same number or more of positive reactions, the lowest positive reaction was registered as the MEC. In Tables 6a and 6b, the individual STSs and MECs are given. Friedman test was used for statistical calculations to compare the reactivity response for STS and MEC.

3.8.2 Study II

Student t-test paired analysis was used for the statistical analyses.

3.8.3 Study III

The answers to the questionnaire were compared between the GLS patients and the control group. Fisher's exact test, two-sided, was used for the patch test results and Pearson's chi-square test was used to evaluate the difference between the two groups. Any p-value of < 0.05 was considered statistically significant.

3.8.4 Study IV

Age differences were analysed using Student's t-test. Statistical difference when comparing the nickel-allergic and gold-allergic individuals was evaluated with Fisher's exact test.

Differences between the total population investigated and the two allergic groups were calculated using the chi-square test with Yates' correction. Significant associations with gold were investigated using univariable and multivariable models based on logistic regression.

4 Results

4.1 Study I

Figure 6.
Triplicate applications of the same serial dilution were applied to either the right side or the left side of the back.

4.1.1 Reproducibility with regard to horizontal change i.e. with regard to the left and the right side of the back

The dilution series used for patch testing were labelled A, B, and C, or A', B', and C', depending on the location (Figure 6). The individual STS and MEC values are given for the different dilution series for each individual, in Table 6a and 6b. There was no statistically significant difference when analysing STS and MEC regarding whether the patients had been tested on the most lateral part of the left side (A) or on the most lateral part of the right side (A'), and similarly, there were no significant differences when comparing B and B' or C and C'. Furthermore, no significant differences were found regarding whether there was the same reactivity on the middle of the back and on the lateral part, i.e. whether the STS results of test panel A differed significantly from those of test panel B or test panel C and with regard to the right side, A' was equivalent anatomically to C', and C to A'. The A, B, and C panels were compared on D3 and D7 (Figure 7).

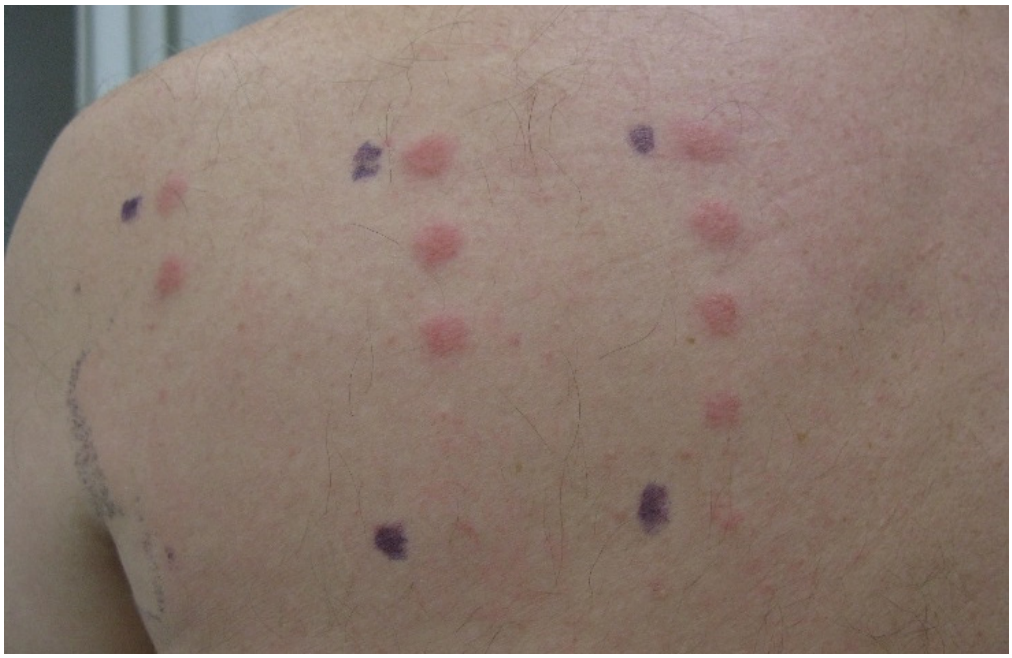

Figure 7.
The patch test reactivity pattern seen in some subjects when they were patch tested with exactly the same dilution series in triplicate.

Table 6a.
Minimal eliciting concentration (MEC) and summarised test score (STS) for patients tested with serial dilutions of gold sodium thiosulfate.

Reading	Day 3						Day 7					
Site	A/A'		B/B'		C/C'		A/A'		B/B'		C/C'	
Subject	MEC	STS	MEC	STS	MEC	STS	MEC	STS	MEC	STS	MEC	STS
1*	2.0	3.5	0.63	5.0	0.63	3.0	2.0	3.5	2.0	4.5	0.63	3.0
2*	0.063	10.0	0.063	13.0	0.063	8.5	0.063	9.0	0.063	9.0	0.063	9.0
3*	0	0	6.3	2.5	2.0	3.5	6.3	1.5	2.0	5.0	2.0	7.0
4*	0.63	6.5	0.63	8.5	0.63	8.5	0.2	9.5	0.2	11.0	0.063	12.0
5*	0	0	0	0	2.0	2.0	0	1.0	0	1.5	2.0	4.0
6*	0.2	8.5	0.63	7.5	0.63	7.5	0.63	5.5	0.63	6.5	0.63	7.0
7*	0.063	8.0	0.063	9.5	0.063	11.5	0.063	9.0	0.063	10.0	0.063	9.0
8*	2.0	3.5	6.3	2.5	2.0	2.5	2.0	3.5	2.0	3.0	2.0	3.5
9*	0	0	6.3	1.5	0	0.5	0	0	0	0	6.3	1.0
10* [1]	0.2	12.0	0.063	13.0	0.063	14.0	0.2	10.0	0.063	12.0	0.063	13.0
11* [1]	0.63	4.0	0.63	3.0	2.0	2.0	2.0	4.5	0.63	5.5	2.0	2.5
12* [1]	6.3	1.0	0	0.5	0	0	2.0	3.0	6.3	1.0	6.3	1.0
13**	0.63	4.5	0.2	6.5	0.063	7.0	0.63	5.0	0.2	7.0	0.02	9.0
14**	0.02	12.5	0.02	10.0	0.02	11.0	0.02	9.0	0.02	8.0	0.02	8.5
15**	0	0	2.0	1.0	0	0	0	0	2.0	1.5	0	0
16**	0.063	9.0	0.063	10.5	0.063	9.5	0.063	8.0	0.02	12.0	0.063	10.5
17** [1]	0.063	11.0	0.02	12.0	0.063	10.0	0.063	6.5	0.02	8.0	0.02	8.0
18** [1]	0.63	4.5	0.63	3.5	0.63	3.0	0.2	5.5	0.63	4.0	0.63	4.5
19** [1]	0.63	2.0	2.0	1.0	0	0	0.63	2.5	0	0.5	0	0

*6.3%, 2.0%, 0.63%, 0.2%,and 0.063% aq **2.0%, 0.63%, 0.2%, 0.063%, and 0.02% aq. [1] A', B', and C': tested on the right side of the back.

Table 6b.
Minimal eliciting concentration (MEC) and summarised test score (STS) for patients tested with serial dilutions of nickel sulfate hexahydrate.

Reading	Day 3						Day 7					
Site	A/A'		B/B'		C/C'		A/A'		B/B'		C/C'	
Subject	MEC	STS	MEC	STS	MEC	STS	MEC	STS	MEC	STS	MEC	STS
20	**1.6**	**4.5**	**0**	**0**	**5.0**	**4.0**	**1.6**	**5.5**	**5.0**	**3.0**	**1.6**	**6.0**
21	**5.0**	**3.0**	**5.0**	**3.0**	**16.0**	**3.0**	**16.0**	**2.5**	**5.0**	**3.5**	**5.0**	**2.5**
22	**1.6**	**8.5**	**1.6**	**6.0**	**1.6**	**6.0**	**1.6**	**6.5**	**1.6**	**5.5**	**1.6**	**6.5**
23	**0.5**	**6.0**	**1.6**	**5.5**	**5.0**	**4.0**	**1.6**	**5.5**	**1.6**	**5.5**	**5.0**	**2.0**
24	**1.6**	**6.0**	**5.0**	**4.5**	**16.0**	**2.5**	**1.6**	**5.0**	**5.0**	**5.5**	**16.0**	**3.0**
25	**1.6**	**5.5**	**5.0**	**6.0**	**0.5**	**9.0**	**1.6**	**5.0**	**1.6**	**6.0**	**0.5**	**8.0**
26	**0.5**	**5.5**	**0.5**	**5.5**	**0.5**	**7.0**	**0.5**	**4.0**	**0.5**	**4.0**	**0.5**	**5.5**
27	**5.0**	**5.0**	**1.6**	**8.0**	**0.5**	**8.5**	**5.0**	**6.0**	**1.6**	**7.0**	**0.5**	**8.5**
28 [1]	**5.0**	**3.0**	**5.0**	**2.0**	**16.0**	**1.0**	**1.6**	**3.0**	**5.0**	**2.0**	**5.0**	**3.5**
29 [1]	**0**	**0.5**	**0**	**0.5**	**0**	**0**	**0**	**0.5**	**16.0**	**1.0**	**16.0**	**1.0**
30 [1]	**5.0**	**5.0**	**1.6**	**5.5**	**1.6**	**5.5**	**5.0**	**5.0**	**1.6**	**5.5**	**1.6**	**6.5**

*16.0%, 5.0%, 1.6%, 0.5%, and 0.16% aq. [1] A', B', C': tested on the right side of the back.

4.1.2 Analysis of reproducibility of allergic reactions at simultaneous patch testing

Taking into consideration the high degree of reproducibility regarding anatomical position, (see above), we also analysed the reproducibility at simultaneous patch testing and dose.

For each step of reactivity (+, ++, +++), the degree of reproducibility with regard to whether or not any allergic reaction (independent of intensity) could be reproduced is shown. The two allergens are shown separately. The figures, however, show a very similar pattern, indicating that as the degree of reactivity decreases, the reproducibility becomes lower (Figure 8a and 8b).

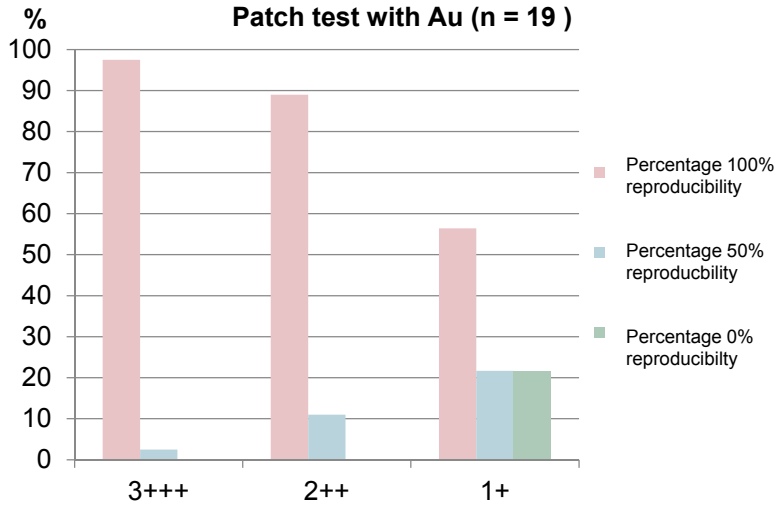

Figure 8a.
The reproducibility pattern for gold based on the intensity of the allergic reaction (+, ++, +++) at D3 when each subject was tested with three identical dilution series at the same time. For each contact-allergic reaction, the levels of reproducibility at that concentration step, that is, the three identical patch tested doses, were compared. The only comparison being made was whether there was 100% reproducibility, that is, all three showed allergic reactions (+, ++, +++), even if the reactivities of the reactions were not identical, or whether there was 50% reproducibility, that is, two of three reactions were positive, or whether there was 0% reproducibility, that is, if there was only one positive reaction and/or (+) or negative reaction.

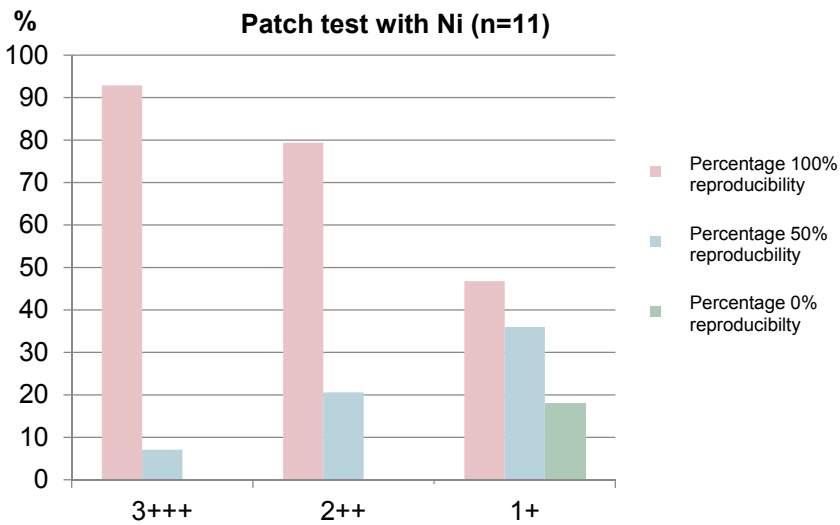

Figure 8b.
The reproducibility pattern for nickel based on the intensity of the allergic reaction (+, ++, +++) at D3 when each subject was tested with three identical dilution series at the same time. For each contact-allergic reaction, the levels of reproducibility at that concentration step, that is, the three identical patch tested doses, were compared. The only comparison being made whether there was 100% reproducibility, that is, all three showed allergic reactions (+, ++, +++), even if the reactivities of the reactions were not identical, or whether there was 50% reproducibility, that is, two of three reactions were positive, or 0% reproducibility, if there was only one positive reaction and/or (+) or negative reaction.

4.1.3 Patch test reactivity with regard to patch test dose

Another way of indicating the reproducibility and how this changes with reactivity is to look at the reactivity pattern at each concentration step (each dose) (Figure 9a and 9b).

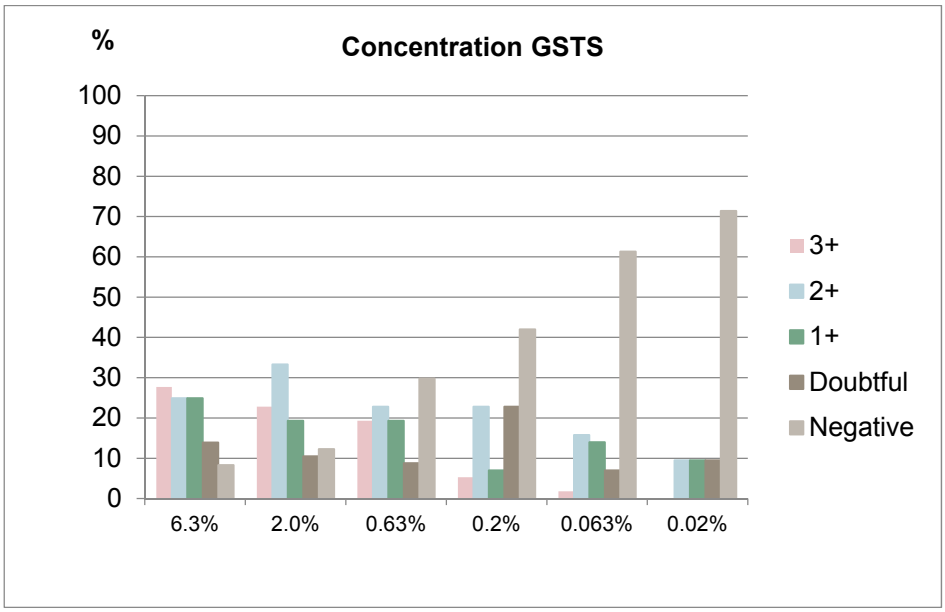

Figure 9a.
Patch test reaction pattern on D3 (with % of total number patch tested defined according to the ICDRG as negative, doubtful, +, ++, or +++) for each concentration of GSTS used for patch testing.

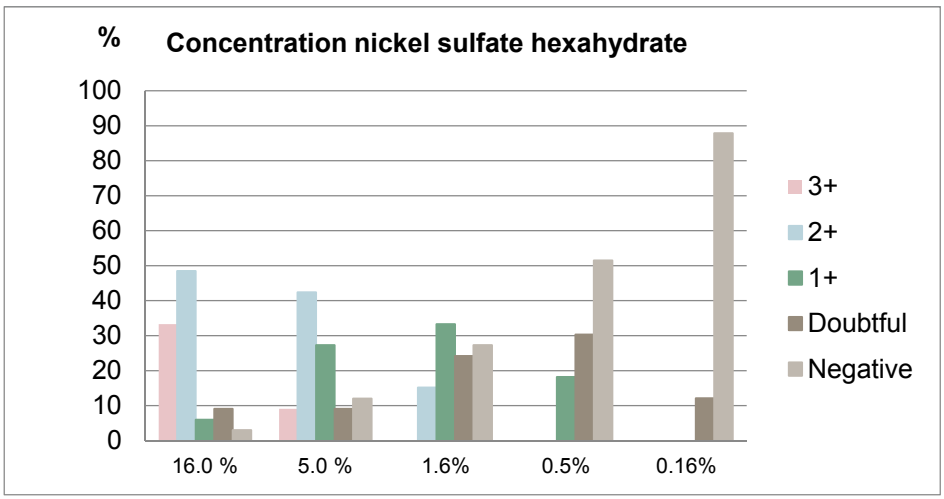

Figure 9b.
Patch test reaction pattern on D3 (with % of total number patch tested defined according to the ICDRG as negative, doubtful, +, ++, or +++) for each concentration of nickel sulfate hexahydrate used for patch testing.

4.2 Study II

In vivo analyse of the metals nickel and gold was performed before the metal discs were applied to the skin. Nickel was discovered on the skin before provocation, but there was no significant increase in nickel—either on the skin or in the blood—during the provocation.

There was a significant increase in the amount of gold on the skin, but this was not detected as an increase in blood—as a sign of systemic uptake. The results of gold and nickel release on the skin at different time intervals are shown in Figure 10a and 10b.

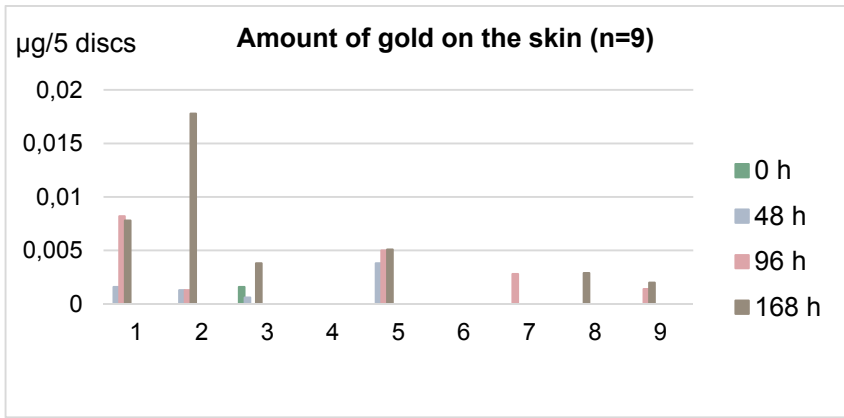

Figure 10a.
The amount of gold found on the skin before provocation (0 hours) with 16 gold discs where five were removed after 48 hours, another five were removed after 96 hours, and finally six were removed after 168 hours.

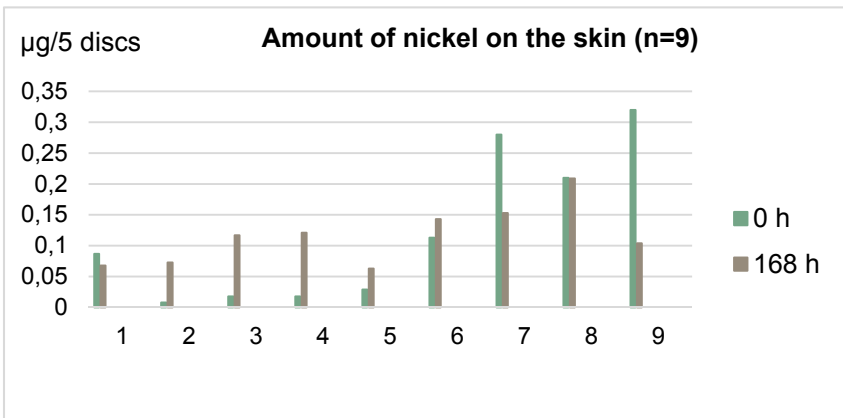

Figure 10b.
The amount of nickel found on the skin before provocation (0 hours) and after provocation (168 hours) with 5 stainless steel discs.

4.3 Study III

The answers to the questions in the questionnaire are shown in Paper III. There was a difference between the two groups in the answers regarding symptoms from the skin and from the mucosa. 10 of 41 of the women in the GLS group reported that they had itch, flush and swelling after skin contact with gold jewellery, compared to 4 of 40 in the control group ($p=0.045$). With regard to symptoms from skin when in contact with metals apart from gold, the two groups did not differ.

Regarding symptoms from the genital mucosa, 39 females in the GLS group reported this, as compared to 4 in the control group ($p < 0.001$). Twice as many women in the GLS group reported symptoms from the oral mucosa: 13 of 38 as compared to 6 of 37 ($p = 0.073$).

Table 7a.
Reactions in Patch test series based on the Swedish baseline series and a modified dental series.

Patch test preparations	Conc (%)	GLS n=41	Controls n=40	P-value
Metals				
Mercury	0.5	2	0	
Copper sulfate	2			
Palladium chloride	2	2	1	
Aluminium chloride hexahydrate	2	1	0	
Tin	50			
Titanium	50			
Calcium titanate	10			
Silver sulfate	10			
Ammonium hexachloroplatinate	0.1 *			
Titanium nitride	5			
Gold sodium thiosulfate	2	6	9	
Mercury	1.6 *	2	0	
Zinc chloride	1			
Manganese chloride	2			
Potassium dichromate	0.5			
Cobalt chloride (hexahydrate)	1			
Nickel sulfate (hexahydrate)	5	6	11	0.181
Corticosteroids				
Tixocortol pivalate	0.1			
Budesonide	0.01			
Fragrances				
Myroxylon pereirae	25	4	8	0.225
Fragrance mix II	14	0	1	
Fragrance mix I	8	2	4	
Lichen acid mix	0.3	0	3	0.115
Lyral	5	3	0	0.240
Eugenol	2			
Antibiotic				
Neomycin sulfate	20			
Fungistat, antiinfective				
Quinoline mix	6			
Flavor				
Carvone L_form	5	4	1	
Topical anaesthetics				
Caine mix II	10	1	2	
Rubber chemicals				
Black rubber mix	0.6			
Mercapto mix	2			
Thiuram mix	1	1	0	
Resins				
Colophonium	20	1	1	
Epoxy resin	1			
p-tert-Butyl phenol formaldehyde resin	1			

Conc.: w/w % in petrolatum, except * w/v % in water.
When no values are given, p > 0.3.
Any p-value < 0.05 was considered statistically significant.

Table 7b.
Reactions in patch test series based on the Swedish baseline series and a modified dental series.

Patch test preparations	Conc (%)	GLS n=41	Controls n=40	P-value
Preservatives				
Formaldehyde	2*	2	1	
Formaldehyde	1*			
Quaternium-15	1			
Paraben mix	16			
Diazolidinyl urea	2*			
CL + ME – isothiazolinone, Kathon CG	0.02*	1	0	
Methyldibromo glutaronitrile	0.5			
Sodium metabisulfite	2	1	1	
Desinfections				
Glutaraldehyde	0.2			
Plastic - related substances				
N, N- Dimethyl – p – toluidine	5			
2-Hydroxy – 4 – Methoxybenzophenone	10			
Benzoylperoxide	1	1	1	
Ethyl p-toluenesulfonamide	0.1			
p-Tolyldiethanolamine	2			
Methylhydroquinone	1			
Camphoroquinone	1			
Tinuvin P	1			
Plant				
Sesquiterpene lactone mix	0.1			
Emulsifier				
Amerchol L 101	100	1	0	
Stabilizer				
Ethylendiamine dihydrochloride	1	1	0	
Miscellaneous				
para -phenylendiamine	1	1	1	
Canada balsam	25	1	0	

Conc w/w % in petrolatum
Conc.: w/w % in petrolatum, except * w/v % in water.
When no values are given, p > 0.3.

The patch test results are shown in Table 7a and 7b. The results showed that 24 of 41 (59%) of the women with GLS and 23 of 40 (58%) in the control group had at least one positive patch test reaction, but the GLS group had positive reactions to more allergens (21 of 69) than the control group (14 of 69).

4.4 Study IV

The rate of contact allergy to nickel was 19% for all individuals tested (27% in women and 5% in men). The rate of contact allergy to gold was 14% for all individuals tested (18% in women and 8.4% in men). Suspected gold-allergic individuals had significantly higher mean age than those with contact allergy to nickel. Gold allergy was associated with female sex, higher age, atopy, and facial dermatitis (Table 8).

Table 8.
The descriptive characteristics of the population. Atopic dermatitis, mucosal symptoms (i.e. rhinoconjuncitivitis and/or asthma), hand dermatitis, and facial dermatitis in the total population investigated and in the nickel- (Ni) and gold-allergic (Au) individuals.

Individuals	The total patch tested population	Nickel allergic (Ni)	Gold allergic (Au)	Au vs Ni	Tested Ni vs Ni	Tested Au vs Au
Total number	13106	2490 (19.0%)	1883 (14.4%)			
Atopic dermatitis	584 (4.4%)	191 (7.7%)	123 (6.5%)	p = 0.16	p = 0.0001	p = 0.0001
Mucosal symptoms	1182 (9.0%)	338 (13.6%)	297 (15.8%)	p = 0.042	p = 0.0001	p = 0.0001
Hand dermatitis	4006 (30.6%)	856 (34.4%)	598 (31.8%)	p = 0.070	p =0.0001	p = 0.0001
Facial dermatitis	2320 (17.7%)	521 (20.9%)	433 (23.0%)	p = 0.10	P =0.0001	p = 0.0001

5 Discussion

5.1 Study I

The aim was to investigate the reproducibility of the patch test technique regarding where the allergen is patch tested and how strong the reactivity is[103].

Using a highly standardised patch test technique with the same test system, defined doses as serial dilutions, and a test reaction classification with additional grades, a good reproducibility of the patch test technique was found independently of where on the upper back the patch test chambers were placed [104]. However, it was clear that when the reactivity was lower the reproducibility also decreased. We found that for 3+ reactions, for example, the reproducibility was almost 100%—whereas as the reactivity decreased, the reproducibility also decreased in a dose-dependent manner.

The results shown in Figure 9a and 9b do not support the results from the analysis of reproducibility over time. Excited skin syndrome is defined as many positive patch test reactions where particularly many of the weak + reactions are false-positive and will result in negative reactions when re-tested on a later occasion[105, 106]. This demonstrates that a non-reproducible 1+ reaction is not equivalent to a false-positive reaction, as has been argued, since we know that these patients are actually allergic. The findings are of interest for the general standardisation of the patch test technique.

In this study, the test substances were the same. Differences were not found because of possible cross-reactivity/increased reactivity due to chemically similar or exactly the same hapten, as has previously been stated as a possible reason for non-reproducible test results [100].

5.2 Study II

The aim was to determine whether metal objects actually release detectable amounts of gold and nickel onto the skin, when in prolonged contact in an occlusive environment, and to investigate whether any systemic uptake from this exposure could be detected[107, 108].

The initial experiments to verify release of nickel and gold from the metal objects were performed similarly to previous experiments [66, 109].

We know that when patch testing with GSTS at 2% in petrolatum in a Finn chamber with an area of 0.5 cm^2, this is equivalent to a surface concentration of about 300 $\mu g/cm^2$ gold ions. *In vitro* analysis of gold release in cysteine was 10.71 $\mu g/cm^2$, about 30 times less. On the skin, the metal release after 168 hours varied between 0 and 0.0178 µg (mean value 0.00027 $\mu g/cm^2$) with a total disc surface of 15.7 cm^2. This corresponds to a release of about 40,000 times less than the release from the gold disc *in vitro* (10.71 $\mu g/cm^2$). The stainless steel disc released 0.0092 $\mu g/cm^2$ Ni in cysteine and 0.0020 $\mu g/cm^2$ in artificial sweat.

It must be taken into consideration that the values appeared to increase with time. Regarding nickel, there was no significant difference before or after provocation, but the exposure was also much less, since the aim of the study was really to prove release of gold onto skin. We did not try to optimize nickel release, in order not to run the risk of sensibilization [110]. It was interesting to find that in many of the subjects, nickel was present in higher amounts on the skin prior to provocation—indicating the fact that nickel is a ubiquitous substance [44]. No detectable systemic uptake was found. Even if individuals are non-contact allergic to gold, there seems to be a large difference in the amount of gold released in different individuals. Further knowledge about the uptake of metals on the skin would of course be of interest, and this could be performed using the tape stripping technique, which has already been done with nickel [69, 111].

The data indicate a possible relationship between dose and time i.e. that as the exposure time increases, the amount of gold on the skin increases. The study was limited, but it did reflect the usual clinical situation, that a person even with a found systemic gold source in for example a gold implant can wear gold jewellery but there may still be a release of metal from an object in prolonged contact.

5.3 Study III

The aim was to investigate whether contact allergy, especially contact allergy to metals, is more frequent in patients with lichen sclerosus et atrophicus. We found a high rate of contact allergy with a frequency of almost 60% in the females in both the GLS group and the control group. The frequency of contact allergy in women with GLS has not been investigated extensively. The particular aim of the study was to explore the possibility that contact allergy to dental materials might be of possible clinical importance for GLS as a manifestation of systemic allergic contact dermatitis. The hypothesis was also that the patients with GLS would present with more contact allergies to allergens frequently used in the genital area. To study this, a control group, age-correlated, was invited to participate. The study design was unique, as it eliminated bias with regard to patch test reading. Regarding contact allergy to substances known to cause possible systemic reactions, the patients with GLS were not over-represented. In our findings, there was not a significant over-representation of metal allergy in the GLS group, indicating the significance of metal allergy as such as the etiological factor

for GLS. This does not preclude the importance of a metal allergy and systemic effects as a factor in deterioration of the disease. When we looked at dental materials only, apart from gold, there was a trend of an over-representation of dental allergens in the GLS group; this patient group also reported more symptoms from the oral mucosa. The results of this study did not indicate an increased contact allergy rate for allergens that could be correlated to topically used medicaments/products or to allergens that are known to give rise to local reactions. A possible explanation for the high frequency of contact allergy in the control group also could of course be selection bias due to the fact that when given information on the study, subjects with skin or mucosal problems may have been more inclined to participate.

The number of positive patch test reactions was almost the same in the two groups, but the women in the GLS group had positive patch test reactions to several more substances than the controls.

5.4 Study IV

The aim was to retrospectively investigate the dermatitis patients who had been patch tested in Malmö between 1995 and 2014, with regard to contact allergy to gold and nickel (Figure 11).

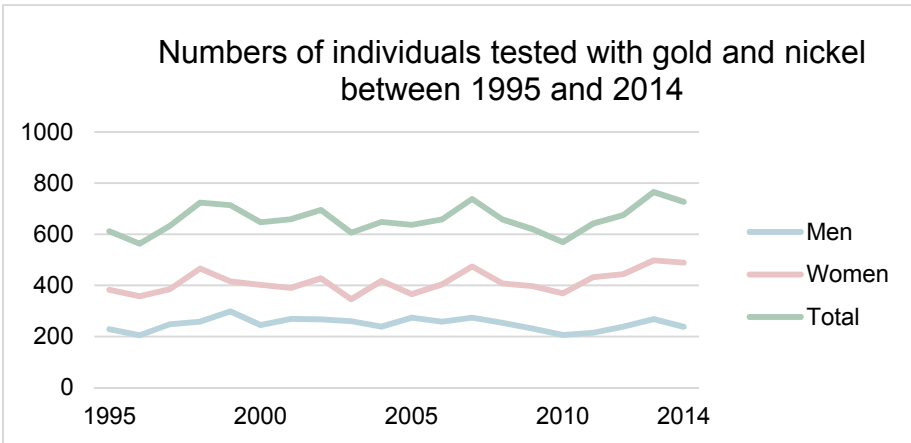

Figure 11.
Numbers of individuals tested with gold and nickel at the Department of Occupational and Environmental Dermatology, Malmö, between 1995 and 2014.

The frequency of gold allergy in the study was 14% and the frequency of nickel allergy was 19%. In the Eden study, which investigated contact allergy in a large European general population, the frequency of contact allergy to nickel was about 15%—with the lowest prevalence in Sweden (8.3%). Considering that the population investigated in here was selected due to the fact that they were referred for suspected allergic contact

dermatitis, we did not find our nickel allergy numbers to be surprising [91, 112, 113]. For both gold and nickel, we found that the allergy was more frequent in females (the female-to-male ratio in the total material was 1.7 to 1) (Table 8).

A correlation to female sex has been found previously [52, 89, 114-118]. Atopic dermatitis was over-represented in the nickel-allergic group, which has also been shown previously [90, 117]. The facial localisation of dermatitis has been discussed previously, but nickel allergy is more often associated with hand dermatitis [6, 119], which was also the case in this study. Contact allergy to gold has previously been associated with facial dermatitis, particularly eyelid dermatitis, where the exposure has been very clearly identified [54, 59, 120].

Contact allergy to both gold and nickel in this Swedish material appears to be decreasing slightly in frequency. The nickel directive [49, 121] may of course be of importance for the rate of contact allergy to nickel. The directive has been in use in the EU since 1994, and the number of nickel-allergic patients would be expected to be greater in the elderly groups, as these individuals have lived for a longer period without the regulation. When we scrutinised the numbers, however, this was not the case. Nickel allergy was most common in the 30- to 34-year age group, and then the number declined in the higher age groups. The prevalence of gold allergy also appears to be declining, with reduced use of gold in the oral mucosa (Camilla Ahlgren, pers. comm.). Gold was often used in dental restorations, but it is a very expensive material and other alternatives are more frequently used today [60].

Considering the reactivity at D3/4 and how there is an increase in positive reactions to gold at D7 and a slight but definite decline in positive reactions to nickel at D7, there is a clear argument for the use of consecutive readings at D3/4 and at D7 (Figure 12).

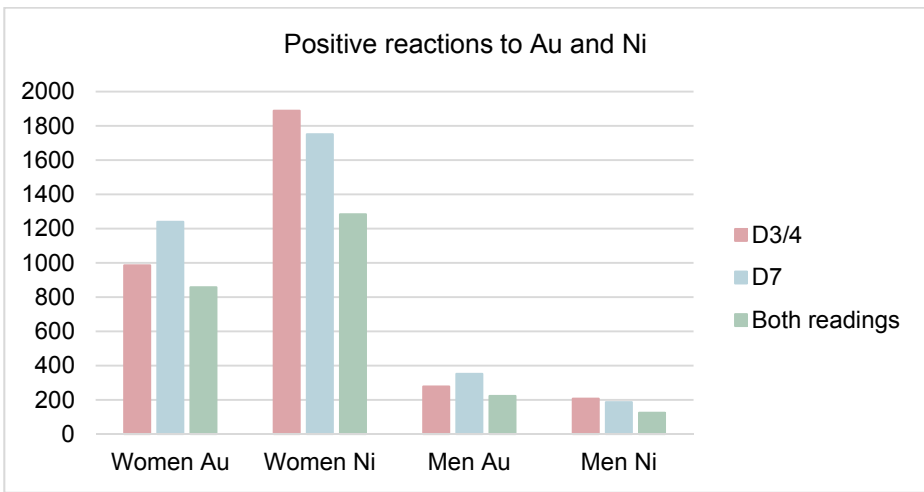

Figure 12.
Positive reactions to Au and Ni at D3/4 and D7 and both readings.

6 Summary and concluding remarks

Patch testing is still the gold standard for evaluation of contact allergy. Diagnosis of allergic contact dermatitis is a three-step procedure [20, 122].

- The patient must have a contact allergy that is correctly established.
- The patient must be exposed to the allergen or possibly a cross-reacting substance.
- Upon removal of the exposure, the dermatitis should disappear/improve significantly and re-appear on re-exposure.

Gold is an allergen that has been controversial, and it still is to some extent, due to several factors already described. In this thesis, we wanted to further investigate some of the questions surrounding gold as a contact allergen and also the difficulties of patch testing, particularly with metals.

We were able to prove that reproducibility of patch testing does not vary according to which part of the upper back is tested.

The reactivity of the reaction decreases when the patch test dose is reduced, which is a major reason for a patient not having reproducible reactions. It should, however, be noted that even with the same reactivity or dose, the reproducibility is still not 100%. This perhaps indicates local differences in the immunobiology of the skin barrier.

When we want to define the relevance, we often use spot tests to prove release of the contact allergen found.

Study II showed that even if we know that metal is released from an object, it is not necessarily detected on the skin—even after prolonged contact. A significant increase in gold was detected, but this was not the case with nickel after provocation with stainless steel. There was even a difference in metal release in the *in vitro* experiments, where the release of nickel in cysteine was much lower than the release of gold. In artificial sweat, there was a low *in vitro* release of nickel.

Thus, we cannot make any comparisons between the two findings on skin. An interesting finding was that nickel was detectable even before provocation, and no increase was actually found during the provocation. The release of gold was signficantly different. When studying individuals who were exposed to gold, there was a large difference between individuals; 2 out of 9 individuals had no detectable amounts on their skin even after 168 hours. From the study, one can hypothesise that metal release is related to factors in the skin such as the characteristics of the sweat. These findings

are of importance regarding metal allergy in general and gold allergy in particular, and for the discussion on what is a relevant dermatitis in relation to an allergy that has been found.

In study III, we wanted to find further proof for the clinical relevance of metal allergy, especially gold allergy. Here, the aim was to investigate possible association with a systemic allergic contact dermatitis. Gold allergy has been associated with systemic symptoms and with oral lichenoid reactions, and particularly with local reactions in proximity to a gold dental implant [7, 8, 22, 30, 123]. More diffuse symptoms have also been associated with allergy to metals such as gold [8].

In this study, we did not find any association between genital lichen sclerosus and contact allergy. The study was, however, limited in size and choice of patients. It would be of interest to investigate this better with a provocation study involving patients with lichen ruber planus, the actual disease mimicked in oral lichenoiod reactions. In such a study, the effect of removing the allergen could also be investigated.

In study IV, we wanted to improve our general knowledge of contact allergy to gold, and investigate possible associations with basic characteristics of the patients such as atopy, sex and age. A better knowledge of possible associations with contact allergy may improve our ability to decide when we can recommend patch testing with gold. In the study there were associations between gold allergy and age, sex, atopy, and facial dermatitis.

7 Popular scientific summary in Swedish

Kontaktallergi är en typ IV reaktion, de kliniska manifestationerna visar sig oftast som eksem. Kontaktallergi konstateras genom epikutantesting, en provokations-undersökning där patienten får små kammare med de allergen man önskar testa, klistrade på huden på ryggen. Testen sitter på i 48 timmar och avläses på dag 3 eller dag 4 samt dag 7. Om man är allergisk för det ämne som testas utvecklas en eksemreaktion som man kan följa vid avläsningarna. Hur vi testar, vilka ämnen, mängder, koncentrationer och hur vi avläser resultatet bygger dels på vetenskap dels på beprövad erfarenhet. Självklart är det viktigt att tekniken kontinuerligt utvärderas och standardiseras. Testningen är en icke invasiv undersökning där ämnena sätts på huden, men den kräver flera besök för att diagnos skall kunna ställas. Man har försökt finna *in vitro*- testmetoder t.ex. lymfocyt-transformationstest, dvs. ett blodprov där man analyserar specifika T-cellers aktivitet vid exponering för kontaktallergi-framkallande ämnen *in vitro*. Metoden fungerar på gruppnivå på en del allergen t.ex. metallsalter men fortfarande är epikutantest den rekommenderade metoden för att diagnosticera kontaktallergi, vilket gör standardisering ännu viktigare.

Metallallergi, tillhör våra vanligaste kontaktallergier vid testning. Vanligast är nickelallergi som testas i vår bas-serie, den standardserie som är rutin, och som alla patienter testas med. Den näst vanligaste kontaktallergin är guldallergi. Guld är dock ett kontaktallergiframkallande ämne som inte testas rutinmässigt, huvudsakligen beroende på reproducerbarheten och otillräcklig kunskap om den kliniska relevansen.

Relevansbedömning vid guldallergi är svårare än för andra kontaktallergiframkallande ämnen. Detta beror på att guldallergi vid testning kan visa sig sent och att en enskild guld-allergisk testreaktion kan kvarstå på huden upp till ett år. Dessutom kan eventuellt guld som tillförs via mag-tarmkanalen, förutom hudförändringar ge upphov till influensaliknande symtom.

Med nuvarande kunskap om betydelsen av metallallergi hos individer med implantat är guldallergi den viktigaste kontaktallergin.

För vissa patientgrupper kan en kontaktallergi mot guld vara synnerligen viktigt att känna till, bland annat har man funnit en relation mellan lichenoida reaktioner i munslemhinnan och kontaktallergi mot guld. Guldallergi, liksom nickelallergi, är också känt för att kunna ge systemiska reaktioner det vill säga reaktioner på lokaler som inte

primärt är i kontakt med allergenet, som till exempel vid implantat, utan distributionen sker via hud, slemhinna eller blod.

I denna avhandling har vi velat undersöka vissa basala begrepp som är viktiga vid epikutantestning, att finna vetenskapliga bevis för dessa samt få mer kunskap om kontaktallergenet guld.

I studie I undersöktes reproducerbarheten vid epikutantestning dvs. om vi reagerar lika mycket på höger som vänster sida av ryggen samt centralt respektive lateralt. Vi undersökte också hur reproducerbarheten för en reaktion sjunker när reaktiviteten blir lägre. Vi fann att det inte är någon skillnad mellan var på ryggen man testar och att man har god reproducerbarhet för högre koncentrationer/doser vid en högre reaktivitet samt lägre när reaktiviteten sjunker. Detta ger ytterligare kunskap om svagt positiva/tveksamma reaktioner och att dessa inte behöver vara falskt positiva bara för att de inte är reproducerbara vid omtestning.

I studie II undersöktes om metaller avges från metallföremål som är i närkontakt med huden under längre tid. I denna studie undersökte vi guld respektive nickel och fann en frisättning av guld, ju längre kontakttid desto mer guld på huden. Vi fann dock inget systemiskt upptag i undersökningen.

I studie III undersökte vi eventuella systemiska effekter av metaller hos patienter med genital lichen sclerosus et atrophicus samt om dessa patienter på grund av sin påverkade slemhinna har större risk att få kontaktallergi. Vi fann inte att så var fallet och inte heller någon ökad frekvens av metallallergi. Dock rapporterade dessa kvinnor signifikant mer besvär som klåda, rodnad och svullnad från huden efter kontakt med guld vilket inte angavs när det gällde annan metall. Kvinnorna med GLS hade även signifikant mer besvär från den genitala slemhinnan jämfört med kontroll-patienterna. Studien var begränsad i antal men stöder inte att kontaktallergi för metallen guld skulle ge ökade genitala besvär.

I studie IV studerades uppgifter, lagrade i databasen DALUK, gällande de patienter som epikutantestats mellan åren 1995 och 2014 med bas-serie och tilläggsserie, som i Malmö även innehåller guld. I studien önskade vi jämföra dem som uppvisat positiv reaktion för guld respektive nickel, samt övriga individer med avseende på atopi, handeksem, ansiktseksem, ålder och kön. Vi fann att guldallergi är vanligt även om frekvensen tycks sjunka något över tid. Guldallergi är vanligare bland kvinnor än bland män och vanligare med stigande ålder. Mer kunskap behövs för att förbättra vår förmåga att avgöra när vi ska rekommendera att epikutantesta med guld då denna allergi kan ge så skiftande och diffusa symtom.

Acknowledgements

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References

1. Gell P G H, R R A. Coombs. Clinical aspects of immunology. 1963, Oxford: Blackwell.
2. Lachapelle J M, Maibach H I, Patch Testing Methodology. In *Patch Testing and Prick Testing: A Practical Guide Official Publication of the ICDRG.* Berlin, Springer, 2009 33-70.
3. Averbeck M, Gebhardt C, Emmrich F, Treudler R, Simon J C. *Immunologic principles of allergic disease.* J Dtsch Dermatol Ges, 2007: **5** 1015-1028.
4. Menné T, Veien N K. *Systemic Contact Dermatitis*, in *Textbook of Contact Dermatitis*. Rycroft R J G et al. (eds.) Berlin, Springer, 2001 355-366.
5. Andersen K E, Hjorth N, Menne. The baboon syndrome: systemically-induced allergic contact dermatitis. *Contact Dermatitis* 1984: **10**: 97-100.
6. Christensen O B, Möller H. External and internal exposure to the antigen in the hand eczema of nickel allergy. *Contact Dermatitis* 1975: **1**: 136-141.
7. Möller H, Björkner B, Bruze M. Clinical reactions to systemic provocation with gold sodium thiomalate in patients with contact allergy to gold. *Br J Dermatol.* 1996: **135**: 423-427.
8. Möller H, Ohlsson K, Linder C, Björkner B, Bruze M. The flare-up reactions after systemic provocation in contact allergy to nickel and gold. *Contact Dermatitis* 1999: **40**: 200-204.
9. Malinauskiene L, Isaksson M, Bruze M. Systemic contact dermatitis in a gold-allergic patient after treatment with an oral homeopathic drug. *J Am Acad Dermatol* 2013: **68**: 58.
10. Bloch B. The role of idiosyncrasy and allergy in dermatology. *Archives of Dermatology and Syphilology* 1929: **19**: 175-197.
11. Bruze M, Isaksson M, Edman B, Björkner B, Fregert S, Möller H. A study on expert reading of patch test reactions: inter-individual accordance. *Contact Dermatitis* 1995: **32**: 331-337.
12. Svedman C, Isaksson M, Björk J, Mowitz M, Bruze M. 'Calibration' of our patch test reading technique is necessary. *Contact Dermatitis* 2012: **66**: 180-187.
13. Wilkinson D S, Fregert S, Magnusson B, Bandmann H J, Calnan C D, Cronin E, Hjorth N, Maibach H J, Malalten K E, Meneghini C L, Pirilä V Terminology of contact dermatitis. *Acta Derm Venereol.,.* 1970: **50**: 287-292.
14. Johansen J D, Aalto-Korte K, Agner T, Andersen K E, Bircher A, Bruze M, Cannavó A, Giménez-Arnau A, Gonçalo M, Goossens A, John S M, Lidén C, Lindberg M, Mahler V Matura M, Rustemeyer T, Serup J, Spiewak R, Thyssen J P, Vigan M, White I R, Wilkinson M, Uter W. *European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice.* 2015: **73**: 195-221.
15. Wentworth A B, Yiannias J A, Keeling J H, Hall M R, Camilleri M J, Drage L A, Torgerson R R, Fett D D, Prakash A V, Scalf L A, Allen E M, Johnson J S, Singh N, Nordberg Linehan D L, Killian J M, Davis M D. Trends in patch-test results and

allergen changes in the standard series: a Mayo Clinic 5-year retrospective review (January 1, 2006, to December 31, 2010). J Am *Acad Dermatol.*2014: **70**: 269-275.
16. Lindberg M, Matura M. Patch Testing In *Contact Dermatitis*, D.J. Johansen, J.P. Frosch, and J.-P. Lepoittevin, (eds): Berlin, Springer, 2011: 439-464.
17. Uter W, Gefeller O, Mahler V. Does a short patch test training course have an impact? *Contact Dermatitis.* 2016: **74**: 317-319.
18. Belsito D V. Patch testing: after 100 years, still the gold standard in diagnosing cutaneous delayed-type hypersensitivity. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M.* 1997: 195-202.
19. Mendelow A Y, Forsyth A, Florence A T, Baillie A J. Patch testing for nickel allergy. The influence of the vehicle on the response rate to topical nickel sulphate. *Contact Dermatitis* 1985: **13**: 29-33.
20. Bruze M, Condé-Salazar L, Goossens A, Kanerva L, White I R. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis* 1999: **41**: 241-250.
21. Bruze M, Isaksson M, Gruvberger B, Frick-Engfeldt M. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis* 2007: **56**: 281-285.
22. Ahlgren C, Bruze M, Möller H, Gruvberger B, Axéll T, Liedholm R, Nilner K. Contact allergy to gold in patients with oral lichen lesions. *Acta Derm Venereol*, 2012: **92**: 138-143.
23. Neill S M, Lewis F M. Non-Infective Cutaneous Conditions of the Vulva. In *Ridley's The Vulva*. Wiley-Blackwell. 2009: 85-144.
24. Liebowitch M, Staughton R, Neill S, Barton S, Marwood R. *Inflammatory diseases. An Atlas of Vulval Disease*. 1997, Martin Dunitz Ltd, London.: London.
25. Marangon Junior H, Souza P E, Soares R V, Gomez R S, Pereira G H, Horta M C. Oral Lichen Sclerosus: A Rare Case Report and Review of the Literature. *Head Neck Pathol.* 2016.
26. Marren P, Millard P, Chia Y, Wojnarowska F. Mucosal lichen sclerosus/lichen planus overlap syndromes. *Br J Dermatol.* 1994: 131: 118-123.
27. Evans A V, Fletcher C L, Owen W J, Hay R J. Oesophageal lichen planus. *Clin Exp Dermatol.* 2000: **25**: 36-37.
28. Kreuter A, Kryvosheyeva Y, Terras S, Moritz R, Möllenhoff K, Altmeyer P, Scola N, Gambichler T. Association of autoimmune diseases with lichen sclerosus in 532 male and female patients. *Acta Derm Venereol.* 2013: **93**: 238-241.
29. Meyrick Thomas R H, Ridley C M, McGibbon D H, Black M M. Lichen sclerosus et atrophicus and autoimmunity--a study of 350 women. *Br J Dermatol.* 1988: **118**: 41-46.
30. Ahnlide I, Ahlgren C, Björkner B, Bruze M, Lundh T, Möller H, Nilner K, Schütz A. Gold concentration in blood in relation to the number of gold restorations and contact allergy to gold. *Acta Odontol Scand.* 2002: **60**: 301-305.
31. Lewis F M, Shah M, Gawkrodger D J. Contact sensitivity in pruritus vulvae: patch test results and clinical outcome. *Am J Contact Dermat.* 1997: **8**: 137-140.
32. Rustemeyer T, Van Hoogstraten I M V, Von Blomberg B M E, Gibbs S, Scheper R. *Mechanisms of Irritant and Allergic Contact Dermatitis.* In Contact Dermatitis. D.J. Johansen, J.P. Frosch, and J.-P. Lepoittevin, (eds.) Berlin, Springer, 2011: 43-90.
33. De Groot A C. Test concentrations and vehicles for 4350 Chemicals. *3rd ed.* Wapserveen, the Netherlands:acdegroot publishing, 1994

34. Smith Pease C K, Basketter D A, Patlewicz G Y. Contact allergy: the role of skin chemistry and metabolism. *Clin Exp Dermatol.* 2003: **28**: 177-183.
35. Bos J D, Meinardi M M. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Exp Dermatol.* 2000: **9**: 165-169.
36. Bruze M, Hedman H, Björkner B, Möller H. The development and course of test reactions to gold sodium thiosulfate. *Contact Dermatitis.* 1995: **33**: 386-391.
37. Isaksson M, Bruze M, Late patch-test reactions to budesonide need not be a sign of sensitization induced by the test procedure. *Am J Contact Dermat*. 2003: **14**: 154-156.
38. Isaksson M, Lindberg M, Sundberg K, Hallander A, Bruze M. The development and course of patch-test reactions to 2-hydroxyethyl methacrylate and ethyleneglycol dimethacrylate. *Contact Dermatitis.* 2005: **53**: 292-297.
39. Frick-Engfeldt M, Isaksson M, Zimerson E, Bruze M. How to optimize patch testing with diphenylmethane diisocyanate. *Contact Dermatitis.* 2007: **57**: 138-151.
40. Thyssen J P, Menne T. Metal allergy--a review on exposures, penetration, genetics, prevalence, and clinical implications. *Chem Res Toxicol.* 2010: **23**: 309-318.
41. Thyssen J P, Linneberg A, Menné T, Johansen J D. The epidemiology of contact allergy in the general population--prevalence and main findings. *Contact Dermatitis.* 2007: **57**: 287-299.
42. Lidén C. Nickel. In Handbook of Occupational Dermatology, L. Kanerva, P Elsner, J E Wahlberg, H I Maibach (eds): Berlin, Springer, 2000: 524-533.
43. Bruze M, Andersen K E. Gold--a controversial sensitizer. European Environmental and Contact Dermatitis Research Group. *Contact Dermatitis.* 1999: **40**: 295-299.
44. Elsome A. Metals and the Skin: Topical Effects and Systemic Absorption. In: *Applied Organometallic Chemistry.* R H Guy, J J Hostýnek, R S Hinz, C R Lorence Marcel Dekker (eds):New York New York 2000:**14**: 398-399.
45. Frosch P J, Menné T, Lepoittevin J P. *Contact dermatitis 4th ed.* Frosch P J, Menne T, Lepoittevin J P. (eds): Berlin, Springer, 2006. 1136 p
46. *Nickel / Committee on Medical and Biologic Effects of Environmental Pollutants*. 1975, Washington DC: National Academy of Sciences.
47. Drasch G, Muss C, Roider G. Gold and palladium burden from dental restoration materials. *J Trace Elem Med Biol.* 2000: **14**: 71-75.
48. *European Environmental and Contact Dermatitis Research Group. Nickel Contact Dermatitis from Consumer Products. Documentation and Evaluation of Preventive Aspects.* 1990.
49. European Communities. European Dir. 94/27/EC of 30 June 1994 amending for the 12th time Dir. 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of dangerous substances. Official J Eur Communities. 1994:37:1–2.
50. Thyssen J P. Nickel and cobalt allergy before and after nickel regulation--evaluation of a public health intervention. *Contact Dermatitis.* 2011: **65** Suppl 1: 1-68.
51. Richardson C, Hamann C R, Hamann D, Thyssen J P. *Mobile Phone Dermatitis in Children and Adults: A Review of the Literature.* Pediatr Allergy Immunol Pulmonol, 2014. **27**(2): p. 60-69.
52. Ekqvist S, Svedman C, Möller H, Kehler M, Pripp C M, Björk J, Gruvberger B, Holmström E, Gustavsson C G, Bruze M. High frequency of contact allergy to gold in patients with endovascular coronary stents. *Br J Dermatol.* 2007: **157**: 730-738.

53. Svedman C, Ekqvist S, Möller H, Björk J, Pripp C M, Gruvberger B, Holmström E, Gustavsson C G, Bruze M. A correlation found between contact allergy to stent material and restenosis of the coronary arteries. C*ontact Dermatitis.* 2009: **60**: 158-164.
54. Björkner B, Bruze M, Möller H, Salemark L. Allergic contact dermatitis as a complication of lid loading with gold implants. *Dermatitis.* 2008 **19**: 148-153.
55. Svedman C, Möller H, Gruvberger B, Gustavsson C G, Dahlin J, Persson L, Bruze M. Implants and contact allergy: are sensitizing metals released as haptens from coronary stents? *Contact Dermatitis.* 2014: **71**: 92-97.
56. Wiesenfeld D, Ferguson M M, Forsyth A, MacDonald D G. Allergy to dental gold. *Oral Surg Oral Med Oral Pathol.* 1984: **57**: 158-160.
57. Scalf, L A, Fowler J F Jr, Morgan K W, Looney S W. Dental metal allergy in patients with oral, cutaneous, and genital lichenoid reactions. *Am J Contact Dermat*. 2001: **12**: 146-150.
58. Kanerva L. Skin Disease from Dental Materials. In: *Textbook of Contact Dermatitis 5th edn*. R.J.G. Rycroft, T Menné , P J Frosch, J P Leppoittevin. (eds): Berlin, Springer 2001: 841-881.
59. McDaniel L M, Couch S M. Allergic Eyelid Dermatitis as the Sole Manifestation of Gold Hypersensitivity. *Ophthal Plast Reconstr Surg.* 2016
60. Roach M. Base metal alloys used for dental restorations and implants. *Dent. Clin. North Am.* 2007: **51**: 603-627.
61. Möller H, Svensson A, Björkner B, Bruze M, Lindroth Y, Manthorpe R, Theander J. Contact allergy to gold and gold therapy in patients with rheumatoid arthritis. *Acta Derm Venereol.* 1997: **77**: 370-373.
62. Svensson A, Möller H, Björkner B, Bruze M, Leden I, Theander J, Ohlsson K, Linder C. Rheumatoid arthritis, gold therapy, contact allergy and blood cytokines. *BMC Dermatol*. 2002: **2**: 2.
63. Richards D G, McMillin D L, Mein E A, Nelson C D. Gold and its relationship to neurological/glandular conditions. *Int J Neurosci.* 2002: **112**: 31-53.
64. Bruze M. (In Swedish) Den yrkesdermatologiska verksamheten i Malmö efter oktober 1990. I Jubileumsskrift. Yrkes-och miljödermatologisk verksamhet i södra Sverige firar 50 år. 2010: 16-19. (ISBN # 978-91-7261-221-1) https://www.skane.se/Public/SUS_extern/Verksamheter/ Hudkliniken/YMDA/dokumentYMDA_ jubileumsskrift.pdf.
65. Hamann D, Thyssen J P, Hamann C R, Hamann C, Menné T, Johansen J D Spiewak R, Maibach H, Lundgren L, Lidén C. Jewellery: alloy composition and release of nickel, cobalt and lead assessed with the EU synthetic sweat method. *Contact Dermatitis.* 2015: **73**: 231-238.
66. Svedman C, Gruvberger B, Dahlin J, Persson L, Möller H, Bruze M. Evaluation of a method for detecting metal release from gold; cysteine enhances release. *Acta Derm Venereol.* 2013: **93**: 577-578.
67. Milheiro A, Muris J, Kleverlaan C J, Feilzer A J. Influence of shape and finishing on the corrosion of palladium-based dental alloys. *J Adv Prosthodont.* 2015: **7**: 56-61.
68. Liden C, Skare L, Lind B, Nise G, Vahter M. Assessment of skin exposure to nickel, chromium and cobalt by acid wipe sampling and ICP-MS. *Contact Dermatitis.* 2006: **54**: 233-238.
69. Hostynek J J, Dreher F, Nakada T, Schwindt D, Anigbogu A, Maibach H I. Human stratum corneum adsorption of nickel salts. Investigation of depth profiles by tape stripping in vivo. *Acta Derm Venereol Suppl*. 2001:**212**: 11-18.

70. Liden C, Skare L, Nise G, Vahter M. Deposition of nickel, chromium, and cobalt on the skin in some occupations - assessment by acid wipe sampling. *Contact Dermatitis.* 2008: **58**: 347-354.
71. Isaksson, M, Gruvberger B, Frick-Engfeldt M, Bruze M. Which test chambers should be used for acetone, ethanol, and water solutions when patch testing? *Contact Dermatitis.* 2007: **57**: 134-136.
72. Frick-Engfeldt M, Gruvberger B, Isaksson M, Hauksson I, Ponten A, Bruze M. Comparison of three different techniques for application of water solutions to Finn Chambers(R). *Contact Dermatitis.* 2010: **63**: 284-288.
73. Macfarlane A W, Curley R K, Graham R M, Lewis-Jones M S, King C M. Delayed patch test reactions at days 7 and 9. *Contact Dermatitis,* 1989: **20**: 127-132.
74. Jonker M J, Bruynzeel D P. The outcome of an additional patch-test reading on days 6 or 7. *Contact Dermatitis.* 2000: **42**: 330-335.
75. Fregert S. *Manual of Contact Dermatitis*. 2 ed. Copenhagen: PJ Schmidt, Vojens. 1981:138
76. Mowitz M, Zimerson E, Svedman C, Bruze M. Stability of fragrance patch test preparations applied in test chambers. *Br J Dermatol.* 2012: **167**: 822-827.
77. Frick M, Zimerson E, Karlsson D, Marand A, Skarping G, Isaksson M, Bruze M. Poor correlation between stated and found concentrations of diphenylmethane-4,4'-diisocyanate (4,4'-MDI) in petrolatum patch-test preparations. *Contact Dermatitis.* 2004: **51**: 73-78.
78. Elgart M L, Higdon R S. Allergic contact dermatitis to gold. *Arch Dermatol.* 1971: **103**: 649-653.
79. Fox J M, Kennedy R, Rostenberg A Jr. Eczematous contact-sensitivity to gold. *Arch Dermatol.* 1961: **83**: 956-959.
80. Fregert S, Kollander M, Poulsen J. Allergic contact stomatitis from gold dentures. *Contact Dermatitis.* 1979: **5**: 63-64.
81. Goh C L. Occupational dermatitis from gold plating. *Contact Dermatitis.* 1988: **18**: 122-123.
82. Forster H W Jr., Dickey R F. A case of sensitivity to gold-ball orbital implant; eczematous contact-type dermatitis due to 14-karat gold. *Am J Ophthalmol*. 1949: **32**: 659-662.
83. Kligman A M. The identification of contact allergens by human assay. III. The Maximization Test: a procedure for screening and rating contact sensitizers. 1966. *J Invest Dermatol.* 1989: **92**:151; discussion 152S.
84. Fowler J F Jr. Selection of patch test materials for gold allergy. *Contact Dermatitis.* 1987: **17**: 23-25.
85. Sabroe R A, Sharp L A, Peachey R D. Contact allergy to gold sodium thiosulfate. *Contact Dermatitis.* 1996: **34**: 345-348.
86. Bruze M, Björkner B, Möller H, *Patch testing with gold sodium thiosulfate.* In: Jadassohn Centenary Congress, 1996. 9-12 October in London: Abstract No. 49, p 14.
87. Fowler J Jr., Taylor J, Storrs F, Sherertz E, Rietschel R, Pratt M, Mathias CG, Marks J, Maibach H, Fransway A, DeLeo V, Belsito D. Gold allergy in North America. *Am J Contact Dermat*. 2001: **12**: 3-5.
88. Bruze M, Björk AK, Engfeldt M, Persson L, Svedman C *A seborrheic dermatitis-like allergic contact dermatitis from gold in a spectacle frame*. In manuscript 2016.
89. Björkner B, Bruze M, Möller H. High frequency of contact allergy to gold sodium thiosulfate. An indication of gold allergy? *Contact Dermatitis.* 1994: **30**: 144-151.

90. Torres F, das Graças M, Melo M, Tosti A. Management of contact dermatitis due to nickel allergy: an update. *Clin Cosmet Investig Dermatol.* 2009: **2**: 39-48.
91. Diepgen T L, Ofenloch RF, Bruze M, Bertuccio P, Cazzaniga S, Coenraads PJ, Elsner P Goncalo M, Svensson Å, Naldi L. Prevalence of contact allergy in the general population in different European regions. *Br J Dermatol.* 2016: **174**: 319-329.
92. Schittenhelm A, Erhardt W. Anaphylaxiestudien bei Mensch und Tier. *Zeitschrift für die gesamte experimentelle Medizin.* 1925: **45**: 75-88.
93. Bruze M, B jörkner B, Möller H. Practical Contact Dermatitis. A handbook for the Pratcitioner. 1995: New York: McGraw-Hill.
94. Dooms-Goossens A, Naert C, Chrispeels MT, Degreef H. Is a 5% nickel sulphate patch test concentration adequate? *Contact Dermatitis.* 1980: **6**: 232.
95. Hindsen M, Bruze M, Christensen O B. Individual variation in nickel patch test reactivity. *Am J Contact Dermat.* 1999: **10**: 62-67.
96. Edman B.*Computerized patch test data in contact allergy*. In: Department of Dermatology, Malmö General Hospital. 1988: Lund University: Lund.
97. Simonetti V, Manzini B M, Seidenari S. Patch testing with nickel sulfate: comparison between 2 nickel sulfate preparations and 2 different test sites on the back. *Contact Dermatitis.* 1998: **39**: 187-191.
98. van Strien G A, Korstanje M J. Site variations in patch test responses on the back. *Contact Dermatitis.* 1994: **31**: 95-96.
99. Ale S I, Maibach H I. Reproducibility of patch test results: a concurrent right-versus-left study using TRUE Test. *Contact Dermatitis.* 2004: **50**: 304-312.
100. Duarte I, Lazzarini R, Buense R, *I*nterference of the position of substances in an epicutaneous patch test battery with the occurrence of false-positive results. *Am J Contact Dermat.* 2002: **13**: 125-132.
101. Magnusson B, Hersle K. Patch test methods. II. Regional variations of patch test responses. *Acta Derm Venereol.* 1965: **45**: 257-261.
102. Barany E, Bergdahl I A, Schütz A, Skerfving S, Oskarsson A. Inductively coupled plasma mass spectrometry for direct multi-element analysis of diluted human blood and serum. *Journal of analytical atomic spectrometry,* 1997: **12**: 1005-1009.
103. Memon A A, Friedmann P S. Studies on the reproducibility of allergic contact dermatitis. *Br J Dermatol.* 1996: **134**: 208-214.
104. Lachapelle J M. A left versus right side comparative study of Epiquick patch test results in 100 consecutive patients. *Contact Dermatitis.* 1989: **20**: 51-56.
105. Bruynzeel D P, van Ketel W G, von Blomberg-van der Flier M, Scheper R J. Angry back or the excited skin syndrome. A prospective study. *J Am Acad Dermatol.* 1983: **8**: 392-397.
106. Mitchell J C. The angry back syndrome: eczema creates eczema*. Contact Dermatitis.* 1975: **1**: 193-194.
107. Rapson W S. Skin contact with gold and gold alloys*. Contact Dermatitis*. 1985: **13**: 6-65.
108. Rapson W S. Effects of biological systems on metallic gold. *Gold Bulletin.* 1982: **15**: 19-20.
109. Liden C, Nordenadler M, Skare L. Metal release from gold-containing jewelry materials: no gold release detected. *Contact Dermatitis*. 1998: **39**: 281-285.
110. Erfani B, Liden C, Midander K. Short and frequent skin contact with nickel*. Contact Dermatitis.* 2015: **73**: 222-230.

111. Hostynek J J, Dreher F, Pelosi A, Anigbogu A, Maibach H. Human stratum corneum penetration by nickel. In vivo study of depth distribution after occlusive application of the metal as powder. *Acta Derm Venereol Suppl.* 2001: **212**: 5-10.
112. Naldi L, Cazzaniga S, Gonçalo M, Diepgen T, Bruze M, Elsner P, Coenraads P J, Svensson Å, Bertuccio P, Ofenloch R. Prevalence of self-reported skin complaints and avoidance of common daily life consumer products in selected European Regions. *JAMA Dermatol, 2014:* **150**: 154-163.
113. Fall S, Bruze M, Isaksson M, Lidén C, Matura M, Stenberg B, Lindberg M. Contact allergy trends in Sweden - a retrospective comparison of patch test data from 1992, 2000, and 2009. *Contact Dermatitis.* 2015: **72**: 297-304.
114. Lidén C, Bruze M, Menné T. Metals Carola Lidén, Magnus Bruze, Torkil Menné. In: *Contact Dermatitis*. P.J. Frosch, T. Menné, J-P Lepoittevin (eds.): Berlin, Springer, 2006: 537-568.
115. Tsuruta K, Matsunaga K, Suzuki K, Suzuki R, Akita H, Washimi Y, Tomitaka A, Ueda H. Female predominance of gold allergy. *Contact Dermatitis.* 2001: **44**: 55-56.
116. McKenna K E, Dolan O, Walsh M Y, Burrows D. Contact allergy to gold sodium thiosulfate. *Contact Dermatitis*. 1995: **32**: 143-146.
117. Meding B. Differences between the sexes with regard to work-related skin disease. *Contact Dermatitis.* 2000: **43**: 65-71.
118. Vahter M, Åkesson A, Lidén C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. *Environmental Research.* 2007: **104**: 85-95.
119. Boonstra M B, Christoffers W A, Coenraads P J, Schuttelaar M L. Patch test results of hand eczema patients: relation to clinical types. *J Eur Acad Dermatol Venereol.* 2015: **29**: 940-947.
120. Oh J E, Lee H J, Choi Y W, Choi H Y, Byun J Y. Metal allergy in eyelid dermatitis and the evaluation of metal contents in eye shadows. *J Eur Acad Dermatol Venereol.* 2016.
121. *Commission Directive 2004/96/EC of 27 September 2004 amending Council Directive 76/769/EEC as regards restrictions on the marketing and use of nickel for piercing post assemblies for the purpose of adapting its Annex I to technical progress.* , in *Official Journal of the European Union*. 2004.
122. Bruze M. What is a relevant contact allergy? *Contact Dermatitis.* 1990: **23**: 224-225.
123. Ahlgren C, Ahnlide I, Björkner B, Bruze M, Liedholm R, Möller H, Nilner K. Contact allergy to gold is correlated to dental gold. *Acta Derm Venereol.* 2002: **82**: 41-44.