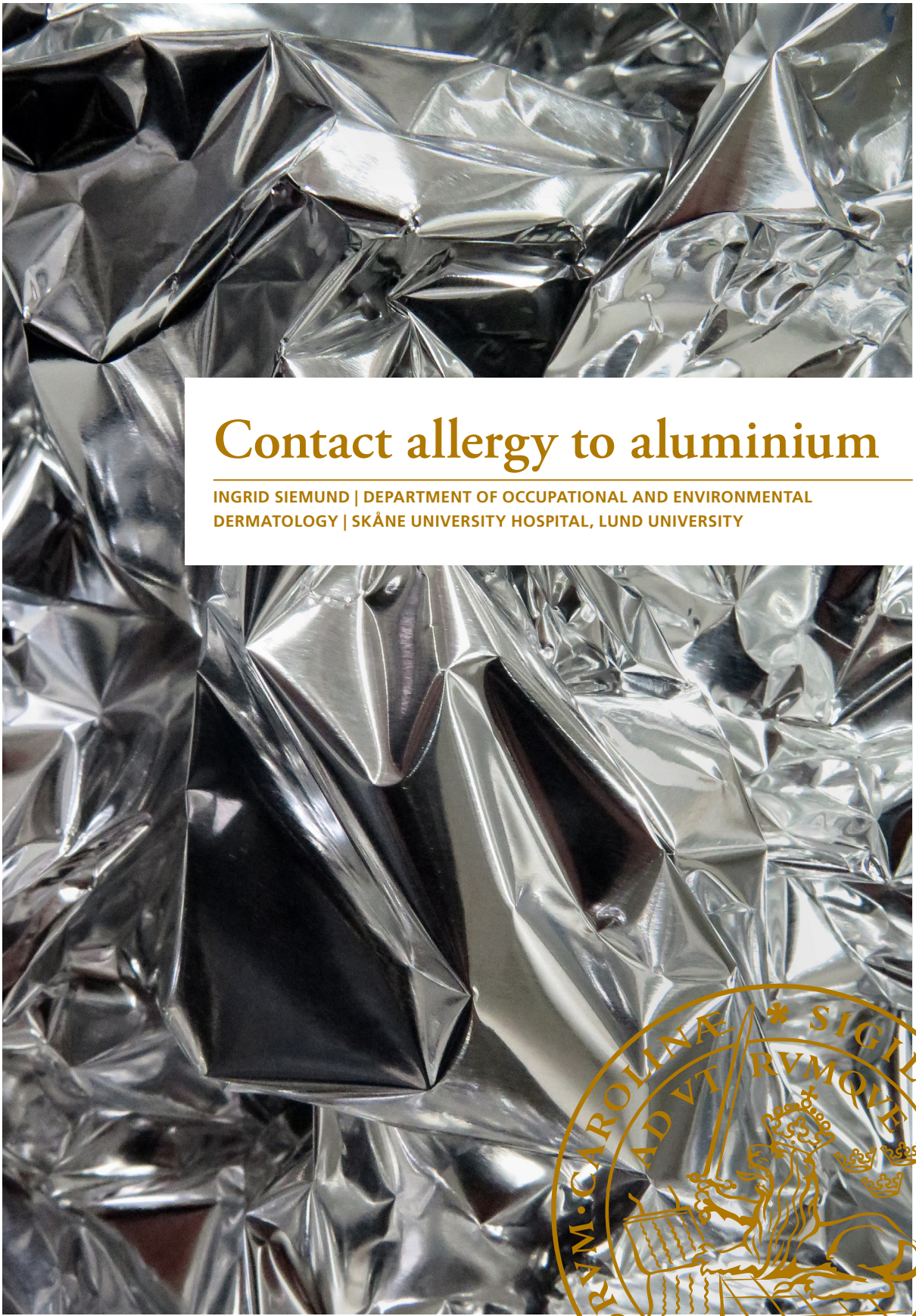
Contact allergy to aluminium

Siemund, Ingrid

2017

Document Version:
Publisher's PDF, also known as Version of record



Contact allergy to aluminium

INGRID SIEMUND | DEPARTMENT OF OCCUPATIONAL AND ENVIRONMENTAL DERMATOLOGY | SKÅNE UNIVERSITY HOSPITAL, LUND UNIVERSITY

Contact allergy to aluminium

Contact allergy to aluminium

Ingrid Siemund

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden. To be defended at Aulan Kvinnokliniken, Jan Waldenströms gata 47, plan 3, Skåne University Hospital, Malmö, on Friday 8 September 2017 at 9.00 am.

Faculty opponent

Professor Lennart Emtestam, Unit for Infection and Dermatology,
Department of Medicine, Huddinge Stockholm

Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION
Department of Occupational and Environmental Dermatology, Skåne University Hospital Malmö, Sweden	Date of issue September 8, 2017
Author(s) Ingrid Siemund	Sponsoring organization

Title and subtitle: Contact allergy to aluminium

Abstract

Contact allergy and atopic dermatitis may be present in the same patient. Known comorbidities of atopic dermatitis are allergic asthma and allergic rhinoconjunctivitis, both of which can be treated with allergen-specific immunotherapy (ASIT). Persistent itching nodules and contact allergy to aluminium are known adverse reactions after ASIT with aluminium-containing allergen extracts as well as after immunisation with aluminium-containing vaccines.

The aims of this thesis were: i) to investigate the presence of contact allergies in atopic individuals with and without childhood eczema, before and after ASIT (Paper I), ii) to investigate whether ASIT with aluminium-containing allergen extracts during one year induces persistent itching nodules and contact allergy to aluminium (Paper II), iii) to provide increased knowledge about establishing contact allergy to aluminum (Paper III), and iv) to study contact allergic reactions to aluminium over time (Paper IV).

Paper I and paper II are based on a prospective study in atopic individuals suffering form allergic asthma and/or allergic rhinocunjunctivitis. The study participants were treated with ASIT with aluminium-containing allergen extracts during one year. They were patch tested with aluminium chloride hexahydrate before and during ASIT. At the end of the study all participants were patch tested with aluminium and the baseline series. Paper III reports on aluminium-allergic volunteers who were patch tested with six different aluminium compounds and an empty Finn chamber, and also tested intradermally with aluminium chloride hexahydrate. In paper IV the results of repeated patch testing with aluminium chloride hexahydrate and aluminium lactate in aluminium-allergic volunteers are presented.

The findings were as follows: i a) a lower number of contact allergies was found in individuals treated with ASIT, i b) a higher number of contact allergies was found in individuals with a history of childhood eczema, ii a) contact allergy to aluminium was found in those treated with ASIT but ASIT was not shown to be a risk factor, ii b) contact allergy to aluminium and itching nodules seemed to be more common in children and in those with a history of atopic dermatitis, iii a) patch testing with aluminium chloride hexahydrate 2.0% petrolatum (pet.) and an empty Finn chamber, as well as the intradermal test are insufficient to detect aluminium allergy, iii b) most positive reactions were noted to aluminium chloride hexahydrate 10% pet., iv a) patch test reactivity to aluminium varies over time, iv b) an aluminium-allergic individual may have a false-negative reaction to aluminium.

Key words: Adverse reactions, aluminium, atopic dermatitis, baseline series, ASIT, contact allergy, itching nodules, patch testing.

Classification system and/or index terms (if any)

Supplementary bibliographical information	Language English
ISSN and key title 1652-8220 Lund University, Faculty of Medicine Doctoral Dissertation Series 2017:113	ISBN 978-91-7619-495-9

Recipient's notes	Number of pages	Price
	Security classification	

Signature Ingrid Siemund Date 2017-07-25

Contact allergy to aluminium

Ingrid Siemund

LUND
UNIVERSITY

Department of Occupational and Environmental Dermatology
Lund University
Skåne University Hospital, Malmö, Sweden
Malmö 2017

Coverphoto by

Erik Zimerson

Copyright by

Ingrid Siemund

Department of Occupational and Environmental Dermatology
Skåne University Hospital, Lund University
SE 205 02 Malmö, Sweden

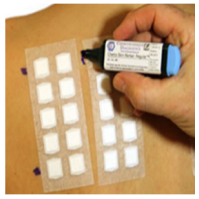
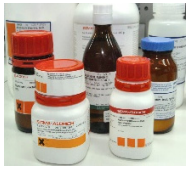
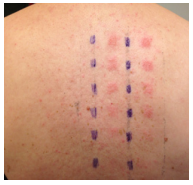
ISBN 978-91-7619-495-9
ISSN 1652-8220
Lund University, Faculty of Medicine Doctoral Dissertation Series 2017:113

Printed in Sweden by Media-Tryck, Lund University
Lund 2017

MADE IN SWEDEN

To Fredrik, Philip and Jakob

Content

Paper	Objective	Method	Illustration	Main findings/Conclusions
I. Contact allergy in atopic individuals in relation to allergen-specific immunotherapy	To compare the number of contact allergies between groups of study participants with and without a history of atopic dermatitis (AD), before and after one year of allergen-specific immunotherapy (ASIT).	Children and adults with allergic asthma and/or rhinoconjunctivitis, treated or untreated with ASIT, were patch tested with a baseline series.		The higher number of contact allergies detected in individuals with a history of AD indicated that AD may be a risk factor for type IV sensitisation. The lower number of contact allergies in patients exposed to ASIT suggested an immunomodulatory effect on type IV sensitisation.
II. Does allergen-specific immunotherapy induce contact allergy to aluminium?	To investigate whether ASIT with allergen preparations containing aluminium hydroxide induces contact allergy to aluminium and persistent itching nodules.	Three study groups, consisting of children and adults, were patch tested with aluminium chloride hexahydrate at baseline and during ASIT. A control group was included. All groups were patch tested at the end of the study in a randomised fashion.		Contact allergy to aluminium was found, but ASIT was not found to be a risk factor. Allergy to aluminium, and itching nodules after ASIT, appeared to be more common in children and in those with atopic dermatits.
III. Establishing aluminium contact allergy	To investigate different aluminium compounds and patch test concentrations to find an optimal patch test preparation	Six different aluminium compounds and an empty Finn chamber were used to patch test individuals with known contact allergy to aluminium.		Aluminium chloride hexahydrate 2.0% petrolatum (pet.) and an empty Finn chamber were insufficient for detection of aluminium allergy. Aluminium chloride hexahydrate 10% pet. gave the highest number of positive reactions.
IV. Variation in aluminium patch test reactivity over time	To investigate a possible variation in patch test reactivity to aluminium over time.	Individuals with known contact allergy to aluminium were patch tested 4 times over 8 months with equimolar dilution series of aluminium chloride hexahydrate and aluminium lactate.		Patch test reactivity to aluminium varied over time. Aluminium-allergic individuals may have false-negative reactions, which is why retesting with aluminium should be considered when there is a strong suspicion of aluminium contact allergy.

List of publications

This thesis is based on the following papers, which are referred to in the text by their Roman numerals. The papers are reprinted at the end of the thesis, with permission.

I. **Contact allergy in atopic individuals in relation to allergen-specific immunotherapy** Siemund Ingrid, Hindsén Monica, Netterlid Eva, Güner Nuray, Bruze Magnus, Eur J Dermatol 2016, 26: 271-80.

II. **Does allergen-specific immunotherapy induce contact allergy to aluminium?** Netterlid Eva, Hindsén Monica, Siemund Ingrid, Björk Jonas, Werner Sonja, Jacobsson Helene, Güner Nuray, Bruze Magnus. Acta Derm Venereol 2013, 93: 50-56.

III. **Establishing aluminium contact allergy** Siemund Ingrid, Zimerson Erik, Hindsén Monica, Bruze Magnus. Contact Dermatitis 2012, 67: 162-70.

IV. **Individual variation in aluminium patch test reactivity over time** Siemund Ingrid, Mowitz Martin, Zimerson Erik, Bruze Magnus, Hindsén Monica. Contact Dermatitis 2017, Version of Record online: 11 Jul 2017 | DOI: 10.1111/cod.12836

Abbreviations

ACD	allergic contact dermatitis
AD	atopic dermatitis
aq	aqua
Al	aluminium
APC	antigen-presenting cell
ASIT	allergen-specific immunotherapy
CD	contact dermatitis
D	day
DAMPs	danger-associated molecular patterns
DC	dendritic cell
ESCD	European Society of Contact Dermatitis
ICDRG	International Contact Dermatitis Research Group
Ig	immunoglobulin
IL	interleukin
INF-γ	interferon-γ
MEC	minimal eliciting concentration
MHC	major histocompatibility complex
NALP3	NACHT, LRR, and PYD domains-containing protein 3
NOD-like receptors	nucleotide oligomerisation domain-like receptor
TA, TB, TC, TD	test occasion A, B, C, D
T_c	T-cytotoxic
TGF-β	transforming growth factor beta
T_h	T-helper
TLR	toll-like receptor
TNF-α	tumour necrosis factor alpha
T_{reg}	T-regulatory
TSLP	thymic stromal lymphopoetin
PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptor
pet	petrolatum
STS	summarised test score
vs	versus
w/v	weight/volume
w/w	weight/weight

1. Introduction

The central theme of this thesis is contact allergy to aluminium, which is part of the overall medical area of allergology. The work presented in the thesis touches on the fields of different types of allergies, especially in individuals with an atopic constitution, of allergen-specific immunotherapy with aluminium-containing allergen extracts and its possibly adverse reactions, persistent itching nodules, and contact allergy to aluminium in particular. To facilitate for the reader, an introduction is given in this chapter to atopy, different types of allergies, diagnostic tools, allergen-specific immunotherapy and its side effects, and also to aluminium—including its properties, uses, and ways of exposure.

1.1 Nomenclature

In 2001, a task force of the EAACI (the European Acadamy of Allergology and Clinical Immunology) published a revised nomenclature for allergic terms to improve the definitions and therefore communication in the field of allergy (1, 2). The authors distinguish between allergic and non-allergic hypersensitivity, which means hypersensitivity with an underlying immunological or non-immunological mechanism, and between IgE-mediated and non-IgE-mediated allergic hypersensitivity.

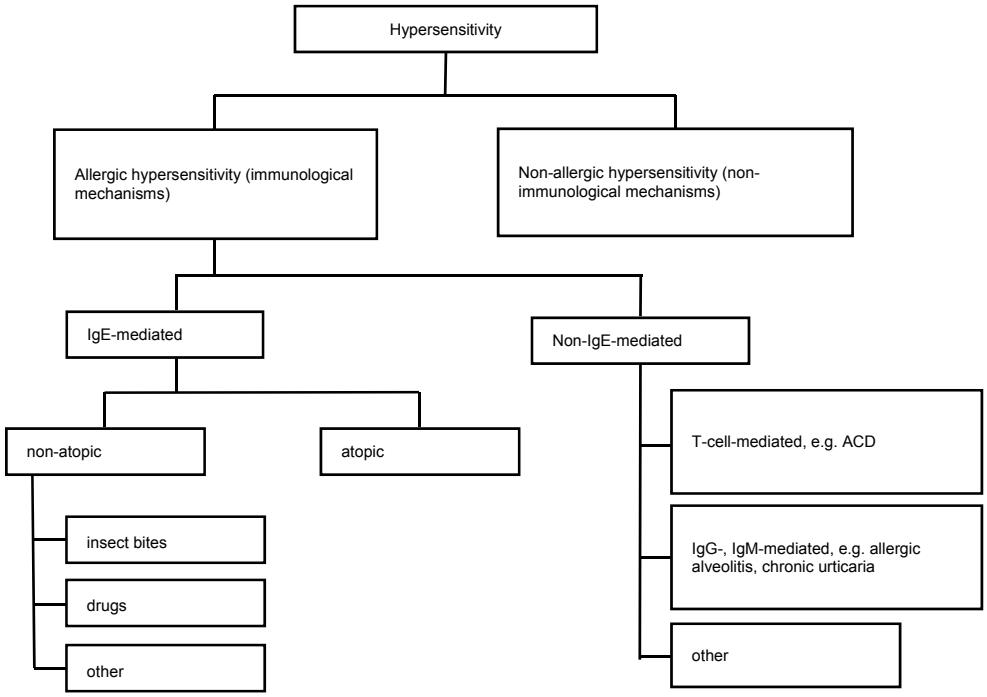


Figure 1.
Clinical classification of hypersensitivity, modified after Johansson et al. (2001) (3).

In 2003, the entire nomenclature of allergic diseases was updated by the World Allergy Organisation (WAO) (2) based on the mechanisms of allergic reactions. Special attention was given to the definitions of skin disorders, some of which are summarised by Tanno et al. as follows (4):

Allergy

"…a hypersensitivity reaction initiated by proven or strongly suspected immunologic mechanisms IgE-mediated or non-IgE-mediated. The triggers are substances that the subject has been previously exposed to and sensitised."

Sensitisation

"…when an underlying immune mechanism is proven by an in vivo or in vitro procedure method, such as the presence of a specific IgE or T lymphocyte to an allergen…."

Atopy

"personal and/or familial tendency, usually in childhood or adolescence, to become sensitised and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins."

Atopic diseases

"…typical symptoms of asthma, rhinoconjunctivitis, or eczema in atopic patients. These clinical presentations can happen isolated or in combination…."

These terms are used in this thesis. The term "atopic dermatitis (AD)" is used synonymously with the term "atopic eczema", as it has been shown that "atopic dermatitis" is the term that is most commonly used in studies (5).

1.2 Atopy and atopic diseases

In 1923, Coca and Cooke, both American allergists, introduced the term "atopy", which is derived from the ancient Greek and means "not in the right place" or "strange" (6). They described a qualitatively abnormal response to environmental substances in people, which was observed especially within families. In their original definition of "atopy", Coca and Cooke included only hay fever and bronchial asthma (6). In 1933, Wise and Sulzberger wrote for the first time about "confusing types of localised and generalised lichenifaction, generalised neurodermatitis, or manifestation of atopy" and named it "atopic dermatitis". Even though the term "atopy" is quite new, these clinical conditions have been reported for thousands of years in the litterature, e.g. "noisy breathing" documented by Huang Ti in 2698 B.C., or the first-described atopic individual Emperor Octavianus Augustus, who suffered from itchy skin, seasonal rhinitis, and tightness of the chest (7, 8). Also, the family history is documented—including his grandson's (Emperor Claudius') rhinoconjunctivitis and the suspected dander allergy of his great-nephew, Britannicus.

The term "allergy" was coined for the first time by the Viennese paediatrician Clemens von Pirquet (9). The altered responsiveness of the human organism was the main interest of his work. On 24 July 1906, Pirquet officially introduced the term allergy into the medical profession, initially as a purely clinical expression to denote both host-protective and host-injurious reactions of the human organism (9). "Allergy" comes from the ancient Greek words *allos* meaning "other" and *ergon* meaning "work". Today, an allergy denotes a hypersensitivity reaction caused by a strictly immunological mechanism and triggered by a substance—or occasionally by physical factors—that the person has tolerated earlier.

In 1963, Gell and Coombs, both British immunologists, described a quite simple working classification of four types of allergic reactions (Table 1) (3).

Table 1.
Classification of four types of allergic reactions, described by Coombs and Gell, modified after Averbeck et al. (2007) (3)

Reactions	**Antibody-mediated**			**Cell-mediated**
Types	Type I	Type II	Type III	Type IV
Mechanism	Immediate, IgE-mediated	Humoral, cytotoxic	Immune complex-mediated	Delayed, T-cell-mediated
Symptoms	Allergic rhinoconjunctivitis Allergic asthma Protein contact dermatitis	Drug-induced cytopenia	Allergic vasculitis Allergic alveolitis	Drug-induced exanthema Contact allergy

Four years later, in 1967, immunoglobulin E was identified as a new class of human immunoglobulin (10-12). The authors documented: "In studies of immediate-type hypersensitivity the binding of IgE for a given allergen in various human sera correlated with the ability of these sera to passively sensitise human skin to that allergen."

Today, allergies are one of the most common causes of chronic disease in Europe (13). European studies have reported that up to 30% of the population suffer from allergic rhinoconjunctivitis, while up to 20% suffer from asthma and up to 15% have allergic skin disorders. It is difficult to exactly determine the prevalence of the atopic diseases, especially that of AD, because of a high variation in clinical features and its intermittent course. In 45% of children, the onset of AD occurs during the first 6 months of life, in 60% during the first year, and in 85% before the age of 5 years (14). A Swedish study followed a population-based cohort from birth up to 12 years of age to investigate the development of eczema, asthma, and rhinitis (15). At the age of 12 years, 58% of children had had at least one of these allergic diseases at some time. At least two of these allergic diseases were found in 1.8% children at 1 year of age and in 7.5% at 12 years. In a Danish study, an unselected cohort of eighth-grade schoolchildren was followed into adulthood, to the age of 28−30 years (16). The lifetime prevalence of AD (from birth to 29 years) was assessed to be 34.1%, based on a questionnaire. The point prevalence of AD at 29 years of age was 6.1%, based on clinical examination. Another Danish study, following a birth cohort up to 14 years of age, found that the point prevalence of AD at 14 years was 8.1%, that of rhinoconjunctivitis was 32.8%, and that of asthma was 12.9%. Altogether, 12.1% had more than one atopic disease (17).

The atopic diseases, allergic asthma, allergic rhinoconjunctivitis, and AD are clinically quite well-defined diseases, but as in many other diseases, there is a wide spectrum of manifestations. The diagnosis of AD relies mainly on clinical assessment of the eczematous lesions along with a family history of atopic diseases and a possible overlap of allergic asthma or rhinoconjuntivitis. Efforts have been made to find a

diagnostic classification, and the diagnostic criteria of Hanifin and Rajka are an important milestone in this field (18). However, the best-validated and most widely used criteria for AD are the UK criteria (also known as the Williams criteria) (19):

Mandatory:

An itchy skin condition (or parental report of scratching or rubbing in a child)

Plus 3 or more of the following:

- A history of involvement of the skin creases such as fold of elbows, behind the knees, fronts of ankles, or around the neck (including cheeks in children under 10 years of age)
- A personal history of asthma or hay fever (or history of atopic disease in a first-degree relative in children under 4 years of age)
- A history of a general dry skin in the last year
- Visible flexural eczema (or eczema involving the cheeks/forehead and outer limbs in children under 4 years of age)
- Onset under the age of 2 years (not used if the child is under 4 years old)

Hand eczema and contact allergies are common comorbidities in adult atopic individuals suffering from eczema (20, 21). In 41% of adult atopic subjects, at least one positive patch test reaction was reported by Clemmensen et al. in 2014 (22). Isaksson et al. found contact allergies in 26.8% of atopic children, especially in those with hand and foot eczema (23). An undiagnosed contact allergy can trigger and/or worsen and/or maintain a dermatitis. Contact allergy in atopic individuals is one of the topics in this thesis, and will be discussed later on.

1.3 Contact allergy and allergic contact dermatitis

In this work, contact allergy is defined as an immune reaction of delayed hypersensitivity or type IV allergy and allergic contact dermatitis (ACD) is its clinical manifestation. Thus, contact allergy and ACD are not synonymous concepts. Immediate contact reactions consisting of a type I allergy are commonly caused by proteins, e.g. food allergens. However, these reactions are not discussed in this thesis.

Contact allergy, i.e. the delayed hypersensitivity, is a reaction pattern defined by a sensitisation phase when the immunological memory of the contact sensitiser is established, and an elicitation phase usually starts when the subject is re-exposed to the sensitiser (24). ACD is the disease which may develop and be diagnosed in three steps by (i) the establishment of contact allergy, (ii) the present re-exposure to the

contact sensitiser or exposure to a possible cross-reacting substance, at a concentration exceeding the individual's threshold (25), and (iii) the evidence that exposure explains the dermatitis under investigation with regard to its morphology, localisation, and clinical history. In a multi-centre study performed by the European Dermato-Epidemiology Network (EDEN), the prevalence of contact allergy to at least one allergen in the general population, aged 18−74 years, in different European countries was found to be 27.0% (26). Contact allergies are a common cause of eczema in all age groups and are one of the most common causes of occupational disability (27, 28). In a pre-existing skin disease such as AD, an unrecognized contact allergy may be the reason for recurrence and poor response to treatment. The clinical findings in ACD may vary greatly and may depend on the sensitiser and on its route of administration (24, 29, 30). Eczematous lesions are the most common clinical manifestations, but some allergens cause lichenoid or pustular lesions (30, 31). Oral, percutaneous, or inhalatory exposure may lead to a systemic allergic contact dermatitis (32, 33). Flare-up reactions on previous patch tested areas or at locations of previous ACD may also be a clinical manifestation of a systemic allergic contact dermatitis (34-36).

1.4 Etiopathological aspects

Antigens

The skin and the mucosa are the sites where the reaction of a person to the environment takes place. Most substances do not penetrate the skin and are not sufficiently harmful to initiate an immune response. Allergens leading to allergic asthma or rhinoconjunctivitis are almost always high-molecular-weight and highly water-soluble proteins belonging to quite a small proportion of all possible protein families (≈ 0.35%) (37). As mentioned above, the sensitisation to proteins can also occur by contact with the skin and cause an immediate contact reaction, known as protein contact dermatitis or immunological contact urticaria (38, 39). Allergens that can cause contact allergy consist of approximately 4,300 recognized substances—mostly small, mostly lipophilic, and mostly chemically reactive molecules with a molecular weight of < 500, but molecules of higher molecular weight have also been reported (40-42) However, these molecules are too small to act as antigens themselves. They therefore bind covalently to—or form coordination complexes with—skin proteins to produce antigen complexes (43). Such contact sensitisers are called haptens, i.e. incomplete antigens.

Immune response

The immune system has been classified into two types: innate and acquired immunity (3). The innate immune response occurs in the early phase of the defence, and it reacts non-specifically, without memory and without changing the response when it meets the same pathogen again. Various humoral mechanisms (e.g. complement activation, antimicrobial peptides) and cellular defence mechanisms (e.g. macrophages, mast cells) interact in this system. Characteristic pathogens, also designated pathogen-associated molecular patterns (PAMPs), can be recognized by the cells of the innate immune system. Various pattern recognition receptors (PRRs), e.g. Toll-like receptors (TLRs), mediate the recognition of PAMPs. The acquired immune response works more specifically using antigen-specific humoral components (e.g. antibodies) and cellular components (e.g. B- and T-lymphocyts), and it creates an immunological memory. There are two important subsets of T-cells: T-helper (T_h cells) and cytotoxic T-cells (T_c cells or killer cells), which can be distinguished by the surface markers CD_4^+ and CD_8^+, respectively. Naive CD_4^+ T_h cells become differentiated into T_h1, T_h2, or T_h17 and into T-regulatory cells (T_{reg}) in a cytokine milieu created by APC, a process called "priming". These subsets of T_h cells are again distinguished by the cytokines they produce: T_h1 cells secrete mainly IFN-γ, TNF-α, and IL-2; T_h2 mainly IL-4, -5, -9, -10, and-13; T_h17 mainly IL-17 and -22; and T_{reg} IL-10 and TGF-β. Thus, the T-helper cells conduct the sequences of the immune response, e.g. IL-4 activates B-lymphocytes and from this, the production of IgE. For the CD_8^+ T_c cells, a distinction is made between T_c1 and T_c2 cells. T_c1 cells produce mainly IFN-γ while T_c2 cells produce mainly IL-4 and -5. The B-lymphocytes play an important role in the acquired immune system by producing various immunoglobulins as soluble antibodies: IgG, IgM, IgA, IgD, and IgE. In the presence of T-cells and their cytokines, an altered production of immunoglobulin class can be initiated, called isotype-switch. Both innate immunity and acquired immunity are two branches of one system, which are interacting and collaborating (44, 45).

The dendritic cell plays an interacting role between the innate immunity and adaptive immunity (3). DCs are present in all tissues, but mainly in the skin and mucosa; in the epidermis they are called Langerhans cells and in the dermis they are called dermal DCs. These cells are able to take up and process the antigen, to migrate to the draining lymph nodes, and to present the antigen to T-lymphocytes using specialised receptors on their surface: MHC I and II. In the lymph node, the T-lymphocytes become activated by APCs expressing MHC on their surface, and become differentiated into distinct subsets of T-cells in the presence of cytokines. This first part of the immune reponse is designated the sensitisation phase.

The next step in **type I allergy**, i.e. the IgE-mediated allergic reaction, is called the effector phase. In the sensitisation phase, naive CD_4^+ T_h0 cells have developed into T_h2 cells. By producing various interleukins, e.g. IL-4, -5, and -13, the T_h2 cells now activate IgE-producing B-cells. IgE binds to high-affinity receptor Fc*e*RI, which is

expressed on mast cells and basophils. Upon re-exposure to the allergen, it binds to the allergen-specific IgE and stimulates the mast cells and basophils to release mediators, e.g. histamine and heparin. The symptoms of allergic rhinoconjunctivitis and asthma may be felt within seconds. Memory cells, a subtype of B-lymphocytes, are responsible for the flare-up of symptoms upon re-exposure to the allergen (3, 37, 45).

In this context, the "hygiene hypothesis" should be mentioned. There is a discussion among researchers regarding an explanation for the increasing number of allergies over the last few decades. In modern life, viral and bacterial infections during childhood are decreasing, so the immune response involving production of T_h1 cells is lacking. Thus, unrestricted production of T_h2 cells may occur, leading to more allergies (46, 47).

The mechanism of **type IV allergy**, i.e. the delayed T-cell-mediated allergic reaction, explains the acquisition of contact allergies and its clinical manifestation, ACD. This immune response is not mediated by antibodies but by a cellular mechanism. It is divided into two phases: the sensitisation phase and the elicitation phase. The sensitisation phase lasts from 10−15 days up to several weeks, whereas the elicitation phase usually takes 1−2 days, but for some substances up to 2−3 weeks (48, 49).

After the antigen, i.e. hapten, has penetrated the epidermis, it reacts first with a protein—whereby the Langerhans cells are able to take it up. Upon contact with the hapten, the Langerhans cells become activated. The secretion of cytokines and chemokines (proteins like cytokines) facilitates migration and maturation of the Langerhans cells. They migrate as APCs to the regional lymph nodes and present the hapten to naive T cells, which become activated (3). By binding to MHC II receptors on the APC—and under the influence of, for example, IL-12—the antigens induce differentiation and proliferation of naive T-cells (for example, CD_8^+ T_c cells as cytotoxic cells producing IFN-γ and CD_4^+ T_h cells as regulatory cells producing IL-10). An important result of this process is the subgroup of the hapten-specific T-memory cells, which are released into the blood circulation and into the skin, or rest in the lymph node. After renewed contact between the hapten and the skin, i.e. the elicitation phase, the Langerhans cells take the hapten up and present it to these antigen-specific T-memory cells. CD_8^+ Tc cells and CD_4^+ T_h1 cells are recruited. A cascade of inflammatory events starts and leads mostly to an eczematous reaction in the skin—mostly within a few hours, but it may last for several weeks (48). Many regulatory mechanisms play a role in reducing the inflammatory process, such as the anti-inflammatory cytokines TGF-β and IL-10 secreted by CD_4^+ CD_{25}^+ T_{reg} cells (3, 25, 50).

Increased IgE production is the main characteristic and a clearly underlying mechanism in allergic asthma and rhinoconjunctivitis. In **atopic dermatitis**, the IgE-mediated sensitisation is not as closely linked to it. Various studies have shown that

up to two-thirds of patients with AD have no measurable allergen-specific antibody sensitisation (51). Allergen sensitisation appears to be an epiphenomenon of disease activity rather than a uniform cause of the skin disease.

Since antiquity, it has been known that the atopic diseases are found in families (8). Today, it is known that AD is caused by a combination of genetic and environmental factors. Twin-based studies have clearly shown the role of genetic factors (14, 52, 53). The term "epigenetics" has been introduced to explain how environmental factors may contribute to the pathogenesis of AD. Epigenetics in this context means the phenomenon of changes in gene expression without involving a change in the DNA sequence (54). DNA methylation or histone modification are examples of mechanisms that lead to such changes. Changes in gene expression can happen in response to the environment without altering the underlying DNA sequence. Examples of such environmental factors are tobacco smoke, pollutants, and changes of diet. However, a dysfunctional skin barrier and cutaneous inflammation due to an inappropriate immune response in the skin are the two major causes of developing AD. The impaired epidermal barrier function in AD, due to both immunological and physicochemical abnormalities of the skin, is associated with a high degree of microbial colonisation characterised by reduced microbial diversity and a uniform colonisation by *Staphylococcus aureus* (14, 45, 53). Decreased hydration and increased transepidermal water loss, altered lipid composition, and a raised skin pH may affect lesional and non-lesional skin. A well-known genetic determinant of reduced epidermal function is the null mutation of filaggrin (FLG), which can be found in atopic individuals. Filaggrin deficiency affects the differentiation and function of the keratinocytes, and an association with the development of allergic asthma and allergic rhinoconjunctivitis has been reported (55).

The cutaneous inflammation is a major part in the pathomechanism of AD (14, 53). Already in non-lesional skin, an increased number of T_h2 cells, T_h22 cells, and T_h17 cells can be found, together with a pro-inflammatory cytokine milieu (53). Antigen-specific T-memory cells (T_m) in the skin are able to recall rapid immunological response. Also, epithelial cells—primarily keratinocytes—produce cytokines such as TSLP, IL-25, and IL-33, and in this way contribute to activation of DCs and differentiation of T_h2 cells. It has been shown that a dysfunctional skin barrier generates elevated TSLP, and that IL-25 reduces filaggrin production (56, 57). Moreover, the keratinocytes are also able to respond to PAMPs and other stimuli, with upregulation of both Il-8 and Il-18 mediating a pro-inflammatory response. DCs activated by TSLP stimulate T_h2 cells to produce the pro-inflammatory molecules IL-4, -5, -13, -31, and TNF-α, whereas the anti-inflammatory T_{reg} cytokine IL-10 and the T_h1 cytokine IFN-γ are suppressed. TSLP also induces activation and proliferation of B-lymphocytes.

The acute phase of AD is characterised by the infiltration of T_h2 cells, DCs, eosinophils, and the cytokine milieu of IL-4, -5, and -13 in non-lesional and lesional skin (14, 53). In the chronic phase, the cellular infiltrate is further increased but now includes both T_h2 and T_h1 cells. The T_h17 and T_h22 cells are fewer than in the acute phase.

It should be mentioned that chemokines also play an important role in the pathomechanism of AD. They contribute to the infiltration of macrophages, eosinophils, and T-cells into acute and chronic skin lesions.

1.5 Skin tests for diagnosis of hypersensitivity

Skin tests are most commonly used to support diagnosis in patients who are suspected of having an allergic disease. Skin tests are provocation tests that can be performed through the epicutaneous or intradermal route. When the allergen applied causes a specific skin reaction consistent with an allergy, the individual allergen possibly involved in the allergic disease can be identified (58). A major advantage of skin tests is that the patient can be tested with the actual substance of interest being chosen from a large number of possible allergens—for patch testing, > 4,300 substances (40).

For **type I allergies**, the immediate type of allergic reaction mediated by quick release of IgE, the **skin-prick test** is the method of choice. The allergens to be tested are standardised extracts and they are often commercially available. It is also possible to use test preparations processed from raw materials. A drop of each allergen, most of them water-soluble proteins, is placed on intact skin, usually on the volar part of the forearm, and then the skin is pricked with a special needle, a lancet. A positive control with histamine and a negative control with saline are often included in the test. The test is read after 15−20 minutes using a defined grading scale. In adults and older children, a positive result is normally consistent with a raised weal of at least 3−4 mm in diameter on the skin surrounded by erythema, provided that the negative control is negative. If the negative controls elicit a small reaction, their diameter should be taken into account. Dilution series of the allergen in question can be performed to evaluate the patient's sensitivity to it (59, 60).

For **type IV allergies**, the delayed hypersensitivity mediated mainly by $CD_8^+T_c$ and CD_4^+ T_h-cells, the **epicutaneous patch test** is the golden standard in clinical practice. Josef Jadassohn, a dermatologist at Breslau University in Germany in the late 1890s, is acknowlegded as being the "father of patch testing", a diagnostic tool for finding contact allergy (61). About 30 years later, in 1929, Bruno Bloch, a dermatologist at the University of Zürich, Switzerland, described the test procedure in detail (61). If a patient is suffering from dermatitis of unknown cause or a previously stable dermatitis

that is becoming worse, patch testing may reveal a possible underlying cause. The suspected allergen, applied at a concentration exceeding the patient's threshold on intact skin, causes an eczematous reaction indicating contact allergy (25). Over many years of research and clinical experience, the patch test technique has been developed further and standardised regarding the properties of the allergens and the vehicles, the test concentrations, the doses, and the reading and scoring of the skin reactions (24, 62-65).

The allergens, mostly lipophilic molecules, are mixed at appropriate concentrations with the vehicle and placed in small test chambers, which are attached to adhesive tape. On day (D) 0, the patches are fastened to the patient's upper back. After 48 hours of occlusion time, the patches must be removed again. On D3 or D4 and on D7 the patient has to come back for reading of the test by a trained dermatologist, according to the guidelines (65). As mentioned above, the protocol of patch test reading has also been standardised, but research has shown that the readings may still be subjective (63, 66, 67).

According to the International Contact Dermatitis Research Group (ICDRG), patch test reactions should be scored as follows: (+) meaning doubtful, + meaning weakly positive, ++ meaning strongly positive, +++ meaning extremely positive (65, 68). Redness and infiltration of the test area is the minimum criterion for a positive reaction.

False-positive reactions are positive reactions with a morphology that cannot be distinguished from a contact-allergic reaction, but which are caused by irritation and not by contact allergy. Patch testing with a dilution series and/or patch testing of control subjects may exclude the possibility of a false-positive reaction. The concentration can usually be decreased by a factor of 100 and still cause a moderate skin reaction without losing the possibility of eliciting a positive result if the patient is truly contact-allergic to the substance under test (24).

False-negative reactions are negative reactions defined as failure to elicit a positive patch test reaction despite the fact that the patient being tested has a known contact allergy. There are many reasons for a false-negative reaction: e.g. a patch test concentration that is too low, an unstable substance, an inappropriate vehicle or test chamber, immunosuppressive therapy during patch testing, or the patch test being read too soon (69-71).

Late patch test reactions are positive reactions that appear at the site of a previously negative patch test, but later than on D7. Such reactions may be examples of active sensitisation, but they may also represent late reactions in already sensitised individuals (see below). In these already sensitised individuals, some sensitisers are known to elicit late skin reaction, e.g. corticosteroids, gold, and acrylates. The patch test reactions to these sensitisers may be negative on D3 or D4 but may be positive on

D7 or later. These sensitisers belong to a group where reactions may appear later than on D7, e.g. on D10−14, even in already sensitised individuals (24, 49).

Active sensitisation is the most serious adverse effect of patch testing. If a positive reaction appears 10−20 days afterward in the area of a previously negative patch test reaction or on D3−4 after retesting, active sensitisation may be suspected (63). Patch testing with a serial dilution of the suspected allergen is recommended to substantially reduce the possibility that the positive reaction on D3 or D4 at retesting was due to variation in reactivity (24, 48, 49, 72).

The **intradermal test** is a skin test that can be used to trace both **type I and type IV allergies**, but in tracking a type I allergy it is strictly recommended as a second choice when a skin-prick test has proven negative, due to the higher risk of causing a systemic reaction (59). Regarding type I allergies, it is mostly used in diagnosing drug allergy.

Regarding diagnosis of delayed hypersensitivity, Epstein published the study "Contact dermatitis due to nickel and chromate; observations on dermal delayed (tuberculin-type) sensitivity" in 1956 (73, 74). He was the first to point out the advantages of the intradermal test in comparison to patch testing for detection of chromate sensitivity. For many years, the intradermal or intracutaneous test has been used in parallel with patch testing (75). Various groups of contact allergens have been compared by intradermal testing and patch testing, especially different metals (among them, nickel) (75). The reason for the study of intradermal testing with metals may be that patch test reactions to metals are sometimes difficult to interpret, and nickel allergy is very common. The intradermal test has been found to be particularly useful in disclosing false-positive and false-negative patch test reactions (48, 76-79). The fact that patch testing has been standardised and optimised on the one hand and the drawbacks of intradermal testing—e.g. the technical challenges of giving an injection and the burning skin after injection—on the other hand may be one explanation as to why this test is no longer used in clinical practice. Another explanation may be that it is impractical to handle the test substances used for intradermal testing in everyday clinical practice, at least at the Department of Occupational and Environmental Dermatology in Malmö.

Performance of intradermal testing in the context of contact allergy is not as standardised as patch testing, especially regarding dose, concentration, and reading of the test (24, 62-65). However, the solubility of the allergen to be tested, which is a crucial factor for penetration into the epidermis in patch testing, plays a minor role in this method. The allergen is injected directly into the dermis.

The technique of administering the test is exactly the same for tracking type I or type IV allergy: A dose of 0.1 ml of the diluted allergen is injected intradermally with a needle. A raised papule of about 4 mm indicates that it was correctly done. In

diagnosing type I allergies, the test is read after 15–20 min and a raised weal of > 5 mm is considered to be a positive reaction—or when the diameter of the weal is double the length of the initial papule and surrounded by a typical flare (59).

In diagnosing delayed hypersensitivity, the dose of allergen has varied in different studies, and also the day on which the test was read. R. Herbst stated that a concentration 10–100 times lower than that in a patch test should be used (75). Reading is mainly performed 48 hours after injection, but in some studies reading has also been performed after 24 or 72 hours. The infiltration of the skin can be measured by 2 right-angled diameters or more often as a raised weal of > 4–6 mm consistent with a positive reaction (48, 60, 73, 76-79).

In all skin tests, it is important to remember that a positive test only confirms the sensitisation to an allergen. The tests do not show the clinical relevance of the sensitisation or explain the allergic disease itself.

1.6 Allergen-specific immunotherapy (ASIT) and adverse reactions

Besides allergen avoidance, ASIT is the only curative treatment for allergic asthma and rhinconjunctivitis. ASIT is the administration of gradually increasing quantities of an allergen vaccine to an allergic subject, reaching a dose that is effective in ameliorating the symptoms associated with subsequent exposure to the causative allergen (80, 81).

When Pirquet coined the term allergy in 1906, he used the concepts of immunity, hypersensitivity, and anaphylaxis (81, 82). By comparing horse serum reactions to smallpox vaccination, he declared that “immunity and hypersensitivity can thus be closely related”. The idea was that hay fever and infectious diseases might have the same causative path. An immunisation strategy, which had been implemented to prevent infectious diseases (as shown by vaccination against, for example, diphtheria by Emil von Behring at Koch’s Institute in Berlin in 1890), could also be used for hay fever (83). To produce an antitoxin, pollen was injected into animals; then their serum was used to immunise patients. William Dunbar, who worked at the State Hygienic Institute in Hamburg from 1893, did a lot of investigations on this field, and the antitoxin that he manufactured was called “pollantin”. However, the treatment of hay fever with pollantin failed. Dunbar’s work was followed up by two physicians working at St Mary’s Hospital Medical School in London—Leonard Noon and John Freeman. Noon introduced pre-seasonal immunotherapy by performing investigations of the required dosages of pollen extract in a series of subcutaneous injections. First, therapeutic benefits including guidelines that are still applicable to

some extent, were published by Noon in the Lancet in 1911 (84). Three years later, Freeman reported the first benefits of immunotherapy with grass pollen extract over a three-year period (85). The reagins, "skin-sensitising antibodies", were described by Coca and Grove in 1925, but it was not until 1967 that they were identified and denoted as a new immunoglobulin, now called IgE (10-12, 82).

To reduce the frequency of injections, various depot-like, immunogenic substances were prepared to provide a slow, continuous release of the allergen from injection sites (85). It was already known that bacterial vaccines and modified toxoid had been effective immunogens. Thus, soluble pollen allergens were treated with aluminium precipitation and aluminium adsorption. Only the aluminium-adsorbed pollen extracts were finally accepted as the preferred preparations. In 1949, Egon Bruun published the first controlled study on the efficacy of specific house dust extracts in patients suffering from asthma (81, 82, 86).

Over time, the terminology has changed from desensitisation or hyposensitisation therapy to ASIT (80). Today, ASIT is not only a proven treatment for allergic rhinoconjunctivitis and asthma but also for insect venom allergy, i.e. bee and wasp stings (87). The therapy can be used in children from 6 years of age. The recommanded administration is a 3- to 5-year course starting with a dose-increasing period, usually 7−12 weeks long, followed by a maintenance treatment with injections every 6−8 weeks. The injections are usually given subcutaneousely in the lateral upper arm. The allergen vaccines used in Sweden are commercially available, standardised, aluminium-adsorbed depot preparations (88).

Although ASIT has been used for more than a century to obtain tolerance to a sensitiser and thus remission of the missdirected allergic immune response, its immunological mechansim is not yet entirely understood (87, 89-91). In summary, previous studies speak for a slow reduction in allergen-specific IgE levels and an increasing production of allergen-specific IgG1 and IgG4 antibodies, also called "blocking" immunoglobulins because they compete with IgE for allergen binding and do not trigger histamine release. However, clinical improvement is not always associated with a decline in IgE or rising IgG1 and IgG4 levels, but can often precede it. Less effector cells such as mast cells, eosinophils, and CD_4^+ T-cells get recruited and a shift from T_h2 to T_h1 occurs during the immune response in subjects treated with ASIT. These processes are reflected in the changing levels of mediators. The levels of T_h2 cytokines IL-4, -5, and -13 are reduced in most cases, while those of T_h1 cytokines IFN-γ and IL-12 are raised. CD_4^+ CD_{25}^+ T_{reg} cells produce more IL-10 and TGF-β after ASIT, which are believed to have a crucial anti-inflammatory effect.

As in all medical treatments, **adverse reactions** can also be experienced during ASIT. Local, transitory reactions such as injection site redness and swelling within 15−20 minutes—or a few hours after injection—are almost to be expected. Systemic reactions such as reactivation of the allergic disease or even anaphylactic shock and

death have been reported earlier, but life-threatening reactions are considered to be rare nowadays (80, 92, 93). In a systemic review in 2013, Kim et al. investigated trials regarding ASIT for paediatric asthma and rhinoconjunctivitis. There was one report on anaphylaxis, but no reports involving death. Respiratory symptoms were reported in up to 30% of children, and urticaria in up to 19% (94).

Two other reactions have been reported following ASIT, which are subjects of this thesis: **persistent itching nodules** at the injection site and **contact allergy to aluminium.**

Since 1960, the development of subcutaneous, persistent itching nodules at the injection site has been noted following the use of aluminium-containing vaccines and allergen vaccines, mostly in children or adolescents, but also in adults; in most cases it has been strongly associated with contact allergy to aluminium (95-108). Until 2003, both the long-lasting, itching nodules and contact allergy to aluminium were considered to be rare adverse reactions to both vaccination and ASIT, and mainly appeared in case reports or case series. However, in 2003 Elisabeth Bergfors et al. reported long-lasting itching nodules in 745 of 76,000 children (0.98%) after vaccination with aluminium-adsorbed vaccines, in most cases injected subcutaneously but also intramuscularly (109, 110). The causes of the itching nodules were clinical trials in this geographical area on pertussis vaccination and mass vaccination with aluminium hydroxide-adsorbed vaccine from one single producer. In this study, 455 children with persistent itching nodules were patch tested with aluminium and 352 (77%) were diagnosed as having an aluminium allergy. Later, further studies followed on from investigation of these troublesome skin disorders and contact allergy to aluminium that occurred after use of aluminium-containing vaccines from different manufacturers and after ASIT with aluminium-containing allergen vaccines (111-116).

Interestingly, there have also been studies that could not confirm either the high incidence of itching nodules and contact allergy to aluminium after vaccination with aluminium-containing diphtheria-tetanus vaccines or the relatonship between them (117-120).

Other culprit substances have been detected by patch testing of patients with adverse skin reactions after vaccination (121). Thimerosal (merthiolate), an organic mercurial derivate, is used as a preservative e.g. in vaccines and allergen vaccines, in solutions for intracutaneous skin testing, in cleansing solutions for contact lenses, and in topical medications. However, contact allergy to thimerosal is not thought to have clinical relevance to the present skin disorders and to further vaccinations (122-124). Today, all paediatric vaccines used in the USA and Europe are thimerosal-free, or contain only a minimum amount (81). 2-Phenoxyethanol, another preservative and rare sensitiser, has been related to eczema after vaccination in an 18-month-old child (125, 126). Formaldehyde, a common allergen, is added to vaccines to eliminate the

dangerous effects of bacterial toxins and to prevent the replication of infectious viruses (121). The exacerbation of hand eczema due to formaldehyde in a vaccine has been presented in a case report (127). Neomycin, an antibiotic and also a common sensitiser, is added in small amounts to vaccines to prevent bacterial contamination during manufacture. No reports on eczematous exacerbations following neomycin-containing vaccines have been found. None of these mentioned additives have been related to the adverse events of persistent itching nodules.

1.7 Persistent itching nodules

In 2004, the Brighton Collaboration produced a definition of nodules at the injection site as an adverse event following immunisation, and also guidelines that should be used to document these adverse events in prospective studies (128). The definition was based on the published literature from 1966 to 2002, which mainly consisted of case reports. Because of a lack of information about onset, duration, and size of the nodules in these reports, the authors decided to preclude this information from the case definition. However, onset, duration, and size of the nodules should be assessed in reports on adverse events in prospective studies according to the guidelines. The clinical entity is defined as a "well-demarcated soft tissue mass or lump at the injection site. There may also be tenderness and pruritus in the absence of abscess formation and erythema and warmth." However, this definition is too unspecific to distinguish between the well-known, relatively asymptomatic, transient bumps at the injection site, which have also been described in the literature (106, 128), and the persistent nodules with the predominant symptom of severe itch (81, 115). Intense itch at the injection site may cause local eczema and excoriations, hypertrichosis, and hypo- or hyperpigmentation. Intermittent exacerbations during infections or active allergic rhinitis—during patch testing, but also after vaccination without aluminium-containing vaccines, e.g. against mumps-measle-rubella—have been described (81, 102, 109, 111, 114, 115). Other typical findings are the long delay between vaccination and onset of symptoms, i.e. a median time of 3 months, and the long duration, i.e. a median time of 3–4 years. Recently, the proportion of long-lasting itching nodules in children after aluminium-containing vaccination, both in Sweden and Danmark, was estimated to be 0.8% (115, 116).

Histopathological investigations of these subcutaneous nodules have been done by several pathologists and researchers, using electron microscopy, histochemistry, fluorescence histochemistry, and X-ray microanalysis (101). In summary, a mixed inflammatory cell infiltrate including giant cells has been described, forming a granuloma that almost always contains aluminium crystals, which in some cases are seen in necrotic areas (98, 113, 129).

After the first reports of persistent itching nodules following mass vaccination in the Gothenburg area, it was suggested that the injection technique was the reason for this high incidence of skin lesions (130, 131). However, when the injection technique was changed—i.e. from subcutaneous injection to intramuscular injection, the itching nodules still occurred. Further studies have shown that the injection technique may not be the crucial factor (115, 116). The risk of developing the troublesome skin lesions increases with the number of doses of aluminium-containing vaccine given; this has been shown in a prospective, double-blind study (109, 115, 132).

1.8 Aluminium

13	2
Al	8
Aluminium	3
26.981538	

Symbol: Al

Atomic number: 13

Weight: 26.98

Electron shell structure: 2, 8, 3

Aluminium is the third most abundant element in the Earth's crust after silicon and oxygen; about 8% of the Earth's crust consists of aluminium. However, elemental aluminium is too reactive to be found in its pure form. Pure aluminium almost always forms compounds by bonding to other elements—especially to oxygen, for which it has a high affinity. This may be the reason for it remaining unknown for such a long time, unlike gold and silver (133).

"Alumen" is the Latin name for alum, which denotes aluminium compounds in nature. In1825, the Danish chemist Hans Christian Oersted succeeded in separating small amounts of the metal from its ore, bauxite. Bauxite is the name for any ore composed of a mixture of hydrous aluminium oxides (133, 134). It took many years until the metal could be produced commercially from bauxite. Aluminium remained so scarcely available commercially that for a long time it was much more expensive than silver. Even today, the manufacturing process is highly energy-intensive and expensive. Aluminium is used as the elemental metal or as a salt. The metal aluminium has many advantages; it is soft, non-magnetic, light, corrosion-resistant, and recyclable. Today, only iron, as a metal and an alloy, is more used than the metal aluminium—which, for example, is used in transport, packaging, construction, electronic equipment, and household gods.

Aluminium as a trivalent metal cation, Al^{3+}, can be found in many different **aluminium salts** with various properties, which is why the salts are used in industry,

in cosmetics, in dental restorations, in food, and in medicines (135). For example, aluminium sulphate is used in water purification and paper production, aluminium acetate and aluminium acetotartrate as an astringent in solutions, aluminium chloride hexahydrate and aluminium chlorohydrate in antiperspirants, and aluminium hydroxide in antacids. Aluminium salts such as aluminium hydroxide and aluminium phosphate are added to vaccines and allergen vaccines to enhance the immune response in the individual being treated.

In view of the widespread use of aluminium, exposure to aluminium is unavoidable (136). It has been suggested that the body burden of aluminium may be linked to different diseases. Macrophagic myofasciitis and chronic fatigue syndrome can be caused by aluminium-containing adjuvants in vaccines (137, 138), neurodegenerative diseases such as Alzheimer's disease may be due to aluminium accumulated in the brain, and breast cancer may develop after using aluminium-containing antiperspirants (139).

Macrophagic myfasciitis (MMF) has been described as a disease in adults presenting with ascending myalgia and severe fatigue following exposure to aluminium hydroxide-containing vaccines (140). The corresponding histological findings include aluminium-containing macrophages infiltrating muscle tissue at the injection site. The hypothesis is that the long-lasting granuloma triggers the development of the systemic syndrome. Willhite et al. discussed these findings in a systematic review (139). They stated that an independent definitive characterisation of the proposed MMF could not be found in the literature.

Recent studies have confirmed the findings of elevated levels of aluminium in brain tissue in Alzheimer's disease, and have also found evidence that aluminium shows neurotoxicity at low dosages (141-143). According to Willhite and colleagues, there is still no evidence to implicate aluminium as a high risk factor for Alzheimer's disease in the general population (139).

Many studies investigating the carcinogenity of aluminium have been published, especially breast cancer in women after using aluminium-containing antiperspirants. Recently, it has been shown that aluminium chloride hexahydrate promotes tumor genesis and metastasis in normal murine mammary gland epithelial cells (144). However, epidemiological studies have not found that the use of underarm antiperspirants is associated with an elevated risk of breast cancer in women (139).

1.8.1 Aluminium adjuvants

The term "adjuvant" is derived from the Latin "adjuvare", which means to help or to aid. Adjuvants in relation to vaccines and allergen vaccines are defined as substances that are capable of enhancing the immunogenicity of the vaccine antigen when added

to it. The different modes of function of adjuvants can be summarised as follows: (1) to form a depot of antigen at the inoculation site for slow release of the antigen, (2) to present the antigen to immunocompetent cells, and by that to induce an immune response, and (3) to deliver the antigen to the target organ (145, 146).

The **history of using adjuvants** started with the veterinarian Gaston Ramon in 1925. He was working at the Pasteur Institute when he—by coincidence—detected higher titres of specific antibodies against diphtheria in horses that developed abscesses at the injection site because of another infection (147, 148) Ramon provoked sterile abscesses by injection of a mixture of breadcrumbs (or starch) and an inactivated toxin. The immune response measured as the antitoxin titre was much higher than that to the antigen alone. Almost at the same time, Glenny et al. reported the adjuvant effect of aluminium salts, commonly known as "alum adjuvants". He added potassium alum to diphtheria toxoid and got a precipitate, which induced a higher antigenic response when it was injected into guinea pigs compared to the response to soluble toxoid (149). Since the1920s, aluminium-based adjuvants have been the most common adjuvants used in vaccines for the longest period of time, most of all aluminium hydroxide and aluminium phosphate. There are two methods by which aluminium-based adjuvants have usually been produced. The first one leads to a so-called alum-precipitated vaccine, by adding a solution of alum to an antigen to generate a precipitate of protein aluminate. A so-called aluminium-adsorbed vaccine is prepared by means of the second method. An antigen is added to a preformed aluminium phosphate or aluminium hydroxide gel and binds to the surface of the salt (145, 150). The mode of adsorption has been the commonly used method for producing aluminium-containing vaccines in a standardised manner over the last few decades.

Aluminium hydroxide and **aluminium phosphate** differ in their physical properties, and therefore also in their adjuvant properties (147, 151). Aluminium hydroxide is a crystalline aluminium oxyhydroxide that is positively charged at physiological pH, while aluminium phosphate is an amorphous aluminium hydroxyphosphate that is negatively charged at physiological pH. Aluminium phosphate adjuvant is generally more soluble than aluminium hydroxide adjuvant. Aluminium hydroxide has shown a higher adsorption of antigens at neutral pH than aluminium phosphate. Aluminium hydroxide is therefore considered to be a more potent adjuvant than aluminium phosphate.

Since the discovery of adjuvants, many natural and synthetic substances have been tested in experimental vaccine models in animals and in humans, to find **alternatives to aluminium adjuvants** (145, 146, 148, 152). In 1930, Jule Freund composed a mineral oil-based water-in-oil emulsion, called Freund's adjuvant. The complete Freund's adjuvant also contained heat-killed mycobacteria whereas the incomplete Freund's adjuvant was produced without the bacteria. The latter was studied in

allergen-specific immunotherapy during the late 1940s and early 1950s, but it is no longer used in humans because of its various side effects including possible carcinogenicity. Calcium phosphate is another adjuvant that has been used in routine vaccination in France with good safety over many years, and it is also used in allergen vaccines (81, 146). Tyrosine is one of the amino acids normally found in the human body. Microcrystalline tyrosine has been added to allergen vaccines as an adjuvant for at least 25 years. Good safety and immunostimulatory activity have been reported (81, 146, 153, 154).

Today**, vaccine adjuvants** can be classified into different generations (152, 154). The mineral salts already mentioned, emulsions, and also liposomes and microparticles may be considered as the first generation while the second generation consists of combined adjuvants. Bound to substances of the first generation of vaccine adjuvants, they are components capable of specific immunopotentiation of immune pathways. Adjuvants currently added to human vaccines and licensed for use in Europe/USA comprise aluminium salts, oil-in-water emulsions, virosomes, and AS04 (which is monophosphoryl lipid A (MPL) combined with aluminium salt) (155). MPL, for example, is known as a TLR 4 agonist. In Sweden, mainly vaccines and allergen vaccines with aluminium salts as adjuvants are being used, as shown in Table 2 (personal communication with Eva Netterlid at the Swedish Public Health Agency, March 2017, and (156).

The **activity mechanisms** of many adjuvants including aluminium salts, the oldest adjuvants in use, have still to be clarified (155). Reed and colleagues pointed out that it is difficult to interpret all studies done on the mechanism of action of aluminium adjuvants because of the lack of uniformity in the available reagents classified as aluminium salts. They stated that alum affects antigen uptake, induces “danger signals” (DAMPs; e.g. those released from necrotic cells exposed to alum), recruits different types of innate immune cells, and creates a T_h2 cell response characterised by the release of IL-4 and the production of IgG and IgE antibodies. Aluminium salts were traditionally considered to be depot adjuvants enabling a slow allergen release, thus improving tolerability and promoting allergen uptake. Interestingly, animal studies have demonstrated that if the alum-antigen depot is surgically removed a few hours after injection, the antigen-specific immune response continues unaltered (146, 148, 154). This result is generally interpreted as the capability of alum to directly stimulate the immune system. However, the relationship between induction of the innate immune system and the following processes that lead to a manifested immune response is not understood (154). In the innate immune system, macrophages and dendritic cells induce an inflammatory response by recognition of PAMPs through their PRRs. NOD-like receptors are intracellular PRRs, among which NALP3 is one of the best described. Activated NALP3 forms a multiprotein complex, the NALP3 inflammasome, which contributes to the secretion of pro-inflammatory cytokines such as IL-1β and Il-18. It has been shown that aluminium salts may directly activate

the NALP3 inflammasome (157). However, Klimek et al. pointed out that the results are conflicting and that the role of aluminium salts in activation of the adaptive immune response remains elusive (154).

Table 2.
Vaccines and allergen vaccines adsorbed to aluminium adjuvant (156), which are commonly used in Sweden (revised from the thesis of Eva Netterlid (2010) and after personal communication with her at the Swedish Public Health Agency, March 2017)

Commercial name	Vaccine against	Producer	Aluminium adjuvant	Amount of Al (mg Al^{3+})/dose
Alutard SQ	Type I allergic disease	Allergologisk Laboratorium København (ALK) Nordic	Al hydroxide ($Al(OH)_3$)	3.3/100,000 SQ-E/ml
Boostrix®	Diphtheria, tetanus, pertussis (DTP),	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$) Al phosphate ($AlPO_4$)	0.3 0.2
Boostrix®polio	DTP, polio	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$) Al phosphate ($AlPO_4$)	0.3 0.2
Cervarix®	Human papillomavirus (HPV) type 16,18	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$)	0.5
DiTeBooster®	Diphtheria, tetanus (DT)	Scandinavian Biopharma	Al hydroxide ($Al(OH)_3$)	0.5
DiTeKiBooster®	DTP			0.5
Encepur®	Tick-borne encephalitis (TBE)	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$)	0.3–0.4 (adult) 0.15–0.2 (child)
Engerix®-B	Hepatitis (Hep) B	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$)	0.5 (adult) 0.25 (child)
HBVAXPRO®	Hep B	Merck Sharp & Dohme (MSD)	Al hydroxide phosphate sulphate	0.5 (adult) 0.25 (child)
Hexyon®	DTP, polio, *Haemophilus influenzae* type b (Hib), Hep B	Sanofi AB	Al hydroxide ($Al(OH)_3$)	0.6
Gardasil®	HPV type 6, 11, 16, 18	MSD	Al hydroxide phosphate sulphate	0.225
Havrix®	Hep A	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$)	0.5 (adult) 0.25 (child)
Infanrix® Polio	DTP, polio	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$)	0.5
Infanrix® Polio + Hib	DTP, polio, Hib	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$)	0.5
Infanrix® hexa	DTP, polio, Hib, hep B	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$) Al phosphate ($AlPO_4$)	0.5 0.32
Pentavac®	DTP, polio, Hib	Sanofi Pasteur	Al hydroxide ($Al(OH)_3$)	0.3
Prevenar13®	Pneumococcal disease	Pfizer	Al phosphate ($AlPO_4$)	0.125
Synflorix®		GlaxoSmith Kline		0.5
Tetravac®	DTP, polio	Sanofi Pasteur	Al hydroxide ($Al(OH)_3$)	0.3
Twinrix®	Hep A, B	GlaxoSmith Kline	Al hydroxide ($Al(OH)_3$) Al phosphate ($AlPO_4$)	0.025 (child) 0.05 (adult) 0.2 (child) 0.4 (adult)

1.8.2 Contact allergy to aluminium

Aluminium acts not only as an adjuvant, stimulating the immune system either to fend off infections or to tolerate antigens, it also acts as a sensitiser—causing contact allergy and ACD. In general, metal allergies are very common. There exist more than 50 metals, and many more metal compounds and alloys, but only a few metals are on the top list of sensitisers (nickel, chromium, and cobalt) (158). These three metals are included in the European and North American baseline patch test series (159). Thyssen et al. reported that the prevalence of contact allergy to nickel in the general population is estimated to be up to 17% in women and up to 3% in men. It is 1–3% for cobalt and chromium (159). Mahler et al. presented trends in contact sensitisation in Germany by analysing data from patch test clinics from 2010 to 2012 (160). They found a one-year prevalence of contact allergy to nickel of 14.9–15.3%, to cobalt of 4.4–5.8%, and to chrome of 2.5–3.0%. There have also been reports of other metal sensitisers such as gold, palladium, and aluminium causing contact allergy (31, 109, 161-163). No figures on the prevalence of contact allergy to aluminium are available.

In view of its ubiquitous environmental presence and widespread use, aluminium must be considered to be a weak allergen (158). In everday life, humans may be exposed to aluminium by skin contact, inhalation, and ingestion. In 2007, Krewski et al. published a comprehensive review on human exposure to aluminium and its potential health risks (135), which was revised and updated by Willhite et al. in 2014 (139). The original review concluded that daily exposure in humans ranges from "as little as less than 0.06 mg Al/day as a result of inhaling air to as high as 3,500–5,300 mg Al/day as a result of consuming aluminium antacids". Pineau et al. performed an experimental study using Franz diffusion cells to measure the in vitro transdermal uptake in humans from topical aluminium chlorohydrate (ACH), which is an active ingredient of antiperspirants (164). Skin biopsies from five healthy Caucasian volunteers were treated with either 14.5% "roll-on" ACH emulsion (≈ 4.55 mg/cm^2), 21.2% ACH "stick" preparation (≈ 3.1 mg/cm^2), or 38.5% ACH "aerosol" preparation (≈ 2.59 mg/cm^2). After 24 hours of contact with the skin, the highest mean aluminium concentration measured in the horny layer was 2.24–4.43 µg/cm^2, irrespective of which ACH formulation was used. In the stripped epidermis treated with the "stick" preparation at 21.2%, a mean aluminium concentration of 9.42 ± 7.82 µg/cm^2 was measured. In the receptor fluid, the aluminium concentration was less than 0.1µg/cm^2—corresponding to only 0.012% of the aluminium applied.

A metal must be ionised to be able to act as a contact allergen, then it has to undergo haptenisation to be immunogenic and to initiate an immune response (159, 165). Most studies on immune mechanisms have been performed with nickel as a typical allergen, and some with cobalt, chrome, palladium, or beryllium as allergen, but as far as I know, no such studies have been performed with aluminium (165). The various mechanisms identified have varied, depending on the metal allergen, suggesting the

existence of overlapping and unique mechanisms. Once inside the skin, the metal ions must bind to proteins to become immunologically reactive. It has been shown that nickel and cobalt can bind to histidine residues and interact with TLR 4, which belongs to the pattern recognition receptor family expressed by different cells, e.g. dendritic cells and macrophages. Its activation leads to an intracellular signaling pathway and production of inflammatory cytokines, thus activating the innate immune system. The metal-induced adaptive immune response differs in the patterns of T_h cell polarization induced by nickel, cobalt, and palladium. T_h17-, T_h22-, and IL-9 producing T_h 9 cells have been added to the list of contributory cells besides T_h1, T_h2 and T_c1, all of which are involved in the mechanism of ACD caused by metals.

The most important routes of exposure and sensitisation to aluminium are through aluminium-containing vaccines (98, 101-103, 105, 166) and allergen vaccines (97, 99, 100, 106), which, as described in sections 1.6 and 1.7, is often associated with persistent itching nodules. Since the surprising results of the mass vaccination trial in Gothenburg, when 77% of 645 children with itching nodules developed contact allergy to aluminium, several reports have been published, indicating an increasing awareness of these clinical issues (107, 109, 112-116, 129, 167). One Swedish study showed a statistically significant association between contact allergy to aluminium and persistent itching nodules in children treated with ASIT (112). Nodules were overrepresented in patients with contact allergy to aluminium (6/8 versus (vs.) 7/29; $p = 0.013$).

Other routes of sensitisation reported in the literature are the prolonged use of aluminium-containing antiperspirants (168, 169), topical medication (170), and tattooing of the skin with aluminium-containing pigments (171, 172). Most of the patients experienced eczematous reactions, whereas tattooing caused granulomas. Even though aluminium is used extensively in industry, only a low number of cases of occupational skin sensitisation to aluminium have been reported (173, 174).

Systemic allergic contact dermatitis in the form of flare-up reactions after re-exposure to aluminium has been documented: pruritic nodules at present and previous injection sites, eczema at the site of vaccination as well as at typically atopic localisations after vaccination with aluminium-containing vaccines and/or patch testing with aluminium, and also after use of aluminium-containing toothpaste (97, 102, 103, 175).

1.8.3 Diagnosis of contact allergy to aluminium

Patch testing with an aluminium salt is the method of choice when contact allergy to aluminium is suspected. Traditionally, patch testing is performed with aluminium chloride hexahydrate 2% pet. and an empty Finn chamber, which is made of elemental aluminium. However, for a few years an increase in the concentration to

10% pet. has been recommended (167). Positive patch test reactions usually appear on D3 and D4, and some even later (D7) (112). Thus, patch tests with aluminium should always be read twice, on D3 or D4 and on D7.

Contact allergy to aluminium may accidentally be detected when patch testing is performed with Finn chambers as they are made of elemental aluminium (176-178). On the other hand, there have also been reports of negative reactions to an empty Finn chamber although patch testing simultaneously confirmed an aluminium allergy (101, 167, 169). However, if there is a strong suspicion of contact allergy to aluminium, chambers made of plastic should be used for patch testing.

A number of aluminium salts at various concentrations, both in pet. and in water, have been used for patch testing since many years, but most of them appeared only in case reports and/or case series (Table 3).

Table 3.
Aluminium compounds and test concentrations used in aluminium patch testing according to reports in the literature from 1980 until 2010

Aluminium compounds	Vehicles	Concentration(s) (% w/w)	References
Aluminium chloride hexahydrate ($AlCl_3 6H_2O$)	aq.; pet.	0.1; 0.5; 1.0; 2.0; 5.0; 10.0	Clemmensen 1980 (97); Fischer 1982 (168); Kotovirta 1984 (176); Meding 1984 (170); Frost 1985 (99); Veien 1986 (100); Cox 1988 (102); Castelain 1988; Tosti 1990 (177); Cosnes 1990 (103); Veien 1993 (175); Dwyer 1993 (178); Lopez 1994 (106); Garcia-Patos 1995 (179); Hemmer 1996 (180); Skowron 1997 (181); Bergfors 2003 (109), 2005 (111); Netterlid 2004 (119); Bruze 2008 (167); Netterlid 2009 (112); Brodbaker 2009 (182); Garg 2010 (169)
Aluminium hydroxide ($Al(OH)_3$)	aq.; pet.	0.5; 10.0	Böhler-Sommeregger 1986 (101); Lopez 1994 (106); Garcia-Patos 1995 (179); Hemmer 1996 (180); Skowron 1997 (181)
Aluminium subacetate ($C_4H_7AlO_5$)	aq.	1.0; 2.0	Clemmensen 1980 (97); Kotovirta 1984 (176); Tosti 1990 (177); Lopez 1994 (106)
Aluminium acetotartrate ($C_6H_7AlO_8$)	aq.	1.0	Fischer 1982 (168); Meding 1984 (170); Cosnes 1990 (103)
Aluminium sulphate ($Al_2(SO_4)_3$)	aq.	2.0	Clemmensen 1980 (97)
Aluminium acetate ($C_6H_9AlO_6$)	aq.	2.0; 1.3	Cox 1988 (102); Cox 1988 (166); Castelain 1988 (183); Cosnes 1990 (103); O'Driscoll 1991 (184); Hemmer 1996 (180)
Aluminium sulphide (Al_2S_3)	aq.	2.0	Castelain 1988 (183); Tosti 1990 (177)
Potassium aluminium sulphate ($AlK(SO_4)_2\ 12H_2O$)	aq.; pet.	0.98; 1.98; 3.93	Hemmer 1996 (180)
Aluminium phosphate (Al_3PO_4)	pet.	0.25; 0.5; 1.0	Hemmer 1996 (180)
Aluminium oxide (AlO_3)	aq.; pet.	10.0	Skowron 1997 (181)
Aluminium powder	aq.; as is	2.0	Clemmensen 1980 (97); Castelain 1988 (183)
Aluminium metal/sheet			Fischer 1982 (168); Kotovirta 1984 (176); Meding 1984 (170)
Empty Finn chamber			Clemmensen 1980 (97); Fischer 1982 (168); Kotovirta 1984 (176); Meding 1984 (170); Böhler-Sommeregger 1986 (101); Veien 1986 (100); Castelain 1988 (183); Cox 1988 (102); Cox 1988 (166); Cosnes 1990 (103); Tosti 1990 (177); O'Driscoll 1991(184); Dwyer 1993 (178); Lopez 1994 (106); Hemmer 1996 (180); Bergfors 2003 (109), 2005 (111); Netterlid 2004 (119); Bruze 2008 (167); Netterlid 2009 (112); Brodbaker 2009 (182); Garg 2010 (169)

Intradermal testing is recommended in doubtful cases of metal allergy (78), and it has also been used in diagnosing aluminium allergy (97, 106, 168, 177). As test preparation, 0.1−0.5% aluminium hydroxide in sodium chloride (0.5%) or in water (168) was used, and reading of the test was performed after 2−3 days.

To the best of my knowledge, there are no in vitro methods for diagnosing aluminium allergy.

It is not possible to estimate the prevalence of aluminium allergy in the general population due to a relatively low number of reports and a lack of data. At the Department of Occupational and Environmental Dermatology, Malmö, Sweden, aluminium chloride hexahydrate and aluminium lactate have been tested at various concentrations in the baseline series for several years. Frequencies of allergic reactions to aluminium compared to other metal allergies in 2016 are shown in Figure 2.

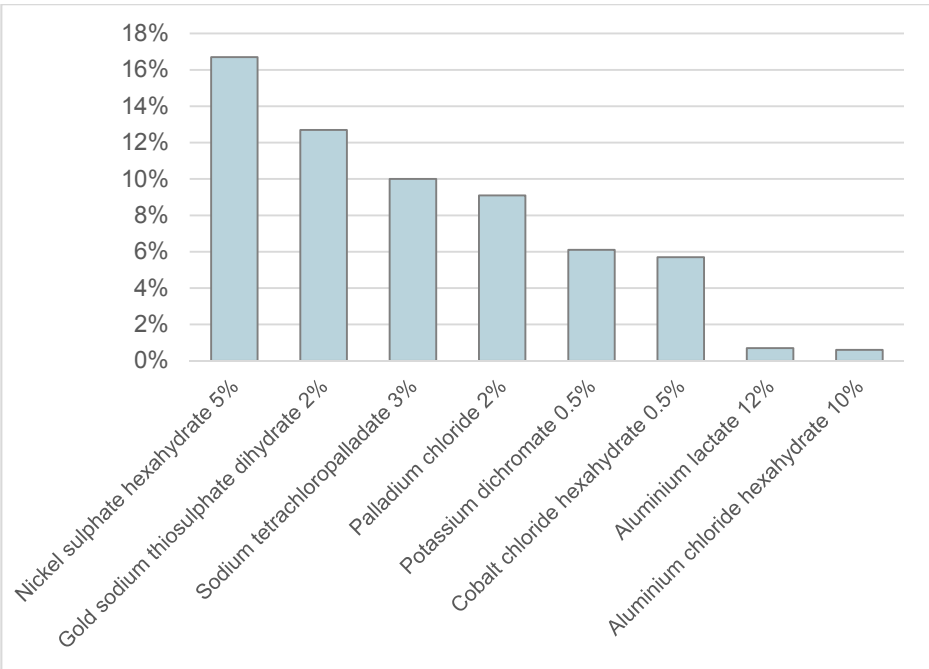


Figure 2.
Metal allergies in Malmö, Sweden, in 2016

2. Aims

In this thesis, the overall aim was to improve our basic knowledge of aluminium as a contact allergen. More specifically, the purposes of the studies included in this thesis were as follows:

- to determine the presence of contact allergies in individuals with allergic asthma and/or rhinoconjunctivitis, and to compare the number of contact allergies between study groups of children and adults, with and without a history of atopic dermatitis
- to investigate whether allergen-specific immunotherapy (ASIT) with allergen extracts containing aluminium hydroxide as adjuvant induces contact allergy to aluminium and itching nodules in children and adults
- to compare various aluminium compounds and concentrations to find an optimal patch test preparation
- to investigate possible variation in patch test reactivity to aluminium chloride hexahydrate and to aluminium lactate over time, to compare the patch test reactivity between various subgroups (e.g. atopic or non-atopic individuals, treated with ASIT or not treated with ASIT), and to investigate whether there is a correlation between test reactivity to aluminium chloride hexahydrate and to aluminium lactate.

3. Materials and methods

Detailed descriptions of the materials and methods are given in the individual papers. This section is an overview. Papers I and II are based on a prospective, randomised, controlled single-blind study of children and adults. Papers III and IV were experimental studies with adult volunteers.

3.1 Study populations

3.1.1 Studies I and II

Studies I and II were based on one trial, in which 202 children and 349 adults with allergic rhinoconjunctivitis, and/or asthma, and/or allergy to insect venom, who were scheduled to start ASIT in the autumn of 2007 and 2008 at 14 medical units in southern Sweden, were invited to participate. Of the 551 individuals who were asked to take part, 248 participated (45%), 86 (35%) children (56 males and 30 females; mean age 12 years, range 5−17 years) and 162 (65%) adults (69 males and 93 females; mean age 33 years, range 18−74 years).

3.1.2 Studies III and IV

In study III, 21 subjects (7 males and 14 females; mean age 48 years, range 23−81 years) and in study IV, 21 subjects (7 males and 14 females; mean age 49 years, range 29−72 years), who had all been diagnosed with contact allergy to aluminium earlier, were enrolled. Only a minority of these volunteers participated in both studies. In addition, 20 adult volunteers who had been referred to the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, for suspected allergic contact dermatitis, participated as controls in study III.

3.2 Study designs

3.2.1 Studies I and II

The trial, which led to papers I and II, was designed to determine the presence of contact allergy in atopic individuals after one year of ASIT with allergen preparations containing aluminium hydroxide, and to compare the numbers of contact allergies between different study groups. Another aim was to determine the number of individuals who developed contact allergy to aluminium and persistent itching nodules over one year of ASIT.

The participants who started with ASIT in the autumn of 2007 were all designated as being exposed, and they were randomly divided into three subgroups with different schedules of patch testing (Fig. 3) This randomisation refers to study II and is not valid for study I. Before the start of ASIT and at different time intervals during the ASIT, depending on which subgroup the subjects belonged to, they were patch tested with aluminium chloride hexahydrate, with the last testing for all of them after one year. These patch tests, performed before and during ASIT, were done to be able to exclude a possible sensitisation to aluminium by the patch test itself in the exposed individuals. The tests were read by the participants themselves. Each result was noted in a protocol, which was placed in a sealed envelope. At the end of the study, after one year of ASIT, a control group of children and adults about to start ASIT in the autumn of 2008 was included and labelled as being unexposed. By comparative investigation of the controls, it was possible to determine whether there was an altered or increased environmental exposure to aluminium to explain a possible sensitisation to aluminium. All study persons, the exposed subjects after one year of ASIT, and the unexposed subjects before the start of ASIT, were patch tested with allergen extracts used for ASIT, a baseline series routinely used to detect contact allergy, and with aluminium chloride hexahydrate. When patch testing and reading, both exposed and unexposed study persons were randomly mixed. The reading dermatologist was blind as to whether the patch tested subject had been treated with ASIT for one year or had not yet started it; he/she was also blind regarding all the data collected and regarding the results of the physical examination.

Before taking part in the trial, all the participants filled out a questionnaire regarding atopic diseases, metal sensitivity, piercing, use of antiperspirants, aluminium-containing medication, vaccinations, and other sources of aluminium exposure.

At the same time, before the start of ASIT, all the study persons, or their parents/guardians, were asked about itching. The exposed subjects were even asked about itching after one year of ASIT, before the final patch test was done. Regarding development of subcutaneous itching nodules, an examination by visual inspection

and palpation of the injection sites, i.e. both upper arms, was performed at the start of the study and in the exposed subjects also after one year of ASIT before the final patch test, still without knowing the group to which the volunteers belonged.

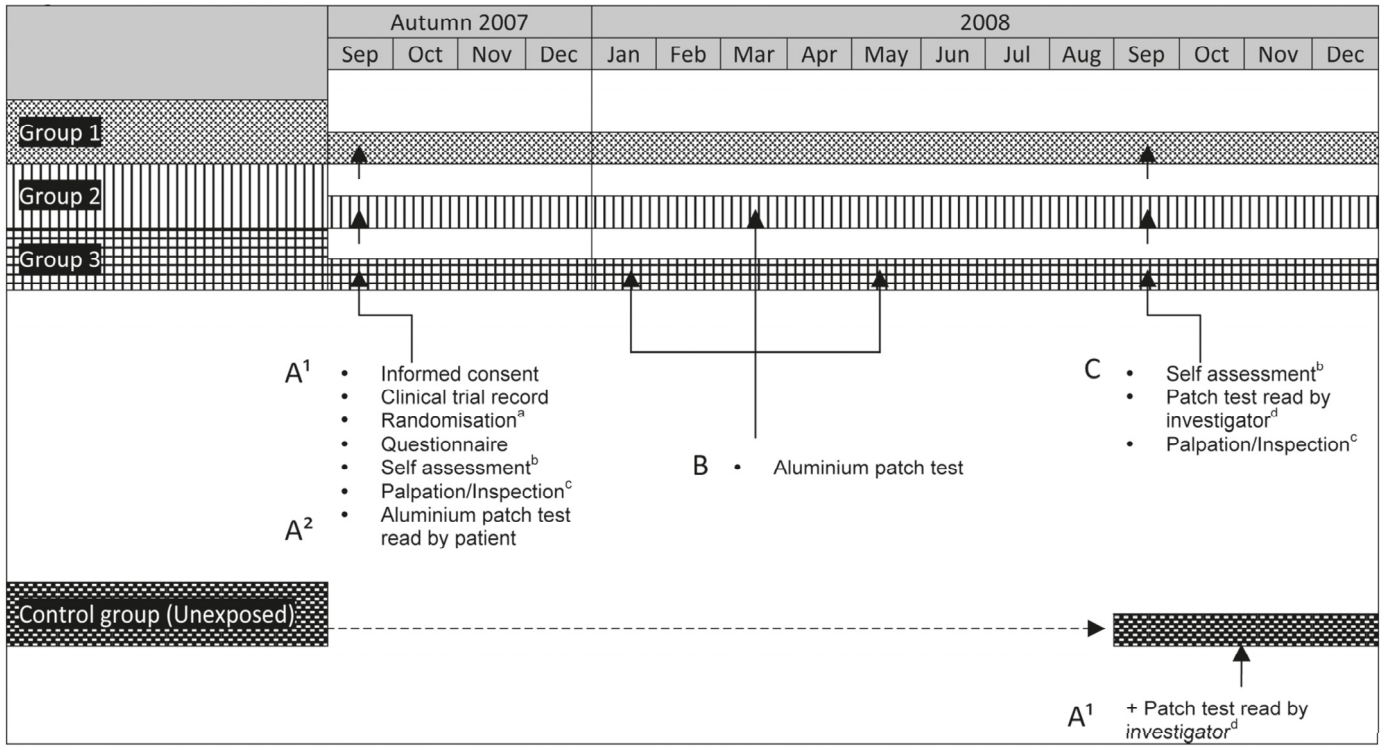


Figure 3.
Study design of papers I and II.

[a] Randomly assigned database sequence numbers in order to facilitate blind assessment and randomised grouping.
[b] The subject's assessment of itching.
[c] Palpation of possible nodules and inspection of signs of pruritus (scratch mark) by the investigator.
[d] Patch test with the European baseline series supplemented with aluminium preparations and antigen extracts.

3.2.2 Study III

In study III, 21 volunteers with known contact allergy to aluminium were patch tested with 6 different aluminium compounds in concentrations equimolar to a dilution series of aluminium chloride hexahydrate, and an empty Finn chamber (Table 5). At the same time, 19 of 21 volunteers were tested intracutaneously with aluminium chloride hexahydrate in saline. The reading of the patch tests was done on D3 or D4 and on D7; the intradermal test was only read on D3.

3.2.3 Study IV

In study IV, patch testing was performed in 21 volunteers with known aluminium allergy. The individuals were patch tested with equimolar dilution series of aluminium chloride hexahydrate and aluminium lactate over a period of 8 months with an interval of approximatly 2.5 months between test occasions (TA, TB, TC, and TD). The reading of the patch tests was done on D3 or D4 and on D7. The reading dermatologist was blind regarding the order of the places on which the dilution series were applied to the individual's back. Before each test occasion, all study persons filled out a questionnaire with questions about atopic diseases, metal sensitivity, use of antiperspirants, immunomodulating medication, and additional vaccinations. Women also answered regarding the stage of their menstrual cycle.

Three dermatologists read all the tests, both in study I and in study II; two of them read all the tests in studies III and IV. The dermatologists reading all test reactions were calibrated in their patch test reading through long experience in joint patch test reading.

3.3 Chemicals, test preparations, and test materials

The main chemicals, allergen extracts, and test materials used in the studies are listed in Table 4. All aluminium test preparations and arrangements to test the study persons were done by the same personnel in the laboratory at the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö.

Table 4.
The main chemicals, allergen extracts, and test materials used in the studies, and the manufacturers/suppliers

Studies I and II	Alutard SQ 3-trees ((alder, birch, hazel), 100,000 SQ-E/ml	ALK-Abelló A/S, Hœrsholm, Denmark
	Alutard SQ 5-grass pollen mixture, 100,000 SQ-E/ml	
	Alutard SQ Mugwort (Artemisia vulgaris), 100,000 SQ-E/ml	
	Alutard SQ Cat dander, 100,000 SQ-E/ml	
	Alutard SQ Dog dander, 100,000 SQ-E/ml	
	Alutard SQ 2- Dermatophagoides mites, 100,000 SQ-E/ml, 100,000 SQ-E/ml	
	Alutard SQ Honeybee venom, 100,000 SQ-E/ml	
	Alutard SQ Yellow jacket venom, 100,000 SQ-E/ml	
	Aluminium chloride hexahydrate	MPBiomedicals, Inc., Eschwege, Germany
	All test preparations in the European baseline series	Chemotechnique Diagnostics, Vellinge, Sweden
Studies III and IV	Aluminium chloride hexahydrate, 99%	Sigma Aldrich, Steinheim, Germany
	Alum (Aluminium potassium sulphate dodecahydrate, ≥ 98%)	Sigma Aldrich, Steinheim, Germany
	Aluminium lactate (aluminium L-lactate, 97%)	Sigma Aldrich, Steinheim, Germany
	Aluminium hydroxide (reagent grade)	Sigma Aldrich, Steinheim, Germany
	Aluminium phosphate, 97%	Alfa Aesar GmbH, Karlsruhe, Germany
	Aluminium acetotartrate, 50 % in water	Apoteket, Produktion och Laboratorier, Göteborg, Sweden
	Aluminium chloride hexahydrate at 1.0 µmol/ml (0.24 mg/ml) and 10.0 µmol/ml (2.4 mg/ml) in saline (aluminium chloride hexhydrate 0.24 mg and 2.4 mg, sodium chloride, 9 mg; aqua for injection)	Apoteket APL, Umeå, Sweden
Studies I, II, and III	Finn chamber, made of elemental aluminium; diameter 8 mm	Epitest Ltd Oy, Tuusula, Finland
Studies I, II, III, and IV	Petrolatum IQ chambers made of additive-free polyethylene plastic and mounted on a non-woven adhesive tape	Apoteket, Skåne University Hospital, Malmö, Sweden Chemotechnique Diagnostics, Vellinge, Sweden

3.3.1 Studies I and II

The study persons were patch tested with the European baseline series supplemented with allergen extracts used for ASIT, each containing aluminium hydroxide (3.3 mg/ml), with aluminium chloride hexahydrate 2.0% pet. and 10.0% pet., and an empty Finn chamber (Table 4). At the final patch test, subjects showing a doubtful reaction on D3 or D4 were also tested with aluminium chloride hexahydrate 20.0% pet..

3.3.2 Study III

All study persons were patch tested with 6 different aluminium compounds in equimolar concentrations to a dilution series of aluminium chloride hexahydrate 20.0 % pet. and with an empty Finn chamber. Additionally, aluminium chloride hexahydrate 10.0% pet. without any other aluminium compound in an equimolar concentration was tested in all subjects. Petrolatum was chosen as the vehicle because of the insolubility of some of the aluminium compounds in water. Only 7 of the 21 subjects were also patch tested with aluminium acetotartrate in water (Table 5), as aluminium acetotartrate was not available when the study started. 19 of the 21 subjects were tested intradermally on the same day as when the patch test was applied: 14 of the 19 with 0.1 ml of aluminium chloride hexahydrate in saline at a concentration of 1.0 µmol/ml (0.24 mg/ml), and 5 of the 19 with 0.1 ml of aluminium chloride hexahydrate in saline at 10.0 µmol/ml (2.4 mg/ml).

20 subjects, serving as controls, were patch tested with the 6 different aluminium compounds only at the highest equimolar concentrations (Table 5).

Table 5.
Equimolar dilution series of the aluminium compounds used for patch testing in study III

Test preparation	Aluminium chloride hexahydrate $AlCl_3 6H_2O$ MW 241 petrolatum (% w/w)	Aluminium hydroxide $Al(OH)_3$ MW 78 petrolatum (% w/w)	Aluminium phosphate $AlPO_4$ MW 122 petrolatum (% w/w)	Aluminium lactate $Al(C_3H_5O_3)_3$ MW 294 petrolatum (% w/w)	Alum $AlK(SO_4)_2 12H_2O$ MW 474 petrolatum (% w/w)	Aluminium acetotartrate $C_6H_7AlO_8$ MW 234 water (% w/v)
Concentration	20.0[a]	6.5[a]	10.0[a]	24.0[a]	39.0[a]	25.0[a]
	10.0					
	6.3	2.1	3.2	7.7	12.0	8.8
	2.0	0.65	1.0	2.4	3.9	2.9
	0.63	0.21	0.32	0.77	1.2	0.91
	0.20	0.065	0.10	0.24	0.39	0.29

[a] Stock solutions were diluted by a factor of √10. MW, molecular weight. w, weight; v, volume.

3.3.3 Study IV

All study persons were patch tested with two equimolar dilution series with aluminium chloride hexahydrate and aluminium lactate, both in petrolatum, which was chosen as the vehicle because of the insolubility of aluminium lactate in water (Table 6).

Table 6.
Equimolar dilution series of aluminium chloride hexahydrate $AlCl_36H_2O$ and of aluminium lactate $Al(C_3H_5O_3)_3$

Aluminium chloride hexahydrate $AlCl_36H_2O$ pet. (% w/w)	Aluminium lactate $Al(C_3H_5O_3)_3$ pet. (% w/w)	Corresponding aluminium concentration (% w/w)
(32)[a]	(38)[a]	3.5
10.0[b]	12[b]	1.1
3.2	3.8	0.35
1.0	1.2	0.11
0.32	0.38	0.035
0.10	0.12	0.011
0.032	0.038	0.0035
0.010	0.012	0.0011

[a] Hypothetical minimal eliciting test concentration (MEC) in patients with contact allergy to aluminium but showing negative reactions to 10% (12%) and lower concentrations in this study.
[b] Stock solutions were diluted by a factor of √10.
pet., petrolatum; wt, weight.

3.4 Patch testing and intradermal testing

3.4.1 Patch test technique

In all our studies, patch testing was performed with IQ chambers (Table 4) mounted on non-woven adhesive tape to fasten the patch tests on the upper back of the participants. IQ chambers were chosen because they are made of additive-free polyethylene plastic. Finn chambers, which are often used in routine patch testing, are made of elemental aluminium and—as an empty chamber—are often used to diagnose contact allergy to aluminium. For the test preparations in petrolatum, an amount of 30 mg (37 mg/cm^2) of each allergen preparation was applied to each IQ chamber (65); of the liquid test preparations, a volume of 25 µl (31 µl/cm^2) of each allergen solution was applied with a micro-pipette to each chamber (64).

In studies III and IV, the participants were patch tested with dilution series of different aluminium salts, as described above. The chambers with the dilution series of one aluminium salt were placed in a row of decreasing concentration, i.e. the highest concentration was always placed at the top and the lowest at the bottom, and in study III with an empty Finn chamber at the end.

In study IV, on each test occasion the two patches with the equimolar dilution series of aluminium chloride hexahydrate and aluminium lactate were applied to a new area of the individual's upper back randomly according to a Latin square table (185). In this way, none of the subjects were tested twice on the same area.

Within the test area, the order of application of the two patches was also randomised on each test occasion, and the dermatologist reading the tests was kept blind.

3.4.2 Reading of patch test

In all the studies, the patch tests were removed by the participants themselves after 48 h and read by the dermatologists on D3/D4 and D7. The strongest reaction on D3/D4 or on D7 was used for statistical analysis. The patch test reactions were scored according to International Contact Dermatitis Research Group guidelines (ICDRG) (68). In studies III and IV, we used additional grading: strong + and ++ reactions were graded +(+) and ++(+), respectively (185).

As mentioned above, in study II the patch tests with aluminium chloride hexahydrate before and during ASIT were read by the study person on D3 according to a self-assessment protocol. Only the patch test with aluminium chloride hexahydrate at the end of the study was read by the dermatologists on D3/D4 and D7 according to the ICDRG guidelines. The test with aluminium chloride hexahydrate 20.0% pet. was only read once, on D7, i.e. 3 or 4 days after application of the additional test.

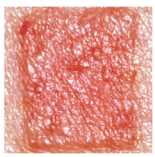
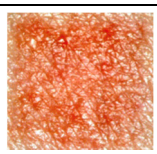
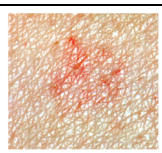
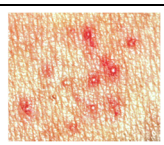
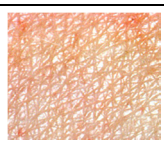
Foto av testområdet (Photograph of the test area)	**Beskrivning** (Description)	**Kryssa för det foto som stämmer bäst med din egen test** (Select the picture that best corresponds to your own test)
	Rodnad som täcker hela testområdet. Det kan också finnas små vätskeblåsor eller knottror. (Redness that covers the entire test area. There may also be small vesicles or papules.)	☐
	Rodnad som täcker hela testområdet. Det finns inga vätskeblåsor eller knottror. (Redness that covers the entire test area. There are no vesicles or papules.)	☐
	Rodnad som inte täcker hela testområdet. (Redness that does not cover the entire test area.)	☐
	Enstaka knottror eller blåsor inom testområdet. Viss rodnad kan finnas men täcker inte hela testområdet. (Occasional small bumps or vesicles within the test area. Redness may be present, but does not cover the entire test area.)	☐
	Testområdet liknar den normala otestade huden som finns runtomkring. (The test area is similar to the normal untested skin round about.)	☐

Figure 4.
Part of self-assessment form regarding patch test with aluminium chloride hexahydrate

3.4.3 Intradermal testing in study III

An intradermal testing (78) was performed in 19 of 21 study persons on the same day on which the patch test with dilution series of various aluminium compounds was applied. 14 of the 19 were tested with 0.10 ml aluminium chloride hexahydrate in saline at a concentration of 1.0 µmol/ml (0.24 mg/ml) and 5 of the 19 were tested with 0.10 ml aluminium chloride hexahydrate in saline at 10.0 µmol/ml (2.4 mg/ml). The injection site was the volar aspect of the forearm, and a weal of ≥ 4 mm in diameter was raised when 0.1 ml fluid was injected. The dermatologist read the skin reaction on D3/D4. A red and infiltrated area of ≥ 4 mm was considered to be a positive test.

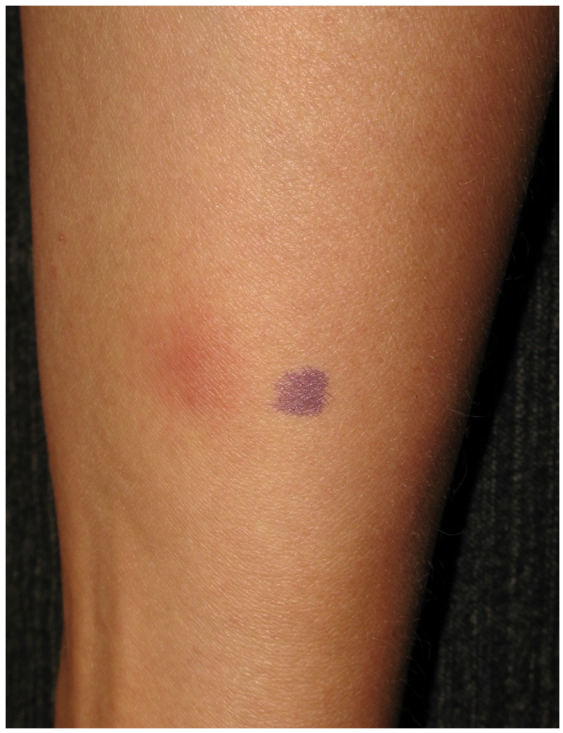


Figure 5.
Positive skin reaction to an intradermal test

4. Ethics

In all studies, the volunteers were informed about the nature of the different tests and possible adverse reactions. Written informed consent was obtained from the participants or the parents/ guardians. All the studies were approved by the Regional Ethical Review Board, Lund, Sweden. The investigation described in papers I and II was registered in the ISRCTN database (www.isrctn.org; no: ISRCTN57796160). All the photographs and drawings in this thesis were published with the consent of the parents and children.

5. Statistics

For statistical calculations in studies I, II, and III, any doubtful reactions were regarded as being negative.

Study I

Firstly, a descriptive data analysis was performed. The absolute and relative frequencies for categorical variables (presented as numbers and percentages) have been reported, as are mean and 95% confidence interval. For normally distributed variables, we used parametric tests exclusively. Comparisons of two independent groups with regard to numerical outcome variables were performed using the independent samples t-test, e.g. the number of contact allergies in exposed and unexposed groups. Chi-square test and Fisher's exact test, two-sided, were used to analyse the association between categorical variables, e.g. childhood eczema (yes/no), contact allergy (yes/no) in exposed/unexposed groups. Multivariate (backward stepwise selection method with probability for the removal of 0.10) linear regression analysis was used to determine the association of variables with the number of contact allergies. Factors suspected of being predictive of the number of contact allergies in multivariate analysis were gender, age, atopic dermatitis, and ASIT. The analyses were performed using SPSS version 22.0 (SPSS Inc., IBM Corp., Armonk, NY, USA).

Study II

The non-parametric Mann-Whitney U-test was used when comparing ordinal and continuous outcome variables between 2 independent groups, e.g. exposed individuals vs. controls. When comparing categorical variables with binary outcome, e.g. frequencies of positive and negative test results, two-sided Fisher's exact test was used. Two-sided McNemar's test was used to compare 2 proportions estimated from paired observations, e.g. baseline vs. follow-up observation. Power calculation at the design phase was conducted in StatX-act-6 (Cytel Software Corp., Cambridge, MA, USA). All data analyses were performed using SPSS version 17.0 for Windows (SPSS Inc.).

Study III

Two-sided McNemar test was used to compare the number of positive reactions to aluminium chloride hexahydrate 2.0% pet. and to aluminium lactate 2.4% pet. For

comparison of the positive patch test results between the 2 independent groups (study group vs. control group), regarding one aluminium compound at the highest concentration, two-sided Fisher's exact test was used.

Study IV

To increase the possible sensitivity of the statistical analysis, the patch test reactions were transformed to numerical values as follows (185): negative = 0, (+) = 0.5; + = 1, +(+) = 1.5, ++ = 2, ++(+) = 2.5, and +++ = 3. The patch test results were calculated in two ways: (1) the lowest concentration eliciting at least a + reaction was registered as MEC (minimal eliciting concentration), and (2) the scores for all skin reactions were summed and registered as the STS (summarised test score). The positive test reactions shown by a study person 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 all other situations, the concentration above the first negative or doubtful reaction was noted as MEC. If a participant did not show any positive reaction to the dilutions of the salt being tested, the MEC was estimated to be the theoretically next higher patch test concentration according to the increasing steps of the dilutions.

A Friedman test was run to compare the reactivity response for MEC and STS values for aluminium chloride hexahydrate and aluminium lactate from test occasions A to D (TA to TD). Mann-Whitney U-test was used to compare means of MEC and STS values between different subgroups on each test occasion, e.g. women vs. men, atopics vs. non-atopics, subjects treated with ASIT vs. subjects not treated with it, subjects with additional vaccinations vs. subjects with no additional vaccinations. Fisher's exact test, two-sided, was used for contingency tables. Spearman's rank-order correlation coefficient (r_s) was calculated to evaluate the covariation of test reactivity and the concomitant reactivity to both aluminium salts on TA to TD. The covariation of both salts was analysed as correlation between the changes in the MEC values from TA to TB, from TB to TC, and from TC to TD and in the same manner between the changes in the STS values. The concomitant reactivity to both salts was analysed by comparing MEC and STS for each study person on each test occasion, as well as mean MEC and mean STS.

In all statistical calculations resulting in any p-value, the differences were considered to be statistically significant when $p < 0.05$.

6. Results

Paper II has been part of the thesis of Eva Netterlid (81), where the same results are reported.

In the study covered by papers I and II, a total of 551 atopic individuals at 14 medical units in southern Sweden were invited to take part in the study. Of those 551 individuals, 248 study persons (45%) were included, 162 adults and 86 children. Of these, 205 (83%), 127 adults and 78 children, were patch tested at the end of the study with the baseline series supplemented with aluminium and the allergen extracts used for ASIT.

Comparing basic data on the study participants and the answers to the questionnaire, there were no significant differences between the study groups; nor were there any relevant differences between the groups when comparing the information in the case report forms collected by the investigators.

6.1 Study I

The number of contact allergies diagnosed in the study persons varied between zero, one, two, and three allergies. Contact allergy to at least one allergen was diagnosed in 72 of 205 participants (35%). A history of AD was documented in 81 of 197 (41%), and 35 (43%) of these study persons were diagnosed with contact allergy to at least one allergen. In 116 of 197 individuals with no history of AD, we found 34 subjects with at least one contact allergy (29%). Thus, more individuals with a history of AD had at least one contact allergy than those without AD (35/81 vs. 34/116; $p = 0.049$). A summary of the positive reactions to each test preparation in our baseline series in the different groups and in the whole study group of the patch tested participants is given in Tables 7–9.

Table 7.
Numbers and proportion (%) of positive patch test reactions in children according to contact sensitiser[a] and in the study group "children"

	Exposed group (51*)						**Unexposed group (27*)**						**All children (78*)**					
Childhood eczema	Yes (23*)		No (26*)		U (2*)		Yes (14*)		No (11*)		U (2*)		Yes (37*)		No (37*)		U (4*)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Potassium dichromate	1	4.3	-	-	-	-	2	14.3	-	-	-	-	3	8.1	-	-	-	-
Cobalt chloride	-	-	-	-	-	-	-	-	1	9.1	-	-	-	-	1	2.7	-	-
Nickel sulphate	2	8.7	-	-	1	50	1	7.1	-	-	-	-	3	8.1	-	-	1	25
Colophony	1	4.3	-	-	-	-	-	-	-	-	-	-	1	2.7	-	-	-	-
Textile disperse dye mix	1	4.3	-	-	-	-	-	-	-	-	-	-	1	2.7	-	-	-	-
para-tertiary Butyl phenol-formaldehyde resin	-	-	-	-	-	-	-	-	1	9.1	-	-	-	-	1	2.7	-	-
Formaldehyde	-	-	-	-	-	-	-	-	1	9.1	-	-	-	-	1	2.7	-	-
Fragrance mix I	3	13.0	-	-	-	-	-	-	-	-	-	-	3	13.0	-	-	-	-
Amerchol L 101	6	27.3	1	3.8	-	-	2	14.3	1	9.1	-	-	8	21.6	2	5.4	-	-
Thimerosal	-	-	-	-	-	-	1	7.1	-	-	-	-	1	2.7	-	-	-	-
Tixocortol pivalate	-	-	-	-	-	-	1	7.1	-	-	-	-	1	2.7	-	-	-	-
Total, n	14		1		1		7		4		0		21		5		1	

[a] Results after patch testing with European baseline series.
*Number of study persons.
n, number of positive patch test reactions; U, unknown history of childhood eczema.

Table 8.
Numbers and proportion (%) of positive patch test reactions in adults according to contact sensitiser[a] and in the study group "adults"

	Exposed group (82*)						Unexposed group (45*)						All adults (127*)					
Childhood eczema	Yes (27*)		No (53*)		U (2*)		Yes (17*)		No (26*)		U (2*)		Yes (44*)		No (79*)		U (4*)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Potassium dichromate	1	3.7	3	5.7	-	-	1	5.9	2	7.7	-	-	2	4.5	5	6.3	-	-
Paraphenylene diamine	-	-	2	3.8	-	-	-	-	1	3.8	-	-	-	-	3	3.8	-	-
Thiuram mix	-	-	-	-	-	-	-	-	1	3.8	-	-	-	-	1	1.3	-	-
Neomycin sulphate	1	3.7	-	-	-	-	-	-	-	-	-	-	1	2.3	-	-	-	-
Cobalt chloride	2	7.4	1	1.9	-	-	1	5.9	1	3.8	1	50	3	6.8	2	2.6	1	25
Nickel sulphate	6	22.2	8	15.1	-	-	4	23.5	8	30.8	1	50	10	22.7	16	20.3	1	25
Lyral[b]	1	3.7	-	-	-	-	-	-	-	-	-	-	1	2.3	-	-	-	-
Colophony	-	-	2	3.8	-	-	-	-	-	-	-	-	-	-	2	2.5	-	-
Parabens	-	-	-	-	-	-	-	-	1	3.8	-	-	-	-	1	1.3	-	-
Textile disperse dye mix	-	-	1	1.9	-	-	-	-	1	3.8	-	-	-		2	2.5	-	-
Myroxylon pereirae	-	-	-	-	-	-	2	11.8	-	-	-	-	2	4.5	-	-	-	-
para-tertiary Butyl phenol-formaldehyde resin	-	-	-	-	-	-	2	11.8	-	-	-	-	2	4.5	-	-	-	-
Fragrance mix II	-	-	1	1.9	-	-	-	-	-	-	-	-	-	-	1	1.3	-	-
Formaldehyde	-	-	-	-	-	-	-	-	2	7.7	-	-	-	-	2	2.5	-	-
Fragrance mix I	1	3.7	-	-	-	-	-	-	1	3.8	-	-	1	2.3	1	1.3	-	-
Ethylene diamine dihydrochloride	-	-	1	1.9	-	-	-	-	-	-	-	-	-	-	1	1.3	-	-
Amerchol L 101	1	3.7	1	1.9	-	-	3	17.6	1	3.8	-	-	4	9.1	2	2.5	-	-
Thimerosal	-	-	-	-	-	-	-	-	2	7.7	-	-	-	-	2	2.5	-	-
Budesonide	1	3.7	-	-	-	-	-	-	-	-	-	-	1	2.3	-	-	-	-
Methyldibromo-glutaronitrile	-	-	1	1.9	-	-	1	5.9	1	3.8	-	-	1	2.3	2	2.5	-	-
Total, n	14		21		0		14		22		2		28		43		2	

[a] Results after patch testing with European baseline series.
[b] INCI name: hydroxyisohexyl 3-cyclohexene carboxaldehyde.
*Number of study persons.
n, number of positive patch test reactions; U, unknown history of childhood eczema.

Table 9. Numbers and proportion (%) of positive patch test reactions in the study persons according to contact sensitiser[a] and in the study group "all study persons"

	Exposed group (133*)						Unexposed group (72*)						All study persons (205*)					
Childhood eczema	Yes(50*)		No (79*)		U (4*)		Yes (31*)		No (37*)		U (4*)		Yes (81*)		No (116*)		U (8*)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Potassium dichromate	2	4.0	3	3.8	1	25	3	9.7	2	5.4	-	-	5	6.2	5	4.3	1	12.5
Paraphenylene diamine	-	-	2	2.6	-	-	-	-	1	2.7	-	-	-	-	3	2.6	-	-
Thiuram mix	-	-	-	-	-	-	-	-	1	2.7	-	-	-	-	1	0.9	-	-
Neomycin sulphate	1	2.0	-	-	-	-	-	-	-	-	-	-	1	1.2	-	-		-
Cobalt chloride	2	4.0	1	1.3	-	-	1	3.2	2	5.4	1	25	3	3.7	3	2.6	1	12.5
Nickel sulphate	8	16.0	8	10.4	-	-	5	16.1	8	21.6	1	25	13	16.0	16	13.8	1	12.5
Lyral[b]	1	4.0	-	-	-	-	-	-	-	-	-	-	1	1.2	-	-	-	-
Colophony	1	2.0	2	2.6	-	-	-	-	-	-		-	1	1.2	2	1.7	-	-
Parabens	-	-	-	-	-	-	-	-	1	2.6	-	-	-	-	1	0.9		-
Textile disperse dye mix	1	2.0	1	1.3	-	-	-	-	1	2.6	-	-	1	1.2	2	1.7	-	-
Myroxylon pereirae	-	-	-	-	-	-	2	6.5	-	-	-	-	2	2.5	-	-		-
para-tertiary Butyl phenol-formaldehyde resin	-	-	-	-	-	-	2	6.5	1	2.7	-	-	2	2.5	1	0.9		-
Fragrance mix II	-	-	1	1.3	-	-	-	-	-	-	-	-	-	-	1	0.9	-	-
Formaldehyde	-	-	-	-	-	-	-	-	3	8.1	-	-	-	-	3	2.6	-	-
Fragrance mix I	4	8.0	-	-	-	-	-	-	1	2.7	-	-	4	4.9	1	0.9	-	-
Ethylene diamine dihydrochloride	-	-	1	1.3	-	-	-	-	-	-	-	-	-	-	1	0.9		-
Amerchol L 101	7	14.3	2	2.6	-	-	5	16.1	2	5.4	-	-	12	14.8	4	3.5		-
Thimerosal	-	-	-	-	-	-	1	3.2	2	5.4	-	-	1	1.2	2	1.7	-	-
Tixocortol pivalate	-	-	-	-	-	-	1	3.2	-	-	-	-	1	1.2	-	-	-	-
Budesonide	1	2.0	-	-	-	-	-	-	-	-	-	-	1	1.2	-	-	-	-
Methyldibromo-glutaronitrile	-	-	1	1.3	-	-	1	3.2	1		-	-	1	1.2	2	1.7		-
Total, n	28		22		1		21		26		2		49		48		3	

[a] Results after patch testing with European baseline series.
[b] INCI name: hydroxyisohexyl 3-cyclohexene carboxaldehyde.
* Number of study persons. n, number of positive patch test reactions. U, unknown history of childhood eczema.

Comparing the number of contact allergies in the groups of unexposed and exposed participants, with and without a history of AD, we found the statistical results shown in Table 10.

Table 10.
Numbers of positive patch test reactions in the study groups, including statistical comparisons

Children	**Exposed group (51*)**			**Unexposed group (27*)**			**All children (78*)**		
Childhood eczema	Yes(23*)	No(26*)	U(2*)	Yes(14*)	No(11*)	U(2*)	Yes(37*)	No(37*)	U(4*)
n	14	1	1	7	4	0	21	5	1
p-value: Yes vs. No	***p* < 0.001**			*p* > 0.3			***p* = 0.002**		
p-value: Exposed vs. Unexposed	*p* > 0.3								
Adults	**Exposed group (82*)**			**Unexposed group (45*)**			**All adults (127*)**		
Childhood eczema	Yes(27*)	No(53*)	U(2*)	Yes(17*)	No(26*)	U(2*)	Yes(44*)	No(79*)	U (4*)
n	14	21	0	14	22	2	28	43	2
p-value: Yes vs. No	*p* > 0.3			*p* > 0.3			*p* > 0.3		
p-value: Exposed vs. Unexposed	***p* = 0.004**								
All study persons	**Exposed group (133*)**			**Unexposed group (72*)**			**All study persons (205*)**		
Childhood eczema	Yes(50*)	No(79*)	U(4*)	Yes(31*)	No(37*)	U(4*)	Yes(81*)	No(116*)	U(8*)
n	28	22	1	21	26	2	49	48	3
p-value: Yes vs. No	***p* = 0.013**			*p* > 0.3			*p* > 0.3		
p-value: Exposed vs. Unexposed	***p* = 0.004**								

* Number of study persons.
n, number of positive patch test reactions.
U, unknown history of childhood eczema.

In the study persons with a history of AD, significantly more contact allergy was found in three study groups than in those without: in the group of exposed children, in the whole group of children, and in the group of all study persons who were exposed (Table 10).

In the study persons exposed or unexposed to ASIT, independently of the history of AD, we found significantly more contact allergy in the group of unexposed adults and in the group of all unexposed study persons than in the groups of exposed adults and all exposed study persons (Table 10).

By comparing the numbers of positive reactions to the individual sensitisers patch tested in the study persons (Table 9), we found significantly more individuals with contact allergy to Amerchol L101 in the group of all study persons with a history of AD than in those with no history (12/81 vs. 4/116; $p = 0.007$).

Based on the multivariate linear regression analysis including gender, age, AD, and ASIT, age ($p = 0.006$), AD ($p = 0.024$), and ASIT ($p = 0.009$) were identified as significant independent predictors of the number of contact allergies. For age and AD there was more contact allergy, whereas for ASIT there was less.

6.2 Study II

Contact allergy to aluminium

Assessment of baseline values regarding the prevalence of contact allergy to aluminium revealed a significant difference between the exposed group and the unexposed group (0/133 vs. 4/72; $p = 0.01$). After one year of ASIT, 4 (3%) of the exposed individuals developed contact allergy to aluminium (0/133 vs. 4/133; $p = 0.12$), but this difference was still not statistically significant. Figures on contact allergy to aluminium are given in Table 11. Contact allergy to aluminium was diagnosed in 8 of 205 study participants (3.9%), four in the exposed group and four in the unexposed group (4/133 vs. 4/72; $p > 0.3$). 7 out of 8 tested positive to aluminium chloride hexaydrate 10.0% pet., and 5 tested positive to 2.0% pet. None of the aluminium-allergic subjects showed a positive reaction to the empty Finn chamber (Table 11). To diagnose contact allergy to aluminium, a second reading on D7 was necessary in 3 patients, 2 of whom had a doubtful reaction on D3. The median age of those who were contact-allergic to aluminium was 15.5 (range 9−31) years and that of those with no contact allergy to aluminium was 27.0 (5-74) years, with no significant difference ($p = 0.11$).

Comparison of the three randomised groups regarding contact allergy to aluminium did not show any statistically significant difference between those who had been patch tested with aluminium once, twice, or three times (2/44, 1/43, 1/46; $p > 0.3$).

Six of the eight aluminium-allergic individuals had AD, and 4/6 belong to the exposed group. Thus, contact allergy to aluminium was over-represented among the exposed individuals with AD (4/50 vs. 0/79; $p = 0.021$), which was not the case in the unexposed individuals (2/31 vs. 2/37; $p > 0.3$).

Table 11.
Results of patch tests and information concerning atopic dermatitis and self-reported deodorant intolerance in the 8 participants with contact allergy to aluminium (81)

			Patch test to aluminium							
Exposed	Sex, F/M	Age, years	2.0%[a]		10.0%[a]		Finn chamber		Atopic dermatitis	Deodorant sensitivity
			Day 3[b)]	Day 7 [b)]	Day 3 [b)]	Day 7 [b)]	Day 3 [b)]	Day 7 [b)]		
	F	18	+	+	++	++	-	-	Yes	No
	F	16	-	-	+	(+)	-	-	Yes	No
	M	12	(+)	+	(+)	+	-	-	Yes	No
	M	9	-	(+)	-	+	-	-	Yes	No
Unexposed	F	15	(+)	-	(+)	+	-	-	No	No
	F	13	+	+	++	+	-	-	Yes	No
	F	31	+	-	-	-	-	-	No	Yes
	F	27	+	+	++	++	-	-	Yes	Yes

(+) = doubtful patch test reaction; + and ++ = positive patch test reactions with an allergic morphology; - = negative patch test reaction.
[a] Aluminium chloride hexahydrate in petrolatum.
[b] Day of reading.
[c] If the result from the reading on D3 was questionable, a retest was conducted with aluminium chloride hexahydrate (20.0% pet.) on the same day and read only once, i.e. on D4.
F, female; M, male; NT, not tested.

Subcutaneous nodules and pruritus

Investigation by the physician before ASIT and after one year of treatment revealed a significant increase in the number of nodules (0/130 vs. 23/130; $p < 0.001$). Comparison of the number of nodules in unexposed individuals at baseline with the number of nodules in exposed individuals after one year of ASIT also showed a significant difference (0/72 vs. 23/130; $p < 0.001$). No association was found between AD and the development of nodules in the exposed individuals (9/39 vs. 18/61; $p > 0.3$).

In the exposed group, significantly more study persons reported pruritus and significantly more individuals were judged by the investigator to have scratch marks on at least one arm after one year of ASIT than at baseline (56/94 vs.12/94; $p < 0.001$; and 10/98 vs. 2/98; $p = 0.039$). Furthermore, a significant difference was found when we compared unexposed individuals having pruritus at baseline with exposed individuals having pruritus after one year of ASIT (0/68 vs.10/98; $p = 0.006$). Self-assessement of pruritus on the arms was also evaluated by comparison of the subgroups. Comparing unexposed individuals at baseline and exposed individuals after one year of ASIT, there was a statistically significant difference (4/50 vs. 56/94; $p < 0.001$). There was also a numerical association, but not a statistically significant one, between self-reported pruritus on at least one arm after one year of ASIT and AD (27/36 vs. 37/65; $p = 0.087$). Finally, a statistically significant association was found between investigator-assessed nodules and pruritus after one year of treatment *($p < 0.001$)* (Table 12).

Table 12.
Development av nodules and pruritus during one year of ASIT. The *p*-values were obtained from McNemar's exact test (intra-group comparisons) and from Fisher's exact test (inter-group comparisons)

Groups	Presence of Nodules				Presence of Pruritus							
	Baseline	After 1 year	*p*-value		Baseline		After 1 year		*p*-value			
			Intra	Inter					Intra[c]		Inter[d]	
					Sa[a]	Inv[b]	Sa[a]	Inv[b]	Sa[a]	Inv[b]	Sa[a]	Inv[b]
Exposed	0/130	23/130	< 0.001 (0/130 vs 23/130)		12/94	2/98	56/94	10/98	**< 0.001** (12/94 vs 56/94)	**0.039** (2/98 vs 10/98)		
Unexposed	0/72	NAe			4/50	0/68	NA[e]	NA[e]				
Exposed vs unexposed: i) Baseline vs Baseline				> 0.3 (0/72 vs. 0/130)							> 0.3 (4/50 vs. 12/94)	> 0.3 (0/68 vs. 2/98))
ii) Baseline vs 1 Year				**< 0.001** (0/72 vs. 23/130)							< 0.001 (4/50 vs. 56/94)	0.006 (0/68 vs. 10/98)

[a] Self-assessed
[b] Investigator-assessed
[c] Comparisons baseline vs after 1 year within the group of the exposed
[d] Comparisions between exposed (treated wiyh ASIT) and unexposed (not-treated with ASIT)
[e] NA not assessed

6.3 Study III

Patch testing

A summary of all patch test reactions and reactions to the intradermal testing is given in Table 13. In the 21 study participants, 15 (71%) had a positive reaction to at least one dilution of aluminium chloride hexahydrate. The responses were strongest on D3, but one individual only had a positive reaction to aluminium chloride hexahydrate on D7. Twelve of the 21 individuals (57%) had a positive reaction to the highest concentration of aluminium chloride hexahydrate 20.0% pet. and 4 of the 21 (19%) reacted to it at 2.0% pet.. Fourteen of the 21 (67%) were found to have contact allergy to aluminium chloride hexahydrate 10.0% pet. (Fig. 6). One of them had a positive reaction to this test preparation alone. Testing with aluminium lactate gave positive reactions in 13 of the 21 subjects (62%). It was the dilution at 7.7% that verified all these positive reactions. Both higher and lower concentrations of aluminium lactate gave fewer positive reactions. However, 10 of the 21 subjects (48%) had a positive reaction to the dilution at 2.4% (Fig. 6). Thus, significantly more individuals reacted to aluminium lactate 2.4% pet. than to aluminium chloride hexahydrate 2.0% pet. *(p = 0.03)*. Alum and aluminium hydroxide each gave positive reactions in 2 of the 21 individuals, aluminium phosphate in 1 of the 21, and aluminium acetotartrate in 3 of 7 individuals. None of the 21 individuals had a positive reaction to an empty Finn chamber. In all, none of the 6 aluminium compounds alone verified that all individuals had a contact allergy to aluminium. Aluminium chloride hexahydrate, aluminium lactate, and aluminium acetotartrate together showed that 19 of the 21 individuals (91%) had an aluminium allergy. Two of the 21 study participants did not react to any of the 6 aluminium compounds.

Intradermal testing

Of the 21 individuals who were previously diagnosed with aluminium allergy, 19 were tested intracutaneously: 1 out of 14 had a positive reaction to the lower concentration of aluminium chloride hexahydrate (1.0 mol/ml) and 2 out of 5 had a positive reaction to the higher concentration (10 mol/ml) (Table 13). These 3 patients were also positive in patch testing.

Controls

When 20 consecutive subjects were patch tested with 5 different aluminium compounds at the highest concentration, we noted that 1 patient had a positive reaction to aluminium acetotartrate 25.0% pet.. On comparison of the patients with regard to how many positive reactions they had to aluminium lactate at 24.0%, there

was a statistically significant difference between the group with previously known aluminium allergy and the controls (12/21 subjects vs. 0/20 controls; $p < 0.001$). There was no statistically significant difference either for alum 39.0% pet. (2/21 subjects vs. 0/20; $p > 0.3$), for aluminium hydroxide 6.5% pet. (2/21 subjects vs. 0/20; $p > 0.3$), for aluminium phosphate 10.0% pet. (2/21 subjects vs. 0/20 controls; $p > 0.3$), or for aluminium acetotartrate 25.0% pet. (3/7 subjects vs. 1/20 controls; $p > 0.3$). Aluminium chloride hexahydrate 20.0% pet. was not patch tested in the controls because it had been included in the baseline series at the department in Malmö since 2005

.

Table 13.
The results of patch testing[a] and intradermal testing[b]

Substance, Vehicle, MW	Concentration, %	Patient no.																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Aluminium chloride hexahydrate pet. (% w/w) $AlCl_3 6H_2O$ MW 241	20.0	++	-	+	++(+)	++	(+)	(+)	+	++	++	-	-	-	+[c]	++	(+)	-	(+)	+++	++	+++
	10.0	+[c)]	-	+	+(+)	++	(+)[c]	-	+	++	++	+	++	-	++[c]	++	(+)	-	-	++	++	+
	6.3	+	-	(+)	(+)	-	+[c]	-	+	+	(+)	(+)	-	-	+[c]	+	(+)	-	-	++	+	++
	2.0	(+)	-	+	(+)	-	(+)[c]	-	(+)	(+)	-	-	-	-	-	(+)	-	-	(+)	+	+	+++
	0.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	-	-	+	(+)	++
	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aluminium lactate pet. (% w/w) $Al(C_3H_5O_3)_3$ MW 294	24.0	++	-	+	++	-	+	-	+	-	-	(+)	-	(+)	++	++	+	-	++	++	++	++
	7.7	++	-	+	++	-	+	-	+	-	-	(+)	-	+	++	+	+	-	++	++	+	++
	2.4	+	-	+	+	(+)	+	-	+	-	-	-	-	(+)	++	-	(+)	-	+	+	+	+
	0.77	(+)	-	-	(+)	(+)	-	-	-	-	-	-	-	-	(+)	-	-	-	(+)	-	(+)	+
	0.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	-	-	(+)	-
Alum pet. (% w/w) $AlK(SO_4)_2$ $12H_2O$ MW 474	39.0	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	(+)	-	-	-	-	++
	12.0	(+)	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	++
	3.9	(+)	-	-	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	++
	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
	0.39	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
Aluminium hydroxide pet. (% w/w) $Al(OH)_3$ MW 78	6.5	-	-	-	-	-	-	-	-	-	-	-	++	-	+[c]	-	-	-	-	-	-	-
	2.1	-	-	-	(+)	-	-	-	-	-	-	-	(+)	-	++[c]	-	-	-	-	-	-	-
	0.65	-	-	-	(+)	-	-	-	-	-	-	-	(+)	-	+[c]	-	-	-	-	-	-	-
	0.21	-	-	-	(+)	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
	0.065	-	-	-	(+)	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
Aluminium phosphate pet. (% w/w) $AlPO_4$ MW 122	10.0	-	-	(+)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3.2	-	-	(+)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.0	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	(+)	-	-	-	-	-
	0.32	-	-	-	(+)	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
	0.1	-	-	-	(+)	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
Aluminium acetotartrate water (% w/vol) $C_6H_7AlO_8$ MW 234	25.0	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	+	-	+
	8.8	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	(+)	-	-
	2.9	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	-	-	(+)	-	(+)
	0.91	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	+	-	+	-	(+)
	0.29	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	(+)	-	+	-	(+)
Finn chamber		-	-	-	-	-	-	-	-	-	-	(+)	-	-	-	-	(+)	-	-	-	-	-
Aluminium chloride hexahydrate[d]	1 µmol/ml	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	NT	NT	NT	NT	NT	NT	NT
	10 µmol/ml	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	NT	-	-	NT	Pos	Pos

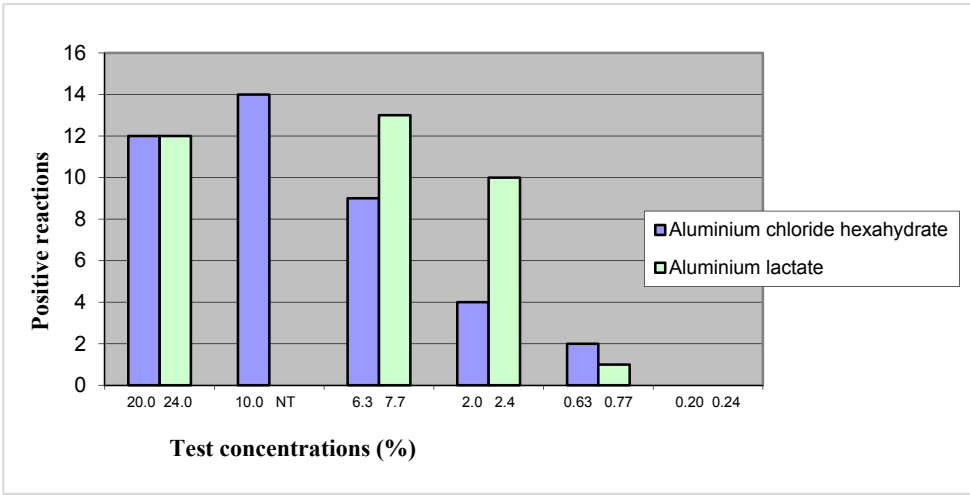
MW, molecular weight; NT, not tested; Pet., petrolatum; Pos, positive reaction. The strongest recorded reaction on D3 or D7 is given.

[a] Patch testing was performed with different aluminium compounds at concentrations equimolar to a dilution series of aluminium chloride hexahydrate, and an empty Finn chamber.

[b] Intradermal testing was performed with aluminium chloride hexahydrate in saline.

[c] The strongest reaction was recorded on D7. All other recorded reactions were strongest on D3.

[d] Used with intradermal testing.



NT, not tested.

Figure 6.
The number of positive reactions to aluminium chloride hexahydrate and to aluminium lactate at different equimolar concentrations.

6.4 Study IV

Test reactivity in all study persons on 4 test occasions

Twenty-one individuals completed the study. The results of the study participants' answers to the questionnaires on each test occasion are recorded in Table 15. MEC and STS, as calculated for all test reactions to aluminium chloride hexahydrate and to aluminium lactate on each test occasion, are shown in Table 16. Four of the 21 study persons did not respond with any positive reaction throughout this study, but 17 of the 21 had a positive reaction to at least one test salt on at least 1 test occasion (Table 16; Fig. 7). Eleven study participants did not react to aluminium chloride hexahydrate on at least 1 occasion (and up to a maximum of 3 occasions). Fifteen study participants did not show any reaction to aluminium lactate on 1 test occasion (and up to all test occasions). In 6 of the 21 subjects, contact-allergic reactions to aluminium chloride hexahydrate and/or aluminium lactate were seen on all 4 test occasions. Two out of the 6 had a positive reaction to both aluminium chloride hexahydrate and aluminium lactate on all 4 test occasions.

Differences in patch test reactivity

Twenty-one individuals went through 4 serial dilution tests with both aluminium chloride hexahydrate and aluminium lactate on 4 test occasions, i.e. 84 serial dilution tests with each test salt. Thirty-six of 84 serial dilution tests with aluminium chloride hexahydrate (43%) and 49 of 84 with aluminium lactate (58%) were negative. The range of reactivity varied between negative reactions to aluminium chloride hexahydrate 10.0% pet. and/or to aluminium lactate 12.0% pet. and a positive reaction to aluminium chloride hexahydrate 0.1% pet. and/or to aluminium lactate 0.12% pet. (Fig. 7). The highest individual difference noted in test reactivity to aluminium chloride hexahydrate/aluminium lactate was 320 times when comparing the 2 most divergent MECs (person nos. 10, 14, 15; Fig. 7). There were no statistically significant differences between the distribution of MEC and STS values on the 4 test occasions; nor did we find any significant difference by comparing test reactivity estimated as mean MEC and mean STS for both aluminium salts on the 4 test occasions. The results of the comparison between various study subgroups concerning MEC and STS values for both test salts, each separately, are shown in Table 17. Lower MEC values and at the same time higher STS values in one subgroup indicate stronger test reactivity compared to the other (i.e. opposite) subgroup. Comparing atopics and non-atopics, numerically—but not statistically—all MEC values for aluminium chloride hexahydrate and for aluminium lactate were lower and all STS values for both salts were higher in atopics than in non-atopics. The minimum MEC of aluminium chloride hexahydrate ($p = 0.03$) and of aluminium lactate ($p = 0.03$) and also the MEC of aluminium lactate on TA ($p = 0.045$) and TB ($p = 0.05$) were statistically significantly lower in atopics than in non-atopics. The STS for aluminium chloride hexahydrate on TB ($p = 0.04$) and TC ($p = 0.045$), the STS for aluminium lactate on TB ($p = 0.01$) and the sum of STS values for both salts ($p = 0.02$; $p = 0.01$) were statistically significantly higher in atopics than in non-atopics (Table 17). In individuals who had been treated with ASIT earlier as compared to those who had not been treated with it, we mainly found numerically (but not statistically significantly) lower MEC values and at the same time higher STS values for both salts (Table 17).

Concomitant reactivity to aluminium chloride hexahydrate and aluminium lactate

By pairwise comparison between the MEC values and between the STS values of both test salts on each test occasion, statistically significant strong correlations were found. Figure 8 shows the statistically significant strong correlation between the test reactivity to aluminium chloride hexahydrate and to aluminium lactate by using the means of MEC ($r_s = 0.86$; $p < 0.001$) and the means of STS ($r_s = 0.92$; $p < 0.001$), each averaged over the 4 tests.

Table 14.
Information on study participants

Partici-pant no.	Sex, F/M	Age, years	Time since diagnosis of aluminium allergy, in months	Patch test preparation in % petrolatum when diagnosed with aluminium allergy					
				$AlCl_3$ 6 (H_2O)				$Al(C_3H_5O_3)_3$	
				20	15	10	2	12	2.4
1	F	60	51	++	*	*	-	*	*
2	F	38	26	*	*	*	+	*	*
3	M	58	8	*	*	(+)	+	*	*
4	M	42	20	*	*	++	++	++	++
5	F	68	80	+	+	*	*	*	*
6	F	51	70	+	+	*	*	*	*
7	M	72	226	*	*	*	+++	*	*
8	F	31	13	*	*	+	+	-	-
9	F	70	69	*	*	*	+	*	*
10	F	41	44	++	*	*	++	*	*
11	M	29	48	+	*	*	-	*	*
12	F	52	178	*	*	*	+++	*	*
13	F	38	13	*	*	+	+	*	*
14	F	43	21	*	*	++	++	++	-
15	F	23	1	*	*	++	*	++	++
16	F	51	36	+	*	*	+	*	*
17	M	53	51	++	*	*	+	*	*
18	M	53	77	+++	-	*	*	*	*
19	F	35	70	++	++	*	*	*	*
20	M	62	91	+	*	*	*	*	*
21	F	68	214	*	*	*	+	*	*

$AlCl_3 6(H_2O)$, aluminium chloride hexahydrate; $Al(C_3H_5O_3)_3$, aluminium lactate; F, female; M, male.
* Not tested.

Table 15.
Information on the study participants according to questionnaire results

No.[a]	Anti-perspirants Yes/No	Rash by anti-perspirants	Atopy[b] Yes/No	Skin symptoms[c] during the study	ASIT[d] Yes/No	No. of additional vaccinations				Sun exposure ≥ 7 days				Menstruation during				Imm. med[f]
						before TA[e]	after TA[e] before TB[e]	after TB[e] before TC[e]	after TC[e] before TD[e]	before TA[e]	after TA[e], before TB[e]	after TB[e], before TC[e]	after TC[e], before TD[e]	TA[e]	TB[e]	TC[e]	TD[e]	
1	No	-[g]	Yes	Yes	No	5	3	1	0	No	Yes	No	No	Pm[h]	Pm[h]	Pm[h)]	Pm[h)]	No
2	Yes	Yes	Yes	Yes	Yes	0	0	0	0	No	No	No	No	No	Yes	No	No	No
3	Yes	Yes	No	Yes	No	2	0	0	0	No	No	No	No	Male	Male	Male	Male	No
4	Yes	Yes	Yes	Yes	No	1	2	0	0	No	No	Yes	No	Male	Male	Male	Male	No
5	Yes	Yes	No	Yes	No	0	0	0	0	No	Yes	No	No	Pm[h]	Pm[h]	Pm[h)]	Pm[h)]	No
6	No	-[g]	Yes	No	No	0	0	0	0	No	No	Yes	No	No	No	No	No	No
7	No	-[g]	Yes	No	No	Un[i]	0	0	0	No	No	No	No	Male	Male	Male	Male	No
8	Yes	Yes	Yes	Yes	Yes	2	0	0	0	No	No	No	No	No	No	No	No	No
9	Yes	Yes	No	No	No	6	0	0	0	No	No	No	No	Pm[h]	Pm[h]	Pm[h)]	Pm[h)]	No
10	Yes	Yes	Yes	No	Yes	0	0	0	0	No	No	No	No	IUD[j]	IUD[j]	IUD[j)]	IUD[j)]	No
11	Yes	No	No	No	No	Un[i]	0	0	0	No	No	No	No	Male	Male	Male	Male	No
12	Yes	Yes	Yes	No	Yes	3	0	0	0	No	No	No	No	No	No	No	No	No
13	Yes	Yes	Yes	No	No	5	0	0	0	No	No	No	No	IUD[j]	IUD[j]	IUD[j)]	IUD[j)]	No
14	Yes	No	Yes	No	No	Un[i]	1	0	0	No	No	No	No	No	No	No	No	No
15	Yes	No	Yes	Yes	Yes	5	0	0	0	No	Yes	Yes	No	Yes	No	Yes	Yes	No
16	No	-[g]	Yes	Yes	No	0	0	0	0	No	No	No	No	No	No	No	No	No
17	Yes	No	No	Yes	No	3	0	0	0	No	No	No	No	Male	Male	Male	Male	No
18	Yes	Yes	No	Yes	No	2	0	0	0	No	No	No	No	Male	Male	Male	Male	No
19	Yes	Yes	No	Yes	No	2	0	0	0	No	No	No	No	Un	No	No	No	No
20	Yes	No	No	No	No	Un[i]	0	0	0	No	No	No	No	Male	Male	Male	Male	No
21	Yes	No	Yes	Yes	Yes	1	0	0	0	No	No	No	No	Pm[h]	Pm[h]	Pm[h)]	Pm[h)]	No

[a] Study participant number. [b]Atopy: having had childhood eczema and/or having type 1 allergy. [c] mild to moderate skin symptoms such as eczema, rosacea, itch. [d]ASIT, allergen-specific immunotherapy. [e] TA, TB, TC, TD, test occasions A–D. [f] Imm. med, immuno-modulating medication. [g] -, no answer. [h] Pm, post-menopausal. [i] Un, unknown. [j] IUD, intrauterine device.

Table 16.
Patch test reactivity in all the study participants

MEC[a] (%) and STS[b] on test occasions A–D, strongest on D3 or D7, for dilution series of:																
	Aluminium chloride hexahydrate								Aluminium lactate							
No.[c]	TA[d] MEC[a]	TA[d] STS[b]	TB[e] MEC[a]	TB[e] STS[b]	TC[f] MEC[a]	TC[f] STS[b]	TD[g] MEC[a]	TD[g] STS[b]	TA[d] MEC[a]	TA[d] STS[b]	TB[e] MEC[a]	TB[e] STS[b]	TC[f] MEC[a]	TC[f] STS[b]	TD[g] MEC[a]	TD[g] STS[b]
1	3.2	2.5	3.2	3.5	3.2	2	10	2	1.2	7	3.8	2.5	1.2	5	1.2	2
2	3.2	4.5	3.2	2.5	1	5	3.2	3.5	0.38	7.0	> 12	1.5	3.8	3	1.2	4.0
3	> 10	1	3.2	2	> 10	1	10	1	3.8	1.5	3.8	2.0	> 12	1	> 12	1.5
4	> 10	1	> 10	0	10	2	> 10	0.5	> 12	0	12	1.5	> 12	0.5	12	1.5
5	> 10	0	10	1	> 10	0.5	> 10	0.5	> 12	0	> 12	0	> 12	0	> 12	0
6	> 10	0	3.2	2.5	> 10	0.5	> 10	0	> 12	0	> 12	0	> 12	0	> 12	0
7	1	3.5	> 10	1	3.2	2.5	10	1.5	3.8	4.0	3.8	2.5	> 12	1.5	12	1.5
8	> 10	0.5	10	1	10	1.5	10	2.5	> 12	0	> 12	0	> 12	1.5	> 12	0
9	> 10	1	> 10	0.5	10	1	> 10	0	> 12	1	> 12	0	> 12	0	> 12	0
10	0.1	11.5	3.2	4	3.2	3	0.1	7	0.12	9.5	> 12	1.5	> 12	1	0.38	5.5
11	1	5	10	1.5	3.2	2	> 10	0.5	> 12	1	> 12	0	3.8	2	12	1.5
12	0.32	10	0.32	9.5	0.1	10.5	0.1	13	0.38	10.5	0.38	8.5	0.38	11.5	0.38	11.5
13	1	2.5	0.32	8.5	3.2	2.5	0.32	9.5	3.8	2.5	1.2	4	> 12	0	1.2	3
14	3.2	4.5	0.1	12	3.2	5.5	> 10	0	3.8	2	1.2	5.5	0.38	5.5	> 12	0
15	3.2	6.5	10	2	> 10	0.5	10	1.5	0.12	10.5	1.2	3	> 12	1.5	> 12	0.5
16	1	4	10	2.5	3.2	2.5	> 10	0.5	> 12	1	12	2.5	12	2	3.8	2.5
17	3.2	3	3.2	2.5	3.2	4.0	3.2	4.5	> 12	3.0	> 12	1	12	1.5	3.8	2.5
18	> 10	1	> 10	0	> 10	0	> 10	0	> 12	0	> 12	0	> 12	0	> 12	0
19	> 10	0.5	> 10	0	> 10	0	> 10	0	> 12	0	> 12	0	> 12	0	> 12	0
20	> 10	0	> 10	0	> 10	0	> 10	0	> 12	0	> 12	0	> 12	0	> 12	0
21	> 10	0.5	> 10	0	> 10	0	> 10	0	> 12	0	> 12	0	> 12	0	> 12	0

[a] MEC, minimal eliciting concentration. [b] STS, summarised test score. [c] Study participant number; [d-g] Test occasions A, B, C, D.

Table 17.
Differences in MEC (minimal eliciting concentration) and STS (summarised test score) for aluminium chloride hexahydrate (AlC) and aluminium lactate (All) between subgroups

Test occasions (T) A, B, C, D	*p*-value[a]	Female (n[b] = 14) vs. Male (n = 7)				Atopics (n = 13) vs. Non-atopics (n = 8)				Additional vaccinations (n = 13) vs. No additional vaccinations (n = 5) (3 unknown)			
		AlC		All		AlC		All		AlC		All	
		MEC	STS	MEC	STS	MEC	STS	MEC	STS	MEC	STS	MEC	STS
TA	*p* =	≥ 0.3 10.5 vs. 12.0	≥ 0.3 11.3 vs. 10.4	0.26 9.9 vs. 13.2	≥ 0.3 11.8 vs. 9.5	0.08 9.1 vs. 14.1	0.10 12.7 vs. 8.1	**0.045** 8.9 vs. 14.5	0.10 12.8 vs. 8.1	≥ 0.3 10.1 vs. 7.9	≥ 0.3 9.4 vs. 9.7	≥ 0.3 9.7 vs. 9.1	≥ 0.3 9.5 vs. 9.4
TB	*p* =	0.15 9.6 vs. 13.9	0.08 12.7 vs. 7.6	≥ 0.3 10.6 vs. 11.8	≥ 0.3 11.7 vs. 9.6	0.14 9.4 vs. 13.6	**0.04** 13.2 vs. 7.4	**0.05** 9.0 vs. 14.3	**0.01** 13.6 vs. 6.8	≥ 0.3 9.9 vs. 8.5	≥ 0.3 8.9 vs. 11.2	0.17 8.4 vs. 12.3	≥ 0.3 10.0 vs. 8.3
TC	*p* =	≥ 0.3 10.5 vs. 12.0	≥ 0.3 11.6 vs. 10.1	≥ 0.3 10.5 vs. 11.9	≥ 0.3 11.4 vs. 10.3	0.08 9.1 vs. 14.1	**0.045** 13.1 vs. 7.6	≥ 0.3 10.2 vs. 12.3	0.08 12.9 vs. 7.9	≥ 0.3 9.9 vs. 8.6	≥ 0.3 9.1 vs. 10.5	≥ 0.3 9.5 vs. 9.4	≥ 0.3 9.6 vs. 9.3
TD	*p* =	≥ 0.3 10.5 vs. 12.1	≥ 0.3 11.5 vs. 10.1	≥ 0.3 10.7 vs. 11.6	≥ 0.3 11.0 vs. 11.9	0.14 9.4 vs. 13.6	0.10 12.8 vs. 8.1	0.09 9.2 vs. 13.9	0.12 12.7 vs. 8.3	≥ 0.3 9.5 vs. 9.6	≥ 0.3 9.4 vs. 9.9	≥ 0.3 10.2 vs. 7.8	≥ 0.3 8.9 vs. 11.1
MMEC[c]	*p* =	≥ 0.3 10.1 vs. 12.8		≥ 0.3 10.2 vs. 12.6		**0.03** 8.7 vs. 14.8		**0.03** 8.7 vs. 14.7		0.25 10.4 vs. 7.1		≥ 0.3 9.7 vs. 8.9	
SSTS[d]	*p* =		0.26 12.1 vs. 8.7		≥ 0.3 11.9 vs. 9.1		**0.02** 13.5 vs. 6.9		**0.01** 13.6 vs. 6.8		≥ 0.3 9.2 vs. 10.2		≥ 0.3 9.5 vs. 9.6

Test occasions (T) A, B, C, D	*p*-value[a]	ASIT (n = 6) vs. No ASIT (n = 15)				Atopics with ASIT (n = 6) vs. Atopics without (n = 7)			
		AlC		All		AlC		All	
		MEC	STS	MEC	STS	MEC	STS	MEC	STS
TA	*p* =	≥ 0.3 9.0 vs. 11.8	0.11 14.4 vs. 9.6	0.06 6.8 vs. 12.7	0.11 14.4 vs. 9.6	≥ 0.3 6.8 vs. 7.1	0.23 8.4 vs. 5.8	0.18 5.3 vs. 8.4	0.23 8.4 vs. 5.8
TB	*p* =	≥ 0.3 9.6 vs. 11.6	≥ 0.3 12.5 vs. 10.4	≥ 0.3 11.0 vs. 11.0	≥ 0.3 12.2 vs. 10.5	≥ 0.3 7.3 vs. 6.8	≥ 0.3 6.5 vs. 7.4	≥ 0.3 8.0 vs. 6.1	≥ 0.3 6.1 vs. 7.7
TC	*p* =	≥ 0.3 9.4 vs. 11.6	≥ 0.3 12.6 vs. 10.4	≥ 0.3 10.7 vs. 11.1	0.27 13.5 vs. 10.0	≥ 0.3 7.0 vs. 7.0	≥ 0.3 6.9 vs. 7.1	≥ 0.3 7.3 vs. 6.8	≥ 0.3 7.3 vs. 6.7
TD	*p* =	**0.04** 6.6 vs. 12.8	0.06 15.1 vs. 9.4	≥ 0.3 9.2 vs. 11.7	0.30 13.3 vs. 10.1	0.14 5.2 vs. 8.6	0.18 8.6 vs. 5.6	≥ 0.3 6.7 vs. 7.3	≥ 0.3 7.7 vs. 6.4
MMEC[c]	*p* =	≥ 0.3 9.4 vs. 11.6		0.13 7.7 vs. 12.3		≥ 0.3 7.3 vs. 6.8		≥ 0.3 5.8 vs. 8.0	
SSTS[d]	*p* =		0.13 14.3 vs. 9.7		0.10 14.6 vs. 9.6		≥ 0.3 7.8 vs. 6.3		≥ 0.3 8.1 vs. 6.1

[a] $p < 0.05$ was considered statistically significant (values shown in bold); [b] n, number of study participants; [c]MMEC, minimum of MEC during TA to TD; [d]SSTS, sum of STS during TA to TD.

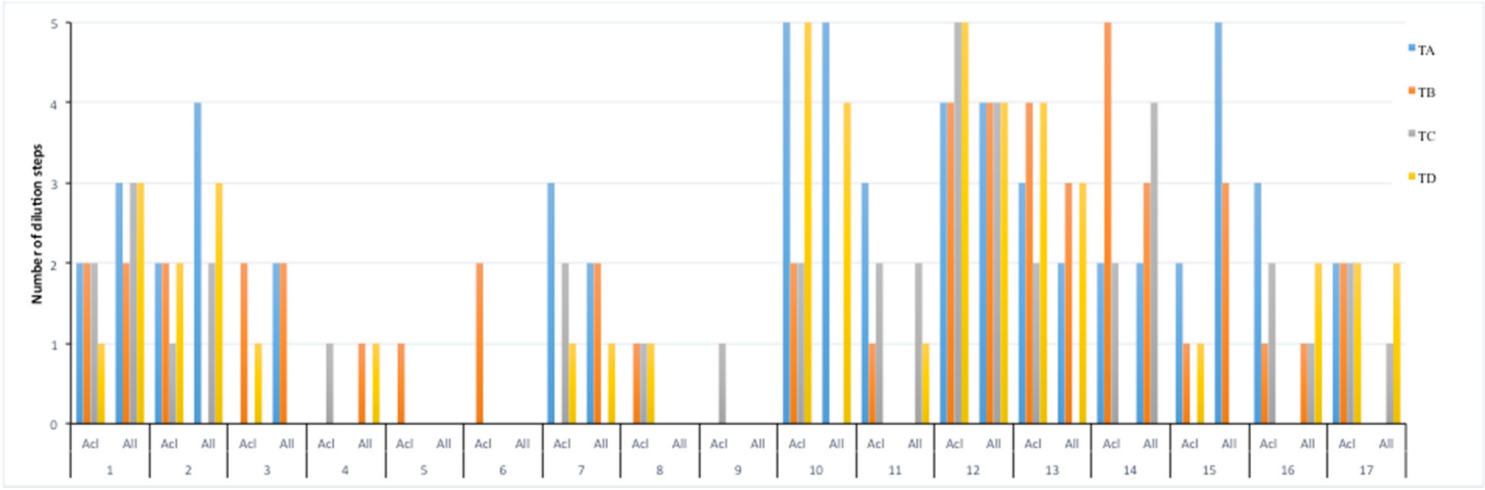
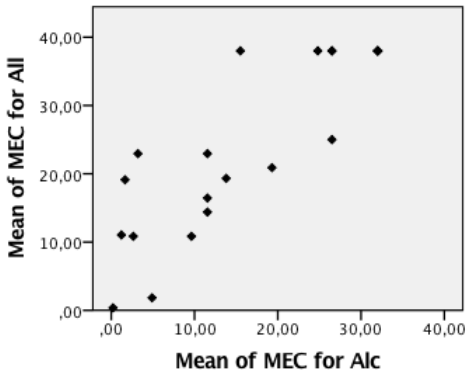
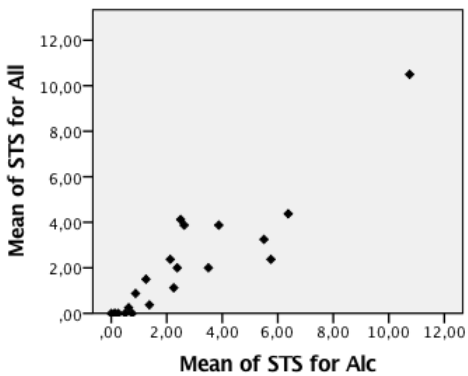


Figure 7.
Variation of aluminium patch test reactivity in 17 aluminium-allergic participants showing at least one positive reaction on four test occasions. The minimal eliciting concentration (MEC) is expressed as a scale of dilution steps of equimolar concentrations of aluminium chloride hexahydrate (Acl)) and aluminium lactate (All), for example, step 1 corresponds to Acl 10% pet. and to All 12.4% pet., and step 2 to Acl 3.2% pet. and All 3.8% pet. TA-TD, test occasions A-D.



r_s= 0.86

p< 0.0001



r_s= 0.92

p< 0.0001

Figure 8.
Concomitant patch test reactivity calculated as correlation between the means of MEC (minimal eliciting concentration) and STS (summarised test score) for aluminium chloride hexahydrate (Alc) and aluminium lactate (All) for each study participant, averaged over all four tests.

7. Discussion

7.1 Study I

The first aim of this research and the major aim of the study in paper I was to determine the presence of contact allergies in atopic individuals by patch testing with the European baseline series. The numbers of contact allergies were compared between study groups (children, adults, and all participants), with and without a history of AD, before and after treatment with ASIT over one year.

Two hundred and five atopic individuals suffering from AA and/or AR participated and finished the study. There were more children with a history of AD (50%) than adults (36%). The same prevalence of history of childhood eczema (50%) was reported by young individuals with atopic diseases such as AA and/or ARC who were patch tested with the European baseline series in another Swedish study (186). A prevalence of self-reported childhood eczema of 13.7% in adults was noted in a Swedish population-based study (21). The difference between children and adults regarding the prevalence of childhood eczema in this study may be explained by recall bias in the adults, which has also been shown in a prospective cohort study of children by Mortz et al. (187). At school age, the prevalence of AD was 34.1%; 15 years later, it was 23.6% in the same cohort. An explanation of the high prevalence figures for AD in this study (compared to population-based figures) may be the selected study population, consisting of individuals suffering from severe atopic diseases, and the fact that having a concomitant atopic disease such as AA and/or AR is one of the most important factors for remembering having had childhood eczema (187).

There is an on-going debate on whether individuals with AD have an altered prevalence or increased risk of contact allergy compared to non-atopic individuals (22, 188, 189)

In this study, the main findings were that age and childhood eczema were associated with an increased risk of becoming sensitised while ASIT is associated with a decreased risk. Previous studies have shown that contact allergies increase with age and exposure to contact sensitisers (190-192). Thus, age would be expected to be an independent risk factor for contact sensitisation. In comparing the number of positive patch test reactions in individuals with a history of AD and in those with no such

history, we found significantly more contact allergies in the study groups "exposed children", "all children", and "all exposed participants" (Table 10). Population-based, cross-sectional studies have found a positive and significant association between AD and contact sensitisation (188, 193). Also, studies based on selected populations, i.e. on patients who were referred because of suspicion of ACD, have demonstrated that AD is significantly associated with higher rates of positive patch test reactions, both in children (194, 195) and adults (196, 197). In the Swedish study mentioned above involving young individuals with atopic diseases such as AA and/or AR, contact allergy to at least one sensitiser was found in 14 of 30 subjects (47%) with a history of AD and in 5 of 31 subjects with no such history (16%) ($p = 0.013$) (186).

However, there have also been trials—epidemiological, clinical, and experimental—that have shown an inverse correlation between AD and contact allergy (198-201), which has been explained by an impaired cell-mediated immunity in patients suffering from AD. Today, it is known that innate immunity plays an important role in contact sensitisation and that both T_h1 and T_h2 are involved in ACD. Severe AD has been associated with both lower (198, 201) and higher numbers of contact allergies—and also with multiple sensitisations (22, 195, 202). Whether patients suffering from AD who have shown less contact allergies are truly not sensitised, or whether disease activity suppresses the elicitation of contact sensitisations at the time of patch testing, has not been clarified. Uehara et al. performed a sensitisation trial with dinitrochlorobenzene (DNCB) in patients with AD. Thirty-three per cent of 24 subjects with severe AD, 95% of 86 subjects with moderate AD, and 100% of 40 subjects with mild AD showed positive reactions to DNCB (198). After treatment of the AD in 20 subjects who did not react on the first occasion, 18 subjects (with now controlled dermatitis) showed positive reactions to DNCB. The authors concluded that the suppressed DNCB contact sensitisation seen in subjects with severe AD was a result of the skin disease rather than of the atopic constitution per se. Nevertheless, other trials have not detected any differences in frequencies of contact sensitisation between atopic and non-atopic patients (26, 203). In a recent systematic, Hamann et al. did not find an overall relationship between AD and contact sensitisation (189).

In paper I, the atopic individuals themselves were divided into subgroups to demonstrate the results of patch testing of individuals with a history of AD, and also to determine the frequency of contact allergy in atopics with only AR and/or AA. In 1992, Lammintausta et al. patch tested atopic subjects who were suffering from AD and those with only AR and/or AA (190). In the latter group, the rate of contact allergy was 25−30%, which is similar to our results (29%) and—in Lammintausta's report—was similar to that in healthy controls (25%). Netterlid et al. found contact allergy in 16% young atopic individuals suffering from AA and/or AR but with no history of AD (186).

Today, AD is mainly considered to be a disease of a disturbed skin barrier. The positive association between AD and contact allergy/ACD is often explained by repeated use of emollients and topical medications because of dry skin and inflammation (195, 204). In paper I, all individuals with a history of AD showed significantly more contact allergy to Amerchol L101 than individuals with no history of AD (Table 9). Amerchol L101 is produced from lanolin and is a common ingredient of many skin care products. Lanolin allergy is often associated with AD, and the prevalence of lanolin allergy has been shown to be higher in atopic individuals than in non-atopics (23, 194, 205, 206).

However, the most interesting and important result in paper I was that the number of contact allergies was significantly higher in the subgroups "unexposed adults" and "all unexposed study participants" than in those who were exposed, i.e. treated with ASIT for one year (Table 10). This observation can only be explained by an—to my knowledge—unknown immunological mechanism of ASIT, which suppresses the elicitation of contact allergy. It means that strongly positive patch test reactions may become weaker, moderately positive patch test reactions may turn to doubtful reactions, and weakly positive reactions may present without any morphological features suggestive of contact allergy, i.e. false-negative reactions.

Apart from avoidance of allergen, ASIT is the only curative therapy for treatment of AR and AA. The benefits of ASIT in patients with AD and type I sensitisation have been reported, but they are still controversial. In 2013, Bae et al. performed a systematic review and meta-analysis to assess the efficacy of ASIT in patients with AD (207). They concluded that ASIT, applied subcutaneously, has a significant efficacy as a long-term treatment (> 12 months) for severe AD. Recently, the Cochrane collaboration published a systematic review on "Specific allergen immunotherapy for the treatment of atopic eczema" (208). In the studies reviewed, children and adults who were allergic to house dust mites, grass pollen, and other inhaled allergens participated. Immunotherapy in these trials was administered subcutaneously, intradermally, orally, or sublingually. Three studies found better results in patients suffering from AD and from allergy against house dust mites (209-211). In one trial, children and adults were treated with ASIT subcutaneously (209); in the second one, children were treated with sublingually administered ASIT (SLIT) (210), and in the third one adults were treated with SLIT (211), all of them over one year. However, the Cochrane collaboration group stated that only limited evidence was found that ASIT may also be an effective therapy for AD. The quality of evidence was deemed to be low, mainly due to the low number of studies and participants included—and due to methodological concerns in the studies. Concerning the review done by Bae et al. (207) (see above), the Cochrane group judged that "the outcomes are due to unconventional approaches for extracting and combining data from the included trials" (208).

The immune mechanism of ASIT is not completely understood. Overall, it is described as a shift of immune response from a T_h2 to a T_h1 pattern, the generation of regulatory T-cells, a reduction of the ratio of allergen-specific IgE:IgG4, a reduction in mediator release, and probably an increased release of inhibitory mediators (91, 207).

According to the results of this study (paper I), there may be an explanation for the ability of ASIT to reduce symptoms of AD. At least in theory, the improvement may in part be due to a reduced capacity to elicit co-existing contact allergy. In the clinical appearance of eczema, the possible co-occurrence of both AD and ACD is a commonly acknowledged phenomenon (212).

Hyposensitising immunotherapy of ACD has been reported in both experimental and clinical trials (213). Animal studies have focused on the prevention of future sensitisation; clinical studies, however, have concentrated on reducing existing hypersensitivity. Animal studies have shown that extracutaneous administration of a hapten reduces the risk of sensitisation to a hapten by skin exposure. In humans, hyposensitisation to nickel was performed when nickel-allergic subjects ingested nickel-containing capsules over six weeks (214). This was the first double-blind, placebo-controlled study of nickel hyposensitisation. A decrease in patch test reactivity was observed. No conclusions about the effectiveness of nickel immunotherapy could be drawn.

In conclusion, paper I shows that contact allergy is common in atopic individuals, with significantly more contact allergies in those who have a history of AD. Patch testing should be considered in atopic individuals suffering from dermatitis that is difficult to control. Irrespective of there being a history of AD, atopic individuals with type I allergies treated with ASIT showed a significantly lower frequency of contact allergy than those who were not treated with ASIT. The question of whether ASIT not only induces an immunological hyposensitisation to allergens causing type I allergies, but also to allergens causing type IV allergies—and thus leading to an improvement in AD—can only be answered from further prospective, randomised controlled studies.

7.2 Studies II, III, and IV

The second aim of this work and the major aim of the study in paper II was to investigate whether ASIT with allergen extracts containing aluminium hydroxide as adjuvant induces contact allergy to aluminium and itching nodules in children and adults.

Four exposed participants showed contact allergy to aluminium after one year of ASIT, which is known to be one of the main routes of sensitisation to aluminium (97, 99, 100, 106, 112, 179, 215) besides vaccination (98, 101, 105, 109, 111, 114-116, 181) Even though this is a small number, the aluminium allergy is considered to be induced by ASIT since there were no patch test reactions to aluminium when the exposed group was tested with aluminium chloride hexahydrate 10% pet. at baseline before the start of ASIT. There was nothing to indicate that patch testing with aluminium during the trial influenced the development of contact allergy to aluminium.

To the best of my knowledge, there have been no other prospective, randomised controlled studies in the literature to which the incidence of aluminium allergy during ASIT can be compared. However, the previously mentioned retrospective study, performed by Netterlid et al., investigated young individuals with AA and/or AR who had been treated or had not been treated with ASIT. Contact allergy to aluminium was found in 8 of 37 exposed individuals (21.6%) but in none of the unexposed group (0/24; p =0.02) (112). A possible explanation for this difference may be that more injections led to a higher total dose of aluminium injected, and also later follow-up with respect to the start of ASIT. This is supported by the finding of at least three children who have been diagnosed with contact allergy to aluminium after the end of this study. However, aluminium allergy has been detected in very young children after only a few routine vaccinations (114-116, 166, 216). Bergfors et al. reported a prevalence of aluminium allergy of 95% in 2013 (114) and 85% in 2014 (115) in children with persistent itching nodules after aluminium-containing vaccination. However, the median age of the study participants at epicutaneous testing in those studies was 3.3 and 2.6 years, respectively. The median age of the children in the present study was 12 years. It has been suggested that age may play an opposite role in contact allergy to aluminium compared to other contact allergies, as discussed in paper I.

Surprisingly, contact allergy to aluminium was also diagnosed in 4 unexposed individuals (i.e. not treated with ASIT) before the start of ASIT. The control group was recruited to enable a blind patch test reading at the end of the study, and to investigate whether a source of aluminium exposure other than the allergen exposure could have induced aluminium allergy. It should be noted that the main comparison of the investigation was based on groups (i.e. exposed and unexposed) that were not randomised. Systematic differences between the groups with respect to environmental aluminium exposure, such as vaccination with aluminium-containing vaccines, cannot be excluded.

Nevertheless, paper II showed contact allergy to aluminium in 8 of 205 atopic individuals with AA and/or AR (3.9%). Six of those 8 reported having a history of

childhood eczema, four of the exposed aluminium-allergic individuals and two of the unexposed.

Whether the development of contact allergy to aluminium found in this study was due to ASIT, to previous vaccination, to a new exposure in the environment, or to some other reason can only be speculated on. One possible reason could be differences in the vaccines that had been given at different ages, as there was a tendency to be of lower age in the aluminium positive group (Table 11). In 1996, the pertussis vaccines containing aluminium hydroxide as an adjuvant were reintroduced in the Swedish vaccination program in combination with diphtheria and tetanus vaccine (81). Before 1996, except for large pertussis vaccine trials in 1991−1994, all the children in Sweden were offered a combined diphtheria and tetanus vaccine using aluminium phosphate as adjuvant. In the report from the Gothenburg area, all 352 children with contact allergy to aluminium had undergone vaccination with aluminium hydroxide-adsorbed vaccines (109). In 2002, the booster dose of the diphtheria-tetanus vaccine using aluminium phosphate was replaced with a vaccine using aluminium hydroxide as adjuvant (119). The use of other vaccines, such as hepatitis B vaccine and HPV vaccine, containing adjuvants based on aluminium hydroxide has increased in recent years (Table 2). One of the unexposed individuals who tested positive to aluminium had recently received three doses of HPV vaccine, and another unexposed patient with contact allergy to aluminium had received three doses of hepatitis B vaccine. Thus, it has been suggested that the different chemical and physical properties of aluminium hydroxide and aluminium phosphate—apart from differences in the given amounts of aluminium and possible differences in the manufacturing process of vaccines—may be a possible explanation for differences in the bioavailability of aluminium ions in the tissue, and thus for the increasing frequency of contact allergy to aluminium and even itching nodules (81, 147, 149). In some recent studies, it has been found that children with contact allergy to aluminium and persistent itching nodules had been immunised against pneumococcal infections with a new a vaccine containing aluminium phosphate as adjuvant (Table 2) (114, 115). However, it was not possible to determine whether there is a difference in causing contact allergy to aluminium and persistent itching nodules compared to vaccines containing aluminium hydroxide.

In paper II, a high number of exposed individuals developed subcutaneous persistent itching nodules at the injection sites and, compared to baseline, there was a statistically significant difference (0/130 vs. 23/130; $p < 0.001$). The proportion of persistent itching nodules in children found after routine vaccination has been reported to be 0.8% (115, 116). That the development and the frequency of persistent itching nodules is associated with the amount of aluminium used in different allergen extracts has been shown in a 3-year, prospective double-blind study of patients treated with ASIT (132). Theoretically, the development of nodules could be ascribed to either the ASIT or an environmental factor unrelated to ASIT.

However, the unexposed group investigated at the same time in a blind manner did not show any nodules, strongly suggesting that ASIT caused the nodules (Table 12). The development of nodules was not associated with the presence of AD in this study.

At baseline, pruritus, assessed indirectly by signs of scratching and registered by the investigator, was noted in a few individuals in both the exposed and unexposed groups (Table 12). During one year of ASIT, significantly more exposed individuals developed signs of scratching on the upper arms ($p = 0.039$). These scratch marks can be interpreted as signs of severe pruritus. Thus, regarding self-assessed pruritus a higher number of individuals in both the exposed group and the unexposed group noted having pruritus at baseline. A statistically significant increase in self-assessed pruritus in exposed individuals was found after one year of ASIT ($p < 0.001$). The higher number of individuals with pruritus after treatment could theoretically be explained by ASIT or an unknown cause unrelated to the therapy. However, the comparison between the exposed individuals treated for one year and the unexposed individuals just before the start of treatment regarding the presence of pruritus showed statistically significant differences, indicating that ASIT caused the pruritus. Furthermore, there was some indication that development of self-reported pruritus, but not investigator-assessed pruritus, on the upper arms was associated with atopic dermatitis (p=0.087).

In conclusion, paper II showed a proportion of atopic individuals with contact allergy to aluminium of 3.9%. The result does not necessarily imply that ASIT was a risk factor for induction of contact allergy to aluminium. Aluminium allergy was over-represented in young individuals and in those with AD. A significant development of nodules and pruritus was noted in atopic individuals during ASIT. We found weak evidence for the association between AD and the development of pruritus, but not the development of nodules.

If contact allergy is strongly suspected in an individual but the patch test reaction to the allergen in question is negative, a false-negative reaction must always be considered. As mentioned under section 1.5, there are many causes for a possible false-negative reaction—including a patch test concentration that is too low, unstable substances, and an inappropriate test chamber. Also, the very basic question of whether patch testing has been performed with the appropriate allergen compound should be considered. There may also be some "extrinsic" factors to which the individual to be tested is exposed, e.g. UV light, or some that are "intrinsic" to the individual being tested, e.g. hormonal changes, that may influence patch test reactivity and that may be a possible explanation for false-negative reactions (185, 217). Studies III and IV were performed to investigate these questions with regard to contact allergy to aluminium.

The third aim of this work and the major aim of the study in paper III was to compare various aluminium compounds and concentrations to find an optimal patch test concentration.

Traditionally, contact allergy to aluminium has been diagnosed by patch testing with aluminium chloride hexahydrate 2.0% pet. and an empty Finn chamber. However, different aluminium compounds that can be used in patch testing to demonstrate contact allergy to aluminium have been reported in the literature (Table 3). To trace contact allergy to aluminium, aluminium chloride hexahydrate 10.0% pet. was first recommended for patch testing by our research group at the Department of Occupational and Environmental Dermatology in Malmö (40, 167).

In paper III, the highest test concentration for patch testing with aluminium chloride hexahydrate was 20.0% pet. This seems a very high concentration compared to other allergens in the baseline series for routine patch testing. However, when aluminium chloride hexahydrate 20.0% pet. is patch tested (IQ chamber with 30 mg of preparation), the surface concentration of aluminium becomes 0.83 mg Al/cm^2. This is a quite high surface concentration, but it is in the same order of magnitude as when other metals are tested. Nickel sulphate hexahydrate ($NiSO_46H_2O$) 5% pet. is included in the Swedish baseline series. When this preparation is tested (small Finn chambers with 20 mg of preparation), the surface concentration of nickel used is 0.45 mg Ni/cm^2. On the other hand, this high concentration of aluminium chloride hexahydrate is not remarkable if we consider reports in the literature about treatment of axillary hyperhidrosis with antiperspirants containing aluminium chloride hexahydrate in concentrations in ethanol of up to 25−35% (218, 219).

Interestingly, the highest number of positive reactions was not noted for aluminium chloride hexahydrate at 20.0% pet. but at 10.0% pet. (Figure 6). Another aluminium salt, aluminium lactate, which, to the best of my knowledge, has never been used in patch testing before but which is also used as an ingredient of antiperspirants and toothpaste, showed a similar pattern of elicitation to that of aluminium chloride hexahydrate. More patients reacted positively to aluminium lactate 7.7% pet. than to 24.0% pet. (Figure 6). Concerning contact allergy, the dose of an allergen per unit skin area is one of the most important factors for both sensitisation and elicitation (64, 220). The aluminium allergen does not appear to work in this way. A possible explanation may be the astringent effect of aluminium salts. The test concentration of aluminium chloride hexahydrate at 20.0% and the equimolar concentration of aluminium lactate at 24.0% may impair penetration through the epidermis compared to the corresponding lower test concentrations.

However, the most surprising result in paper III was that significantly more patients reacted to aluminium lactate 2.4% pet. than to the equimolar concentration of aluminium chloride hexahydrate 2.0% pet. (4/21 vs 10/21; p = 0.031). We can only speculate about the reason for this result. Aluminium lactate is more lipophilic than

aluminium chloride hexahydrate, and might be able to penetrate the human skin easier than aluminium chloride hexahydrate.

Another interesting observation was made in this study. 8 of 21 subjects had previously been diagnosed as having a contact allergy to aluminium because of a positive reaction to aluminium chloride hexahydrate 2.0% pet.. Only three of these 8 subjects (38%) now showed a positive reaction at this concentration. This means that in 5 of the 8 subjects (63%), the aluminium allergy could not be verified with the same test concentration as used before. But 3 of 5 subjects who had earlier shown a positive reaction to aluminium chloride hexahydrate 2.0% pet. now showed positive reactions to higher concentrations of aluminium chloride. Finally, when including other aluminium compounds in patch testing, the aluminium allergy in these eight patients was reproducible. This phenomenon may indicate that the reactivity to aluminium decreases over time. This finding was also described in a follow-up study by Gente Lidholm et al. (110). One hundred and eighty-six of the 241 children (77%) had no reaction to aluminium chloride hexahydrate 2.0% pet., even though they had reacted positively to it several years before. Another explanation might be individual variation in patch test reactivity to aluminium, which has been demonstrated in nickel-allergic women, and this was investigated in study IV (185).

None of the subjects in paper III showed a positive reaction to an empty Finn chamber. Finn chambers are coated with polypropylene foil, which could explain the negative reactions. However, this was not the case in this study. It has been reported that individuals have shown positive reactions to an aluminium salt and, at the same time, have reacted positively or negatively to an empty Finn chamber made of elemental aluminium (101, 106, 112, 114-116, 167, 169, 180). There have been three other studies or case reports on simultaneous testing with aluminium chloride hexahydrate 2.0% pet., 10.0% pet., and with an empty Finn chamber (Table 18). Including the present study, 11 out of 288 subjects (3.9%) had a positive reaction to aluminium chloride hexahydrate 2.0% pet. and 30 of the 288 individuals (10.4%) reacted to 10.0% pet. In the whole population of 288, only one person (0.3%) showed a positive reaction to aluminium chloride hexahydrate 2.0% pet. and also showed a positive reaction to an empty Finn chamber (112). These results may support the hypothesis that only individuals with a strong contact allergy to aluminium have positive reaction to aluminium in its elemental form.

Table 18.
Studies/case reports on patch testing with aluminium chloride hexahydrate (Al Cl_3 $6H_20$) 2.0% pet. and 10.0% pet. and with an empty Finn chamber

Test preparation / Study or case report	$AlCl_3 6H_20$[a] 2.0% pet.	$AlCl_3 6H_20$[a] 10.0% pet.	Empty Finn chamber	No. of subjects with positive reaction/ study population
Paper III in this thesis	4/21	14/21	0/21	14/21
Bruze 2008 (167)	0/1	1/1	0/1	1/1
Netterlid 2009 (112)	2/61	8/61	1[b]/61	8/61
Paper II in this thesis	5/205	7/205	0/205	8/205
Total number of subjects with positive reaction	11/288	30/288	1/288	

[a] $AlCl_3$ $6H_2O$: aluminium chloride hexahydrate.
[b] This subject reacted to $AlCl_3 6H_20$ 2.0% pet. but not to $AlCl_3 6H_20$ 10.0% pet.

The intradermal test has previously been regarded as a valuable method to complement patch testing in diagnosing metal allergy (78, 79, 221), and also to be a more sensitive method (73, 76). Intracutaneous testing in the present study was performed with aluminium chloride hexahydrate at 1.0 µmol/ml in saline, a concentration that has been used in intradermal testing with other metal salts. We noted only 1 of 14 subjects with a positive reaction. The concentration of aluminium chloride hexahydrate was therefore increased to 10 µmol/ml for testing of the other 5 subjects. The higher concentration gave 2 positive reactions of the 5 possible (Table 13). These three individuals also showed positive reactions to aluminium chloride hexahydrate 2.0% pet. and/or to the equimolar concentration of aluminium lactate 2.4% pet. in patch testing. Thus, only 3 of 19 individuals with a strong contact allergy to aluminium could be confirmed by the intradermal test.

Regarding patch testing with the other aluminium compounds, only a few positive reactions to aluminium hydroxide and aluminium phosphate were noted. These aluminium compounds are most frequently used as adjuvants in allergen vaccines and other vaccines (81). Both salts are insoluble in water, which might be the reason for showing quite a low number of positive reactions. With aluminium acetotartrate we noted positive reactions in 3 out of 7 subjects, one of whom reacted to this compound only. Aluminium chloride hexahydrate, aluminium lactate, and aluminium actetotartrate together confirmed aluminium allergy in 19 out of 21 of the study participants (91%).

Aluminium lactate 12.4% pet., the concentration equimolar to aluminium chloride hexahydrate 10.0% pet., was not used in patch testing in paper III. After the study was performed, aluminium lactate at 12.4% pet. was added to the baseline series at our department in Malmö (Figure 9).

In conclusion, paper III showed that patch tests with aluminium chloride hexahydrate 2.0% pet. and an empty Finn chamber—and also the intradermal test with the salt

and doses used—are insufficient methods to demonstrate contact allergy to aluminium. None of the six aluminium compounds alone verified the previously diagnosed aluminium allergy in all 21 patients. Aluminium lactate 2.4% pet. gave significantly more positive reactions in patch testing than the equimolar concentration of aluminium chloride hexahydrate 2.0% pet.. Aluminium chloride hexahydrate 10.0% pet. gave the highest number of positive test reactions.

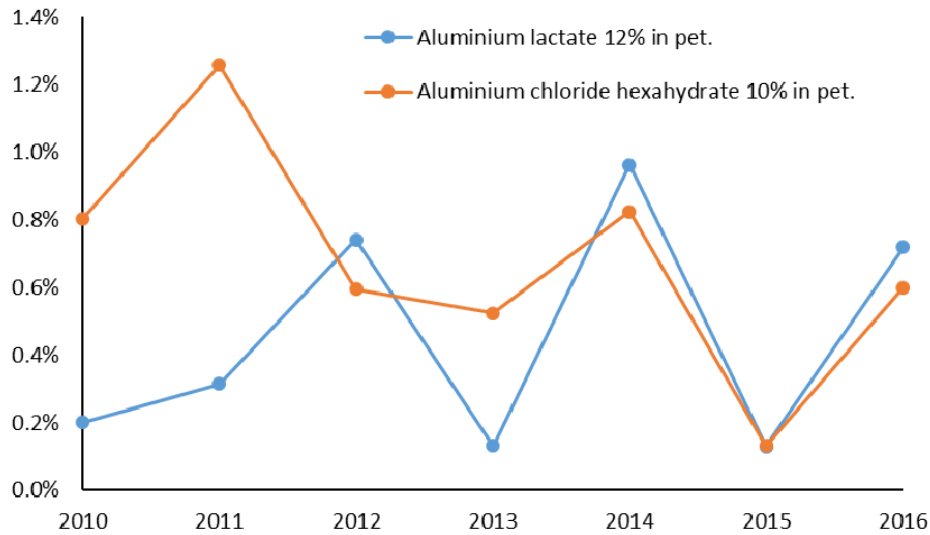


Figure 9.
Frequencies of contact allergy to aluminium chloride hexahydrate 10% pet. and to aluminium lactate 12.4% pet. at the Department of Occupational and Environmental Dermatology, Malmö, from 2010 to 2016.

The fourth aim of this work and the major aim of the study in paper IV was to investigate possible variation in patch test reactivity to aluminium chloride hexahydrate and to aluminium lactate over time, to compare the patch test reactivity between various subgroups (e.g. atopic/non-atopic individuals, treated with ASIT/not treated with ASIT), and to investigate whether there was a correlation between test reactivity to aluminium chloride hexahydrate and to aluminium lactate.

When patch testing all 21 aluminium-allergic study participants with serial dilutions of both test salts, we noted many negative reactions (Table 16; Figure 7), indicating poor patch test reproducibility. A contact-allergic reaction to at least one test salt could be reproduced on all 4 test occasions in only 6 of the 21 subjects. Fifteen of the 21 did not react to aluminium chloride hexahydrate and 19 of the 21 did not react to aluminium lactate on at least one occasion.

Hindsén et al. tested 30 nickel-allergic women and Rosholm Comstedt et al. tested 15 palladium-allergic women on 4 different occasions (185, 222). Two nickel-allergic

females and one palladium-allergic female did not react on one occasion. Other trials investigating the reproducibility of contact allergy to various metals found negative test results when retesting metal-allergic individuals. In 2001, Lee and Maibach published a review on patch test follow-ups years after the initial test (223). Concerning contact allergy to metals, the authors reported on gold sodium thiosulphate, nickel, and cobalt (221, 224, 225). However, the participants were retested only once and the frequency of positive reactions that could not be reproduced ranged from 4% to 42%. Björk et al. (226) retested persons with contact allergy to gold (n = 19) and to nickel (n = 12) with serial dilutions in triplicate applications. In all 19 gold-allergic subjects and in 11 out of 12 nickel-allergic subjects, contact allergy could be reproduced but the reproducibility varied with the degree of patch test reactivity. The authors found that the reproducibility decreased in a dose-dependent fashion when the previous reactivity had been low, e.g. a 1+ reaction.

Regarding contact allergy to aluminium, there have only been a few trials on reproducibility (110, 180). As mentioned before, Gente Lidholm et al. performed a follow-up study of children sensitised to aluminium after vaccination with an aluminium hydroxide-adsorbed vaccine. Aluminium allergy decreased significantly when retesting aluminium-allergic children after several years with aluminium chloride hexahydrate 2% pet.. One hundred and eighty-six of 241 retested children (77%) no longer reacted to aluminium. The authors observed that the chance of having a negative retest was higher with increasing age and a longer time interval between the first vaccination and the second patch test. The median age at retest was 13.3 years and the median time interval from first vaccination with a vaccine produced by Statens Serum Institute (Denmark) to retest was 11.9 years.

Interestingly, we found a similar tendency in our study. In those 4 subjects who only showed negative reactions (nos. 18−21), more than 60 months since diagnosis of aluminium allergy had passed (Table 14). Of the 17 subjects (nos. 1−17) who showed at least one positive reaction, there were only 5 individuals who had been diagnosed with aluminium allergy more than 60 months previously, but 12 subjects had been diagnosed with the allergy less than 60 months previously (4/0 vs. 5/12; $p = 0.02$). This result supports the notion that the time since a previous patch test has an effect on its reproducibility.

Thirteen of 21 participants had reacted positively to aluminium chloride hexahydrate 2.0% pet. earlier (Table 14). Nine of these 13 reacted positively on TA with a MEC for aluminium chloride hexahydrate of ≤ 3.2%, which is the closest concentration to 2.0% in this study. If these had been the only concentrations tested and if there had been only one retest, the aluminium allergy would have "disappeared" in 4 of the 13 subjects. In the 9 individuals who had positive reactions on TA, there were 4 with a positive reaction to aluminium chloride hexahydrate 3.2% pet. and 5 responded to

the concentrations < 3.2% pet.. Thus, the proportion of "loss of allergy" would have been 69% or 61%, respectively, if retesting had been performed on only one test occasion with a test concentration of 3.2% pet. or the concentrations < 3.2% pet.. This result would be similar to that of Gente Lidholm et al.—as far as comparisons are meaningful, in view of the limited sample size in the present study. Looking at all 4 test occasions, 10/13 individuals showed positive reactions to concentrations of aluminium chloride hexahydrate ≤ 3.2% pet. and 3/13 did not; 7/13 had positive reactions to concentrations of aluminium chloride hexahydrate < 3.2% pet. whereas 6/13 did not. Hence, the proportion of reproducibility of aluminium allergy increased with repeated patch testing in paper IV.

Considering the extent of exposure to aluminium, contact allergy to aluminium is rare, which is why this metal is considered to be a weak allergen (158). Another reason for not being able to detect aluminium allergy may be that there are false-negative reactions. As discussed previously, there are many reasons to explain a possibly false-negative patch test reaction such as individual variation in test reactivity, as has been shown in both nickel-allergic and palladium-allergic subjects (185, 222). The variation in individual test reactivity to both aluminium salts in 17 participants of this study can be seen in Figure 7. Thirty-six of 84 serial dilution tests of aluminium chloride hexahydrate (43%) were negative, and 49 out of 84 with aluminium lactate were negative (58%). The highest intra-individual difference, assessed as MEC for both test salts, was 320-fold. This intra-individual variation is in the same order of magnitude as the factor of 250 found in nickel-allergic women by Hindsén et al. (185).

The possible causes of variation in test reactivity, e.g. methodological and immunological factors, have been discussed in the literature (185, 222, 226, 227). Our study was designed to reduce or eliminate these factors. The same experienced personnel prepared and applied all patch tests, and the same experienced dermatologists read all tests in a blind way. Regional differences have been shown in duplicate patch test studies (228, 229). We chose symmetrical parts of the patient's upper back as the test region, as it is the preferred site. The serial dilution tests were applied in random order according to a Latin square, which made blind reading possible. Recently, Björk et al. confirmed previous findings that there is a high reproducibility between the right and left sides of the upper back (226). A more crucial factor that may have influenced test reactivity is the vehicle used in this study: petrolatum. Aluminium lactate is insoluble in water at a concentration of 12.4%. Petrolatum was therefore used as vehicle for the aluminium patch tests in paper IV. Since polar substances such as metal salts are insoluble in petrolatum, uneven distribution of the salts may have occurred (222). Consequently, concentration gradients in the test preparations may have affected further dilution—and through this, also variation in test reactivity. However, we consider it unlikely that this possible factor would account for more than a minor part of the variation in test

reactivity shown in this study. It is known that patch testing during summertime can result in weaker test reactions, which means that a strong patch test reaction in wintertime may become weaker in summertime (217). We started this trial in the autumn—but because of the methodological set-up, we also had to patch test the participants during late spring. However, no statistically significant differences in test reactivity (assessed as MEC and STS for both salts) during the time of the study could be found. No single factor could be found that would explain the variation in patch test reactivity to aluminium documented in paper IV.

When comparing patch test reactivity between various subgroups, the results suggested a stronger aluminium allergy, i.e. a lower MEC and a higher STS, in atopic individuals than in non-atopic individuals (Table 17). None of the volunteers in this study were suffering from severe eczema during patch testing. However, atopic subjects may have an abnormal skin barrier, which probably facilitates the penetration of the allergen, especially of water-soluble metal salts, and may be one explanation for a stronger allergic reaction (204). A similar result has been found when comparing subjects treated with ASIT and those who are not treated with it. Individuals treated with ASIT suffer from severe type I allergies, and thus also from severe atopic disease. As might be expected, since they are atopics treated with ASIT, they have a stronger allergy than those in the no-ASIT subgroup consisting of non-atopics, and atopics who are not treated with ASIT and who probably have a weaker atopic disease than those treated with ASIT.

In paper III, significantly more volunteers reacted positively to aluminium lactate 2.4% pet. than to the equimolar concentration of aluminium chloride hexahydrate 2.0% pet. (p = 0.03). Aluminium lactate therefore seemed to be more suitable for patch testing, which could not be confirmed in the present study. However, we found a strong correlation between aluminium chloride hexahydrate and aluminium lactate when analysing the concomitant test reactivity by comparing MEC, STS, and mean responses of the study persons investigated on each test occasion (Figure 8). These results suggest that it was the aluminium part that we were testing with in both the aluminium lactate and aluminium chloride hexahydrate preparations. However, the results in paper IV are not suitable for basing any new recommendations for patch testing with aluminium test preparations on.

In conclusion, aluminium-allergic individuals may have false-negative reactions, which is why retesting with aluminium should be considered when there is a strong suspicion of ACD caused by aluminium. Aluminium lactate may be as reliable a test salt as aluminium chloride hexahydrate when patch testing a person who is strongly suspected of having ACD caused by aluminium. The results of paper IV support the previous recommendation to use aluminium chloride hexahydrate 10% pet. for patch testing.

7.3 Clinical implications

In individuals suffering from **persistent nodules**, the severe itch is difficult to treat and may affect both the patient and his or her family for a long time. Topical steroids and/or a colloid bandage can be given to relieve the itching. In severe cases, intralesional steroid injections with (for example) the corticosteroid triamcinolone acetonide would be another treatment option.

It is important to spread knowledge about these subcutaneous nodules, to avoid anxiety and unnecessary diagnostic and therapeutic measures, e.g. surgery. Persistent itching nodules are not dangerous and are self-limiting, i.e. most of them disappear with time (115).

Individuals with **contact allergy to aluminium** may develop ACD when using aluminium-containing products such as antiperspirants, sunscreens, or other skin care products. They can also get skin problems after tattooing with aluminium-containing pigments. As far as I know, type I allergy to aluminium has not been reported, which is why anaphylactic reactions due to aluminium allergy would not be expected. Aluminium-containing vaccines are used in national childhood vaccination programs around the world to protect against dangerous diseases. Despite the research with other adjuvants, it is unlikely that aluminium salts will be replaced in the foreseeable future.

There are no recommendations to refrain from further **vaccinations** when a child suffers from itching nodules or has been sensitised to aluminium. By agreement with the parents/guardians, the vaccination may be postponed to a later date if the child still has significant symptoms from the nodules. However, all vaccines within the national vaccination program should be given before the child finishes school (Personal communication with Eva Netterlid at the Swedish Public Health Agency in May 2017).

Regarding **ASIT** in aluminium-allergic patients, allergen extracts that do not contain aluminium can be selected instead. In appropriate cases, the immunotherapy can be given sublingually.

Patients suspected of having contact allergy to aluminium should be investigated with a patch test. Based on the results of studies III and IV of this thesis, **patch testing** with aluminium chloride hexahydrate 2.0% pet. and an empty Finn chamber is insufficient to demonstrate contact allergy to aluminium. Patch testing with aluminium chloride hexahydrate 10.0% pet., which gave the highest number of positive aluminium reactions, is recommended in clinical practice. The reading of the patch test should always be done twice, i.e. on D3 or D4 and again on D7, in order to not miss clinically relevant contact allergy to aluminium. Aluminium-allergic

individuals may have false-negative reactions, which is why retesting with aluminium should be considered if there is a strong suspicion of contact allergy to aluminium.

8. Summary and concluding remarks

Contact allergy, defined as type IV allergy, and AD may be present in the same patient. The clinical manifestation of a contact allergy may appear as eczematous lesions mimicking AD. There is an on-going debate as to whether AD is associated with contact allergy, as the findings in the literature are controversial. The comorbidities of AD are AA and AR caused by IgE-mediated immunological reactions, defined as type I allergies. ASIT is the only causative treatment of type I allergy with a long-term effect. It is an immunomodulating therapy with allergen extracts adsorbed to an aluminium salt, and it is given as repeated subcutaneous injections in the upper arm—often over three years. In patients with both AD and type I allergies, beneficial effects of ASIT have been reported. Whether or not ASIT for type I allergy also influences type IV allergy is unknown.

Subcutaneous, persistent itching nodules at the injection site and contact allergy to aluminium are known side effects of ASIT. These side effects have also been reported after vaccination with aluminium-containing vaccines. ASIT and vaccinations are the main sensitisation routes in contact allergy to aluminium. The golden standard for diagnosis of contact allergy is patch testing. Aluminium allergy has traditionally been diagnosed by patch testing with aluminium chloride hexahydrate 2% pet. and an empty Finn chamber. Our research group at the Department of Occupational and Environmental Dermatology in Malmö has recommended increasing the test concentration to 10% pet., to minimise the risk of false-negative reactions. In general, contact allergy is considered to be lifelong. Studies on the reproducibility of patch testing—especially in metal allergies—have shown varied results. Negative reactions in repeated patch tests in subjects who are already known to have a contact allergy may be explained by individual variation in patch test reactivity, which may result in false-negative reactions.

In this thesis, I wanted to:

- determine the presence of contact allergies in individuals with AA and/or AR, and to determine whether there is an association between a history of AD and contact allergy
- investigate whether ASIT with allergen extracts containing aluminium hydroxide as adjuvant induces contact allergy to aluminium and itching nodules
- compare various aluminium compounds and concentrations to find an optimal patch test preparation
- investigate a possible variation in patch test reactivity to aluminium chloride hexahydrate and to aluminium lactate over time, and whether there is a association between the test reactivity to aluminium chloride hexahydrate and to aluminium lactate

Papers I and II were based on the same study population of atopic individuals with AA and/or AR, and scheduled to be treated with ASIT. Surprisingly, we found that the number of contact allergies was significantly higher in those not yet treated with ASIT compared to those treated with ASIT over one year. To my knowledge, this result has never been reported before and it may indicate that ASIT not only has an immunomodulatory effect on type I allergy but also on type IV allergy. Hypothetically, the effect of ASIT might be that the threshold for elicitation of contact allergy gets higher—which means that weak patch test reactions might no longer be detected.

This hypothesis can only be proved by more research, for example a prospective, controlled study with individuals who are treated or not treated with ASIT, and who get patch tested with the baseline series before and after therapy.

Fifty percent of children and 36% of adults reported having had AD. These individuals showed a significantly higher number of contact allergies than those who had never suffered from AD—a result, which supports the results of other studies that have shown an association between contact allergy and AD. The abnormal skin barrier in atopic individuals, which facilitates the penetration of low-molecular-weight substances such as contact sensitisers, may be an explanation—as well as the more frequent use of skin products in those who have a skin disease.

Contact allergy and AD may be present in one individual at the same time. Contact allergy may lead to a flare-up of AD. On the other hand, the clinical picture of ACD can be consistent with the pattern of AD also in individuals who have never had an AD (230, 231). There is a need for more research to clarify the role that exposure and/or contact sensitisation plays in AD. Clinicians should always consider patch testing in atopic individuals suffering from dermatitis, which is difficult to control.

In subjects treated with ASIT for one year, there was a significant increase in subcutaneous, itching nodules and pruritus relative to baseline. Neither the development of nodules nor that of pruritus was significantly associated with AD. Contact allergy to aluminium was found in exposed individuals after one year of treatment. However, the results do not support nor exclude the possibility that ASIT is a risk factor for aluminium sensitisation.

The results in paper II differed from the results in the Gothenborg studies (demonstrating a high frequency of nodules and contact allergy to aluminium) published by Bergfors et al. (109, 114, 115). One can only speculated about the reasons for these differences. In the Gothenborg studies, the median age at patch testing was under six years, as compared to 12 years in the children in our study. Another retrospective study done by our group also found a higher frequency of nodules and aluminium allergies in adolescents treated with ASIT over three years (112). The fact that at least three children were diagnosed with aluminium allergy after the study in paper II had finished supports the suggestion that a longer follow-up time might have led to less ambiguous results. After the report by Bergfors et al. in 2003, it was suggested that the reason for these unexpected results might be found in the manufacturing process of vaccines, since the pertussis vaccine was produced by one single manufacturer, or in the various physico-chemical properties of the aluminium salts (81). However, itching nodules and contact allergy to aluminium have recently been reported after immunisation with vaccines from different manufacturers (114, 115). Variations in the sensitisation capacity of aluminium hydroxide and aluminium phosphate, which are commonly used in vaccines and/or allergen extracts, could probably be investigated using sensitisation trials. To my knowledge, no such trials have ever been done.

In paper III, we did not find the highest number of positive reactors to aluminium at the highest test concentrations of aluminium chloride hexahydrate 20.0% pet. or aluminium lactate 24.0% pet., but to aluminium chloride hexahydrate 10% pet. It has been suggested that aluminium salts may have an astringent effect, hampering penetration of the sensitiser. Another explanation may be an immunomodulatory effect of aluminium itself. Furthermore, more subjects had positive reactions to aluminium lactate 2.4% pet. than to the equimolar concentration of aluminium chloride hexahydrate 2.0% pet.. It was not possible to reproduce the known aluminium allergy in all individuals using any of the six aluminium salts alone. It would be interesting to investigate whether there is any difference in skin penetration of the various aluminium salts using an animal model.

Another surprising result in paper III was the high number of negative reactions to the intradermal test, which contrasts with the test results using other metal salts, such as nickel and gold. Again, our results may be explained by an immunomodulatory effect of aluminium itself.

It has been reported that aluminium allergy may wane or disappear with time. In paper IV, a possible individual variation in patch test reactivity was investigated. The results showed an individual variation in test reactivity—including temporary negative reactions—to aluminium in aluminium-allergic individuals. We also found a strong association between test reactivity to aluminium chloride hexahydrate and to aluminium lactate. Clinicians should be aware that there might be false-negative reactions. Thus, when there is a strong clinical suspicion of aluminium allergy, retesting should be considered if the first test was negative.

Individuals who have been sensitised to aluminium may develop skin problems after the use of aluminium-containing products such as antiperspirants, sun protection preparations, and medications such as antiseptics and eardrops. Questions regarding the clinical relevance of contact allergy to aluminium have not been investigated in this thesis, but remain to be answered. Can an ACD be elicited by airborne aluminium in an aluminium-allergic individual—for example, by industrial work? Can systemic ACD, e.g. hand eczema, be elicited through intake of aluminium-containing drugs? These questions can only be answered after performing controlled studies with both aluminium-allergic and non-allergic participants.

9. Popular scientific summary in Swedish

Med kontaktallergi betecknas en sen allergisk reaktion som klassificeras som typ IV reaktion. Att vara kontaktallergisk mot ett ämne betyder att man blivit sensibiliserad för ämnet vid tidigare kontakt och då utvecklat den immunologiska intoleransreaktionen. En förnyad kontakt med ämnet kan leda till en hudreaktion hos den kontaktallergiska personen, vilket oftast yttra sig som ett eksem och kallas för kontaktallergiskt eksem. Misstänkta kontaktallergier utreds med hjälp av epikutantestning. Små kammare fyllda med de allergenberedningar som önskas testas fästs på patientens rygg. Testerna avlägsnas efter 48 timmar och eventuella hudreaktioner avläses efter 3 eller 4 dagar och andra gången efter 7 dagar. Utredningen kan kompletteras med en intradermal test som framförallt används vid misstänkta metallallergier. En liten mängd av en lösning av metallsalt injiceras i huden; en eventuell hudreaktion avläses efter 3 dagar. Hos 27% av den europeiska befolkningen förekommer kontaktallergi mot åtminstone ett allergiframkallande ämne.

Det finns många olika typer av eksem. Det vanligast förekommande är böjveckseksem som också kallas för atopiskt dermatit (AD). Up till 20% av barnen och up till 10% av vuxna i industrialiserade länder lider av AD. Förutom att de lider av AD kan de också besväras av allergisk astma (AA) och/eller allergisk rinokonjunktivit (AR). Både AA och AR är manifestationer av IgE-medierade allergier, så kallade typ I eller slemhinneallergier. Personer med IgE-medierade allergier kallas för atopiker. En kontaktallergi kan trigga AD och ett kontaktallergiskt eksem kan imitera AD. En bakomliggande kontaktallergi bör alltid misstänkas vid sviktande behandling av AD.

Sedan många år pågår det en diskussion bland forskare och läkare huruvida atopiker som lider eller har lidit av AD har högre risk att utveckla kontaktallergi jämfört med icke-atopiker. Det finns studier som bekräftar hypotesen och andra som förkastar den, men även studier som inte visar någon skillnad.

Förutom undvikandet av allergenet finns det för atopiker som lider av svår AA och/eller AR bara en kausal behandling med långvarig positiv effekt: allergen specifik immunoterapi (ASIT), även kallat hyposensibileringsbehandling eller allergivaccination. Vid den traditionella ASIT ges upprepade injektioner av

allergenextrakt under 3 års tid. De allergenextrakt som huvudsakligen används idag och även de flesta vacciner innehåller aluminiumsalter som ett hjälpmedel, även kallat adjuvans, för att förstärka det immunologiska svaret och för att minska på mängden allergen.

Det har visat sig att både ASIT och vaccinationer är huvudorsaken till sensibilisering för aluminium och således utveckling av kontaktallergi mot aluminium. Aluminium är en av de vanligast förkommande metallerna och finns t.ex. i läkemedel, dentala material, kosmetika och solskyddsmedel. Sedan 80-talet har enstaka individer med aluminiumallergi och kvarstående kliande knutor efter vaccination med aluminiuminnehållande vacciner eller aluminiuminnehållande allergenextrakt rapporterats. Både kvarstående kliande knutor och aluminiumallergi ansågs vara sällsynta. Under 1990-talet utfördes i Göteborgsområdet flera kliniska prövningar av ett nytt kikhostevaccin. Vaccinet innehöll aluminiumhydroxid som adjuvans. 645/76 000 (0,8%) vaccinerade barn utvecklade långvariga kliande knutor i huden. Dessa barn erbjöds epikutantestning med aluminium. 352/ 455 (77%) barn med kliande knutor i huden var kontaktallergiska mot aluminium.

De övergripande syftena med avhandlingen har varit

- att kartlägga kontaktallergier hos atopiska patienter med och utan eksem i barndomen, före och efter hyposensibiliseringsbehandling.
- att undersöka om ASIT med aluminiuminnehållande allergenextrakt under ett år inducerar kontaktallergi mot aluminium och kliande knutor.
- att genom kliniska studier tillföra ökade kunskaper om utredning av kontaktallergi mot aluminium och hur kontaktallergiska aluminium-reaktioner varierar över tid.

Delarbeten I och II utgår från samma studiepopulation med barn och vuxna som har svår AA och/eller AR och därför får behandling med ASIT. I delarbete I visas att antalet kontaktallergier är fler hos de individer som ännu inte påbörjat ASIT vilket antyder att ASIT inte enbart påverkar typ 1 allergier utan möjligen också påverkar intensiteten av befintliga kontaktallergier med följd att de inte kan detekteras vid epikutantestningen. Intressant är fynden att det är de allergiska individerna som förutom astma och/eller rinit även har atopisk dermatit som visar ett högre antal kontaktallergier jämfört med dem som inte har atopisk dermatit. I delarbete II påvisas starkt samband mellan ASIT och uppkomsten av kliande knutor och klåda. Aluminiumallergi påvisades efter ett års behandling med ASIT men någon ökning av aluminiumallergi kunde inte noteras under den korta observationstiden. Studien kunde varken påvisa eller utesluta att ASIT är en riskfaktor for utveckling av aluminiumallergi.

Kontaktallergi mot aluminium har huvudsakligen diagnostiserats genom epikutantestning med saltet aluminiumkloridhexahydrat i koncentrationen 2.0%. I delarbete III epikutantestades redan aluminiumallergiska frivilliga med sex olika aluminiumsalter i spädningsserier och dessutom utfördes intradermal testning. Resultaten är anmärkningsvärda eftersom flest kontaktallergiska reaktioner inte erhölls med de högsta epikutantestkoncentrationerna. Dessutom reagerade endast ett fåtal individer positivt vid den intradermala testningen vilket skiljer sig från testning med andra metaller som nickel, krom och guld. Dessa resultat är sannolikt uttryck för att aluminium förutom adjuvanseffekten vid vaccination även har en immunologisk påverkan på den allergiska reaktionen. Bäst och likvärdiga testresultat erhölls med aluminiumkloridhexahydrat och aluminiumlaktat.

Det har rapporterats att kontaktallergin för aluminium kan försvinna efter några år. Dessa fynd skulle också kunna vara resultatet av en variation i testreaktivitet över tid. Denna hypotes undersöktes i delarbete IV där aluminiumallergiska individer testades med spädningsserier av två aluminiumsalter vid fyra olika tillfällen under ett knappt år. Hypotesen bekräftades. En positiv reaktion för aluminium kan följas av en negativ vid nästa testtillfälle för att återigen vara positiv vid ett tredje testtillfälle.

Sammantaget har resultaten av de olika delarbetena stor betydelse för förståelsen av biverkningar vid ASIT och för den nationella och internationella diagnostiken av kontaktallergi och allergiskt kontakteksem för aluminium. Delarbete II fastslår att ASIT ger kliande knutor och klåda. Påvisandet att atopiska individer som behandlas med ASIT demonstrerar färre kontaktallergier visar på en möjlig framtida behandlingsmetod för individer med eksem med en multifaktoriell bakgrund. Resultaten av delarbete III innebär en ny rekommendation beträffande testning med aluminium med ökad möjlighet att diagnostisera aluminiumallergi som följd. Konsekvensen av delarbete IV är att man vid misstanke på aluminiumallergi och negativt testutfall bör överväga testa om patienten.

10. Acknowledgements

I would like to express my sincerest thanks to all those who have helped and supported me in my work for this thesis. I am particularly grateful to:

Magnus Bruze, my supervisor, for always believing in me, for always being positive, optimistic, and supportive—and for your consistent encouragement and guidance over the years. You have inspired me by your enthusiasm and your scientific knowledge. I appreciate it a great deal that you have always been accessible from anywhere in the world. I am very grateful to be part of your research group.

Monica Hindsén, for always being helpful and supportive, and for your encouragemant, optimism, and friendship over the years. I thank you for helping me with enrolling and patch testing volunteers in all the studies.

Erik Zimerson and Martin Mowitz, for your skilful guidance and helpfulness with everything concerning chemistry, for your constructive support in performing studies, and for being critical readers of the manuscripts.

Eva Netterlid, for invaluable help and support—especially during the writing process—and for advice and friendship.

Nuray Güner, for skilful help with the statistical analysis.

Lena Persson and Karin Olsson, for their kindness and skilful technical assistance.

Åke Svensson, Gustav Christensen, and Marléne Isaksson, for supporting me and giving me the opportunity to complete my work.

All my colleagues and friends at the Department of Occupational and Environmental Dermatology and at the Department of Dermatology and Venereology in Malmö and in Lund, for always being helpful and supportive, and for all our conversations about medicine and life. My special thanks to *Lena Svensson* for always giving kind support in administrative matters.

All the volunteers who participated in the studies.

I would especially like to thank my friends who have been with me through various parts of my life: *Julie Christiansen, Ineze Hauksson, Meirav Holmdahl, Katarina Lundquist, Solveig Klingberg, Mia Soller, Björn* and *Marlene Wullt*. Thank you for just being there.

My parents, my sisters and brothers—for your love, support, and help in many ways.

Roger, for being helpful and supportive—especially with technical issues.

My children, *Fredrik, Philip, and Jakob*, for your love and understanding. You made me appreciate the essential things in life.

The financial support of the Skåne County Council Research and Development Foundation, Consul Th. C. Bergh Foundation, Hudfonden, and Trans-Ver-Sals Stipendium are gratefully acknowledged.

11. References

1 Johansson S G, Hourihane J O, Bousquet J, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001: **56**: 813-824.

2 Johansson S G, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Immunology* 2004: **113**: 832-836.

3 Averbeck M, Gebhardt C, Emmrich F, et al. Immunologic principles of allergic disease. *J Dtsch Dermatol Ges* 2007: **5**: 1015-1028.

4 Tanno L K, Calderon M A, Smith H E, et al. Dissemination of definitions and concepts of allergic and hypersensitivity conditions. *World Allergy Organ J* 2016: **9**: 24.

5 Kantor R, Thyssen J P, Paller A S, et al. Atopic dermatitis, atopic eczema, or eczema? A systematic review, meta-analysis, and recommendation for uniform use of 'atopic dermatitis'. *Allergy* 2016: **71**: 1480-1485.

6 Coca A, Cooke R. On the classification of the phenomena of hypersensitiveness. *J Immunol* 1923: **10**: 445-464.

7 Ring J. Erstbeschreibung einer 'atopischen Familien-Anamnese' im Julisch-Claudischen Kaiserhaus: Augustus, Claudius, Britannicus. *Der Hautarzt* 1985: **36**: 470-474.

8 Ring J. Atopy: condition, disease, or syndrome? In: *Handbook of Atopic Eczema*, 2nd edn, J Ring, B Przybilla and T Ruzicka (eds): Berlin Heidelberg, Springer-Verlag, 2006: 3-9.

9 Pirquet C V. Allergie. *Münchmed Wochenschr* 1906: **53**: 1457-1458.

10 Ishizaka K, Ishizaka T, Terry W D. Antigenic structure of gamma-E-globulin and reaginic antibody. *J Immunol* 1967: **99**: 849-858.

11 Johansson S G. Raised levels of a new immunoglobulin class (IgND) in asthma. *Lancet* 1967: **2**: 951-953.

12 Wide L, Bennich H, Johansson S G. Diagnosis of allergy by an in-vitro test for allergen antibodies. *Lancet* 1967: **2**: 1105-1107.

13 Papadopoulos N G, Agache I, Bavbek S, et al. Research needs in allergy: an EAACI position paper, in collaboration with EFA. *Clin Transl Allergy* 2012: **2**: 21.

14 Bieber T. Atopic dermatitis. *Ann Dermatol* 2010: **22**: 125-137.

15 Ballardini N, Kull I, Lind T, et al. Development and comorbidity of eczema, asthma and rhinitis to age 12: data from the BAMSE birth cohort. *Allergy* 2012: **67**: 537-544.

16 Mortz C G, Andersen K E, Dellgren C, et al. Atopic dermatitis from adolescence to adulthood in the TOACS cohort: prevalence, persistence and comorbidities. *Allergy* 2015: **70**: 836-845.

17 Christiansen E S, Kjaer H F, Eller E, et al. The prevalence of atopic diseases and the patterns of sensitization in adolescence. *Pediatr Allergy and Immunol* 2016: **27**: 847-853.

18 Hanifin J M, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockholm)* 1980: Suppl 92: 44–47.

19 Williams H C, Burney P G, Pembroke A C, et al. The U.K. Working Party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994: **131**: 406-416.

20 Stenberg B, Meding B, Svensson A. Dermatology in public health--a model for surveillance of common skin diseases. *Scand J Public Health* 2010: **38**: 368-374.

21 Bingefors K, Svensson A, Isacson D, et al. Self-reported lifetime prevalence of atopic dermatitis and co-morbidity with asthma and eczema in adulthood: a population-based cross-sectional survey. *Acta Derm Venereol* 2013: **93**: 438-441.

22 Clemmensen K K, Thomsen S F, Jemec G B, et al. Pattern of contact sensitization in patients with and without atopic dermatitis in a hospital-based clinical database. *Contact Dermatitis* 2014: **71**: 75-81.

23 Isaksson M, Olhardt S, Rådehed J, et al. Children with atopic dermatitis should always be patch-tested if they have hand or foot dermatitis. *Acta Derm Venereol* 2015: **95**: 583-586.

24 Bruze M, Conde-Salazar L, Goossens A, et al. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis* 1999: **41**: 241-250.

25 Rustemeyer T, Van Hoogstraten I M V, Von Blomberg B M E, et al. Mechanisms of irritant and allergic contact dermatitis. In: *Contact Dermatitis*, 5th edn, D J Johansen, J P Frosch and J-P Lepoittevin (eds): Berlin Heidelberg, Springer-Verlag, 2011: 43-90.

26 Diepgen T L, Ofenloch R F, Bruze M, et al. Prevalence of contact allergy in the general population in different European regions. *Br J Dermatol* 2016: **174**: 319-329.

27 Diepgen T L, Coenraads P J. The epidemiology of occupational contact dermatitis. *Int Arch Occup Environ Health* 1999: **72**: 496-506.

28 Schwensen J F, Friis U F, Menné T, et al. One thousand cases of severe occupational contact dermatitis. *Contact Dermatitis* 2013: **68**: 259-268.

29 Brasch J, Becker D, Aberer W, et al. Contact dermatitis. *J Dtsch Dermatol Ges* 2007: **5**: 943-951.

30 Pongpairoj K, Ale I, Andersen K E, et al. Proposed ICDRG classification of the clinical presentation of contact allergy. *Dermatitis* 2016: **27**: 248-258.

31 Raap U, Stiesch M, Reh H, et al. Investigation of contact allergy to dental metals in 206 patients. *Contact Dermatitis* 2009: **60**: 339-343.

32 Menné T, Veien N K. Systemic contact dermatitis. In: *Textbook of Contact Dermatitis*, 3rd edn, R J G Rycroft, T Menné, P Frosch and J-P Lepoittevin (eds): Berlin Heidelberg, Springer-Verlag, 2001: 355-366.

33 Andersen K E, Hjorth N, Menné T. The baboon syndrome: systemically-induced allergic contact dermatitis. *Contact Dermatitis* 1984: **10**: 97-100.

34 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**: 423-4277.

35 Möller H, Ohlsson K, Linder C, et al. The flare-up reactions after systemic provocation in contact allergy to nickel and gold. *Contact Dermatitis* 1999: **40**: 200-204.

36 Hindsén M, Bruze M, Christensen O B. Flare-up reactions after oral challenge with nickel in relation to challenge dose and intensity and time of previous patch test reactions. *J Am Acad Dermatol* 2001: **44**: 616-623.

37 Biedermann T. Grundprinzipien von Allergie- und Intoleranzreaktionen. In: *Braun-Falco's Dermatologie, Venerologie und Allergologie*, 6th edn, G Plewig, M Landthaler, W Burgdorf, M Hertl and T Ruzicka (eds): Berlin Heidelberg, Springer-Verlag, 2012: 411-430.

38 Hjorth N, Roed-Petersen J. Occupational protein contact dermatitis in food handlers. *Contact Dermatitis* 1976: **2**: 28-42.

39 Meding B, Fregert S. Contact urticaria from natural latex gloves. *Contact Dermatitis* 1984: **10**: 52-53.

40 De Groot A C. *Patch Testing, Test Concentrations and Vehicles for 4350 Chemicals*, 3rd edn. Wapserveen, acdegroot publishing, 2010.

41 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.

42 Zimerson E, Bruze M. Sensitizing capacity of some trimers in p-tert-butylphenol-formaldehyde resin. *Contact Dermatitis* 2002: **47**: 40-46.

43 Lepoittevin J-P. Molecular aspects in allergic and irritant contact dermatitis. In: *Contact Dermatitis*, 5th edn, D J Johansen, J P Frosch and J-P Lepoittevin (eds): Berlin Heidelberg, Springer-Verlag, 2011: 91-109.

44 Röcken M, Biedermann T. Immunologische Grundlagen. In: *Braun-Falco's Dermatologie, Venerologie ind Allergologie*, 6th edn, G Plewig, M Landthaler, W Burgdorf, M Hertl and T Ruzicka (eds): Berlin Heidelberg, Springer-Verlag, 2012: 21-30.

45 Sonesson A. Aspects of microbial influence on skin disease. Department of Clinical Sciences Lund University, Faculty of Medicine Doctoral Dissertation Series: 2010:103, 2010. Lund.

46 Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. *Allergy* 2014: **69**: 3-16.

47 Strachan D. Siblings, asthma, rhinoconjunctivitis and eczema: a worldwide perspective from the International Study of Astma and Allergies in Childhood. *Clin Exp Allergy* 2015: **45**: 126-136.

48 Bruze M, Hedman H, Björkner B, et al. The development and course of test reactions to gold sodium thiosulfate. *Contact Dermatitis* 1995: **33**: 386-391.

49 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.

50 Lonsdorf A S, Enk A H. [Immunology of allergic contact dermatitis]. *Hautarzt* 2009: **60**: 32-41.

51 Flohr C, Johansson S G, Wahlgren C F, et al. How atopic is atopic dermatitis? *Journ Allergy Clin Immunol* 2004: **114**: 150-158.

52 Schultz Larsen M, Holm V, Henningsen K. Atopic dermatitis. *J Am Acad Dermatol* 1986: **15**: 487-494.

53 Weidinger S, Novak N. Atopic dermatitis. *Lancet* 2016: **387**: 1109-1122.

54 Liang Y, Chang C, Lu Q. The genetics and epigenetics of atopic dermatitis-filaggrin and other polymorphisms. *Clin Rev Allergy Immunol* 2016: **51**: 315-328.

55 Irvine A D, Mclean W H, Leung D Y. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med* 2011: **365**: 1315-1327.

56 Van Den Oord R A, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ* 2009: **339**: 1-12.

57 Homey B. Atopisches Ekzem. In: *Braun-Falco's Dermatologie, Venerologie und Allergologie*, 6th edn, G Plewig, M Landthaler, W Burgdorf, M Hertl and T Ruzicka (eds): Berlin Heidelberg, Springer-Verlag, 2012: 508-528.

58 Tourlas K, Burman D. Allergy Testing. *Prim Care* 2016: **43**: 363-374.

59 Bindslev-Jensen C. Skin tests for immediate hypersensitivity. In: *Contact Dermatitis*, 5th edn, D J Johansen, J P Frosch and J-P Lepoittevin (eds): Berlin Heidelberg, Springer-Verlag, 2011: 511-516.

60 Lachapelle J M, Maibach H I. *Patch Testing and Prick Testing. A Practical Guide Official Publication of the IRCDG*, 2nd edn. Berlin Heidelberg, Springer-Verlag, 2009.

61 Lachapelle J M. Historical aspects. In: *Contact Dermatitis*, 5th edn, D J Johansen, J P Frosch and J-P Lepoittevin (eds): Berlin Heidelberg, Springer-Verlag, 2011: 1-8.

62 Bruze M, Isaksson M, Gruvberger B, et al. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis* 2007: **56**: 281-285.

63 Lindberg M, Matura M. Patch testing. In: *Contact Dermatitis*, 5th edn, J D Johansen, J P Frosch and J-P Lepoittevin (ed)^(eds): Berlin, 2011: 439-464.

64 Frick-Engfeldt M, Gruvberger B, Isaksson M, et al. Comparison of three different techniques for application of water solutions to Finn chambers. *Contact Dermatitis* 2010: **63**: 284-288.

65 Johansen J D, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. *Contact Dermatitis* 2015: **73**: 195-221.

66 Svedman C, Isaksson M, Björk J, et al. 'Calibration' of our patch test reading technique is necessary. *Contact Dermatitis* 2012: **66**: 180-187.

67 Uter W, Gefeller O, Mahler V. Does a short patch test training course have an impact? *Contact Dermatitis* 2016: **74**: 317-319.

68 Fregert S. *Manual of Contact Dermatitis*, 2nd edn. Copenhagen, P J Schmidt, Vojens, 1981: 138.

69 Wilkinson D S, Bandmann H J, Calnan C D, et al. The role of contact allergy in hand eczema. *Trans St Johns Hosp Dermatol Soc* 1970: **56**: 19-25.

70 Frick M, Zimerson E, Karlsson D, et al. 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.

71 Mowitz M, Zimerson E, Svedman C, et al. Stability of fragrance patch test preparations applied in test chambers. *Br J Dermatol* 2012: **167**: 822-827.

72 Bruze M. Simultaneous patch test sensitization to 4 chemically unrelated compounds in a standard test series. *Contact Dermatitis* 1984: **11**: 48-49.

73 Epstein S. Contact dermatitis due to nickel and chromate; observations on dermal delayed (tuberculin-type) sensitivity. *AMA Arch Derm* 1956: **73**: 236-255.

74 Epstein S. Detection of chromate sensitivity: intradermal versus patch testing. *Ann Allergy* 1966: **24**: 68-72.

75 Herbst R A, Lauerma A I, Maibach H I. Intradermal testing in the diagnosis of allergic contact dermatitis. A reappraisal. *Contact Dermatitis* 1993: **29**: 1-5.

76 Marcussen P V. Comparison of intradermal test and patch test using nickel sulfate and formaldehyde *J Invest Dermatol* 1962: **40**: 263-266.

77 Gottmann-Lückrath I E G, Steigleder G K. Vergleichende Untersuchungen mit den Epi- und Intracutantest mit den Metallsalzen von Chrom, Kobalt, Kupfer und Nickel. *Arch Derm Forsch* 1973: **246**.

78 Möller H. Intradermal testing in doubtful cases of contact allergy to metals. *Contact Dermatitis* 1989: **20**: 120-123.

79 Christensen O B, Wall L M. Open, closed and intradermal testing in nickel allergy. *Contact Dermatitis* 1987: **16**: 21-26.

80 Bousquet J, Lockey R, Malling H J. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clinical Immunology* 1998: **102**: 558-562.

81 Netterlid E. Adverse reactions after vaccination and allergen-specific immunotherapy: contact allergy to aluminium and itching nodules. Department of Clinical Sciences, Malmö, Occupational and Environmental Dermatology Unit, Malmö, Lund University, Faculty of Medicine Doctoral Dissertation Series 2010:102, 2010. Lund.

82 Warner J O. A century of immunotherapy. *Pediatr Allergy Immunology* 2008: **19**: 569-570.

83 Cohen S G, Frankland A W, Dworetzky M. Noon and Freeman on prophylactic inoculation against hay fever. *J Allergy Clin Immunol* 2003: **111**: 1142-1150.

84 Noon L, Cantab B, Eng F. Prophylactic inoculation against hay fever. *The Lancet* 1911: **177**: 1572-1573.

85 Cohen S, Evans R I. Allergen immunotherapy in historical perspective. In: *Allergens and Allergen Immunotherapy*, 3rd edn, R F Lockey and D K Ledford (eds): New York, Informa Healthcare USA, 2008: 1-29.

86 Bruun E. Control examination of the specificity of specific desensitization in asthma. *Acta Allergol* 1949: **2**: 122-128.

87 SFFA (Svenska Förening För Allergologi). Allergenspecifik immunterapi. Rekommendationer för läkare och sjuksköterskor. Revidering av tidigare rekommendationer från år 2000. (Allergen-specific immunotherapy-recommendations for physicians and nurses - revised version of previous recommendations from year 2000), 2009.

88 Läkemedelsverket. Läkemedelsfakta (Medical products facts). Swedish. (Online): Läkemedelsverket (Medical Products Agency), 2017. Available at: http://www.lakemedelsverket.se//LMF/?rs=1&type=product; productname =Alutard. (last accessed 2 June 2017).

89 Cantani A. Specific immunotherapy. In: *Pediatric Allergy, Astma & Immunology,* U Heilmann (ed): Berlin Heidelberg, Springer-Verlag, 2008: 911-959.

90 Rak S. Mekanismerna vid allergen-specifik immunterapi klarnar. *Läkartidningen* 2008: **105**: 1935-1937.

91 Weller K, Soost S, Worm M, et al. Atopic dermatitis and allergic rhinitis-do co-effects in therapy exist? *J Dtsch Dermatol Ges* 2012: **10**: 221-236.

92 Lockey R F, Benedict L M, Turkeltaub P C, et al. Fatalities from immunotherapy (IT) and skin testing (ST). *J Allergy Clin Immunol* 1987: **79**: 660-677.

93 Malling H J. Minimising the risks of allergen-specific injection immunotherapy. *Drug Saf* 2000: **23**: 323-332.

94 Kim J M, Lin S Y, Suarez-Cuervo C, et al. Allergen-specific immunotherapy for pediatric asthma and rhinoconjunctivitis: a systematic review. *Pediatrics* 2013: **131**: 1155-1167.

95 Voss H, Tolki U. On an approximately one year old vaccine granuloma in a human. *Zentralbl Bakteriol* 1960: **178**: 291-299.

96 Linse R, Hadlich J, Kirsten D. [Cutaneous foreign body granulomas caused by aluminium-hydroxid after desensitization with Mischpollen-Depotallergen (author's transl)]. *Dermatol Monatsschr* 1979: **165**: 653-657.

97 Clemmensen O, Knudsen H E. Contact sensitivity to aluminium in a patient hyposensitized with aluminium precipitated grass pollen. *Contact Dermatitis* 1980: **6**: 305-308.

98 Fawcett H A, Smith N P. Injection-site granuloma due to aluminum. *Arch Dermatol* 1984: **120**: 1318-1322.

99 Frost L, Johansen P, Pedersen S, et al. Persistent subcutaneous nodules in children hyposensitized with aluminium-containing allergen extracts. *Allergy* 1985: **40**: 368-372.

100 Veien N K, Hattel T, Justesen O, et al. Aluminium allergy. *Contact Dermatitis* 1986: **15**: 295-297.

101 Böhler-Sommeregger K, Lindemayr H. Contact sensitivity to aluminium. *Contact Dermatitis* 1986: **15**: 278-281.

102 Cox N H, Moss C, Forsyth A. Allergy to non-toxoid constituents of vaccines and implications for patch testing. *Contact Dermatitis* 1988: **18**: 143-146.

103 Cosnes A, Flechet M L, Revuz J. Inflammatory nodular reactions after hepatitis B vaccination due to aluminium sensitization. *Contact Dermatitis* 1990: **23**: 65-67.

104 Hütteroth T H, Quast U. Aluminum hydroxide granuloma following hepatitis B vaccination. *Dtsch Med Wochenschr* 1990: **115**: 476.

105 Kaaber K, Nielsen A O, Veien N K. Vaccination granulomas and aluminium allergy: course and prognostic factors. *Contact Dermatitis* 1992: **26**: 304-306.

106 Lopez S, Pelaez A, Navarro L A, et al. Aluminium allergy in patients hyposensitized with aluminium-precipitated antigen extracts. *Contact Dermatitis* 1994: **31**: 37-40.

107 Hindsén M. Contact allergy to aluminium in patients hyposensitized with aluminium-containing hyposensitizing extracts. *Contact Dermatitis* 2005: **53**: 301-302.

108 Maubec E, Pinquier L, Viguier M, et al. Vaccination-induced cutaneous pseudolymphoma. *J Am Acad Dermatol* 2005: **52**: 623-629.

109 Bergfors E, Trollfors B, Inerot A. Unexpectedly high incidence of persistent itching nodules and delayed hypersensitivity to aluminium in children after the use of adsorbed vaccines from a single manufacturer. *Vaccine* 2003: **22**: 64-69.

110 Gente Lidholm A, Bergfors E, Inerot A, et al. Unexpected loss of contact allergy to aluminium induced by vaccine. *Contact Dermatitis* 2013: **68**: 286-292.

111 Bergfors E, Björkelund C, Trollfors B. Nineteen cases of persistent pruritic nodules and contact allergy to aluminium after injection of commonly used aluminium-adsorbed vaccines. *Eur J Pediatr* 2005: **164**: 691-697.

112 Netterlid E, Hindsén M, Björk J, et al. There is an association between contact allergy to aluminium and persistent subcutaneous nodules in children undergoing hyposensitization therapy. *Contact Dermatitis* 2009: **60**: 41-49.

113 Ozdén M G, Kefeli M, Aydin F, et al. Persistent subcutaneous nodules after immunotherapy injections for allergic asthma. *J Cutan Pathol* 2009: **36**: 812-814.

114 Bergfors E, Trollfors B. Sixty-four children with persistent itching nodules and contact allergy to aluminium after vaccination with aluminium-adsorbed vaccines-prognosis and outcome after booster vaccination. *Eur J Pediatr* 2013: **172**: 171-177.

115 Bergfors E, Hermansson G, Nyström Kronander U, et al. How common are long-lasting, intensely itching vaccination granulomas and contact allergy to aluminium induced by currently used pediatric vaccines? A prospective cohort study. *Eur J Pediatr* 2014: **173**: 1297-1307.

116 Salik E, Lovik I, Andersen K E, et al. Persistent skin reactions and aluminium hypersensitivity induced by childhood vaccines. *Acta Derm Venereol* 2016: **96**: 967-971.

117 Mark A, Granström M. The role of aluminium for adverse reactions and immunogenicity of diphtheria-tetanus booster vaccine. *Acta Paediatr* 1994: **83**: 159-163.

118 Rennels M B, Deloria M A, Pichichero M E, et al. Lack of consistent relationship between quantity of aluminum in diphtheria-tetanus-acellular pertussis vaccines and rates of extensive swelling reactions. *Vaccine* 2002: **20** (Suppl 3): 44-47.

119 Netterlid E, Bruze M, Hindsén M, et al. Persistent itching nodules after the fourth dose of diphtheria-tetanus toxoid vaccines without evidence of delayed hypersensitivity to aluminium. *Vaccine* 2004: **22**: 3698-3706.

120 Jefferson T, Rudin M, Di Pietrantonj C. Adverse events after immunisation with aluminium-containing DTP vaccines: systematic review of the evidence. *Lancet Infect Dis* 2004: **4**: 84-90.

121 Heidary N, Cohen D E. Hypersensitivity reactions to vaccine components. *Dermatitis* 2005: **16**: 115-120.

122 Möller H. All these positive tests to thimerosal. *Contact Dermatitis* 1994: **31**: 209-213.

123 Schäfer T, Enders F, Przybilla B. Sensitization to thimerosal and previous vaccination. *Contact Dermatitis* 1995: **32**: 114-116.

124 Wattanakrai P, Rajatanavin N. Thimerosal allergy and clinical relevance in Thailand. *J Med Assoc Thai* 2007: **90**: 1775-1779.

125 Sasseville D. Hypersensitivity to preservatives. *Dermatol Ther* 2004: **17**: 251-263.

126 Vogt T, Landthaler M, Stolz W. Generalized eczema in an 18-month-old boy due to phenoxyethanol in DPT vaccine. *Contact Dermatitis* 1998: **38**: 50-51.

127 Ring J. Exacerbation of eczema by formalin-containing hepatitis B vaccine in formaldehyde-allergic patient. *Lancet* 1986: **2**: 522-523.

128 Rothstein E, Kohl K S, Ball L, et al. Nodule at injection site as an adverse event following immunization: case definition and guidelines for data collection, analysis, and presentation. *Vaccine* 2004: **22**: 575-585.

129 Chong H, Brady K, Metze D, et al. Persistent nodules at injection sites (aluminium granuloma) - clinicopathological study of 14 cases with a diverse range of histological reaction patterns. *Histopathology* 2006: **48**: 182-188.

130 Thierry-Carstensen B, Stellfeld M. Itching nodules and hypersensitivity to aluminium after the use of adsorbed vaccines from SSI. *Vaccine* 2004: **22**: 1845.

131 Trollfors B, Bergfors E, Inerot A. Vaccine related itching nodules and hypersensitivity to aluminium. *Vaccine* 2005: **23**: 975-976.

132 Œsterballe O. Side effects during immunotherapy with purified grass pollen extracts. *Allergy* 1982: **37**: 553-562.

133 Sheasby P G, Pinner R. Aluminium, its properties, alloys and finishes. In: *The surface treatment and finishing of aluminium and its alloys*, 6th edn, Finishing Publications Ltd. and ASM International, 2001: 1-4.

134 Encyclopedia Britannica - An Online Informational Resource. English. 2017 Available: https://global.britannica.com/science/bauxite, (last accessed 3 April 2017).

135 Krewski D, Yokel R A, Nieboer E, et al. Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. *J Toxicol Environ Health B Crit Rev* 2007: **10** (Suppl 1): 1-269.

136 Exley C. Human exposure to aluminium. *Environ Sci Process Impacts* 2013: **15**: 1807-1816.

137 Gherardi R K, Coquet M, Cherin P, et al. Macrophagic myofasciitis: an emerging entity. Groupe d'Etudes et Recherche sur les Maladies Musculaires Acquises et Dysimmunitaires (GERMMAD) de l'Association Francaise contre les Myopathies (AFM). *Lancet* 1998: **352**: 347-352.

138 Exley C, Swarbrick L, Gherardi R K, et al. A role for the body burden of aluminium in vaccine-associated macrophagic myofasciitis and chronic fatigue syndrome. *Med Hypotheses* 2009: **72**: 135-139.

139 Willhite C C, Karyakina N A, Yokel R A, et al. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. *Crit Rev Toxicol* 2014: **44** (Suppl 4): 1-80.

140 Brenner A. Macrophagic myofasciitis: a summary of Dr. Gherardi's presentations. *Vaccine* 2002: **20** (Suppl 3): S5-6.

141 Maya S, Prakash T, Madhu K D, et al. Multifaceted effects of aluminium in neurodegenerative diseases: A review. *Biomed Pharmacother* 2016: **83**: 746-754.

142 Mirza A, King A, Troakes C, et al. Aluminium in brain tissue in familial Alzheimer's disease. *J Trace Elem Med Biol* 2017: **40**: 30-36.

143 Crepeaux G, Eidi H, David M O, et al. Non-linear dose-response of aluminium hydroxide adjuvant particles: Selective low dose neurotoxicity. *Toxicology* 2017: **375**: 48-57.

144 Mandriota S J, Tenan M, Ferrari P, et al. Aluminium chloride promotes tumorigenesis and metastasis in normal murine mammary gland epithelial cells. *Int J Cancer* 2016: **139**: 2781-2790.

145 Gupta R K, Relyveld E H, Lindblad E B, et al. Adjuvants-a balance between toxicity and adjuvanticity. *Vaccine* 1993: **11**: 293-306.

146 Brehler R. [Adjuvants]. *Hautarzt* 2017: **68**: 292-296.

147 Vogel F R, Hem S L. Immunologic adjuvants. In: *Vaccines*, 5th edn, S A Plotkin, W A Orenstein and P A Offit (eds), Saunders Elsevier, 2008: 59-71.

148 Apostolico Jde S, Lunardelli V A, Coirada F C, et al. Adjuvants: Classification, Modus Operandi, and Licensing. *J Immunol Res* 2016: **2016**: 1-16.

149 Baylor N W, Egan W, Richman P. Aluminum salts in vaccines--US perspective. *Vaccine* 2002: **20** (Suppl 3): 18-23.

150 Jones L S, Peek L J, Power J, et al. Effects of adsorption to aluminum salt adjuvants on the structure and stability of model protein antigens. *J Biol Chem* 2005: **280**: 13406-1314.

151 Gupta R K, Bradford E R, Relyveld E, et al. Adjuvant properties of aluminium and calcium compounds. In: *Vaccine Design: The Subunit and Adjuvant Approach*, M F Powell and M J Newman (eds): New York, Plenum Press, 1995: 229-248.

152 Shah R. Overview of vaccine adjuvants: introduction, history, and current status. In: *Vaccine Adjuvants: Methods and Protocols, Methods in Molecular Biology*, C B Fox (ed): NewYork, Springer Science and Business, 2017: 1-8.

153 Baldrick P, Richardson D, Wheeler A W. Review of L-tyrosine confirming its safe human use as an adjuvant. *J Appl Toxicol* 2002: **22**: 333-344.

154 Klimek L, Schmidt-Weber C B, Kramer M F, et al. Clinical use of adjuvants in allergen-immunotherapy. *Expert review of clinical immunology* 2017: **13**: 599-610.

155 Reed S G, Orr M T, Fox C B. Key roles of adjuvants in modern vaccines. *Nat Med* 2013: **19**: 1597-1608.

156 Farmaceutiska Specialiteter i Sverige = FASS; (Swedish medical products facts). Swedish. (Online). 2017. Available at: http://www.fass.se/LIF/startpage/productname; productname= name of the vaccine or substance (last accessed 2 June 2017).

157 Eisenbarth S C, Colegio O R, O'connor W, et al. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 2008: **453**: 1122-6.

158 Lidén C, Bruze M, Thyssen J P, et al. Metals. In: *Contact Dermatitis*, 5th edn, D J Johansen, J P Frosch and J-P Lepoittevin (eds): Berlin Heidelberg, Springer-Verlag, 2011: 643-679.

159 Thyssen J P, Menné T. Metal allergy - a review on exposures, penetration, genetics, prevalence, and clinical implications. *Chem Res Toxicol* 2010: **23**: 309-318.

160 Mahler V, Geier J, Schnuch A. Current trends in patch testing - new data from the German Contact Dermatitis Research Group (DKG) and the Information Network of Departments of Dermatology (IVDK). *J Dtsch Dermatol Ges* 2014: **12**: 583-592.

161 Ekqvist S, Svedman C, Möller H, et al. High frequency of contact allergy to gold in patients with endovascular coronary stents. *Br J Dermatol* 2007: **157**: 730-738.

162 Faurschou A, Menné T, Johansen J D, et al. Metal allergen of the 21st century-a review on exposure, epidemiology and clinical manifestations of palladium allergy. *Contact Dermatitis* 2011: **64**: 185-195.

163 Björk A-K. Patch testing with metals with focus on gold. Department of Clinical Sciences, Malmö, Occupational and Environmental Dermatology Unit, Malmö, Lunds University, Faculty of Medicine Doctoral Dissertation series 2017:28, 2017. Lund.

164 Pineau A, Guillard O, Favreau F, et al. In vitro study of percutaneous absorption of aluminum from antiperspirants through human skin in the Franz diffusion cell. *J Inorg Biochem* 2012: **110**: 21-26.

165 Schmidt M, Goebeler M. Immunology of metal allergies. *J Dtsch Dermatol Ges* 2015: **13**: 653-660.

166 Cox N H, Moss C, Forsyth A. Cutaneous reactions to aluminium in vaccines: an avoidable problem. *Lancet* 1988: **2**: 43.

167 Bruze M, Lundh K, Gruvberger B, et al. Aluminium chloride hexahydrate at 2% is insufficient to trace contact allergy to aluminium. *Contact Dermatitis* 2008: **59**: 183-184.

168 Fischer T, Rystedt I. A case of contact sensitivity to aluminium. *Contact Dermatitis* 1982: **8**: 343.

169 Garg S, Loghdey S, Gawkrodger D J. Allergic contact dermatitis from aluminium in deodorants. *Contact Dermatitis* 2010: **62**: 57-58.

170 Meding B, Augustsson A, Hansson C. Patch test reactions to aluminium. *Contact Dermatitis* 1984: **10**: 107.

171 McFadden N, Lyberg T, Hensten-Pettersen A. Aluminum-induced granulomas in a tattoo. *J Am Acad Dermatol* 1989: **20**: 903-908.

172 Schwarze H P, Giordano-Labadie F, Loche F, et al. Delayed-hypersensitivity granulomatous reaction induced by blepharopigmentation with aluminum-silicate. *J Am Acad Dermatol* 2000: **42**: 888-891.

173 Hall A. Occupational contact dermatitis among aircraft workers. *J Am Med Assoc* 1944: **125**: 179-185.

174 Peters T, Hani N, Kirchberg K, et al. Occupational contact sensitivity to aluminium in a machine construction plant worker. *Contact Dermatitis* 1998: **39**: 322-323.

175 Veien N K, Hattel T, Laurberg G. Systemically aggravated contact dermatitis caused by aluminium in toothpaste. *Contact Dermatitis* 1993: **28**: 199-200.

176 Kotovirta M L, Salo O P, Visa-Tolvanen K. Contact sensitivity to aluminum. *Contact Dermatitis* 1984: **11**: 135.

177 Tosti A, Vincenzi C, Peluso A M. Accidental diagnosis of aluminium sensitivity with Finn Chambers. *Contact Dermatitis* 1990: **23**: 48-49.

178 Dwyer C M, Kerr R E. Contact allergy to aluminium in 2 brothers. *Contact Dermatitis* 1993: **29**: 36-38.

179 Garcia-Patos V, Pujol R M, Alomar A, et al. Persistent subcutaneous nodules in patients hyposensitized with aluminum-containing allergen extracts. *Arch Dermatol* 1995: **131**: 1421-1424.

180 Hemmer W, Wantke F, Focke M, et al. Evaluation of cutaneous hypersensitivity to aluminum by routine patch testing with A1C1(3). *Contact Dermatitis* 1996: **34**: 217-218.

181 Skowron F. Persistent nodules at sites of hepatitis B vaccination due to aluminium sensitization. *Contact Dermatitis* 1997: **39**: 135.

182 Brodbaker E, Pratt M. Contact sensitivity to aluminum. *J Cutan Med Surg* 2009: **13**: 226-229.

183 Castelain P Y, Castelain M, Vervloet D, et al. Sensitization to aluminium by aluminium-precipitated dust and pollen extracts. *Contact Dermatitis* 1988: **19**: 58-60.

184 O'Driscoll J B, Beck M B, Kesseler M E, et al. Contact sensitivity to aluminium acetate eardrops. *Contact Dermatitis* 1991: **24**: 156-157.

185 Hindsén M, Bruze M, Christensen O B. Individual variation in nickel patch test reactivity. *Am J Contact Dermat* 1999: **10**: 62-67.

186 Netterlid E, Hindsén M, Ekqvist S, et al. Young individuals with atopic disease and asthma or rhinoconjunctivitis may have clinically relevant contact allergies. *Dermatitis* 2014: **25**: 115-119.

187 Mortz C G, Andersen K E, Bindslev-Jensen C. Recall bias in childhood atopic diseases among adults in the Odense Adolescence Cohort Study. *Acta Derm Venereol* 2015: **95**: 968-72.

188 Thyssen J P, Linneberg A, Engkilde K, et al. Contact sensitization to common haptens is associated with atopic dermatitis: new insight. *Br J Dermatol* 2012: **166**: 1255-1261.

189 Hamann C R, Hamann D, Egeberg A, et al. Association between atopic dermatitis and contact sensitization: A systematic review and meta-analysis. *J Am Acad Dermatol* 2017: **77**: 70-78.

190 Lammintausta K, Kalimo K, Fagerlund V L. Patch test reactions in atopic patients. *Contact Dermatitis* 1992: **26**: 234-240.

191 Mortz C G, Andersen K E. Allergic contact dermatitis in children and adolescents. *Contact Dermatitis* 1999: **41**: 121-130.

192 Giordano-Labadie F, Rance F, Pellegrin F, et al. Frequency of contact allergy in children with atopic dermatitis: results of a prospective study of 137 cases. *Contact Dermatitis* 1999: **40**: 192-195.

193 Dotterud L K, Smith-Sivertsen T. Allergic contact sensitization in the general adult population: a population-based study from Northern Norway. *Contact Dermatitis* 2007: **56**: 10-15.

194 Herro E M, Matiz C, Sullivan K, et al. Frequency of contact allergens in pediatric patients with atopic dermatitis. *J Clin Aesthet Dermatol* 2011: **4**: 39-41.

195 Mailhol C, Lauwers-Cances V, Rance F, et al. Prevalence and risk factors for allergic contact dermatitis to topical treatment in atopic dermatitis: a study in 641 children. *Allergy* 2009: **64**: 801-806.

196 Shaughnessy C N, Malajian D, Belsito D V. Cutaneous delayed-type hypersensitivity in patients with atopic dermatitis: reactivity to topical preservatives. *J Am Acad Dermatol* 2014: **70**: 102-107.

197 Cronin E, McFadden J P. Patients with atopic eczema do become sensitized to contact allergens. *Contact Dermatitis* 1993: **28**: 225-228.

198 Uehara M, Sawai T. A longitudinal study of contact sensitivity in patients with atopic dermatitis. *Arch Dermatol* 1989: **125**: 366-368.

199 Rees J, Friedmann P S, Matthews J N. Contact sensitivity to dinitrochlorobenzene is impaired in atopic subjects. Controversy revisited. *Arch Dermatol* 1990: **126**: 1173-1175.

200 De Groot A C. The frequency of contact allergy in atopic patients with dermatitis. *Contact Dermatitis* 1990: **22**: 273-277.

201 Thyssen J P, Johansen J D, Linneberg A, et al. The association between contact sensitization and atopic disease by linkage of a clinical database and a nationwide patient registry. *Allergy* 2012: **67**: 1157-1164.

202 Belhadjali H, Mohamed M, Youssef M, et al. Contact sensitization in atopic dermatitis: results of a prospective study of 89 cases in Tunisia. *Contact Dermatitis* 2008: **58**: 188-9.

203 De Waard-Van Der Spek F B, Andersen K E, Darsow U, et al. Allergic contact dermatitis in children: which factors are relevant? (review of the literature). *Pediatr Allergy Immunol* 2013: **24**: 321-329.

204 Thyssen J P, McFadden J P, Kimber I. The multiple factors affecting the association between atopic dermatitis and contact sensitization. *Allergy* 2014: **69**: 28-36.

205 Landeck L, Schalock P, Baden L, et al. Contact sensitization pattern in 172 atopic subjects. *Int J Dermatol* 2011: **50**: 806-810.

206 Warshaw E M, Nelsen D D, Maibach H I, et al. Positive patch test reactions to lanolin: cross-sectional data from the north american contact dermatitis group, 1994 to 2006. *Dermatitis* 2009: **20**: 79-88.

207 Bae J M, Choi Y Y, Park C O, et al. Efficacy of allergen-specific immunotherapy for atopic dermatitis: a systematic review and meta-analysis of randomized controlled trials. *J Allergy Clin Immunol* 2013: **132**: 110-117.

208 Tam H, Calderon M A, Manikam L, et al. Specific allergen immunotherapy for the treatment of atopic eczema. *Cochrane Database Syst Rev* 2016: **2**: 1-77.

209 Sanchez Caraballo J M, Cardona Villa R. Clinical and immunological changes of immunotherapy in patients wit atopic dermatitis: randomized controlled trial. *Isrn Allergy Online* 2012: **2012**: 1-9.

210 Luna-Pech J, Newton-Sanchez O, Torres-Mendoza B, et al. Efficacy of sublingual immunotherapy in the severity of atopic dermatitis in children with allergic sensitisation to dermatophagoides pteronyssinus. *Annals of Allergy, Astma and Immunology* 2013: **111** (5 Suppl 1): A8.

211 Qin Y E, Mao J R, Sang Y C, et al. Clinical efficacy and compliance of sublingual immunotherapy with Dermatophagoides farinae drops in patients with atopic dermatitis. *Int J Dermatol* 2014: **53**: 650-655.

212 Czarnobilska E, Obtulowicz K, Dyga W, et al. A half of schoolchildren with 'ISAAC eczema' are ill with allergic contact dermatitis. *J Eur Acad Dermatol Venereol* 2011: **25**: 1104-1107.

213 Spiewak R. Immunotherapy of allergic contact dermatitis. *Immunotherapy* 2011: **3**: 979-996.

214 Sjövall P, Christensen O B, Möller H. Oral hyposensitization in nickel allergy. *J Am Acad Dermatol* 1987: **17**: 774-778.

215 Nagore E, Martinez-Escribano J A, Tato A, et al. Subcutaneous nodules following treatment with aluminium-containing allergen extracts. *Eur J Dermatol* 2001: **11**: 138-140.

216 Fawcett H A, McGibbon D, Cronin E. Persistent vaccination granuloma due to aluminium hypersensitivity. *Br J Dermatol* 1985: **113** (Suppl 29): 101-102.

217 Bruze M. Seasonal influence on routine patch test results. *Contact Dermatitis* 1986: **14**: 184.

218 Glent-Madsen L, Dahl J C. Axillary hyperhidrosis. Local treatment with aluminium-chloride hexahydrate 25% in absolute ethanol with and without supplementary treatment with triethanolamine. *Acta Derm Venereol* 1988: **68**: 87-89.

219 Solish N, Bertucci V, Dansereau A, et al. A comprehensive approach to the recognition, diagnosis, and severity-based treatment of focal hyperhidrosis: recommendations of the Canadian Hyperhidrosis Advisory Committee. *Dermatol Surg* 2007: **33**: 908-923.

220 Friedmann P S. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. *Br J Dermatol* 2007: **157**: 1093-1102.

221 Bruze M, Björkner B, Möller H. Skin testing with gold sodium thiomalate and gold sodium thiosulfate. *Contact Dermatitis* 1995: **32**: 5-8.

222 Comstedt Rosholm L R, Hindsén M, Frick-Engfeldt M, et al. Variation in patch-test reactivity to palladium and nickel. *Contact Dermatitis* 2016: **75** (Suppl 1): 51-52.

223 Lee E E, Maibach H I. Is contact allergy in man lifelong? An overview of patch test follow-ups. *Contact Dermatitis* 2001: **44**: 137-139.

224 Rystedt I. Evaluation and relevance of isolated test reactions to cobalt. *Contact Dermatitis* 1979: **5**: 233-238.

225 Schubert H, Kohanka V, Korossy S, et al. Epidemiology of nickel allergy: results of a follow-up analysis of patients with positive patch tests to nickel. *Contact Dermatitis* 1988: **18**: 237-239.

226 Björk A-K, Bruze M, Engfeldt M, et al. The reactivity of the back revisited. Are there differences in reactivity in different parts of the back? *Contact Dermatitis* 2017: **76**: 19-26.

227 Masjedi K, Bruze M, Hindsén M, et al. Is the variability of nickel patch test reactivity over time associated with fluctuations in the systemic T-cell reactivity to nickel? *Br J Dermatol* 2009: **161**: 102-109.

228 Lindelöf B. Regional variations of patch test response in nickel-sensitive patients. *Contact Dermatitis* 1992: **26**: 202-203.

229 Strien G A, Korstanje M J. Site variations in patch test responses on the back. *Contact Dermatitis* 1994: **31**: 95-96.

230 Calnan C D, Wells G C. Suspender dermatitis and nickel sensitivity. *Br Med J* 1956: **1**: 1265-1268.

231 Williams J, Cahill J, Nixon R. Occupational autoeczematization or atopic eczema precipitated by occupational contact dermatitis? *Contact Dermatitis* 2007: **56**: 21-26.

Printed by Media-Tryck, Lund 2017 NORDIC ECOLABEL, 3041 0903

LUND UNIVERSITY
Faculty of Medicine

Department of Occupational and
Environmental Dermatology
Lund University, Faculty of Medicine
Doctoral Dissertation Series 2017:113
ISBN 978-91-7619-495-9
ISSN 1652-8220