VACCINE-INDUCED ALUMINIUM ALLERGY AND LONG-LASTING SUBCUTANEOUS ITCHING NODULES

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Sweden 2021

UNIVERSITY OF GOTHENBURG

Gothenburg 2021

Cover illustration: Toxa2x2, ImagePixel

Layout: Annelie Grimm

Drawings: Created by the afflicted children in the follow-ups in which they were invited to describe their symptoms in drawings or by texts.

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and long-lasting subcutaneous itching nodules.

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Paper I	published with permission of John Wiely and Sons
Paper II	published with permission of John Wiely and Sons
Paper III	in manuscript (submitted)
Paper IV	in manuscript (not submitted), published with permission of the authors

ISBN 978-91-8009-494-8 (PRINT)
ISBN 978-91-8009-495-5 (PDF)

Printed in Gothenburg, Sweden 2021
Printed by Stema Specialtryck AB

SVANENMÄRKET
Trycksak
3041 0234

Dedicated to all the afflicted children and their parents
in the Gothenburg Pertussis Vaccine Trials.

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ABSTRACT

Background: Aluminium contact dermatitis is rare even though aluminium is frequently used in antiperspirants and sunscreens. Sensitisation to aluminium is mostly a side effect of aluminium-adsorbed vaccines. These can also induce long-lasting intensely itching subcutaneous nodules (granulomas) at the injection site.

During clinical trials on an acellular aluminium-adsorbed pertussis vaccine in the 1990s in Gothenburg, Sweden, persistent itching nodules were-unexpectedly-reported in 745 of ~ 76 000 vaccinated. Contact dermatitis to aluminium was verified by patch test in 377 children with itching nodules.

Aim: This thesis aims to study the long-term clinical prognosis of itching subcutaneous nodules and aluminium allergy in children who received an aluminium-adsorbed pertussis vaccine in a clinical trial.

Patients and Methods: All 745 vaccinated children with itching nodules in the pertussis vaccine trial were enrolled in a long-term follow-up study (>20 years).

Results: The median duration of itching was 6.6 years. During the follow-up time 637/745 (86%) of the participants experienced full symptom recovery. The remaining were markedly improved. In 186 of 241 (77%) children who were tested twice, aluminium hypersensitisation was no longer detectable. A negative patch test was significantly correlated with loss of itching. 3-7% of the participants who received other aluminium-adsorbed vaccines later in life reported mild and transient itching at the new injection site. The optimal compound to establish aluminium hypersensitivity could not be determined.

Conclusion and recommendations: Vaccine-induced subcutaneous itching nodules associated with aluminium allergy in infants and children can cause great suffering and have a protracted course. However, long-term prospective studies

show that both clinical symptoms and delayed hypersensitivity for aluminium disappear over time. Further vaccination with aluminium-adsorbed vaccines is safe in older children given that the original nodule has vanished and the itching will have resolved or nearly resolved.

Keywords: Childhood vaccine, adverse event, aluminium, aluminium allergy, itching nodules, subcutaneous granulomas, patch test, tolerance

ISBN 978-91-8009-494-8 (PRINT)

ISBN 978-91-8009-495-5 (PDF)

SAMMANFATTNING PÅ SVENSKA

Alla vacciner mot difteri, stelkramp och kikhosta innehåller en liten mängd aluminiumsalt som hjälpmedel (adjuvans) för att öka den immunstimulerande effekten. Även vacciner mot hepatit A och B, humant papillomvirus, fästingburen encefalit och en del pneumokock- och meningokockvacciner innehåller aluminium adjuvans. Globalt ges numer minst 400 miljoner doser av aluminiuminnehållande vaccin till över 100 miljoner barn.

Från 1986 genomfördes flera kliniska prövningar av ett nytt kikhostevaccin i Göteborgsområdet (”Kikhostestudien”). De avslutades 1999 och omfattade då sammanlagt drygt 76 000 barn. Från 1995 rapporterades oväntat att ett stort antal barn (totalt 745, motsvarande ca 1% av alla vaccinerade) fick långvariga besvär av en intensivt kliande ”kula” (nodulus) under huden på platsen för vaccinationen. 241 av dem konstaterades ha blivit kontaktallergiska mot aluminium genom ett lapptest. Symtomen, som var ihållande och svårbehandlade, väckte oro och frågor om fortsatt vaccination av de drabbade barnen.

Syftet med avhandlingen är att öka kunskapen om långvarigt kliande noduli (vaccinationsgranulom) och kontaktallergi mot aluminium genom att studera långtidsprognosen för barn som blivit vaccinerade med aluminiuminnehållande vacciner i Kikhostestudien. Det har skett genom upprepade enkäter, intervjuer, kliniska undersökningar samt totalt tre lapptester. Uppföljningen har pågått under mer än 20 år.

Delarbete I var en uppföljande lapteststudie av 241 barn med kliande noduli och kontaktallergi mot aluminium som påvisats i Kikhostestudien (lapptest I). Vid det förnyade testet 5-9 år senare (lapptest II), fann vi - oväntat - att aluminiumallergi inte längre kunde påvisas hos 77% av de omtestade barnen.

I delarbete II studerades skillnaden i testreaktionen mellan två olika former av aluminium, metallisk aluminium och ett aluminiumsalt (aluminiumklorid hexahydrat i vaselin 2%). Resultaten i lapptest I och II jämfördes på grupp- och individnivå. Metalliskt aluminium visade sig vara mindre känsligt för att påvisa aluminiumallergi jämfört med aluminiumsalt. Styrkan i testreaktionen minskade över tid för båda formerna av aluminium.

Delarbete III var en prospektiv studie (1997-2019) av den kliniska prognosen för kliande noduli hos samtliga 745 barn, nu unga vuxna, som drabbades av sådana efter vaccination i Kikhostestudien. Den kliniska relevansen av aluminiumallergi har värderats genom att följa upp eventuella nya symtom hos de barn som fått ytterligare aluminiuminnehållande vacciner senare i livet. Långtidsuppföljningen visar att vaccinorsakad klåda minskar över tid och saknar klinisk betydelse vid förnyad exponering för andra aluminiuminnehållande vacciner.

I delarbete IV studerades långtidsprognosen för aluminiumallergi genom ett tredje lapptest (2020) av individer som deltog i lapptest II. Ytterligare aluminiumberedningar användes för att undersöka hur man optimalt lapptestar för aluminiumallergi. Studien fick avbrytas i förtid p.g.a. av covid-19-pandemin. De data som ändå kunde inhämtas från 31 av 65 planerade deltagare bekräftar att styrkan på lapptestreaktionerna avtar med tiden och att allergin i de flesta fall är övergående. De olika aluminiumberedningarna som användes i denna studie visade sig vara likvärdiga.

Sammanfattningsvis visar de fyra uppföljande studierna att symtomen från kliande vaccinationsgranulom (noduli) kan bestå under lång tid (flera år) och vara mycket besvärande med periodvis svår klåda och lokala hudförändringar, men att de minskar efter hand och så gott som alltid upphör helt.

Även intensiteten i kontaktallergin mot aluminium avtar med tiden, och hos många kan allergin inte längre påvisas vid lapptest 15-20 år senare.

Fortsatt vaccination med aluminiuminnehållande vacciner kan ske när den ursprungliga kliande kulan har försvunnit eller nästan försvunnit. Återkommande besvär vid förnyad vaccination är sällsynta.

LIST OF PAPERS

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

I. Gente Lidholm A, Bergfors E, Inerot A, Blomgren U, Gillstedt M, Trollfors B. Unexpected loss of contact allergy to aluminium induced by vaccine. Contact dermatitis. 2013;68(5):286-92. doi: 10.1111/cod.12053

II. Gente Lidholm A, Inerot A, Gillstedt M, Bergfors E, Trollfors B. Comparison of reactivity to a metallic disc and 2% aluminium salt in 366 children, and reproducibility over time for 241 young adults with childhood vaccine-related aluminium contact allergy. Contact Dermatitis 2018; Jul;79(1):26-30. doi: 10.1111/cod.12977

III. Gente Lidholm A, Inerot A, Gillstedt M, Bergfors E, Trollfors B. Long-term clinical course and prognosis of vaccine-related persistent itching nodules. Manuscript submitted for publication.

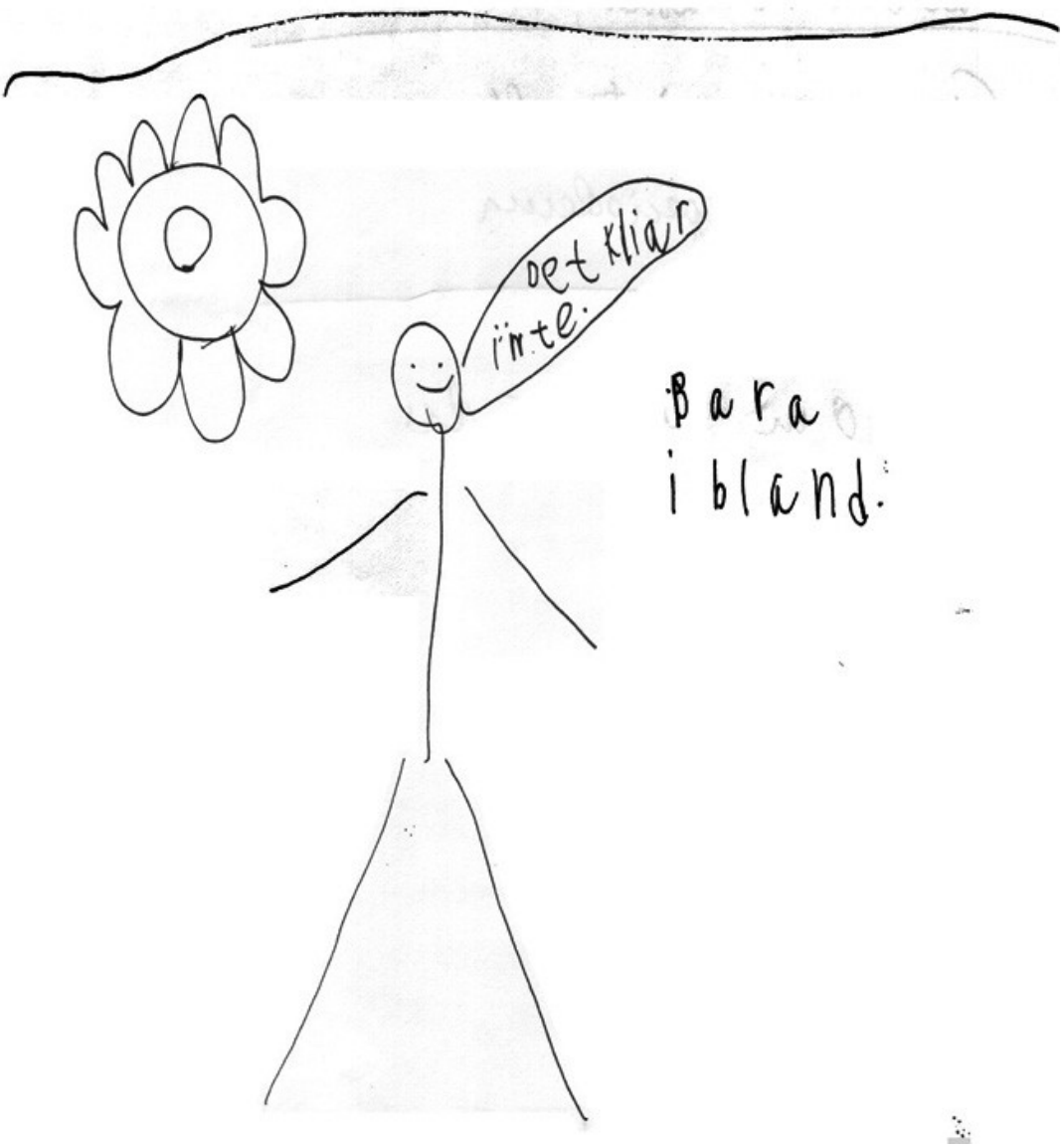
IV. Gente Lidholm A, Inerot A, Gillstedt M, Bergfors E, Trollfors B. Long-term prognosis of vaccine-induced contact allergy to aluminium - third patch-test and different test preparations. In manuscript.

CONTENT

ABBREVIATIONS

ACD	Allergic contact dermatitis
aP	Acellular pertussis vaccine
APCs	Antigen presenting cells
ASIT	Allergen-specific immunotherapy
BCG	Bacillus Calmette–Guérin (BCG) vaccine
CD	Contact dermatitis
CHC	Child Health Center
CI	Confidence interval
Covid-19	Coronavirus disease 2019
DAMP	Damage-associated molecular patterns
DNA	Deoxyribonucleic acid
DT	Diphtheria and tetanus vaccine (combination)
DTP	Diphtheria-tetanus-pertussis vaccine
DTaP	Diphtheria-tetanus-acellular pertussis toxoid vaccine
GSK	Glaxo Smith Kline
HBsAg	Hepatitis B surface antigen
HHE	Hypotonic hyporesponsive episode
HPV	Human papillomavirus
Hib	*Haemophilus influenzae* type b
HIV	Human immunodeficiency virus
ICD	Irritant contact dermatitis
ICDRG	International Contact Dermatitis Research Group
IPV	Inactivated polio vaccine (trivalent)
mRNA	Messenger ribonucleic acid
MHC	Major histocompatibility complex
MI	Methylisothiazolinone
MPA	Medical Products Agency
MSD	Merck Sharp & Dohme Corporation
NIP	National immunisation programme
NK-cell	Natural Killer cell
PACD	Photoallergic contact dermatitis
PAMPs	Pathogen-associated molecular patterns
PCD	Protein contact dermatitis

PICD	Photoirritant (phototoxic) contact dermatitis
PPD	Paraphenylenediamine
PRRs	Pattern-recognition receptors
PT	Pertussis toxin
ROAT	Repeated open application test
ROS	Reactive oxygen species
SBL	Swedish Bacteriological Laboratory Now disused
s.c	Subcutaneous (injection)
SCD	Systemic contact dermatitis
SDRIF	Symmetrical drug-related intertriginous and flexure exanthema
SSI	Statens Serum Institut
TBE	Tick-borne encephalitis
TC-cell	Cytotoxic T-cell
TH-cell	T-helper cell
TCR	T-cell receptor
TLRs	Toll-like receptors
T-reg cell	T-regulatory cell



Drawing by an 8 years old boy saying " It doesn´t itch any longer. Only sometimes." He had – so far - four years´history of itching nodules in his left upper arm where he received three doses of aP vaccine.

INTRODUCTION

CONTACT DERMATITIS

Contact dermatitis (CD) is a clinical manifestation in the skin of an inflammatory reaction caused by direct contact with various substances in our everyday lives and surroundings. The eczematous skin reaction may be either acute or chronic and is divided into different types; allergic contact dermatitis (ACD), irritant contact dermatitis (ICD), protein contact dermatitis (PCD), photoirritant (phototoxic) contact dermatitis (PICD) and photoallergic contact dermatitis (PACD) (1). The clinical manifestation cannot alone verify the type of CD causing the symptoms - an assessment of exposure, clinical examination, patch test to establish ACD, and a prick test to establish PCD are often necessary. Thus, the CD is not synonymous with ACD.

In this thesis, mainly ACD will be discussed. The eczematous skin reaction seen in ACD is triggered by allergens, also called haptens, in persons already sensitised to the substance by repeated skin contacts. ACD is clinically characterised by delayed inflammatory symptoms appearing 24-48 hours after exposure. The immunological reaction is called a delayed hypersensitisation or a type IV reaction. The reaction is one of four overall strategies to guarantee ideal security for the body, see table 1 (2). Thus, the immune response can cause significant damage to the body if overreacting to the threat. The immunological reactions type I-III are immediate reactions within 24 hours and mediated by antibodies, IgE, IgM and IgG.

Table 1

Reactions	**Antibody-mediated**			**Cell-mediated**
Types	**Type I**	**Type II**	**Type III**	**Type IV**
Mecha-nism	Immediate hypersensitiza-tion IgE-mediated	Humoral cytotoxic immune response	Immune complex-mediated immune response	Delayed hypersensitization T-cell-mediated
Symp-toms	- Allergic rhinitis - Bronchial asthma -Protein contact dermatitis	- Drug-induced cytopenia	- Immune complex vasculitis - Exogenous aller-gic alveolitis	- Contact allergy - Drug-induced exanthema

Table 1 Classification of immune responses by Coombs and Gell after modification by Averbeck et al. (2) in the thesis of Ingrid Siemund (64).

The ACD inflammatory reaction starts some day or days after exposure. It is activated by the immunological response type IV reaction and is mediated by antigen-specific T-lymphocytes (3), unlike ICD, in which the particular substance itself damages the skin barrier. ICD occurs almost immediately when the skin is damaged by chemicals, cold, water or friction. The exact mechanism of the inflammatory response in ICD is unclear (4).

ACD can be divided into topical, airborne and systemic exposure. The different exposure pathways are usually suspected from their clinical manifestations. Systemic contact dermatitis (SCD) is an eczematous reaction in the skin appearing after systemic administration of an allergen in a person earlier sensitised to the substance by cutaneous exposure. Typical substances include medication used systemically and topically, plants, spices, and metals such as gold, nickel, cobalt and aluminium (5). Several hypotheses about the immunological mechanism involved have been reported, and they are not yet fully understood. The mechanism seems to be mediated by a type IV hypersensitivity reaction and potentially a type III hypersensitivity reaction (6). Symptoms in SCD are diverse with eczema at the former site of dermatitis, rash at the site of a former positive patch test, vesicular hand dermatitis, pruritic papules on elbows and knees, vasculitis-like lesions and erythroderma. Previously named "baboons syndrome", due to the manifestation of diffuse erythema of the buttocks, which was thought to resemble the baboon's red bottom, symmetrical rash in axillary and inguinal skin folds, is also a manifestation of SCD (7). Today the term "baboons syndrome" has been replaced by SDRIF, symmetrical drug-related intertriginous and flexure exanthema (8).

Drug-induced photosensitive reactions, also called phototoxic reactions, included PACD and PICD. PACD related to the progress of a cutaneous disease caused by exposure to a chemical agent combined with sunlight (9). In the photoallergic reaction, the skin inflammation is caused by an immunological mechanism. The phototoxic reaction on the other hand, is a photochemical event activated by UV light, leading to changes in cell membrane components. The effect is seen after minutes to hours (10). The immunological mechanism of PACD is a type IV reaction caused by light-activated compounds and develops one or two days after exposure (11). Both phototoxic and photoallergic reactions appear in sun-exposed areas. Symptoms such as erythema, oedema, vesicles and bullae with residual hyperpigmentations are seen in phototoxic dermatitis.

In contrast, in allergic photodermatitis, pruritic eczematous eruption with erythema and vesicles are seen in the acute phase and with chronic exposure, lichenification and scaling dominate (11). Common substances for both types of photoreactions are systemic or topical drugs. In studies from 1995 and 2010, sunscreen and its ingredients were identified as the most common causes of photoallergic reactions (12, 13).

Contact urticarial dermatitis and PCD overlap. Urticarial contact dermatitis is a hypersensitivity reaction of type I and is mediated by IgE antibodies produced by mast cells. The type I reaction is immediate and occurs within a few minutes after exposure. It results in hives in the skin area with direct contact to certain allergens, especially latex, and can be generalised urticaria with angioedema, anaphylaxis and even lethal (14). PCD is caused by immediate hypersensitivity to proteins (15). It is seen in contact with proteins, typical in food as in fruit peels (15). Apart from the typical lesions for eczema, urticaria or vesicular eruption occurring a few minutes after contact with the provoking antigen may appear on the skin (16). Symptoms are primarily located on the hands, forearms and faces. The mechanism is unclear and thought to be a combination of type I and a type IV hypersensitivity reaction (16).

ALLERGIC CONTACT DERMATITIS

Prevalence

There are more than 4000 allergens known to cause ACD, among which nickel is the most common (17). The prevalence of ACD in the general population is known mainly from North America and western Europe. In a review of epidemiological studies by Alinaghi et al. (18), the occurrence of contact allergy in the general population between 2007 and 2017 was estimated to be 20.1 % in adults and 16.5% in children. ACD is more than twice as frequent in women as in men, and roughly 5-10% of people develop ACD once yearly. In a study from 2015, approximately 20-25% of the western population in Europe was estimated to be sensitised to at least one allergen (19). The three most common groups of allergens causing ACD are metals, preservatives and fragrances, where nickel is the most frequent allergen with a prevalence of 14.5%. These allergens are found in jewellery, cosmetics, plants and in everyday households products. Due to the regulation of nickel exposure in Denmark in 1991 (20) and later on in 2001 in the European Union, a decrease in cases of nickel contact allergy was seen (21). However, there is still a high prevalence of nickel in the general population. The top ten allergens vary over time due to the exposure pattern and the ingredients used in our surroundings. ACD is among the most frequently seen occupation-related skin diseases, and different legal frameworks regulate the exposure to allergens to prevent disease (19).

Haptens

Allergens or haptens, small reactive molecules less than 500 Da, are mostly too small to be discovered by the immune system (4). However, in the skin a hapten-protein complex is formed which can be recognised. To identify potential contact allergens and their grade of allergenicity, exact knowledge of the chemical structure and its mechanism of reaction must be understood (4). Several of the al-

lergens are not reactive haptens, and thus they require activation. They are converted from pre-haptens or pro-haptens by either an oxidation or metabolic process to form a hapten-protein complex in the epidermis (4). The ability of the allergen to penetrate through the skin is also determined by its factors such as the allergen's molecular weight and lipophilic character (22). Concentration, exposure time, exposure area, localisation of the skin contact, and damaged epithelial barrier are other crucial factors affecting penetration of the allergen into the skin.

Clinical manifestations and treatment

Clinical manifestations such as pruritus, erythema, infiltration, excoriations, oedema, and even vesicles are seen in acute ACD. In the chronic phase, scaling, fissure and lichenification of the skin are seen if the allergen exposure proceeds (23). Other symptoms such as local hypertrichosis (24), aggravated alopecia (25), itching nodules (26) and lichenoid dermatitis (27) can also be observed. The symptoms vary depending on which part of the body is affected and the kind of exposure. Classic anatomical areas with allergens in direct skin contact are the hands, face and eyelids, perianal regions and lower legs (4).

Persistent subcutaneous itching nodules and granulomas are described as a symptom of ACD appearing when the allergen is injected into the tissue. It is reported after sensitisation for cobalt, chrome, aluminium, palladium, beryllium, zirconium, titanium, nickel, zinc, mercury and gold (28-35). For example, persistent subcutaneous itching nodules (granulomas) can be seen in tattoos, ear-piercings and after injections with metal-containing vaccines and anti-rheumatic drugs (28, 36, 37). Dermal nodules in the earlobes were reported in case reports as contact sensitivity to gold after wearing pierced-type gold earrings (38-40). They are regarded to be a consequence of the persistent allergic reaction to the substances (39, 41). Goossens et al. (42) suggested that the reaction is more of a consequence of the individual reaction pattern than the characteristic of the metal.

Treatment of ACD consists primarily of avoiding the allergen. Additional treatments are phototherapy and topical treatment with moisturisers, glucocorticosteroids and other anti-inflammatory ointments on the afflicted areas. Since ACD is considered a lifelong condition, all further exposure to the allergen must be avoided so as not to relapse.

Diagnosis

The diagnosis of ACD is verified by a standardised patch test of the suspected allergens to initiate the elicitation phase of the type IV reaction. The patch test was first suggested by Jadassonh as "the application method" for more than hundred years ago and has since then been further developed (43). The clinical presentation and a typical medical history are necessary to select the patch test materials, evaluate the test findings and their relevance. A few allergens are considered the

most common to cause contact allergies. Therefore, standardised patch tests series have been created. In the European baseline series, which is based on extensive studies on which antigen should be included, approximately 30 test preparations and mixtures are ordered together. The recommendations for the European baseline series are regularly updated along with the change in people's exposure habits to the environment. An addition of a new sensitiser in the European baselines series is proposed when routine testing of patients with suspected ACD has resulted in a contact allergy rate exceeding 0.5-1% (44). The application technique, the occlusion time of the hapten, the vehicle, the concentration, and each allergen's dose are standardised as guidelines for best practice in diagnostic patch testing (23). Several of the sensitisers are commercially available.

There are several different patch test systems. Small aluminium discs in a set of 5 or 10 or a square plastic chamber adjacent to a hypoallergenic adhesive are mainly used. The hapten is dispersed with a vehicle, primarily white soft paraffin (petrolatum) or a water or ethanol solution, depending on the characteristic of the allergen. Specific training is required for the precise technique of applying the allergen. The sensitiser in petrolatum is pipetted from a syringe into the chamber. An exact amount of the dose is applied to fill the chamber without the risk of allergen extrusion. Even though the technique is well trained, there are inter-individual and intra-individual variations of applying the sensitiser (45). For applying liquids, there are other more precise techniques that can be used (23). There are also pre-packaged tests for a limited number of haptens. The dose per area of the allergen is essential for receiving a patch test reaction (elicitation). Recommendations for standard doses are different according to the type of chambers used (23).

The patch test is applied on the upper part of the back day 0. The patient removes it after 48 hours, day 2, and the test is first read after 72-96 hours day 3 or 4, respectively, according to International Contact Dermatitis Research Group (ICDRG) criteria. A second reading is preferably performed around day 7. Substances such as corticosteroids, gold, palladium and acrylates often elicitate reactions later than day 4, and thus a second reading is crucial.

The ICDRG reading criteria of the patch test are based on inspection and palpation of the erythema, infiltrate, papules and vesicles, see table 2 (23). A positive patch test (+, ++, +++) on day 3 or later is regarded as an allergic reaction.

A doubtful reaction, faint erythema (?+), is always interpreted as a negative test even though contact allergy cannot actually be ruled out as the doubtful result could be either a weak allergic response or an irritant reaction. Adding a stronger test concentration or repeating the test later on should be considered if there is a high suspicion of contact allergy to the actual substance.

The morphology of the irritant reaction is diverse and sometimes resembles an actual allergic reaction. A false-positive patch test reaction is an irritant reaction

that, by definition, is morphologically identical to a genuine allergic reaction (44). Some of the patch test allergens, such as chrome and formaldehyde, can cause irritant reactions due to their irritant potential (46). The chosen concentration of the test preparation may also sometimes be very close to the irritancy threshold to avoid negative false test reactions (44). Thus, it can be difficult to distinguish between allergic and irritant reactions. An irritant reaction is commonly seen on day 2 (47), and by reading on day3 or day 4, a false-positive reaction can be avoided.

Many positive patch test reactions could sometimes be observed in individuals with generalised active dermatitis and high hypersensitivity skin that might provoke the test reactions. Since some reactions could then be false positives, a repeated patch test should be performed when the dermatitis is healed. This phenomenon is called "the angry back" or "excited skin syndrome" (48).

The skin reaction in patch testing is dose-dependent (49). It is essential to systematically apply a standardised amount per area of a standardised sensitiser. An optimal test concentration of an allergen should be chosen so that it is as high as possible but without the risk of an irritant reaction in order to avoid difficulties in interpreting the test result and avoiding sensitising the individual in the test situation.

Another testing method is the repeated open application test (ROAT) test, often used in daily clinics (50). It is a way to mimicking a situation of using the substance. In ROAT, the patient applies the product on the same area inside the volar forearm twice a day for two weeks or less if there is an eczematous reaction. A reaction is considered to be a positive test. Besides the patch test and ROAT, the semi-open test, open test and photopatch test are other techniques used to diagnose ACD based on properties of the suspected allergen (23).

Table 2

Symbol	Morphology	Assessment
-	No reaction	Negative reaction
?+	Faint erythema only	Doubtful reaction
+	Erythema, infiltration, possibly papules	Weak positive reaction
++	Erythema, infiltration, papules, vesicles	Strong positive reaction
+++	Intense erythema, infiltrate, coalescing vesicles	Extreme positive reaction
IR	Various morphologies, e.g. soap effect, bulla, necrosis	Irritant reaction

Table 2. Reading criteria of the ICDRG (23, 187, 188).

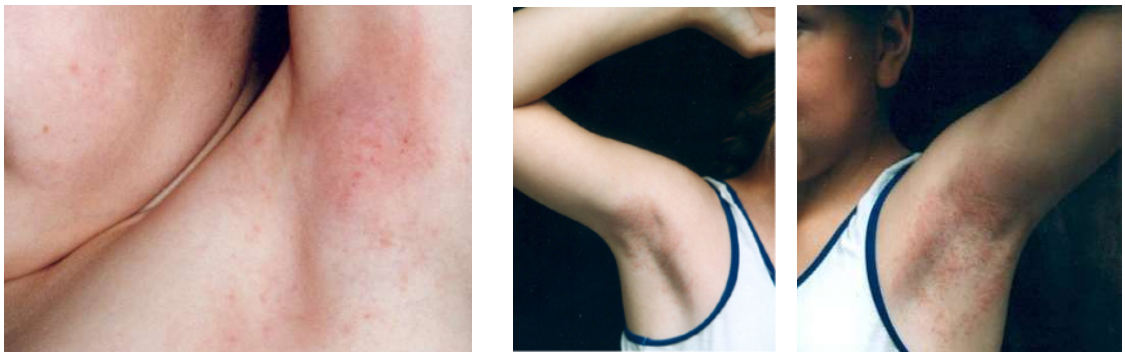
Contact allergy in children

Contact allergy in children is regarded as rare since children have less exposure to allergens such as cosmetics and pharmaceuticals. Lower skin reactivity and sensitisation were also suggested in children younger than 3 years old (51), but the incidence and type of allergens are now primarily considered based on the exposure of allergens in the environment. In a recently published review, a prevalence of 16.5% of ACD in children (< 18 years) in a general population was reported (18). ACD is seen in all ages (52). The most common allergens are the metals nickel, cobalt and chromium. There is no consensus on whether ACD is more common in children with atopic dermatitis (52). Both environmental and genetic factors are thought to be essential in developing ACD. The symptoms of ACD in children are similar to those in adults.

To diagnose ACD in children, patch testing is performed in the same way as in adults, and most often the same test concentrations are used. However, sometimes a lower test concentration is preferable to avoid very strong reactions in children with a high suspicion of ACD. An abbreviated baseline series is commonly used since there are differences between the exposure patterns for children and adults. Depending on the age of the child, the back area could also be limited for space.

Aluminium allergy

Aluminium is the third most common element in the Earth's crust, preceded by oxygen and silicon (53). The atomic number of aluminium in the periodical system is 13, and the ion is highly positively charged, Al3+. Aluminium is very reactive, and therefore never found in its pure form. Its production starts with refining bauxite, a red/brown clay-like soil type found in Africa, Australia, South America and Asia. The Danish chemist Hans Oerstedt managed to refine metal aluminium from bauxite for the first time in 1825. The refining process of aluminium is highly energy-consuming, and most of it can be recycled. Since aluminium has unique properties, namely it is corrosion-resistant, soft, conductive and of low atomic weight, it is widely used in different alloys in the transportation and construction industries as well as in electronics and household items.



Allergic contact dermatitis to aluminium after applying aluminium-containing antiperspirants. Photographs by Elisabet Bergfors published with her consent.

As is the case for other metals, aluminium must be in its ionised form and then haptenisised to act as a hapten (54). Humans are mainly exposed to ionised aluminium in different aluminium salts seen in medications, cosmetics, toothpaste, antiperspirants, sunscreens, tattoos and vaccines. The recommended vaccination schedule for infants in the United States, determined by the United States Food and Drug Administration, allows no more than 4.335 mg of aluminium in vaccines given in the first year of life (55). Even though aluminium is widely used, aluminium is not regarded as a potent allergen (56), and the mechanism of the metal haptenisation is still not fully understood (57).

The prevalence of aluminium allergy has not been well studied (55), although a very large number of metallic discs have been used during long time as test chambers for all the sensitisers in the patch test series. Reactivity caused by the aluminium disc chambers is seldom seen in the test situation, and sensitisation is often incidentally identified. At the end of the 1990s, a retrospective analysis of the frequency of established aluminium allergy in the dental test series was performed in Sweden (Annica Inerot, personal communication). The purpose of the analysis was to update and evaluate the dental screening test series, in which aluminium chloride hexahydrate 2% was included. Test results from a total of 1300 patients from Malmö, Linköping, Jönköping, Stockholm, Umeå, Uppsala and Gothenburg were analysed. None were positive for aluminium chloride hexahydrate 2% in petrolatum, three had doubtful reactions and one had an irritant reaction. In Gothenburg, 208 (mean age 55 years) of the 1300 patients were included and all were negative in the test.

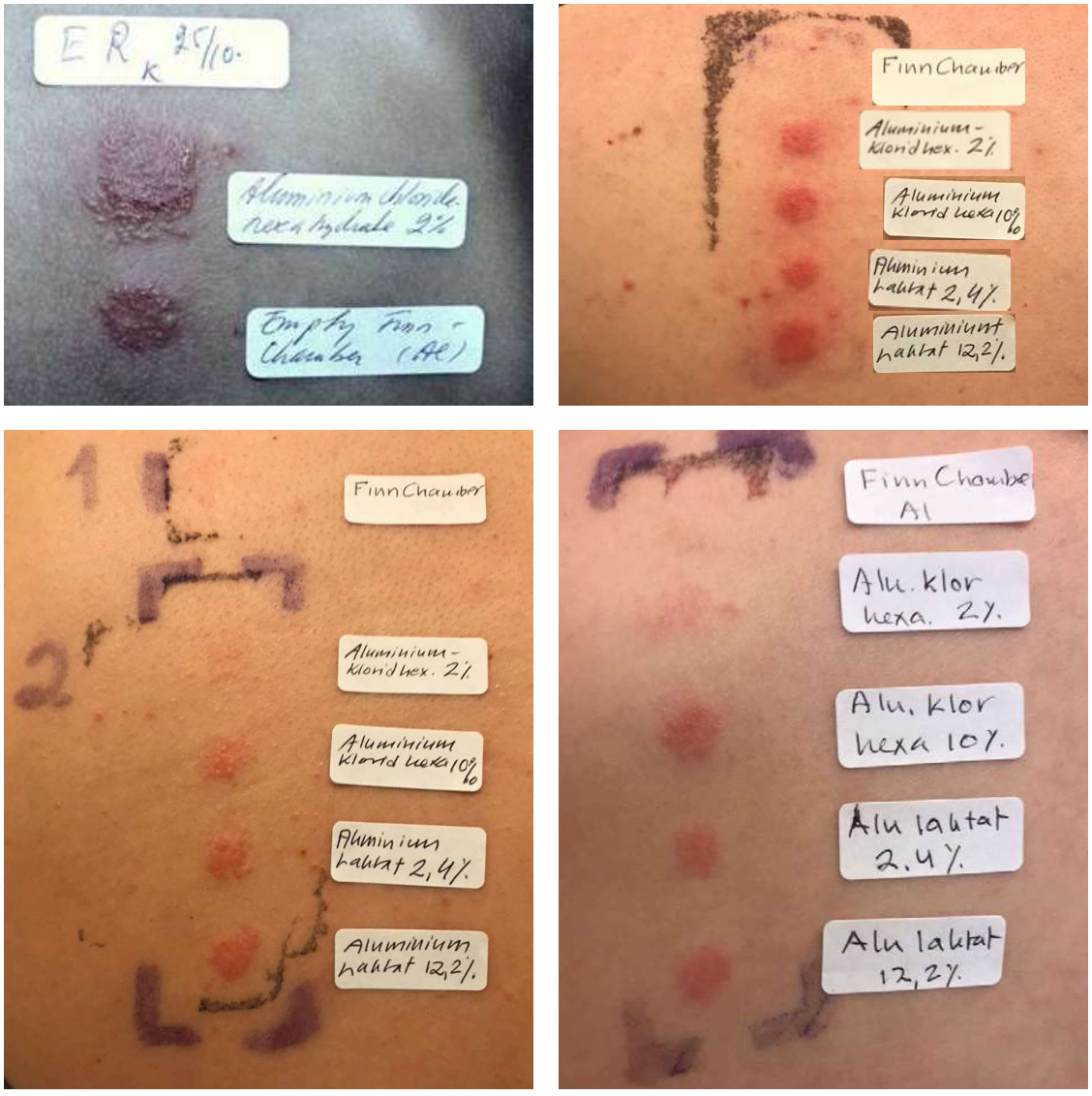
In a meta-analysis of aluminium allergy confirmed by patch testing and without association to vaccination granulomas, the pooled prevalence of adults was 0.36% and 5.61% for children (58). The analyses included 25 articles from 1944-2020 from several parts of the world. The individuals tested were of different ages, and different preparations of aluminium were used (mostly aluminium chloride hexahydrate 2% and 5% in petrolatum). The primary exposure was reported from topical medicaments, metallic aluminium and deodorant. In 10 of the 25 articles, the exposure source of aluminium sensitisation was not known.

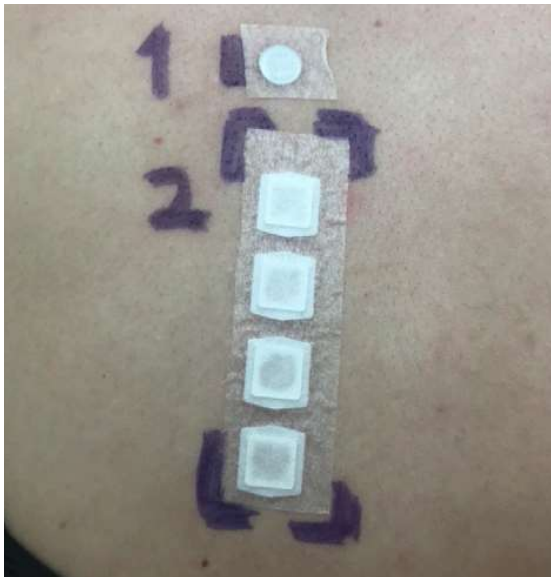
Aluminium patch test

The optimal method for aluminium patch testing has not been investigated in a larger cohort/study. Currently, the commercially available aluminium preparations are aluminium chloride hexahydrate 2% (Chemotechnique Diagnostics®, Malmö, Sweden) and aluminium hydroxide 10% in petrolatum (Chemotechnique Diagnostics®, Malmö, Sweden and SmartPractice, Phoenix, AZ, USA) in petrolatum.

Historically, different forms of aluminium have been used (59-64). Also, intracutaneous testing with aluminium hydroxide in saline or in water has been reported (65-68). Traditionally a metallic disc of elemental aluminium (Finn Chamber®, Ø=8 mm; Epitest Ltd Oy, Tuusula, Finland) and an aluminium salt, aluminium chloride hexahydrate 2% in petrolatum, in a plastic chamber have been utilised. In 2008 and later, a case report (69) and some studies of smaller cohorts (61, 70, 71) were published reporting that the use of aluminium chloride hexahydrate 10% in petrolatum resulted in a larger number of positive reactions than the 2% preparation.

In a review from 2019 (72), three studies on patch testing children for aluminium allergy were evaluated. The authors' conclusion was that aluminium chloride hexahydrate 2% in petrolatum was sufficient to trace contact allergy to aluminium in small children at least before the age of 7 to 8 years. In case of a strong suspicion of ACD caused by aluminium, a retest with aluminium chloride hexahydrate 10% should be performed (73).





Photographs of participants included in the studies in this thesis showing positive patch test results of different aluminium compounds. Photographs by Annica Inerot and Anette Gente Lidholm.

Prognosis of aluminium allergy

ACD is considered to be a lifelong condition. Only a few studies on the issues of loss of aluminium contact allergy are published in which the non-reproducibility of patch test results varies from 19% to 100% (55). In conclusion, the loss of patch test reactions must be interpreted with caution and considered upon the relevance of symptoms by the causative allergen.

THE IMMUNE SYSTEM

The essential features of the immune system are the identification with subsequent elimination of foreign antigens, creation of immunologic memory and developing of tolerance to self-antigens (74). According to the velocity and specificity of action, the immune system is divided into the innate and the adaptive immune system (75). The innate immune system responds rapidly and unspecifically, often leading to more considerable tissue damage, while in comparison the adaptive immune system acts more slowly but with greater specificity. The adaptive immune system is distinguished by memory cells which elicit a dynamic and fast response upon re-exposure to a particular antigen. In humans, the innate and adaptive immune systems are merged and work closely together.

The innate immune system

The innate immune system has a humoral component, with mechanisms including complement activation and use of antimicrobial peptides and effector cells such as macrophages, neutrophils, monocytes and mast cells. These mechanisms are fundamental for the protection of the individual and have been preserved through evolution. The effector cells of the innate immune system recognise highly preserved structures called pathogen-associated molecular patterns (PAMPs), which

are present in large groups of microbes (76). PAMPs include structures such as bacterial and fungal cell-wall components and are not expressed by the host itself. Pattern-recognition receptors (PRRs)/Toll-like receptors (TLRs), located on effector cells such as antigen-presenting dendritic cells, bind to the PAMPs and induces different effector responses such as cytokine and signal release and inflammation (77).

Cytokines are produced by all cells and can induce a broad diversity of events, e.g., cell activation, division, apoptosis and migration (75). Interleukins are cytokines produced by leukocytes and predominately affect other white blood cells. Cytokines interfering with viral replication are called interferons. Chemokines are also members of the cytokine family and have a crucial role in leukocyte migration (75).

Depending on the type of pathogen and its recognition pattern structure, different signals from the innate immune system provide the information to the adaptive immune system leading to activation of relevant effector responses (78). Antigen presenting cells (APCs), especially dendritic cells, have a crucial role in interacting between the innate and adaptive immune systems in the skin by a complex cascade of signals. There are several types and subsets of APCs. These include the dendritic cells in the epidermis (Langerhans cells), dermal dendritic cells, monocytes and macrophages. The APCs take up and process the pathogen and present it on its surface to cells in the adaptive immune system which are then activated. When presenting the pathogens, the APCs use specialised receptors: major histocompatibility complex (MHC) classes I and II. MHC class I mainly present substances produced within the cell (viral or tumour proteins) and MHC II present foreign antigens taken up via endocytosis (75).

The adaptive immune system

The main effector cells of the adaptive immunity response are T-lymphocytes and B-lymphocytes. The B-lymphocytes produce antibodies (immunoglobulins IgG, IgM, IgA, IgD or IgE.) leading to different effects of the immune system such as complement activation, phagocytosis and antibody-dependent cytotoxic attack by natural killer (NK) cells (75). A B-lymphocyte can change its production of immunoglobulin through interaction with T-lymphocytes, a process known as iso-type switch.

There are two different populations of T-lymphocytes: T-helper cells (TH-cells) and cytotoxic T-cells (TC-cells), labelled by two different receptors, CD4+ and CD8+, respectively. The role of TH-cells is to control the immune response by dictating which defence strategy is used against a particular intruder via secretion of different cytokines. In contrast, the cytotoxic T lymphocytes directly cause the death of cells harbouring the pathogen.

In the thymus gland, naïve TH- and TC-cells, mature to recognise and respond to foreign antigens by generating a unique T-cell receptor (TCR) on their surfaces by T-cell receptor gene rearrangement (74) .The cells then migrate into the lymphoid organs (spleen, lymph nodes, and the mucosa associated lymphoid tissue), prepared to encounter the corresponding antigens to their TCRs. The T-cell is regarded naïve until its TCR is stimulated by the specific antigen.

Antigens are processed and then presented to the T-cells by the APCs in the lymphoid organs. The unique TCR on the T-cell recognises the antigen as foreign and interacts with the MHC-receptor expressed on the surface of the APC. Naïve CD4+ TH-cells recognise and interact with APCs MHC class II and naïve CD8+TC-cells interact with APCs expressing MHC class I (79).

This induces intracellular signalling in the T-cell which results in a release of cytokines in the microenvironment and further differentiation of the cell (79). CD4+ TH-cells differentiate into several different types of TH-cells - TH1, TH2, TH17 and T-regulatory (T-reg) cells. The TH-cells are distinguished by their cytokine production and mediate different immune responses. TH1-cells secrete primarily IFN-γ, TNF-α and IL-2. TH2 cells secrete IL-4,-5,-9-10 and -13. IL-17 and -22 are secreted by TH17 cells and T-reg cells secrete mainly IL-10 and TGF-b (80).

The CD8+ TC-cells are subdivided into TC1 and TC2-cells, where TC1-cells mainly produce IFN-γ while TC2-cells primarily produce IL-4 and -5 (80).

Sensitisation and elicitation

ACD occurs in two steps: sensitisation and elicitation. The sensitisation phase lasts from 10-15 days and starts when the antigen penetrates the skin and generates the hapten-protein complex. The penetration of the antigen through the skin stresses the keratinocytes to produce pro-inflammatory cytokines. Also, danger signals are released and is detected by the immune system as tissue damage (81). There are mainly two types of danger signals; the products of the injured tissue created by the antigen invasion called damage-associated molecular patterns (DAMPs) and the products of the invading pathogenic micro-organism which is called pathogen-associated molecular patterns (PAMP) (82). PAMPs include reactive oxygen species (ROS) and the overproduction of ROS is suggested to be the start of the initial allergen sensitisation as well as the development of pathogenic allergic responses (83). Cytokine release from the keratinocytes leads to the activation of dendritic cells, e.g., Langerhans cells that migrate towards the hapten-protein complex. The complex binds to TLRs on the Langerhans cells, stimulating it to release of a cascade of cytokines which leads to antigen uptake, antigen processing and activation of the Langerhans cells cell.

While formation of a hapten-protein complex which subsequently binds to the TLRs is believed to be the most common mode of dendritic cell activation, a few

sensitisers have been described to use another pathway. These sensitisers can bind directly to TLRs without generating a hapten-protein complex. In their ability to ligate and trigger the dendritic cell by themselves, they act more like highly preserved structures, PAMPs. Examples of such sensitisers are the metals nickel, palladium and cobalt which all bind directly to the TLR-4 receptor (84). The TLR-4 receptor is originally known to respond to virulent bacterial antigens e.g. lipopolysaccharides/endotoxins.

The activated APC migrates from the skin to the regional lymph node, where it presents the processed antigen on its surface receptor MHC II to naïve CD4+ T-lymphocytes. The naïve T-lymphocyte is then activated and differentiates into TH1, TH17, Treg and other cells.

The differentiation of T-cells also leads to the formation of a specific subset of hapten-specific T-memory cells with the ability to recognise the same hapten if exposed to it again. Most of the memory cells are released from the lymph nodes into the blood circulation and into the skin.

The elicitation phase typically takes 1-2 days and starts with re-exposure to the allergen in the skin. The APCs are activated, and the hapten-protein complex is taken up, processed and expressed on the surface of the Langerhans cell. It is then recognised by the hapten-specific T-memory cells located in the skin, which in turn recruit the CD8+ TC-cells, CD4+ TH1 and NK-cells, the crucial effector T-cells in ACD. A cascade of cytokines cells leads to an inflammation of the skin.

Tolerance

All cells in the innate and adaptive immune systems derive from the bone marrow in which the B cells are "educated" to recognise the host's own tissues. The majority of T-lymphocytes mature in the thymus, located between the chest and the lungs. The T in T-lymphocytes stands for "thymus". The thymus gland is active until puberty. In the thymus, the lymphocytes mature to respond to foreign antigens while remaining unresponsive to the host's own tissues, a process called thymic selection (85). The T-lymphocytes then migrates to lymph nodes located throughout the body where they fight pathogens.

Chronic tissue inflammation and autoimmunity are caused by an imbalance between pathogenic effector T-cells and T-reg cells. T-reg cells, a subset of CD4+ TH-cells, suppress pathogenic immune responses (86). The majority of T-reg cells originate from naïve CD4+ TH-cells in thymus where they maturate upon recognising self-antigen. These cells are called thymic T-reg cells (85). Some of the T-reg cells are generated outside the thymus from naïve TH-cells and are named peripheral T-reg cells (87). T-reg cells consist of several subpopulations, most likely with different functions in different tissues (86). To date, little is known about T-reg cells residing in human skin.

Immunotherapy of allergic diseases is based on the induction of allergen-specific tolerance. For example, in type I allergies to house dust mites and pollen allergens, hyposensitisation can re-establish allergen tolerance for some years (4). Immunotherapy introduces the allergen via the subcutaneous (SCIT) or sublingual (SLIT) route, gradually in higher doses which leads to a shift in the balance between allergen-specific TH2 cells and T-reg and other regulatory cells (88).

In ACD, the down-regulation of the immune response is defined by effector T-cell death and by Treg cells (4). For type IV allergy, there is no established therapy to induce contact allergen-specific tolerance.

Vaccines

Vaccines are estimated to save approximately 2–3 million lives per year through preventing more than 30 infectious diseases (89). Initially, vaccines were developed empirically, with limited comprehension of how they activated the immune system and elicited immunity. Vaccine history starts at the end of the 18th century when Edward Jenner performed his vaccination trial of smallpox. During the late 19th century and early 20th century, vaccines against bacteria such as anthrax, rabies, diphtheria/tetanus and tuberculosis were introduced. In the middle of the 1950s, a new era started with vaccines against viral diseases. The highly effective polio vaccine was followed by measles, rubella and pertussis vaccines. From the 1980s, vaccines were introduced against varicella, hepatitis A and B, pneumococcal and meningococcal infections, human papillomavirus, herpes zoster, *Haemophilus influenzae* type b and tick-born encephalitis. Lately vaccines against corona virus disease 2019 (covid-19), could be introduced all over the world.

Vaccines have traditionally been injected subcutaneously. In 1997, intramuscular administration was shown to minimise local side effects (90). This is now the standard mode of injection for most vaccines. Also oral administration of some vaccines, e.g., against cholera, polio, rotavirus and typhoid and influenza, is possible (91).

Most vaccines can be divided into live attenuated vaccine, inactivated/killed vaccines or subunit vaccines. The novel vaccines against covid-19 follow completely different designs; mRNA, DNA or vector borne vaccines.

Live attenuated vaccines contain pathogens that have been weakened to be less virulent than their wild-type counterparts (92). These vaccines induce robust cell-mediated and antibody responses and often confer long-term immunity after only 1 or 2 doses (93). The only live attenuated bacterial vaccines used in Sweden are the Bacillus Calmette-Guérin (BCG) vaccine and the oral typhoid vaccine (94). The live attenuated viral vaccines comprise those against measles, parotitis, rubella, varicella and yellow fever.

In subunit vaccines only a part (subunit) of the infecting organism is used. Vaccines with an inactivated toxin as a subunit, toxoid vaccines are used to prevent pertussis, diphtheria and tetanus. Other examples are polysaccharide vaccines, in which an extracellular polysaccharide capsule is used. In this type of subunit vaccine, the polysaccharides are conjugated to a protein to increase the immune response (94). Recombinant vaccines are other subunit vaccines in which the antigen is produced by yeast or bacterial cells, as for hepatitis B virus, where the hepatitis B surface antigen (HBsAg) is used.

Inactivated/killed vaccines need several repeated doses to gain a proper long-lasting immune response. This has led to the development of adjuvants. The adjuvant's role is to elicit a more robust immune reaction by interacting between the innate and adaptive immune response (95).

Adjuvants

The Latin word adjuvant derives from"adjuvare" and means "to aid". Adjuvants used in vaccines and allergen-specific immune therapy (ASIT) are substances with properties to enhance the immunogenicity of the vaccine antigens. Adjuvants are only used for inactivated or killed vaccines (92).

In 1925 the French veterinarian Gaston Ramon detected that horses had higher antibody titres against diphtheria if they had developed an abscess at the injection site due to a simultaneous infection (96). Glenny et al. reported almost simultaneously the immune-enhancing effects of aluminium salts, known as "alum adjuvants" when injecting them together with diphtheria toxoid (97).

Aluminium salts have been used in vaccines as adjuvants to enhance the immune system since the 1920s and are the most widely used adjuvants. For currently used aluminium-adsorbed vaccines in Sweden, see table 3 (98, 99). Two kinds of aluminium-based vaccines are produced by different methods, aluminium-adsorbed vaccine and aluminium precipitated vaccine. Aluminium adsorption is the most common standardised method. In the adsorbed vaccines, an aluminium hydroxide or aluminium phosphate gel is added to the antigen, then binding to its surface (100). These two aluminium salts are the most commonly used and have different characteristics as adjuvants (101). The aluminium hydroxide is positively charged in physiological pH, in contrast to the negatively-charged aluminium phosphate. Aluminium hydroxide is regarded as a more potent adjuvant than aluminium phosphate since it has shown higher antigen adsorption at neutral pH values (101). Other less common aluminium salts used are alum (potassium aluminium sulfate) and mixed aluminium salts (97).

Several other adjuvants have been tested in vaccines for animals and humans but aluminium salts, emulsions and liposomes are the classic (95). Emulsions consist of an oil-in-water or a water-in-oil emulsion and liposomes are spherical lipid

Table 3

Vaccine	Commercial name	Producer	Aluminium adjuvant	Amount of aluminium per dose	Other antigen included
Diphtheria Tetanus Pertussis (acellular)	Infanrix-hexa®	GSK	Al hydroxide	0.5 mg	Polio, Hib, Hep B
	Boosterix®		Al hydroxide	0.3 mg	
	Boosterix Polio®		Al hydroxid + Al phosphate	0.3 mg 0.2 mg	Polio
	Tetravac®	Sanofi AB	Al hydroxide	0.3 mg	Polio
	Triaxis®		Al phosphate	0.33 mg	
	Repevax®		Al phosphate	0.33 mg	Polio
	Hexyon®		Al hydroxide	0.6 mg	Polio, Hib, Hep B
	diTeKiBooster®	Scandinavian Biopharma	Al hydroxide	0.5 mg	
Diphtheria/ Tetanus	diTeBooster®	Scandinavian Biopharma	Al hydroxide	0.5 mg	
Hepatitis A	Havrix®	GSK	Al hydroxide	0.5 mg	
	Avaxim®	Sanofi AB	Al hydroxide	0.3 mg	
	Vaqta®	MSD	Al hydroxide phosphate sulphate	0.45mg (adults, ad) 0.225 mg (children, ch)	
Hepatitis B	Engerix-B®	GSK	Al hydroxide	0.5 mg (ad) 0.25 mg (ch)	
	Fenderix®	GSK	Al phosphate	0.5 mg	
	HBVAXPRO®	MSD	Al hydroxide phosphate sulphate	0.5 mg (ad) 0.25 mg (ch)	
Hepatitis A+B	Twinrix®	GSK	Al hydroxide	0.05 mg (ad) 0.025 mg (ch)	
	Ambirix®		Al hydroxide + Al phosphate	0.05 mg 0.4 mg	
	Fenderix®		Al phosphate	0.4 mg (ad) 0.2 mg (ch)	

Table 3

Vaccine	Commercial name	Producer	Aluminium adjuvant	Amount of aluminium per dose	Other antigen included
Tick-borne encephalitis (TBE)	FSME Vuxen® FSME Junior® Encepur®	Pfizer Bavarin Nordic	Al hydroxide Al hydroxide	0.35 mg (ad) 0.17 mg (ch) 0.3-0.4 mg (ad) 0.15-0.20 mg (ch)	
Japanese encephalitis	IXIARO®	Valneva	Al hydroxide	0.25 mg	
Meningococci (conjugated)	BEXSERO® Trumenba®	GSK Pfizer	Al hydroxide Al phosphate	0.5 mg 0.25 mg	
Pneumococci (conjugated)	Prevenar 13® Synflorix®	Pfizer GSK	Al phosphate Al phosphate	0.125 mg 0.5 mg	
Human papilloma virus (HPV)	Gardasil 9® Cervarix®	MSD GSK	Al hydroxide phosphate sulphate Al phosphate	0.5 mg 0.5 mg	

Table 3. Currently used aluminium-adsorbed vaccines in Sweden. Revised from the thesis of Elisabet Bergfors, 2006 (98), after her permission in 2021, and Fass (99).

layers encapsulating the antigen with the ability of both being a vaccine delivery vehicle and an adjuvant (102). Newer adjuvants with a combination of the classical ones and immunomodulatory molecules are used in the human papillomavirus (HPV) and malaria vaccines (95).

The adjuvants interact and activate the immune system in different ways. The emulsion adjuvant primarily acts by enhancing the antibody response, and the liposomes promote humoral and cell-mediated immune responses (95). Aluminium salts primarily promote antibody responses, with little or no effect on immune response of TH1 and TC-cells but the exact mechanism is unknown (103).

Developing vaccines against human immunodeficiency virus (HIV) and malaria with classical adjuvants has been a challenge since other immunological answers

are necessary. One new approach includes the development of vectored vaccines in which a subunit of a pathogen is carried by non-pathogenic infectious viruses, bacterial or plasmid (92, 104). Another approach is the technique of inserting parts of DNA or RNA in order to encode proteins of the pathogen and promote antigen presentation in human cells to induce an immune response (92). This new technique has been used for novel covid-19 vaccines.

Adverse reactions to aluminium adsorbed vaccines. Persistent itching nodules

Local adverse events at the injection site after vaccinations are common and diverse for all vaccines. Inflammation with erythema and swelling appearing within a day or two after vaccination are seen in 30% of cases and mostly subside after some days (105). This inflammation is often a reaction to the injected antigen-adjuvant solution and is not the same reaction as the persistent itching nodule that may appear some weeks to months after vaccination with aluminium-adsorbed vaccines.

The first report on persistent itching nodules (vaccination granulomas) in 1960 (26) was followed by sporadic case reports during the following decades. Itching nodules were considered as extremely rare events mostly after vaccination with aluminium-adsorbed diphtheria-tetanus and diphtheria-tetanus-pertussis vaccines (106, 107) and antigen extracts used in ASIT (108-110). In the 1990s an unexpectedly high incidence (1%) of itching nodules was reported in clinical trials of a new pertussis vaccine in Sweden (37). Since then an increasing number of reports have been published on cases occurring after vaccination with all aluminium-adsorbed vaccines, except so far, tick-borne-encephalitis (TBE) (111).

The incidence of itching nodules is only described in a few reports on cohorts in Scandinavia (37, 112) and is estimated to be 0.63-1.18% in children receiving aluminium-containing vaccines (111, 113).

Persistent itching nodules are considered to be caused by aluminium salts used as adjuvants in almost all inactivated vaccines. They are strongly associated with aluminium hypersensitivity, verified by a patch test in several studies (37, 62, 63, 114-119). In a study by Bergfors et al. (37), aluminium allergy was demonstrated in 352 of 455 (77%) children with itching nodules after pertussis vaccination. The remaining children who tested negative (23%) were mostly older. In the same study 17 of 211 children without itching nodules after vaccination unexpectedly tested positive for aluminium. On the other hand, no associations were found between vaccination granulomas caused by an aluminium-adsorbed vaccine and patch test verified aluminium allergy in another study (112), and in a review by Jeffersson the evidence could not confirm that aluminium salts in vaccines cause any long-lasting adverse events (120). There was however, a limited amount of comparative data available.

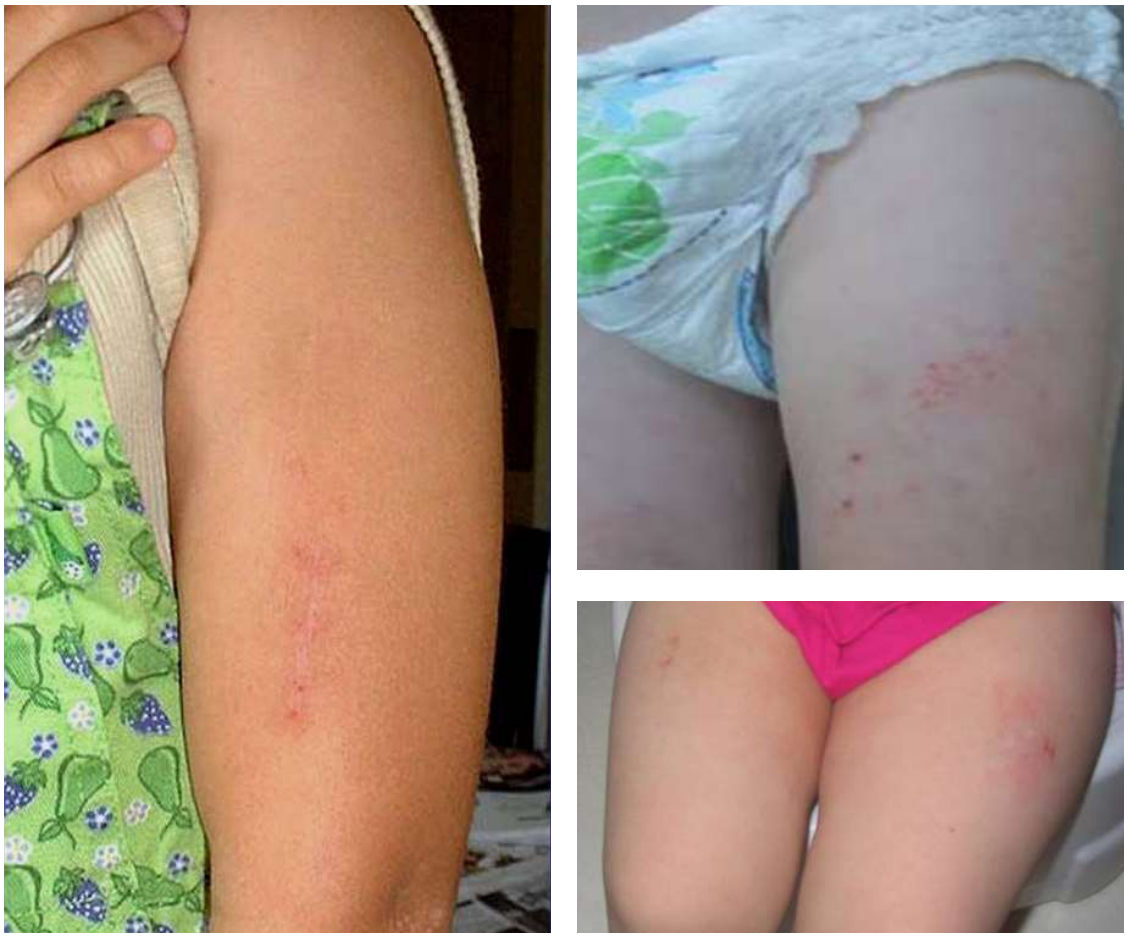
As the name suggests, persistent itching nodules are severely itching and persist for months to years. Common additional symptoms are local hypertrichosis, eczema and hyper- or hypopigmentation at the injection site. The approximate size of the nodule is between 3 and 25 mm and it can be palpated as firm, oblong or round without tenderness (37). A long delay (months) between the vaccination and onset of symptoms is typical (37). The risk for itching nodules increases with the number of doses of aluminium-adsorbed vaccine administrated (37). Exacerbations of the itching nodules are commonly seen during intercurrent infections as well as an experience of itchiness in the former test patch area in those previously tested for aluminium (72). The symptoms can be symptomatically treated with topical steroids under occlusion of hydrocolloid bandage with only temporary effect (98).

The formation of the itching nodule has been hypothesised due to the injection technique. Correctly performed intramuscular injections were thought to give a lower incidence of itching nodules than subcutaneous injections (121) since the risk of deposition of the vaccine subcutaneously should be very small. However, injecting children may be challenging and some of the injected vaccine may be administrated more superficially than intended (122). In the study of Bergfors et al. (37) itching nodules were seen after both subcutaneous and intramuscular injections. These findings are also supported by other studies (111, 113).

The nodules have been examined histopathologically in several studies. Findings include mixed inflammatory cells infiltrated with giant cells and a central necrotic part in which aluminium crystals can be found in special staining (116) and atomic absorption spectrometry(123). The nodules can be found both subcutaneously and in dermal tissue (124).

Through group consensus based on expert opinion and a review of the literature from 1966-2002, the Brighton Collaboration Local Reactions Working Group has tried to develop a case definition and guidelines for the clinical diagnosis of a nodule at the injection site (125). This was performed to improve comparison of vaccine safety data. However, the definition of the nodules for clinical diagnosis found in the literature was too unspecific. Instead, guidelines and a template check list for which data that should be collected were developed.

Other adverse events such as autism and other autoimmune/inflammatory neurological diseases have been suggested to be caused by aluminium adjuvants in the literature. According to the Global Advisory Committee on Vaccine Safety, an advisory board for the World Health Organization, there is no scientific evidence of such harm related to aluminium adjuvant vaccines (126).



Persistent itching subcutaneous nodules after vaccination with aluminium-adsorbed vaccine. Photographs by Elisabet Bergfors published with her consent.

Allergen-specific immunotherapy (ASIT)

Immunotherapy of allergic diseases is based on the induction of allergen-specific tolerance. Most allergen vaccines (extracts) used to treat allergic asthma, rhinoconjunctivitis and insect venom allergy contain aluminium hydroxide as an adjuvant and are given subcutaneously. The dose of the allergen is gradually increased to reach an effective dose to improve the symptoms of the causative allergen (127). During the induction phase, the allergen vaccine is given in approximately 1 dose per week for 15 weeks, followed by less frequent injections over 3 to 5 years. The amount of aluminium injected is 3.3 mg/100.000 SQ-E/ml and accumulated, far higher than the amount in vaccines for pathogens (99).

Persistent itching subcutaneous nodules and aluminium contact allergy have also been reported after ASIT, even though it is considered rare (65, 70, 114, 128, 129). In a study by Netterlid et al. (70), 37 children suffering from asthma and/or allergic rhinitis received hyposensitisation therapy. Itching nodules were found in 13 of them during the treatment. Eight children had a positive patch test for aluminium. Surprisingly, no association between the total dose of aluminium injected and the demonstrated contact allergy was found.

WHOOPING COUGH

Whooping cough, also called pertussis, is caused by *Bordetella pertussis,* one of ten species in the Bordetella genus (130). *B. pertussis* is a Gram-negative aerobic coccobacillus and was first isolated in 1906. It is spread by air droplets exclusively to humans and infection results in respiratory symptoms. Three of the other Bordetella genus species can also cause whooping-cough like symptoms in humans though less severe. *B. parapertussis* is the most common of these 3 species.

The worldwide incidence of pertussis in children younger than 5 years was estimated at 24.1 million cases and around 200 000 deaths in 2014 (131). Morbidity and mortality have decreased dramatically due to vaccination. However, pertussis is still a considerable global health problem, particularly in low-income countries. The disease affects all ages but is most severe in the elderly and in children, especially during the first year of life, and can lead to death. Despite high worldwide vaccination coverage, pertussis is an endemic disease with epidemic outbreaks every 2 to 5 years. Vaccination is crucial in preventing disease but does not control the circulation of the bacteria in the population or its transmission. Under-reporting of the disease is expected because of differences in diagnosis, consulting and recognition of pertussis symptoms.

Before the pertussis vaccine was included in the Swedish childhood vaccination program, approximately 5.000 to 20.000 cases were reported per year (132) and 1.000 people died of whooping cough each year. Following the introduction of the pertussis vaccine, the number of cases decreased to less than 1.000 cases per year at the beginning of the 1970s. During 1979-1996, vaccinations were discontinued due to the vaccine's declining protective effect and suspected severe side effects. Before introducing the new acellular pertussis vaccines, the annual incidence of culture-confirmed B. pertussis was 89–150 per 100.000 person years (133). After the vaccine's reintroduction in 1996, the overall annual incidence dropped to 17–26 per 100,000 person years, nearly 80–90% lower than before the acellular pertussis vaccines were introduced (133). However, since 2014, the number of reported cases has increased three-fold for all ages and still remains at this level. This might partly be explained by an increased number of samples taken. No infant deaths have been reported due to pertussis since 2015 (134).

Pathogenesis

The pathogenesis of *B. pertussis* is not entirely understood. More than 50 virulence factors have been described. The most crucial factors are an exotoxin and surface antigens, causing local damage to the upper and lower respiratory tracts with systemic manifestations. The major toxins contributing to the severity to pertussis disease are pertussis toxin, adenylate cyclase toxin-hemolysin and tracheal cytotoxin (135). Tracheal cytotoxin is the most important toxin as it impairs the

respiratory mucosal by destroying the ciliated cells and thereby impairing the mucocillary clearing (136). Adenylate cyclase toxin-hemolysin inhibits phagocytic cells and their antibacterial activities. Pertussis toxin (PT) has different effects on pathogenesis, and its role is not entirely understood. PT suppresses the protective immune responses, and the detoxified form of PT is an important component of currently used acellular pertussis vaccines. Some of the surface antigens work as adhesins contributing to the interaction between cells (137).

Clinical features

The incubation time for *B pertussis* is 1 to 3 weeks. A person with whooping cough is contagious for approximately 6 weeks. The symptoms are divided into 3 stages: the catarrhal stage, the whooping cough stage and the convalescent stage. In the catarrhal stage, the dominating symptoms are mild, with cold and subfebrility lasting for 2 weeks. During this period, the person is very contagious. In the next phase, the whooping stage, the typical cough dominates, often in attacks and with the characteristic wheezing sound during inhalation between the "whoopings", giving the name to the disease. Infants often get hypoxia and turn cyanotic during the cough attacks and sometimes turn unconscious for some seconds or vomit a thick mucus. The symptoms are worse during evenings and nights as well as after meals. In older, not yet fully vaccinated children, the symptoms are milder with prolonged dry cough. This stage lasts for 1 to 6 weeks before the symptoms gradually decrease, and the disease turns into the convalescent phase for another 2 to 3 weeks.

Infants are most vulnerable to infection, and 70% of infected infants younger than 3 months are hospitalised (138) with complications such as pneumonia, otitis, cerebral hypoxia, encephalopathy and even death.

Adults and the elderly can also be infected, despite earlier vaccination or infection since the immunity response weakens over time. Reported complications of infection in adults are pneumonia, rib fractures, pneumothorax and urinary incontinence in 4 to 23% of infected individuals (139-141).

The diagnosis of whooping cough is best made with PCR of the nasopharyngeal secretions but culture and serology are also available. In Sweden, whooping cough is mandatory to report to the infection control authorities, and infection tracing is performed. There is no cure for the infection, but antibiotics, such as erythromycin and other macrolides, given in the early phase of the disease, can decrease symptoms and reduce contagiousness. According to general advice from the Swedish Public Health Agency to prevent severe illness, all infants less than 6 months old should be treated with antibiotics if they are at risk for pertussis exposure. Children from 6 months to 1 year of age are treated as soon as possible if they have suspected symptoms or if they have come in close contact with diagnosed pertussis.

Prevention

Globally, vaccines provide immunity against pertussis to hundreds of millions of individuals each year. About 85% of infants worldwide received 3 doses of diphtheria-tetanus-pertussis during 2019 (142). During 2019 in Sweden, approximately 97% of all 2-year-olds were fully vaccinated according to the Swedish national immunisation programme's NIP current schedules (143). The current and previous Swedish NIP, are shown in table 4 (144).

In 1953, a whole-cell vaccine against pertussis produced by the Swedish Bacteriological Laboratory (SBL) was introduced in the Swedish NIP for children. The incidence of whooping cough decreased dramatically. In 1979, the whole-cell vaccine was withdrawn due to a gradually impaired protective effect caused by changes in the manufacturing process (145) and suspected neurological side effects. The most dramatic adverse event was a shock-like state, hypotonic hyporesponsive episode (HHE) (146). Within 12 hours after vaccination affected children suddenly became pale, limp and unresponsive for several hours. No persisting brain damage has been observed.

During 1979-1995, 90 000 children were vaccinated against pertussis with various vaccine candidates in different clinical trials, including the Gothenburg Pertussis Vaccine Trials. In 1996 a new acellular pertussis vaccine was reintroduced in the NIP and administered to infants at 3, 5 and 12 months. In 2005 a booster dose was recommended for children at 10 years since pertussis protection was only observed for a limited time. The 10 years' booster was withdrawn in the NIP in 2012 when the recommended age for the fourth dose had been altered to 5-6 years of age. A fifth dose for teenagers 14–16 years old was implemented in 2016. At this time, over 98% of all two years old children in Sweden had received 3 doses and 95% of the scholars in 6th class had received 4 doses of pertussis vaccine (147).

Table 4

Vaccine against	Introduced	Previous program ceased	Program
Smallpox	Early 1800s	1976	Compulsory for the whole population until the disease was eradicated
Tuberculosis	1940s	1975	1 dose for new-borns
Tuberculosis	1940s	1986	1 booster dose at 14–15 years of age
Tuberculosis	1986	Current	Only risk groups
Diphtheria and tetanus	1940s	1986	3 doses between 3 and 12 months of age
Diphtheria and tetanus	1965	1977	1 booster doses at 7–8 years of age
Diphtheria and tetanus	1977	2012	1 booster dose at 10 years of age (children born until 2001)

Table 4

Vaccine against	Introduced	Previous program ceased	Program
Diphtheria and tetanus	1986	Current	3 doses at 3, 5 and 12 months of age
Diphtheria and tetanus	2007	Current	2 booster doses, at 5–6 years of age and 14–16 years of age (children born from 2002)
Whopping cough	1950s	1979	3 doses between 3 and 12 months of age
Whopping cough	1996	Current	3 doses at 3, 5 and 12 months of age
Whopping cough	2005	2012	1 booster dose at 10 years of age (children born until 2001)
Whopping cough	2007	Current	2 booster doses, at 5–6 years of age and14–16 years of age (children born from 2002)
Polio	1957	1986	3 doses between 9 and 18 months of age
Polio	1957	1977	1 booster dose at 7–8 years of age
Polio	1986	Current	3 doses at 3, 5 and 12 months of age
Polio	1977	Current	1 booster dose at 5 years of age
Measles	1971	1982	1 dose after 18 months of age
Rubella	1974	1982	1 dose to girls at 12 years of age
MPR*	1982	2007	2 doses, at 18 months and 12years of age (children born until 2001)
MPR*	2007	Current	2 doses, at 18 months and at 6–8 years (children born from 2002)
Hib**	1993	Current	3 doses at 3, 5 and 12 months
Hepatitis B	1996	Current	Only risk groups
Hepatitis B	2016	Current	Recommended to all infants. 3 doses at 3, 5 and 12 months of age
Pneumokocker	2009	Current	3 doses at 3, 5 and 12 months of age
HPV***	2010	2014	3 doses to girl born in 1999 and later in grade 5–6 (11-12 years of age)
HPV***	2015	2020	2 doses to girls at the age of 10–12 years in grade 5–6
HPV***	2020	Current	2 doses to both girls and boys at the age of 11 years (boys born from 2009) in grade 5
Rotavirus	2019	Current	2 doses at the age of 6 weeks and three months

* MPR = Measles-parotitis-rubella

** Hib = *Haemophilus influenzae* type b

*** HPV = Human papillomavirus

Table 4. Current and previous vaccination programs (144) https://www.folkhalsomyndigheten.se/the-public-health-agency-of-sweden/communicable-disease-control/vaccinations/previous-swedish-vaccination-programmes/

THE GOTHENBURG PERTUSSIS VACCINE TRIALS

For overview of the recommended vaccination schedules in Sweden see table 4. For overview of the Gothenburg Pertussis Vaccine Trials see table 5. For overview of previously and currently used diphtheria-tetanus-pertussis (DTP) vaccines in Sweden see table 6.

Table 5

Study	Vaccine schedule and mode of injection	Age
Efficacy Study 1991-94	DTaP x 3 (s.c) DT x 3 (s.c)	3, 5 and 12 months 3, 5 and 12 months
Booster Study 1997-98 (These children had received 3 doses of DTaP in the Efficacy study)	DTaP (-IPV) x 1 (i.m)	6 years
Control Children Study 1995 (These children had received 3 doses of DT in the Efficacy study)	aP x 3 (s.c)	3-5 years
Mass Vaccination Project 1995-99	DTaP x 3 (s.c/i.m) DTaP+aP+aP (s.c) aP x 3 (s.c/i.m)	3, 5 and 12 months 12, 14 and 20 months 1 - ~ 10 years
Combination Vaccine Study 1997	DTaP-IPV-Hib x 3 (i.m)	3, 5 and 12 months
Routine vaccination after the studies 1999-2000	DTaP x 3 (i.m)	3, 5 and 12 months

Table 5. Vaccination schedules for children in the Gothenburg Pertussis Vaccine Trials and in routine vaccination after the studies. The mode of injection was changed from subcutaneous (s.c) to intramuscular (i.m) in October 1998. Revised from the thesis of Elisabet Bergfors with her permission in 2021 (98).

Table 6

Vaccine	Commercial name	Producer	Aluminium adjuvant	Amount of adjuvant per dose	Period when the vaccine was used
Diphtheria Tetanus Pertussis (Whole cell)	Triple vaccine	SBL	1. Al phosphate 2. Not adsorbed	? 0	1953-1962 1963-1979
Diphtheria Tetanus	Duplex®	SBL	Al phosphate	2.5 mg (primary immunisation) 1.25 mg (booster)	1979-2003
	DT vaccine without aluminium	SSI	Not adsorbed	0	Individually licensed. Not available since 2001
Diphtheria Tetanus Pertussis	DiTeKik®	SSI	Al oxihydrate	Corresponding to 0.5 mg Al per dose	Not used in Sweden since March 2000

Table 6. DTP vaccines with and without Al adjuvant used in Sweden 1953-2001- Revised from the thesis of Elisabet Bergfors, 2006 (98), after her permission in 2021.

The Gothenburg Pertussis Vaccine Trials were initiated in 1986 by two paediatricians, John Taranger and Birger Trollfors, and performed in Gothenburg and nine surrounding municipalities (usually called the Greater Gothenburg area). In 1995, a general practitioner, Elisabet Bergfors, joined the study. The research team was completed by a various number - at most 17- of skilled paediatric nurses.

The vaccine investigated was a new acellular monocomponent vaccine consisting of the pertussis toxoid alone (aP). Several trials had previously been performed in Sweden and other countries to study the efficacy and reactogenicity of different pertussis antigen components. In most of these studies multi-component vaccines were used since it was unknown which pertussis antigen resulted in the best efficacy. In the Gothenburg Pertussis Vaccine Trials, the choice of a monocomponent pertussis toxoid vaccine was supported by the hypothesis that pertussis toxoid was adequate to protect from pertussis (148) and that multi-component vaccines increased the risk of adverse events (149). This is similar to the use of monocomponent toxoid vaccines for diphtheria and tetanus.

The vaccine used in the Gothenburg Pertussis Vaccine Trials was produced by Statens Serum Institut (SSI, Denmark). The hydrogen-peroxide-inactivated pertussis

toxin was manufactured by North American Vaccines Incorporated, Bethesda, Maryland, United States, later acquired by Baxter Healthcare Corporation and the aluminium adjuvant, Alhydrogel® by Superfos, Denmark. The adjuvant used consisted of aluminium hydroxide corresponding to 0.5 mg aluminium per dose. The antigen included in the other study vaccines, diphtheria and tetanus toxoids (DT) and the inactivated polio vaccine were also produced by SSI, compounding the vaccines.

The trials on the mono-component pertussis vaccine included three phase-2 studies (1987, 1988, 1989), which showed the safety and immunogenicity of the vaccine. They were followed by a double-blind-phase-3 **Efficacy study** (1991 to 1994) where infants were randomised to vaccination with either DT or diphtheria-tetanus-acellular pertussis toxoid vaccine (DTaP) at 3, 5 and 12 months of age (150, 151).

Another four studies were performed within the Gothenburg Pertussis Vaccine Trials, the Booster Study (not published), the Control Children Study (not published), the School Children Study (152) and the Combination Vaccine Study (153). In the Booster Study, children who had received aP in the Efficacy Study were offered a booster dose at 10 years of age. Children in the control group in the Efficacy Study who had received DT were offered a catch-up vaccination with aP in the Control Children Study. In the School Children Study serological responses to DTP were observed before and after booster vaccination at 10 years of age and in the Combination Vaccine Study children who received different vaccine combinations of diphtheria-, tetanus-, monocomponent pertussis-, polio- and *Haemphilus influenzae* type b (Hib) vaccines were investigated.

Finally, a large open phase-4 study, the **Mass Vaccination Project,** was initiated with the aim to investigate the epidemiology and transmission of pertussis during the first years after introducing the new monocomponent pertussis toxoid vaccine. The project included approximately 60.000 children in the Greater Gothenburg area and was performed from June 1996 to February 1999 (154).

Three different vaccine schedules were used in the project, depending on the number of previously administrated diphtheria-tetanus (DT) doses and the age at study enrolment; (1) earlier unvaccinated infants were offered a vaccine consisting of diphtheria, tetanus and pertussis toxoids, DTaP, at 3, 5, 12 months of age, (2) children aged 12 months who had received two doses of DT at 3 and 5 months in Child Health Care, were offered one dose of DTaP completed by two doses of aP at age 14 and 20 months; (3) children older than one year, who previously had received three doses of DT vaccine in infancy, were offered three doses of aP alone with intervals of 2 and 6 months (154).

The children participating in the Mass Vaccination Project were born during the 1990s and recruited at Child Health Centers, through advertisements in the press and individual letters (154). Older children were vaccinated upon request. The vaccinations were

performed by the staff of the Mass Vaccination Project in five locations in the Gothenburg area. The vaccines were given subcutaneously as routine but the injections were switched to intramuscular during the last five months of the study. As a rule, infants were vaccinated in the left thigh and older children in the left upper arm.

Since the previously used whole-cell pertussis vaccine was associated with severe adverse events, parents were informed to carefully report suspected vaccine reactions to the staff of the vaccine study at the time of the next vaccination. During the Mass Vaccination Project, intensely itching subcutaneous nodules at the injection site were reported in an unexpectedly high number of children. A structured follow-up was therefore established with regular telephone interviews, questionnaires and physical examinations by the staff of the vaccination team. The children themselves were invited to tell about their symptoms in drawings or text. All children with itching nodules were offered patch testing with aluminium. Child Health Centers were informed and requested to contact the team if they met vaccinated infants/children with itching nodules. The children and their parents were followed regularly, in the beginning every sixth month and later on yearly. Children with severe symptoms were followed more frequently.

The logo of the Gothenburg Pertussis Vaccine Trials. Logo illustration: Johan Lagergård

The aluminium study – patch test I

An association between vaccine-induced itching nodules and delayed hypersensitivity against aluminium was described in several case reports since the 1960s (62, 63, 107). When the number of children with itching nodules in the Mass Vaccination Project unexpectedly increased, and a structured follow-up of them had been initiated in 1997, a patch test with aluminium was offered all of them in order to give the parents proper information on the adverse event and to verify the association with itching nodules in a scientific way. This patch test study, " the Aluminium Study" was a part of the Mass Vaccination Project and was reported in 2003 (37). In this thesis it is called patch test I.

The test procedure and material were the same as in patch test II in this thesis (Paper I). Two traditional aluminium compounds were used; aluminium metal (an empty Finn Chamber®, Epitest, Finland) and aluminium salt (aluminium chloride hexahydrate 2% in petrolatum, Chemotechnique Diagnostics®, Sweden, in

a plastic chamber from the same manufacturer) (37). The applications were made by three of the vaccination team members, after special instruction by the staff in the Occupational and Environmental Dermatology Unit who had special training in patch test technique and long experience.

The patches were placed at the upper part of the back and were removed by the parents after 48 hours. After another 24 hours, the test was read on day 3 by a dermatologist (A.I.) or by one of the physicians (E.B.) in the project according to recommendations of the ICDRG. As only one reading was performed, all parents and their children were asked to pay attention to late reactions and, if that occurred, to contact the team. Before testings the families were interviewed.

At the time of publication of the Mass Vaccination Project (37), 645 children with itching nodules were identified and offered patch testing. 455 of them were tested along with a control group consisting of 265 children without itching nodules. All participants in the control group were siblings to the children with itching nodules and 211 of them had received the same study vaccines in the Mass Vaccination Project. The remaining 54 symptomless siblings had received an aluminium phosphate adsorbed DT vaccine in infancy.

Even though there was a significantly higher rate of positive reactions in children with itching nodules (352/455, 77%) than in their symptomless siblings, surprisingly 8% (17/211) of the siblings were positive in the aluminium patch test. None of the 54 siblings who did not participate in the vaccine trials had positive tests.

Drawing by a 9 years old girl showing the location of the itching nodule that appeared four years earlier after vaccination with three doses of aP.

AIM

This thesis aims to determine the long-term prognosis of vaccine-induced aluminium allergy and the clinical course of subcutaneous long-lasting itching nodules obtained in childhood vaccination within the trials of a new pertussis vaccine in Gothenburg, Sweden. The specific aims of the four studies included were:

To investigate the prognosis of delayed hypersensitivity to aluminium provoked by the aluminium-adsorbed vaccine in childhood vaccination within the Gothenburg Pertussis Vaccine Trials.

To investigate differences in the patch test reactions of the two types of aluminium compounds used in establishing aluminium hypersensitivity in the afflicted children participating in the vaccine trials.

To investigate the long-term clinical prognosis of vaccine-induced itching nodules and aluminium allergy and the outcome of further vaccinations with aluminium-adsorbed vaccines by regular follow-ups of the same cohort during 20 years.

To investigate the prognosis of vaccine-induced delayed hypersensitivity to aluminium by a third patch test in our cohort and define the optimal aluminium preparations to use in these testings.

Drawing by a 3 years old girl with an itching nodule on her left thigh where she received three doses of DTaP in infancy. Her symptoms then continued to about seven years of age.

PATIENTS AND METHODS

Patch test members

Patch test I (mainly during 1998 to 2002 with a few tested until 2005): Annica Inerot (A.I.), Ulla Blomgren (U.B.), Tordis Andrén (T.A), Lisbeth Söderström (L.S), Elisabet Bergfors (E.B.) and Birger Trollfors (B.T.).

Patch test II (2007-2008); Annica Inerot (A.I.), Anette Gente Lidholm (A.G.L.), Ulla Blomgren (U.B.), Elisabet Bergfors (E.B.) and Birger Trollfors (B.T.).

Patch test III (2020); Annica Inerot (A.I.), Anette Gente Lidholm (A.G.L.), Conny Eriksson (C.E), Britt-Marie Ehn (B-M.E), Elisabet Bergfors (E.B.) and Birger Trollfors (B.T.).

Study population

All 4 studies in this thesis are based on the same cohort of 745 children who participated in clinical trials of a new aluminium-adsorbed acellular pertussis vaccine in Gothenburg, Sweden, in the 1990s (151, 154). In all, about 76.000 children aged 3 months to approximately 15 years were included in the trials. From ~1997, an increasing number of subcutaneous, intensely itching nodules on the injection site were reported among the children. The condition was recognised as persistent itching nodules, or aluminium granulomas, induced by aluminium-containing vaccines and associated with contact allergy to aluminium (5, 37, 62, 114-117, 155). Itching nodules were reported in 745 children (455 girls, 290 boys) during the vaccine trials. The first 645 were described in the report, "Unexpectedly high incidence of persistent itching nodules and delayed hypersensitivity to aluminium in children after the use of adsorbed vaccines from a single manufacturer", in 2003 (37). Another 100 cases were identified later and reported in 2013 (Paper I), see table 7.

The 745 children with vaccine-induced itching nodules were further investigated in 3 studies (II-IV).

Table 7

Period	Number of children with reported itching nodules	Number of children tested	Number of children with positive test result	Number of children with negative test result	Report
1998-2002	645	455	352	103	(37)
2003-2005	100	40	25	15	Paper I
Total	745	495	377	118	

Table 7. The results of the first patch test (patch test I) for aluminium hypersensitivity in 745 children with persistent itching nodules in the Gothenburg Pertussis Vaccine Trials.

Paper I

All 377 children who tested positive for aluminium in patch test I were offered a second patch test. Of them, 241 (median age 13.3 years, range 8 to 21 years, 89 boys; 152 girls) were retested in 2007-2008.

Paper II

The patch test results for 366 of the 377 children with positive results in the initial test were further analysed. The reproducibility of test reactions for the 241 children (median age 13.3 years, range 8 to 21 years, 89 boys, 152 girls) who were tested twice was analysed.

Paper III

The clinical course was followed in all 745 children with reported persistent itching nodules in the vaccine trials.

Paper IV

A third patch test (patch test III) was offered to 103 now-adult individuals who participated in the second testing. In total, 65 were included in the study, but only 31 (median age 24.1 years, range 20.8 to 29.0 years, 20 females, 11 males) were tested when the study was interrupted by the covid-19 pandemic. Most of them (20), had positive reactions in the second test, and 11 with negative reactions were tested.

Methods

Paper I

The first study, a follow-up of the initial patch testings within the vaccine trial in 1998- 2002, was performed in October 2007 to May 2008, 5 to 9 years after the first testing.

The test procedure, material and reading criteria were the same as in the initial patch testing (37).

The patch test for aluminium was applied on the upper part of the back, with two different forms: 1) metallic aluminium (empty Finn Chamber® SmartPractice, Phoenix, AZ, USA); and 2) an aluminium salt which was placed in a plastic chamber (IQ Chamber, Chemotechnique Diagnostics®, Malmö, Sweden).

All applications were made by the same staff member (U.B.) and who had received special training in patch test technique and had a long experience in the Occupational and Environmental Dermatology Unit. The patches were removed by the children's parents after 48 hours and were read another 24 hours later by 1 of

the 2 dermatologists (A.G.L., A.I.). The results were interpreted according to recommendations of the ICDRG guidelines. Positive reactions from + to +++ were regarded as a delayed (type IV) hypersensitivity. If the result between aluminium in petrolatum and metallic aluminium differed between positive/negative and the strength of the reaction, the highest score was always used. Doubtful reactions were classified as negative. All the parents were told to contact the study staff if any late reactions appeared.

Before the testings, all children and parents were interviewed regarding ongoing local symptoms, and the injection site was examined. The interviews and clinical examinations were performed by an experienced paediatrician (B.T.) and an experienced general practitioner (E.B.), both of whom were earlier team members of the Gothenburg Pertussis Vaccine Trials and patch test I. The reading dermatologist did not know the results.

Paper II

In order to find out the optimal compound of the 2 traditionally used test preparations of aluminium, the positive patch test reactions to metallic aluminium and aluminium chloride hexahydrate 2% in petrolatum were analysed in detail, retrospectively, in 366 of the 377 children with vaccine-induced persistent itching nodules tested in 1998 to 2002. The remaining 11 children were patch tested in other dermatology clinics and excluded from this analysis.

The second objective of Study II was to compare the individual patch test results to metallic aluminium and aluminium chloride hexahydrate 2% in petrolatum in the group of 241 children tested twice in our studies.

The patch test in 1998 to 2002 was performed in the same way as in study I, except that the readings were then performed by only one of the dermatologists (A.I.).

Paper III

In this study, the long-time clinical course of persistent itching nodules is described, from the onset of the first case in 1993, until the last interview in 2019. In the Mass Vaccination Project, all reported cases were registered as adverse events according to the Helsinki Declaration rules and reported to Swedish Medical Products Agency (MPA) and the vaccine manufacturer. The child was examined by one of the study physicians. The parents were informed of the association between the aluminium adjuvant in the vaccine and the subcutaneous nodule. Symptomatic treatment with weak local steroids and a hydrocolloid bandage (DuoDerm or Comfeel) was offered if needed, and the parents were helped to apply for reimbursement from the Swedish Pharmaceutical Insurance (in Swedish: Läkemedelsförsäkringen).

All data, including the vaccination history, were documented in individual medical records. The children were then followed individually until they were free from symptoms. When the reported number of children with itching nodules rapidly increased, an organized clinical follow-up was initiated and performed mainly by the nurses in the trial staff. This follow-up was comprised of repeated spontaneous and structured interviews by telephone every month to every sixth month, depending on the severity of symptoms, and, if needed, renewed medical consultations with one of the study physicians.

Later, the interviews were replaced by written questionnaires with intervals increasing from 1 to 3 years. The same questions were asked in every contact during the years: *Does the child still have itching at the old vaccination site?* Symptoms were graded in 4 stages: Unchanged – Improved – Nearly recovered – Recovered. "Recovered" was defined as both nodules and itching had vanished for at least 6 months, see table 8 for complete definition (37). Later questions were: *At about what time did the itching cease?* In unclear cases, the end of symptoms was approximated. *Has the child received any other aluminium-containing vaccines, and if so: which? Did the child get another itching nodule at the new vaccination site?* A local warm red swelling occurring a few days after vaccination was interpreted as an unspecific mild local reaction and not as a vaccine-induced long-lasting itching nodule. Information regarding exposure to aluminium-containing products (antiperspirants, sunscreen protectors) was also inquired.

Table 8

State of symptoms	Nodules	Itching
Unchanged symptoms	Unchanged	More or less continuous
Improved	Intermittent or diminished	Free periods for some weeks
Nearly recovered	Vanished or intermittent	Free periods for some months
Recovered	Vanished for ≥ 6 months	None during ≥ 6 months

Table 8. Definitions of symptoms for children with persistent itching nodules who received vaccines produced by SSI in the Gothenburg Pertussis Vaccine Trials and clinic routine (98).

The last questionnaires to all 745 children were sent out in 2011 to 2012, followed by telephone interviews in 2013 and 2014 with those who did not answer the questionnaires. After that, the interviews/questionnaires focused on two groups of study participants: those who reported continued itching at the last contact and those with no further contact since 2008, regardless of symptoms. These interviews were performed by the authors (A.G.L., A.I., E.B., and B.T.)

and by 2 nurses (T.A., L.S.) and a medical laboratory technician (U.B.), almost all of whom had taken part in the clinical trials from the beginning and in patch test I and II. Beyond that, spontaneous contact for information and advice was taken by the parents or the participants themselves during the entire study period. When the children turned 18 years old, they were interviewed themselves instead of their parents.

On both patch test occasions, each individual was interviewed and examined at the original injection site.

Paper IV

In the fourth study, a third patch test was offered to former participants of the vaccine trials with itching nodules who had already been tested twice. It was performed in January 2020 to March 2020, more than 15 years after the first patch test. Participants were divided into 3 groups:

(1) Persons with positive patch tests for aluminium both in 1998-2002 and 2007-2008 (total 55);

(2) Persons with a positive test in 1998-2002 and a negative in 2007-2008 but still having symptoms from their itching nodules at that occasion (total 44);

(3) Persons who had a negative test and were free from symptoms in 2007-2008 but had reacted with a new itching nodule after vaccination with an aluminium-containing vaccine later in life (total 4).

One pregnant woman was excluded.

As in the 2 previous patch test studies I and II (37) (Paper I), the same test material from the same manufacturers was applied on the upper part of the back using aluminium in 2 forms: metallic aluminium (empty Finn Chamber®) and aluminium chloride hexahydrate 2% in petrolatum. In addition, 3 aluminium salt preparations were tested: aluminium chloride hexahydrate 10%, aluminium lactate 2.4% and aluminium lactate 12.2%, all manufactured by Sigma Aldrich as Aluminium chloride 99% and Aluminium L-lactate 95% and then prepared by the Occupational and Environmental Dermatology Unit, at Sahlgrenska University Hospital and University of Gothenburg, Sweden.

All aluminium salts were placed in a plastic chamber, IQ Ultra™, (Chemotechnique Diagnostics®, Malmö, Sweden). Two of our staff members (B-M.E., C.E.) with special training and experience in the Occupational and Environmental Dermatology Unit applied the test patches. The patches were removed after 48 hours by the participants themselves and read on day 3 according to ICDRG guidelines (23) by the same dermatologist as in test II (A.G.L., A.I.). Doubtful reactions which did not fulfil the criteria for a positive reaction were classified as negative. If different reaction strengths of the 5 test preparations were observed, the highest

score was always used, as in patch test I and II, as the maximum score. All participants were asked to contact the study team in case of sign of additional reactions.

The young adults were interviewed before testing. The injection site was inspected and examined for remaining nodules as in previous studies by an experienced paediatrician (B.T.) and an experienced general practitioner (E.B.), both earlier team members of the Gothenburg Pertussis Clinical Trials, see Paper I and (37).

Drawing by an unidentified girl with an itching nodule on her left arm

ETHICAL CONSIDERATIONS

All studies were approved by the Regional Ethical Review Board of the University of Gothenburg. Furthermore, all parents and adolescents and later on young adults received oral and written information about the studies and gave their written consent to participate. Photographs and drawings in the thesis were published with the consent of the participants and their parents.

The registration numbers of the ethical approvals are the following:

Paper I and Paper II: 385-07

Paper III: 623-11

Paper IV: 2019-05005

It is challenging to perform studies in children since they cannot give their consent for participation. The primary ethical consideration of our studies was the concern that reminding the children/adolescents of the itching nodules by repeated examinations, interviews, questionnaires and patch tests might cause unnecessary anxiety. Also, the children's parents were repeatedly reminded of the problems after vaccination which may have strengthened their anger, sorrow and self-accusation of letting their children receive the vaccine. In addition, there may also be a risk that clinically silent hypersensitivities can be sensitised de novo to the numerous applied patch test preparations even although this risk is considered low (23).

It is challenging to study the side effects of vaccines, and it must be stressed that vaccines are one the most important factors to prevent serious diseases. Vaccination has prevented millions of deaths due to serious infectious diseases in children and adults and is essential for public health worldwide. However, we are aware that side effects of vaccines exist, and it is essential to study and report any side effects to maintain confidence in childhood vaccination programs.

Our intention has always been to treat the child and the parents with great respect, and we sincerely believe that the benefits outweighed the risks of participating in these studies.

Drawing by a 5 years old girl with an itching nodule on her left arm where she had received two doses of aP at the age of 1-2 years.

STATISTICS

All doubtful reactions were considered negative, in statistical calculations. All tests were two-sided, and $P<0.05$ was considered statistically significant.

PAPER I

All data were analysed using R version 2.10.1 (The R Foundation for Statistical Computing, Vienna, Austria). A multiple logistic regression was performed with negative patch test as the response variable and time from the first SSI-dose, age at first SSI-dose and itching as predictors.

Fisher's exact test was used for comparing proportions. The exact binomial version of McNemar's test was used to test for differences in the proportion of positive patients in previous tests and the tests in this study. Wilcoxon's rank sum test was used to test differences in age and time from the first SSI-dose between groups.

PAPER II

All data were analysed using R version 3.0.3 (The R Foundation for Statistical Computing, Vienna, Austria).

To test for correlation between 2 numerical variables, Spearman's correlation test was used. Wilcoxon's signed rank test was used to test for pairwise differences in numerical variables. The patch test outcomes were encoded as dummy variables in the above-mentioned tests: Negative → 0, + → 1, ++ → 2 and +++ → 3.

PAPERS III & IV:

All data were analysed using R version 3.5.3, (The R Foundation for Statistical Computing, Vienna, Austria).

A Kaplan-Meier analysis was performed to analyse the proportion of patients with itching over time. Events were defined as contacts where itching had disappeared. Patients were considered lost to follow-up at the last contact with an ongoing itch. In Paper III, the Wilcoxon's rank-sum test was used for the two-sample test. The Fisher's exact test was used for comparing proportions.

Paper IV was mainly a descriptive study. When comparing the number of positive and negative reactions between 2 different patch tests, the exact binomial test was used.

Drawing by a 6 years old girl with an itching nodule on her left arm where she had received three doses of aP vaccine at 2-3 years of age

RESULTS

Paper I

In this study, we aimed to investigate the prognosis of contact allergy to aluminium in children caused by vaccination with a new aluminium-adsorbed pertussis vaccine repeating the patch test more than 5 years after the first test.

Of 495 children tested in patch test I, 377 tested positive against aluminium. By May 2008, 241 (89 boys; 152 girls) children/young adults were tested a second time. The median age was 13.3 years (range 8 and 21 years).

In all, 77% (186/241) participants tested negative in patch test II. Of those who showed a weak positive reaction, +, on the first test, 94.7% (95% confidence interval (CI) 85.4-98.9%) tested negative in the second test, which is significantly more (P = 0.00012) than those with stronger reactions, ++ or +++ (71.7% (95% CI 64.6-78.1)), see figure 1(Paper I). There was no significant difference between those who tested ++ and +++ (P=0.61).

Figure 1

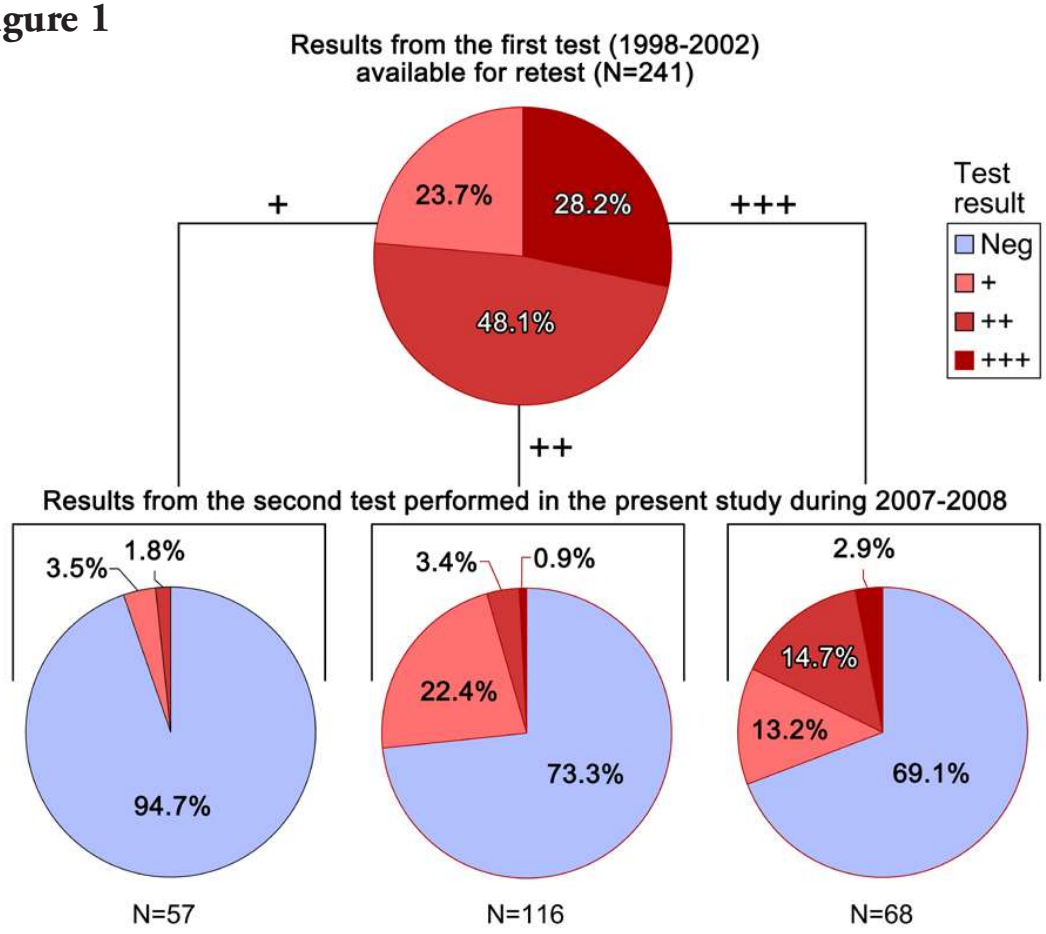


Figure 1 Results of aluminium patch test I (1998 to 2002) in relation to results in aluminium patch test II (2007 to 2008) in 241 participants tested twice (Paper I).

When reading the results from the 241 retested children, 27 cases were classified as doubtful, according to the criteria. They are reported here as negative.

Palpable subcutaneous nodules and itching at the injection site were seen in five of the participants. They all tested negative.

The outcome of patch test reactivity for the 254 children with itching nodules who did not participate in patch test II is unknown. Of those, 118 tested negative in patch test I and 136 tested positive in patch test I. Assuming a worst-case scenario, in which all 254 would have tested positive in patch test II, a significant decrease of patch test reactions would still have been observed (P=0.00011, from 76.2% in the previous study to 62.4% in the current study).

The chance of testing negative in patch test for aluminium was significantly correlated with the disappearance of local itching (P=0.027). Increasing age (P=0.0002) and longer intervals between the first vaccination and the second test (P< 0.0001), also heightened the chance of having a negative test. The actual time interval elapsed in between the first vaccination and the second test, in those with a negative test result and in those with a positive test result, was only half a year. The age at the first SSI dose did not affect the outcomes of the patch test in multivariate analysis considering 3 variables (itching, time from first SSI dose to patch test II, age at first SSI dose).

Paper II

In this retrospective study, data from patch test I were analysed further by comparing the outcomes of patch test reactivity for aluminium chloride hexahydrate 2% in petrolatum and Finn Chamber® in 366 children with positive tests. See outcomes in table 9. Comparing the two different aluminium compounds used in patch test I, aluminium chloride hexahydrate 2% in petrolatum is better for diagnosing aluminium hypersensitivity than an empty Finn Chamber® (metallic aluminium). Also, the higher the strength of the patch test reaction to aluminium chloride hexahydrate 2% in petrolatum, the greater the probability of being positive even to the Finn Chamber® (P < 0.0001).

Table 9

Finn Chamber® \ Aluminium chloride hexahydrate 2%	Negative	+	++	+++	Sum	%
Negative	-	69	40	5	114	31%
+	9	34	77	32	152	42%
++	0	7	39	47	93	25%
+++	0	2	0	5	7	2%
Sum	9	112	156	89	366	100%
%	2%	31%	43%	24%	100%	

Table 9. Overview of the 366 children with positive test reactions in patch test I: Scorings of aluminium chloride hexahydrate 2% in petrolatum and an empty Finn Chamber®.

Another aim was to explore the individual outcomes of the patch test reactivity of the test reactions in 241 children initially tested in 1998 to 2002 and retested in 2007 to 2008. As reported in our earlier study (Paper I), 77% of the children with earlier positive patch test reactions in the first test tested negative in the second test. Four children/adolescents exhibited the same test results on both test occasions, and 2 individuals showed stronger reactions for aluminium chloride hexahydrate 2% in petrolatum in the second test, see individual outcomes in table 10. Analysis of the data shows decreased frequencies of positive reactions in both the empty aluminium Finn Chamber® and the aluminium chloride hexahydrate 2% in petrolatum between patch test I and patch test II in the 241 retested children ($P < 0.0001$).

Table 10

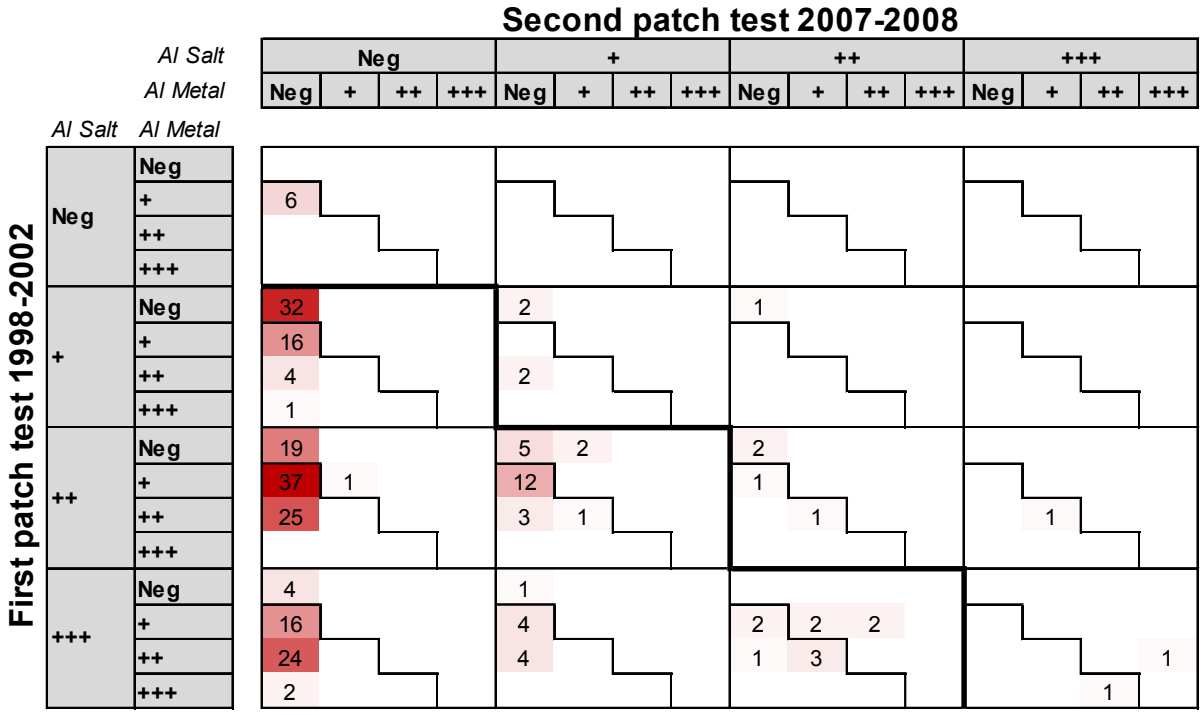


Table 10. Patch tests outcomes in patch test I (1998 to 2002) and II (2007-2008) in 241 children tested twice with an empty Finn Chamber® (Al Metal) and aluminium chloride hexahydrate 2% in petrolatum (Al Salt). The colour gradients in the cells are darker the larger the number.

Paper III

Prognosis

In this long-term follow-up study of the clinical course based on regular interviews, questionnaires, clinical examinations and (at this time) 2 patch tests, the participants were followed for a median time of 15 years (0.1-25.2) from start of symptoms. In all, 86% (637 children/young adolescents, 374 girls, 263 boys) fully recovered from symptoms during this period. Their itching was gradually decreased with a protracted course and with a median duration of 6.6 years (range 0.04-19.2 years).

Of the 108 (81 girls, 27 boys) participants reporting symptoms at their latest contact, 30 children had their last interview before 2008 and were not reachable despite several attempts, since many of them had emigrated. In the last contact of all the 108 participants with symptoms, all but one scored their remaining symptoms as "Improved" or "Nearly recovered". Their median duration of ongoing symptoms was 16.4 years (0.1-25.2 years) in their last contact. In the last physical examination of 241 children in patch test II (2007-2008), remaining nodules were observed in 5 children, local discolouration in 26, hypertrichosis in 5 and visible excoriations in 8 children. Typically, the symptoms gradually decreased and became intermittent over time. However, symptoms were aggravated during periods of infections and this could lead to periods of intense scratching and bleeding of the skin. The nodules subsided before the itching.

Further vaccinations in life

Of the 745 children participating in the study, only 7 children refrained from further DT-booster vaccination, offered at approximately 10 years of age, due to their aluminium allergy. In 11 children, the DT-booster vaccination was received before the clinical trials according to their age and to the Swedish NIP. In 4 children, the vaccination status was unknown.

The 723 remaining children received different DT-booster vaccines: aluminium-free DT-boosters, aluminium-phosphate-adsorbed DT-boosters and commercial aluminium-hydroxide-adsorbed DT-booster.

In all, 3% (23/723) of the participants who received DT-booster vaccinations and 7% (24/332) of those who reported vaccination with other aluminium-adsorbed vaccines later on in life experienced chronic itch at the site of injection. Duration of symptoms was typically much shorter after re-exposure compared to initial exposure.

All 23 participants with recurring itch at the new injection site after DT-booster, received aluminium-containing vaccines and children vaccinated with an aluminium phosphate DT-booster (Duplex®) were mainly those that experienced itch.

For further details of itching status at the old and new injection site and type of DT-booster, see table 11.

Three participants were treated with ASIT, and all of them developed new itching granulomas.

Table 11

	Type of diphtheria / tetanus-booster vaccine			
	Alumini-um-free	**Duplex®**	**Commercial***	**Other****
Total number of participants	115	315	293	22
No itching at original injection site at last contact	96	285	242	14
Still itching at original injection site at last contact	19	30	51	8
Itching at new injection site after DT-booster	0	17	6	-

**Mainly diTeBooster ®. If this was not available, a DTaP combination vaccine was given.*
***DT-booster vaccination received earlier (n=11), denied (n=7) or unknown (n=4).*

Table 11. Status of ongoing itch at the original injection site reported in the last contact of the 745 children participating in the Gothenburg Pertussis Vaccine Trials and status of new itching/local swelling at the new injection site after receiving diphtheria/tetanus-booster vaccination at 10 years of age.

Paper IV

This study aimed to explore the long-term prognosis of vaccine-induced contact allergy to aluminium in childhood by performing a third patch test ≥15 years after the initial test. In total, 103 individuals previously tested twice for aluminium allergy during childhood were invited to participate in the study. Thirty-eight of them declined or were unreachable. Unfortunately, the study had to be cancelled due to the covid-19 pandemic in March 2020. Until then, 31 of the 65 participants planned to be included had been patch tested.

The median time since the first SSI dose and the first, second and third patch tests in 31 participants (20 females, 11 males) was 4.8 years (range 1.9-8.3 years), 11.5 years (range 8.6-15.4 years) and 23.7 years (range 20.5-27.7 years), respectively. The median age was 5.2 years (range 2.2-11.6 years), 11.9 years (range 8.9-17.0 years) and 24.1 years (range 20.8-29.0 years) on each test occasion.

Five aluminium preparations were used, in which 2 of the preparations were the same as in patch test I and II (aluminium chloride hexahydrate 2% in petrolatum

Table 12

Patient number	Group	Sex	Age at third patch test	First patch test (1998-2002)			Second patch test (2007-2008)			Third patch test (2020)							Patient number
				Combined	FinnChamber®	$AlCl_3 6H_2O$ (2%)	Combined	FinnChamber®	$AlCl_3 6H_2O$ (2%)	Combined	Combined FinnChamber® & $AlCl_3 6H_2O$ (2%)	FinnChamber®	$AlCl_3 6H_2O$ (2%)	$AlCl_3 6H_2O$ (10%)	$Al(C_3H_5O_3)_3$ (2.4%)	$Al(C_3H_5O_3)_3$ (12.2%)	
3	1	M	23	++	++	+	+	-	+	-	-	-	-	-	-	-	3
4	1	F	22	++	+	++	++	-	++	++	-	-	-	++	++	++	4
5	1	M	24	+	-	+	++	-	++	++	-	-	-	-	+	++	5
7	1	F	24	+++	++	+++	++	+	++	-	-	-	-	-	-	-	7
8	1	F	23	++	++	+	+	-	+	-	-	-	-	-	-	-	8
10	1	M	21	+++	++	+++	+	-	+	+	+	+	+	+	+	?+	10
11	1	M	24	+++	++	+++	+	?+	+	++	+	-	+	+	++	++	11
12	1	M	23	++	++	++	++	+	++	-	-	-	-	-	-	-	12
14	1	M	22	++	?+	++	+	+	+	-	-	-	-	-	-	-	14
15	1	F	24	+++	?+	+++	+	-	+	++	-	-	-	+	+	++	15
16	1	M	21	+++	+	+++	++	-	++	+	-	-	-	-	-	+	16
17	1	F	22	+++	+	+++	++	-	++	+	?+	-	?+	?+	?+	+	17
18	1	M	25	++	+	++	+	-	+	++	+	-	+	++	-	+	18
19	1	F	25	++	+	++	+	-	+	?+	?+	-	?+	?+	?+	?+	19
21	1	F	26	++	+	++	+	-	+	++	?+	-	?+	+	++	++	21
22	1	F	25	++	++	++	+	-	+	++	-	-	-	++	+	+	22
25	1	F	26	++	++	++	+	+	+	+	+	+	+	+	+	+	25
26	1	M	26	+++	+	+++	++	+	++	-	-	-	-	-	-	-	26
28	1	F	26	+++	++	+++	+	-	+	+	+	+	+	?+	-	-	28
31	1	F	29	++	+	++	+	+	-	-	-	-	-	-	-	-	31
1	2	F	23	+	+	+	?+	-	?+	+	-	-	-	+	+	+	1
2	2	F	23	+	?+	+	-	-	-	-	-	-	-	-	-	-	2
6	2	F	23	+++	+	+++	-	-	-	-	-	-	-	-	-	-	6
9	2	F	24	+	-	+	?+	-	?+	-	-	-	-	-	-	-	9
13	2	M	28	+	+	+	-	-	-	-	-	-	-	-	-	-	13
20	2	F	25	++	+	++	-	-	-	?+	?+	-	?+	?+	-	-	20
23	2	M	26	++	?+	++	-	-	-	+	+	+	+	+	+	+	23
29	2	F	28	+	+	+	-	-	-	++	?+	-	?+	+	++	++	29
30	2	F	29	+++	+++	+	-	-	-	++	+	+	+	+	+	++	30
24	3	F	26	++	-	++	-	-	-	-	-	-	-	-	-	-	24
27	3	F	26	+++	+	+++	-	-	-	-	-	-	-	-	-	-	27

Table 12. The test results in patch test I, II and III in 31 participants with itching nodules from the Gothenburg Pertussis Vaccine Trials. Positive reactions from + to +++ were regarded as delayed hypersensitivity. Doubtful reactions ?+ were regarded as a negative result. The aluminium preparations used were aluminium chloride hexahydrate ($AlCl_3 6H_2O$) and aluminium lactate ($Al(C_3H_5O_3)_3$) in two different concentration, respectively. The strongest positive reaction was used to define the overall score among several compounds. "Combined" in patch test I and II refer to the maximum score of aluminium chloride hexahydrate 2% in petrolatum and an empty Finn Chamber®. The participants were recruited in 3 groups: (1) persons with positive patch tests for aluminium both in 1998 to 2002 and 2007 to 2008; (2) persons with a positive test in 1998 to 2002 (patch test I) and a negative test in 2007 to 2008 (patch test II) but still suffering from itching nodules at the time of patch test II; (3) persons who had a negative test and were free from symptoms in 2007 to 2008 but had reacted with a new itching nodule after receiving an aluminium-containing vaccine given later in life (Paper IV).

and an empty Finn Chamber®). Of the 31 participants who underwent patch test III, 52% (16/31) tested positive, scoring weak (+) or moderate (++). Four participants with a negative patch test II turned positive in patch test III, see table 12 (Paper IV). No additional reactions were reported after day 3.

Comparing the strongest patch test reactivity of aluminium chloride hexahydrate 2% in petrolatum and the empty Finn Chamber® in patch tests II with patch test III, a significant loss of patch test reactions was seen in the third test (P= 0.002).

In patch test III, 5 participants suffered from intermittent itching at the injection site of the SSI dose given in the vaccine trials. Three of those participants also suffered from an intermittent subcutaneous nodule. One of those 3 exhibited a positive patch test, in which all test preparations were positive. None of the 5 participants experienced new symptoms when re-exposed to other aluminium products.

One participant, recovered from the itching, reported local eczema after exposure to aluminium-containing antiperspirants and sunscreens. She also had noticed a new subcutaneous itching nodule after hepatitis B vaccination earlier in life. She tested positive for aluminium in all her 3 test occasions.

A second aim in this study was to investigate the different aluminium preparations to find an optimal compound and test concentration. The preparations used were aluminium chloride hexahydrate 2% and 10% in petrolatum, aluminium lactate 2.4% and 12.2% in petrolatum and an empty Finn Chamber®.

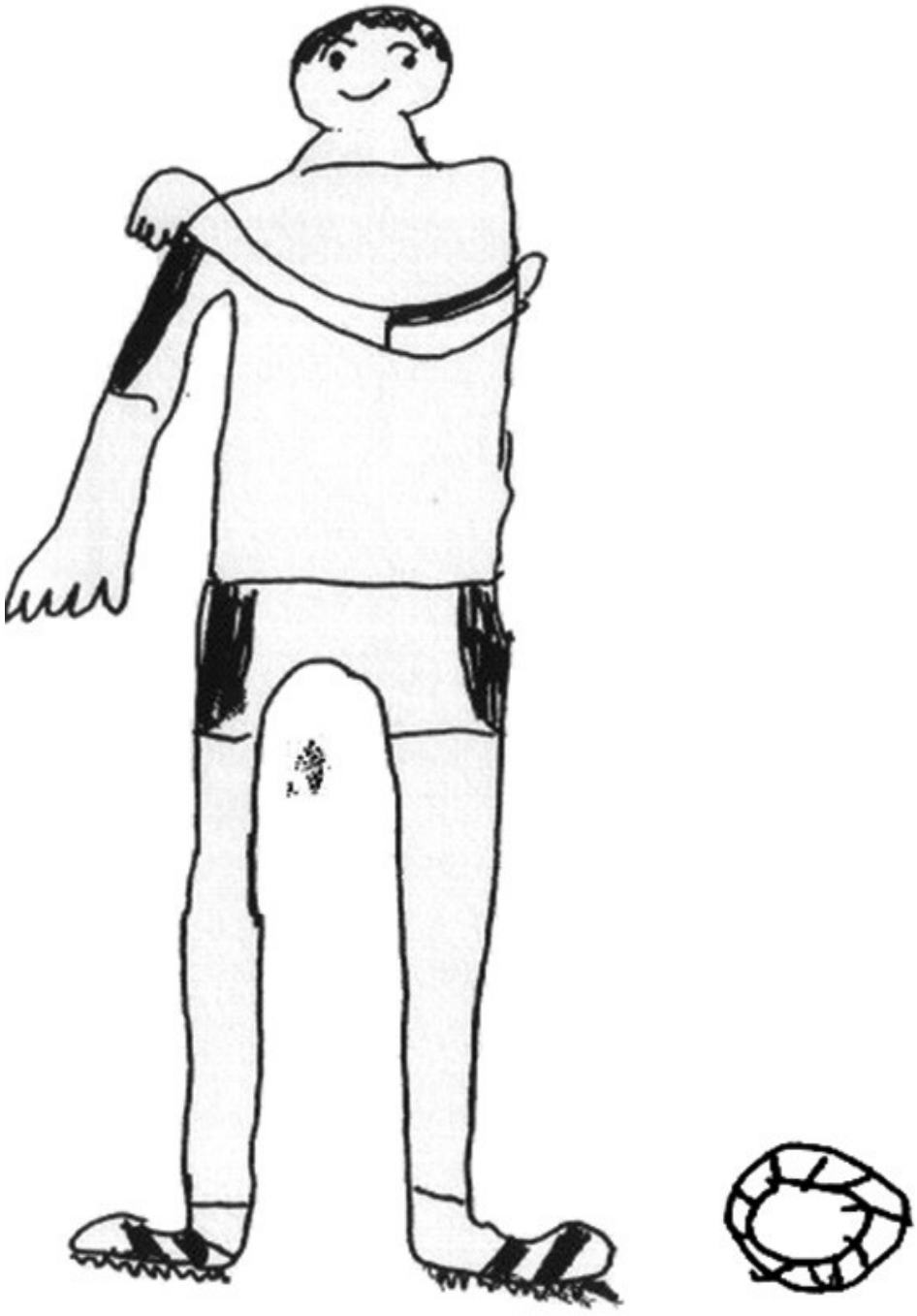
Use of aluminium lactate 12.2% in petrolatum resulted in the highest number of positive reactions (n=14), followed by aluminium chloride hexahydrate 10% in petrolatum (n=12). See the frequency of all positive and negative reactions in table 13.

There were no significant difference found between any of the aluminium formulas and the number of positive reactions in the 31 participants tested.

Table 13

	Patch test result, n (%)	
	Positive	**Negative**
Finn Chamber®	5 (16%)	26 (84%)
$AlCl_3 6H_2O$ (2%)	7 (23%)	24 (77%)
$AlCl_3 6H_2O$ (10%)	12 (39%)	19 (61%)
$Al(C_3H_5O_3)_3$ (2.4%)	12 (39%)	19 (61%)
$Al(C_3H_5O_3)_3$ (12.2%)	14 (45%)	17 (55%)

Table 13. Frequency of positive and negative reactions for all five aluminium preparations used in patch test III in the 31 participants tested. $AlCl_3 6H_2O$ = aluminium chloride hexahydrate, $Al(C_3H_5O_3)_3$ = aluminium lactate



Drawing by a 9 years old boy who had suffered from itching nodules on his left arm after vaccination with three doses of aP vaccine at 1-2 years of age. Now – happily - free from symptoms during the last two years

DISCUSSION

Papers I and II

Prognosis of aluminium allergy

CD is generally considered to be lifelong (156). Therefore, it was surprising to find a high loss of patch test reactions, as observed in 77% of the children with vaccine-induced aluminium allergy, in patch test II. Negative aluminium patch tests were significantly correlated with loss of itching from the persistent itching subcutaneous nodules associated with the allergy. These results indicate that vaccine-induced aluminium allergy acquired in childhood may be temporary.

To our knowledge, there is only one previous study (performed in 1992) on the prognosis of aluminium allergy in children caused by aluminium-adsorbed vaccines (115). In this study, two of four children with aluminium allergy who were retested 1 to 8 years after the initial test had negative reactions in the second one.

Patch test technique

When patch tests I (37) and II were initiated in 1997 and 2007, aluminium chloride hexahydrate 2% in petrolatum was the only commercial preparation available. Using a higher concentration in patch test II was not in question since almost 70% of the children had reacted strongly (++ or +++) to the 2% preparation of aluminium chloride hexahydrate in the first test.

On the other hand, the children were older in patch test II (median 11.5 years, compared to 6 years in the first test), and the use of weak aluminium chloride hexahydrate 2% in petrolatum also this time might have caused some false-negative reactions.

It has been suggested that aluminium chloride hexahydrate 2% in petrolatum is insufficient to detect all cases of aluminium allergy. In a study on optimal aluminium compounds for patch testing, published in 2012 by Siemund et al. (61), 21 persons, all adults, were tested with different aluminium compounds. Only four of them had positive reactions to aluminium chloride hexahydrate 2% while fifteen tested positive to a 10% preparation. No positive reactions were recorded with the empty Finn Chamber®. The conclusion was that aluminium chloride 2% in petrolatum is insufficient to detect all cases of aluminium allergy, and a concentration of 10% was recommended. This is also suggested in other studies (69, 157).

In a review by Bergfors et al. in 2019 (72), the results in three Swedish studies on children with vaccine-induced aluminium allergy, one of which was the aluminium study (patch test I) in the vaccine trial, were compiled. Altogether 459 of 601 children with itching nodules tested positive for either aluminium chloride hex-

ahydrate 2% in petrolatum or an empty Finn Chamber or both. 98, 97 and 100% of the children with verified aluminium allergy in these three studies, respectively, had positive reactions to the 2% aluminium chloride hexahydrate preparation. A high proportion of positive reactions to aluminium chloride hexahydrate 2% in petrolatum was also reported in a Danish study by Salik (113) where 39 of 42 children (93%) with vaccine-induced itching nodules tested positive to this preparation. In France, 10 children were tested with aluminium chloride hexahydrate 2% in petrolatum by Goiset (158) with positive results for all of them. All these studies indicate that aluminium chloride hexahydrate 2% in petrolatum is sufficient to get reliable results in small children (72, 113, 115, 119, 158).

The review mentioned above on three Swedish studies on patch testing children with aluminium chloride hexahydrate 2% in petrolatum (72) also revealed that small children had remarkably strong reactions to this concentration. As many as 69%, 79% and 86% of the tested children, respectively, had ++ or +++ reactions. The younger the children, the stronger were the reactions, especially in the smallest ones. A +++ reaction to aluminium chloride hexahydrate 2% in petrolatum was registered in 65% of one to two years old children as compared with 22% in those aged seven years. In some the most afflicted children reactions were seen rapidly after application so that the test material had to be removed already on day 1. These results, together with other reports on strong reactions in small children patch tested with aluminium chloride hexahydrate 2% in petrolatum, suggest that the 10% preparation should not be used routinely in children (72, 113, 115, 119, 158).

The importance of a defined dose of a sensitiser per area in patch testing was emphasised by Bruze et al. in 2020 (73). The applied amount of a preparation in petrolatum may vary around five times depending on the test technique, implying that patch testing with 50 mg of aluminium chloride hexahydrate 2% in petrolatum can give the same dose/cm^2 as 10 mg of a 10% preparation. Also the application technique might affect the intensity of reaction. Both these factors may have affected the high proportion of +++ reactions reported in the review by Bergfors et al. The general recommendation by Bruze et al. is to use aluminium chloride hexahydrate 10% in petrolatum in a dose of approximately 40 mg/cm^2 for tracing contact allergy to aluminium. In young children, in whom a strong reaction to aluminium is expected, the same dose of aluminium chloride hexahydrate 2% in petrolatum shall be used.

In this context, it should be observed that the guidelines for patch testing were less precise when patch tests I and II were performed than the currently used.

In Paper II, we showed that testing with an empty Finn Chamber® had no advantages compared to testing with aluminium salt for demonstrating aluminium hypersensitivity. This is consistent with findings in other studies (61, 113).

At the time of patch tests I and II in Gothenburg, the children were tested and read according to the then-current guidelines of the ICDRG. The ICDRG was an international research group started in Scandinavia in the 1960s. When the phrase "testing and reading according to the ICDRG" was used in the literature, this could refer to any of a large number of different publications describing slightly different techniques. In 2015, the European Society of Contact Dermatitis (ESCD) published updated guidelines with consideration of many factors involved in patch testing (23). In the ESCD guidelines, the amount of the applied test preparations is defined. The ESCD has standardized the dose of the allergen in each type of test chamber since one of the most crucial factors for sensitisation and elicitation of contact allergy is the "dose per area"(159). When patch test I and II were initiated in 1997 to 2002 and 2007 to 2008, respectively, no recommendations existed regarding the amount of petrolatum preparation to be applied. The plastic chamber available and used in the two first tests, the original IQ Chamber (Chemotechnique Diagnostics®, Sweden) had a volume of 65 µL and an inside area of 81 mm^2 (160). The now commonly used plastic chambers, IQ-ultra® and IQ Ultimate® (Chemotechnique Diagnostics®, Sweden), have the volume of 32 µL and an inside area of 64 mm^2 (160).

The majority of the patch tests in the series here referred to as patch test I were applied by three of the vaccination team members. They were instructed by a clinically experienced staff member from the Department of Occupational and Environmental Dermatology with special training in patch test technique. This staff member applied the majority of the tests in patch test II. It is well known that intra- and inter-individual variation exists, but intra-individual variation is limited (45). The amount of preparation applied in the tests might have varied since it is difficult to repeatedly apply an exact volume/amount of petrolatum as a vehicle.

For practical reasons, only one reading was performed in all patch tests and this took place on day 3. The frequency of reported additional positive reactions on day 4 or later varies in the literature. It is known that approximately 3% to 8.2% of the reactions to allergens in baseline series in Europe are seen first on day 6 or day 7 (161, 162). In patch test II, all parents were told to be observant and contact the study team if there were any signs of additional reactions. Since the parents were vigilant about the vaccine's side effects, we believe they carefully inspected their child's back daily. A few parents contacted the study team, but none of their children had a late reaction.

In patch test II (Paper I), we also wished to offer a renewed test to the children with itching nodules but negative tests in patch test I as a control group. The regional ethics committee indicated in informal contacts that permission to retest children who had never had a positive test result, would be difficult to obtain.

In conclusion, the loss of patch test reactions described in Paper I and II must be interpreted with some caution due to the limitations discussed. However, the loss of itching nodules and the absence of a reaction to aluminium upon retesting previously sensitised children strongly indicate that aluminium allergy may be temporary.

Paper III

Very little was known of the clinical course of vaccine-induced itching nodules before we studied this unique cohort of children.

At least 86% of the participants had a full recovery from symptoms for more than 1 year during a follow-up time of ~ 20 years. An even higher recovery rate is possible since 28% of those still itching have been lost to follow-up since 2008. The subcutaneous nodules were improved before the itching subsided. The median duration of itching was 6.6 years (range 0.04-19.2 years). Others have described the typical protracted course of symptoms after vaccination with aluminium-adsorbed vaccines but mostly in general terms and case reports (116, 163-166).

The long-term follow-up study was not planned in the beginning. However, it started in 1997 within the Mass Vaccination Project when the number of itching nodules at the injection site rapidly increased. The 3 physicians performed the examinations within the Mass Vaccination Project, and the interviews were repeated as structured interviews by telephone or questionnaires performed by the trial staff. The parents and young adults also contacted the vaccination team themselves with questions. In every single contact they were interviewed.

The staff members documented all data in individual medical records and an Excel database called “Superregistret”, initially not created with the purpose of a 20 year long-term follow-up, was designed. Collecting and analysing all the information has been challenging and has only been possible due to the extensive help from physicians and nurses initially involved in the vaccination trials.

There were no validated questionnaires used. However, the same questions were asked in every contact. “Recovered” was defined as follows: both nodules and itching had vanished for at least 6 months. Due to repeated contacts during many years, the duration of itching could be approximated fairly accurately even though there is some uncertainty, especially in study participants with fewer contacts. We have not reported the duration of the nodule itself. The children were frequently examined the first years after the vaccination, but not later on. However in the last examination in 2007 to 2008, only 5 of 241 children still presented with an itching nodule at the vaccination site. In total there were 66 children with remaining itch of the 241 children who were examined.

The reaction to the DT-booster doses administrated at ten years of age is also relatively well known, even if there can be some uncertainty. The DT-booster was

given by the Gothenburg Pertussis Vaccine Trial staff and the School Health Care, which were regularly informed of the adverse events of the vaccine used in the clinical trials. Frequently contacts were also made with those parents who hesitated to vaccinate their children.

The history of further vaccinations with aluminium-adsorbed vaccines can also be challenging to remember in detail, especially when the adolescent answered the questions themselves. Unfortunately, more than half of the participants in this study refrained from further important vaccinations due to their aluminium content.

Only a few of those receiving aluminium-adsorbed DT-booster doses and further vaccination later on in life reported new itching nodules, and then for a much shorter period of time. Similar reactions are described in a few other studies (113, 119).

Surprisingly, a majority of the children suffering from a new itching nodule after DT-booster vaccination were those who received the aluminium-phosphate-adsorbed vaccine (Duplex®). Itching granulomas after Duplex® has not been previously reported.

Itching granulomas after treatment with ASIT are well known and described by others (66, 114, 115, 166). Three in our cohort reported itching nodules when treated with ASIT. The treatment was stopped and the clinical course is not known.

In the 1990s, the general recommendations were to avoid aluminium-adsorbed vaccines as there might be a risk for new problems. The risk for itching nodules increased with the number of doses of aluminium-adsorbed vaccine given, as reported by Bergfors et al. (37). It also seemed to be an increased risk for new persistent itching nodules on the injection site, if the following doses were given at a short interval (2-6 months) from the dose causing the original itching. The risk of getting a new itching nodule decreased with time since only a few children reported symptoms after vaccination with the commercial DT-booster dose at 10 years of age.

Some parents were apprehensive and contacted the staff members repeatedly themselves, possibly leading to over reporting symptoms. Ideally, only planned and continuous follow-up with all participants would have been preferable, regardless of symptoms or not.

Even though we cannot be sure of all the reported data in detail, this study shows that vaccine-induced subcutaneous itching nodules associated with aluminium allergy in infants and children cause great suffering and have a protracted course. Therefore, physicians and parents may be hesitant to further use of aluminium-adsorbed vaccines. Furthermore, this study shows that the itching nodule disappears over time and lacks clinical significance when the participants were re-exposed to other aluminium-containing vaccines.

Paper IV

The main drawback of this study is that it was aborted because of the covid-19 pandemic. Only half of the 65 participants planned to be tested were enrolled, which may have affected the results. The 3 groups recruited were too small to be analysed separately, and their test results are only descriptive. Our hypothesis was to explore whether those still itching at the time of patch test II and who then had negative patch test results with aluminium chloride hexahydrate 2% in petrolatum (group 2) would test positive when using aluminium chloride hexahydrate 10%. Nine of them were tested a third time, of which 4 tested positive in the patch test. All 4 had a positive reaction to aluminium chloride hexahydrate 10% in petrolatum.

Even if no statistically significant difference was found between any of the aluminium formulas and the number of positive reactions, there is a tendency that the higher concentrations of aluminium lactate and aluminium chloride hexahydrate may be preferable in testing for aluminium allergy in adults. The highest number of positive reactions was seen using aluminium lactate.

Three of 5 individuals with doubtful reactions to aluminium chloride hexahydrate 2% in petrolatum in patch test III had positive reactions in at least one of the higher concentrations of the aluminium preparations. They would falsely have been judged as not sensitised without the additional preparations. As earlier mentioned, recent studies by Bruze et al. suggest that the 2% concentration of aluminium chloride hexahydrate may cause false-negative results and that a 10% preparation may be preferable (61, 69, 71).

Only 5 participants were positive in the aluminium Finn Chamber® and all scored positive to other aluminium preparations. Recently reported by Hedberg et al.(167), the amount of aluminium released from an empty Finn Chamber® corresponds to a skin dose of 0.03% to 0.5% of aluminium chloride hexahydrate applied in plastic chambers.

During all 3 patch tests of the cohort, interviews, physical examinations and readings were done by experienced physicians responsible for the Mass Vaccination Project as well as specially trained staff and dermatologists in the Occupational and Environmental Dermatology Unit, Sahlgrenska University Hospital. In total, approximately 10 persons have been involved in more than 700 patch tests, interviews and examinations of the participants. Only a few children had their first patch test performed in other clinics. At the start of patch test II, all the readings were done by the 2 dermatologists together. Even though there may be an intra-individual and inter-individual variation in patch tests (45), the application and the readings procedures in these studies were comparable to those in our regular clinic at the current time for each testing.

Only the single reading on day 3 differs in the study from our daily clinic in which almost always a second reading is performed on day 7. Instead, all participants were asked to contact the study team for another reading if any signs of additional reactions were observed, which no one did. It would have been preferable with a second reading on day 7, at least in those with negative tests and doubtful reactions, since it can be difficult to notice a late reaction on one's back by oneself.

Loss of patch test reactions

Among all 241 individuals who tested positive for aluminium in childhood, 16/31 tested had a detectable hypersensitivity in the third test after more than 15 years. Remarkably, 4 of these individuals had negative results in the second test but switched to positive again in the third.

In the review of Lee et al. (168), several studies describe the loss of patch test reactions of other allergens such as nickel, cobalt and colophony. The proportion of those who had become negative in the test varied from 4% to 59%. The loss of aluminium allergy over time regarding loss of patch test reactions is reported to be between 19% and 100% (55).

Individual variation in test reactivity is a phenomenon that has been reported widely and with great variations in different test methods (169). It has been seen after patch tests repeated several times for nickel, palladium (170, 171), aluminium (172) and other allergens (169, 173). In the study of Siemund et al. (172), the reproducibility of the positive aluminium patch test with the same aluminium preparations increased when the test was repeated 4 times during 8 months.

As recently published (174), the bioavailability of aluminium salt in artificial sweat varies due to sweat composition, pH, metal salt and concentration. In our cohort, the loss of patch test reactions for aluminium increase with the age of the individual. The changed bioavailability might explain these results due to the changing of the sweat composition and pH. However, this could not explain the loss of symptoms when re-exposed to aluminium-containing vaccines, as seen in Paper III. In the study by Dittmar et al. (175), a retrospective analysis was performed on adult patients tested with standard allergen twice between 1995-2016 with the TRUE Test®. In 119 participants, 274 retested positive reactions (33.2%) were transient. Metals and fragrances were reported to be less persistent compared to the other standard allergens.

There is a lack of knowledge regarding the cause of the persistence or loss of positive patch test reactions. Different factors such as methodological (169), impairment of the skin barrier (176), regional skin differences (177), ultraviolet radiation (178), seasonal variations (179), immunomodulation drugs (23), and hormonal variation(180) may influence results. In our studies, none of the patch tests were performed during summertime and, therefore, in most cases, probably

without the influence of ultraviolet radiation. Atopic dermatitis or intake of immunomodulation drugs was not asked for in the interviews.

However, the great loss of patch test reactions combined with the disappearance of itching nodules seen over the years following the Gothenburg Pertussis Vaccine Trials cohort could not possibly be explained only by individual variations in test reactivity.

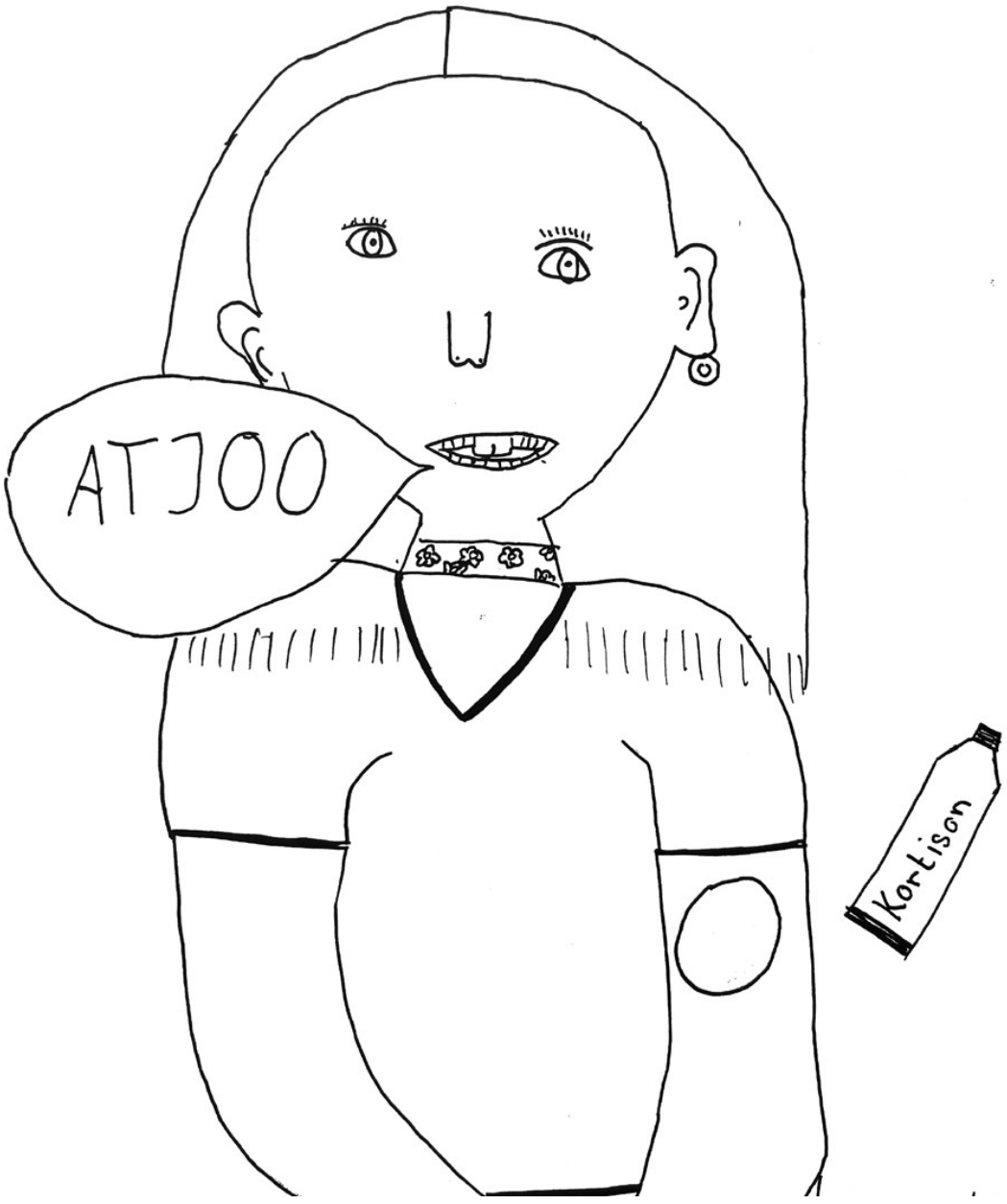
In a Swedish study (181), 4376 adults were patch tested and completed questionnaires inquiring about earlier piercing and orthodontic treatment, which was verified in dental records. There was almost a 2-fold reduced risk of nickel sensitivity in those who used braces before they had any piercing compared to those who did not. In another epidemiologic study of hand eczema (182), junior nurses with orthodontic treatment during childhood showed a lower frequency of nickel allergy than nurses with no history of braces. Nickel-containing alloys may induce tolerance to nickel by oral administration and reduce allergic reactions, which have been seen in both animal and human studies (183, 184). Small quantities of nickel ions are continuously released in the mouth. This might have a tolerogenic effect, inducing specific T-cell tolerance, preventing subsequent development of cutaneous nickel hypersensitivity (185).

A recently published study, in which foreskin from infants of different ages and adults were examined regarding T-cells, showed that very few T-cells are present in the epidermis of human neonates and that the number of T-cells in the epidermis gradually increases during the first year of life (186). They also described that the composition of the epidermal T-cell subsets changed with age, with a majority of CD4+T-cells in new-borns followed later by an increase in the frequency of CD8+ T-cells. They hypothesise that the increase of T-cells in the epidermis and the subset type is driven by antigen exposure and not by age.

We cannot yet explain how or why vaccine-induced aluminium allergy acquired during childhood fades away. As far as we know, the dose-response curve is the same for all ages. Could it be that the subset of early acquired T-cells in the epidermis during childhood are more likely to switch into another type or be suppressed?

Despite limitations in these studies, the negative patch test was significantly correlated with loss of itching at the injection site. In our approximately 20 year long-term follow-up study, Paper III, 86% of the participants had lost their itching, and only a few suffered from new eczema and itching nodules when re-exposed to aluminium-containing products later on in life.

More research in immunology and T-cells combined with further research of how aluminium works as a hapten will hopefully give us the answers behind the decrease in patch test reactivity and disappearance of aluminium CD seen in our cohort.



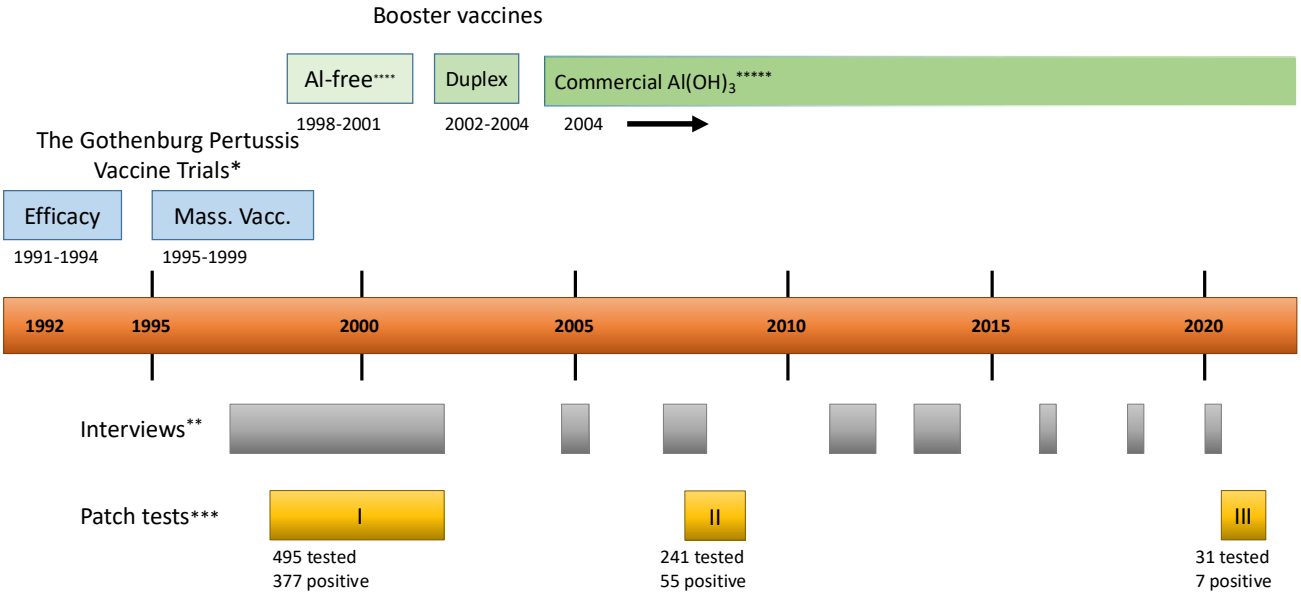
Drawing by a 9 years old girl with itching nodules on her left arm after vaccination with two doses of aP at 5-6 years of age. She illustrates the – not uncommon - recurrence of symptoms during a common cold.

CONCLUSIONS AND RECOMMENDATIONS PAPERS I-IV

Vaccination has prevented millions of deaths in serious infectious diseases in both children and adults and is important for public health worldwide. We are aware that side effects of vaccines occur and think it is important to recognise, study and report them to maintain confidence in childhood vaccination programs. So far, our research group has contributed with 9 publications on adverse events caused by aluminium adjuvants in vaccines since 2003. We understand that parents influenced by large-voiced vaccine opponents in non-scientific social media can be scared and hesitate to vaccinate their children. In choosing what is the best for their child, the discomfort of itching nodules weighs very lightly compared to the disaster that whooping cough, diphtheria or tetanus can imply in a child.

The long-term studies in this thesis, see overview in figure 2, show that clinical symptoms and delayed hypersensitivity for aluminium as determined by patch test disappears over time. Therefore, it can be recommended that further vaccination with aluminium-adsorbed vaccines is safe in older children, given that the original nodule will have vanished and the itching will have resolved or nearly resolved.

Figure 2



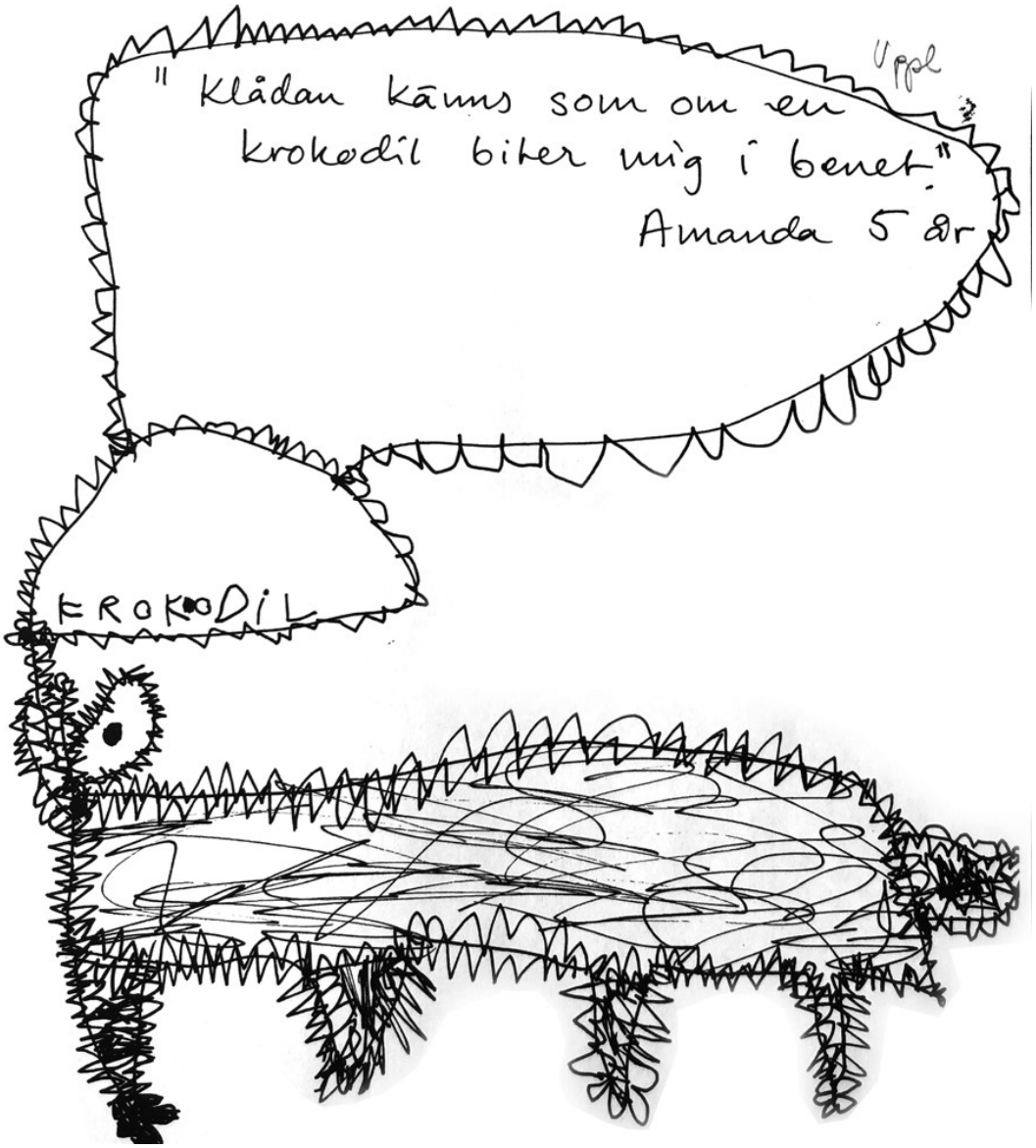
The Gothenburg Pertussis Vaccine Trials included 76.000 children in the Greater Gothenburg area. Persistent itching nodules were reported as an adverse event in 745 children

** **Interviews/questionnaires** were regularly offered to all 745 children with itching nodules.

*** **Patch test I:** 495 children with itching nodules were patch tested for aluminium in which 377 tested positive. **Patch test II:** 241 participant with a previously positive patch test I were tested in which 55 were observed positive; **Patch test III:** 31 participants with a positive patch test I and of which 20 tested positive in patch test II. Of all 31 participants 7 were observed positive in patch test III when tested with aluminium chloride hexahydrate 2% in petrolatum and an empty Finn-Chamber®, the same test preparations as used in patch test I and II. In patch test III another three aluminium preparations were also tested, aluminium chloride hexahydrate 10% in petrolatum an aluminium lactate 2.4% and 12.2% in petrolatum. In total 16 of 31 participants tested positive in test when all 5 test preparations were regarded.

**** **Al-free** = aluminium free
***** **$Al(OH)_3$**= aluminium hydroxid

Figure 2 Schematic overview of the Gothenburg Pertussis Vaccine Trials and the long-term follow-up study of the clinical course in 745 children and the three patch test studies performed during the years1991 to 2020.



Drawing by a 5 years old girl with two, still intensely itching, nodules on her left thigh since vaccination with DTaP in infancy. Her mother writes: "The itching feels as if a crocodile bites me in the leg"

FUTURE PERSPECTIVES

I would like to think that the results of the clinical trials of a new acellular aluminium-adsorbed pertussis vaccine in the 1990s and the children who, unexpectedly, were affected by long-standing itching nodules and contact allergy to aluminium have led to an increased interest in the research fields of aluminium allergy and alternative vaccine adjuvants.

From my perspective, the long-term follow-up study of this cohort is not completed until the remaining participants from the interrupted study have been tested a third time. Then, I would want to re-analyse the patch test outcomes of the different test preparations and, in more detail, study the data of the participants with a negative patch test II and their outcomes in patch test III when using all five test preparations.

We also have several interesting individual records which we would like to publish as case reports.

Further research fields which I see forward to is the mechanism and immunological behaviours of aluminium salts as a hapten as well as better methods of testing for aluminium allergy.

To be further investigated and understood is the persistent itching subcutaneous nodule with negative patch test result commonly seen in adults treated with ASIT.

Om något barn själv vill berätta hur det kändes
när det kliade på armen
eller hur du har det nu
så får du här ett papper att rita eller skriva på

Om jag har tur så kli ar det bara varannan dag.
Ibland är det 2 dagar imellan, medan det ibland är varje dag.
På våren börjar det klia så mycket att jag blir galen och springer runt.
Det är knottror på benet just nu.
Jag vill göra en till aluminiumtest.
Jag vill veta om jag är mindre allergisknu.
Vi köpte en deodorant och vi frågade om det fanns aluminium i. Dom sa nej. Men efterett tag började det stickas i armhålerna. Då kolla vi på ingridienserna. Det var aluminium i allefall.

Text by a nine, "almost ten", years old girl who is still suffering from itching nodules on her left thigh after DTaP vaccination in infancy. "If I am lucky I am itching only every second day." "In spring it will itch so much that I get mad and just have to run around." " There are nodules on my leg now" " I want to know if I am still allergic to aluminium" "After a while (after using an aluminium containing deodortant) it stuck in my armpits".

ACKNOWLEDGEMENT

I would like to express my warmest gratitude to all who have contributed and supported me in this work. In particular I am grateful to:

Annica Inerot, sincere gratitude for always being there and supporting me as a friend and colleague, sharing your wisdom and knowledge with great enthusiasm giving med excellent feedback. I am also so grateful to her wonderful husband Anders who put up with all my Zoom meetings and e-mails during weekends and vacations with the greatest of patience.

Birger Trollfors, my second supervisor for your endless patience and understanding. I cannot find words for your very warm personality, always with the right sense of when to support and how.

Elisabet Bergfors, my co-author who also became my "extra supervisor" and friend over the years. Thank you for your endless sharing of your linguistic skills, work and knowledge of "kikhostestudien".

Martin Gillstedt, my statistician and co-author for all the help with the statistics and figures. It would not have been possible without you!

Ulla Blomgren, Britt-Marie Ehn, Conny Eriksson and **Gabriella Lisengård,** my dear colleagues who booked and tested all the children.

Lina Hagvall, my dear ex-colleague, always keeping my spirits up when needed.

Tordis Andrén and **Lisbeth Söderström** and all the other nurses who participated in the Gotheburg Pertussis Vaccine Trials and collected data through the years.

Jonathan Gente and **Marie Norbäck** for helping me send out all the questionnaires to the children in the trials.

Jenna Anderson, for excellent and rapid linguistic corrections.

Annelie Grimm, for the fantastic help with the layout of this thesis.

Helena Gustafsson, Head of the Dept. of Dermatology and Venereology, Sahlgrenska University Hospital, for your excellent leadership and for making research important in our daily clinical work.

John Paoli, Head of the Dept. of Dermatology and Venereology, Sahlgrenska Academy, for always providing extremely fast mail correspondence and excellent support.

Frida Appelqvist, Mikael Alsterholm, Petra Tunbäck and **Amra Osmancevic,** my dear friends and collegues, for always listening and making me laugh. Thank you for your good advices during the years and for all your help!

All my other collegues at Hudkliniken, for making my working place such a great place!

My second working place, Hallandskustens hudmottagning who make me feel good!

Ella och **Lennart,** my wonderful parents, always supporting me and giving their limitless love. You are the best!

Henrik – my husband and the love of my life, for always loving and supporting me with absolutely EVERYTHING, even Excel. I love you! I am also grateful to his wonderful parents **Ulla och Lennart.**

Carl och **Johan,** my ❤❤, for being the best children in the world!

Johan, my brother and my "extra big brothers" Mikael and Christer and their wonderful families, for sharing my life, always knowing you all are there when I need you.

Finally, sincerest gratitude to all **participants** in these studies and to **their parents.**

Financial support

I am so grateful for the financial support from:

The Research Foundation of the Swedish Asthma and Allergy Association

The Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALFGBG-814381, ALFGBG-932683, ALFGBG-938116)

The Swedish Union of General Practitioners

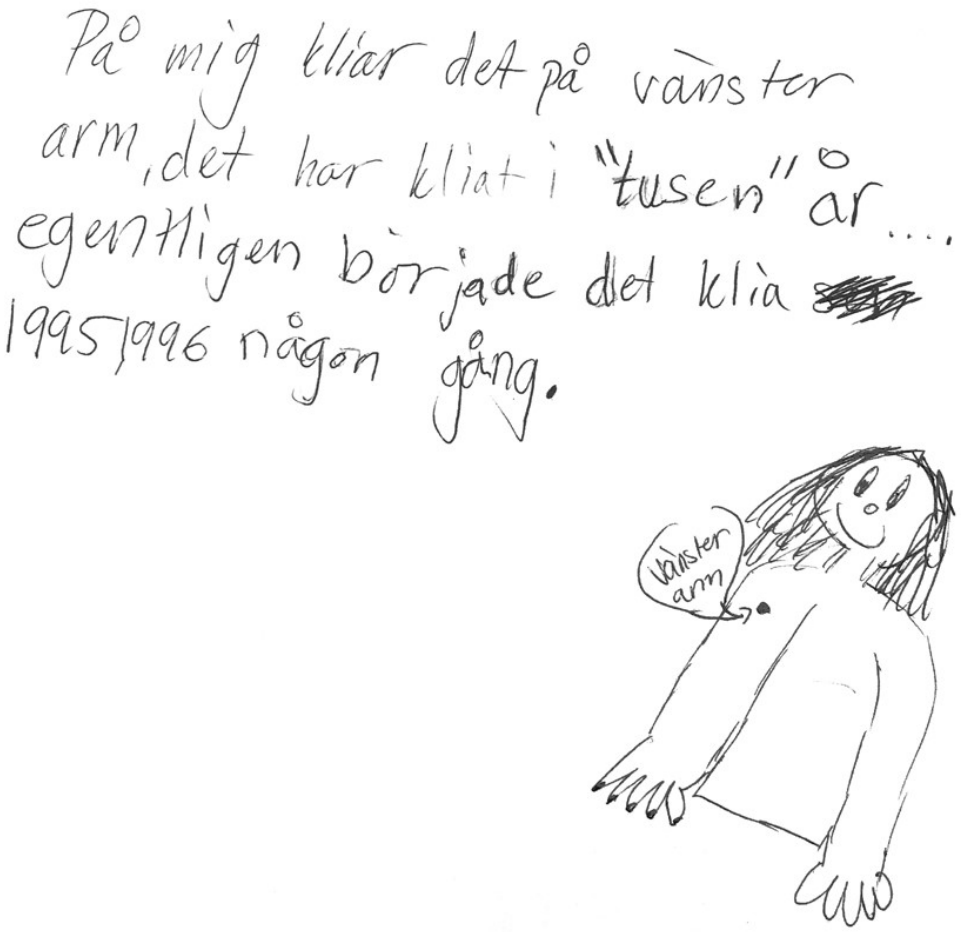
The Petter Silfverskiöld Memorial Foundation

The Swedish Medical Association,

The Gothenburg Medical Association and the

Local Research and Development Council of Göteborg and Södra Bohuslän

The Department of Dermatology and Venereology, Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden



Text and drawing by a 12 years old girl with itching nodules on her left arm after vaccination with aP at 4-5 years of age. "My itching is on the left arm, it has been itching for "a thousand years" ….. actually it started in 1995-1996 some time" (the period when she received the vaccines)

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