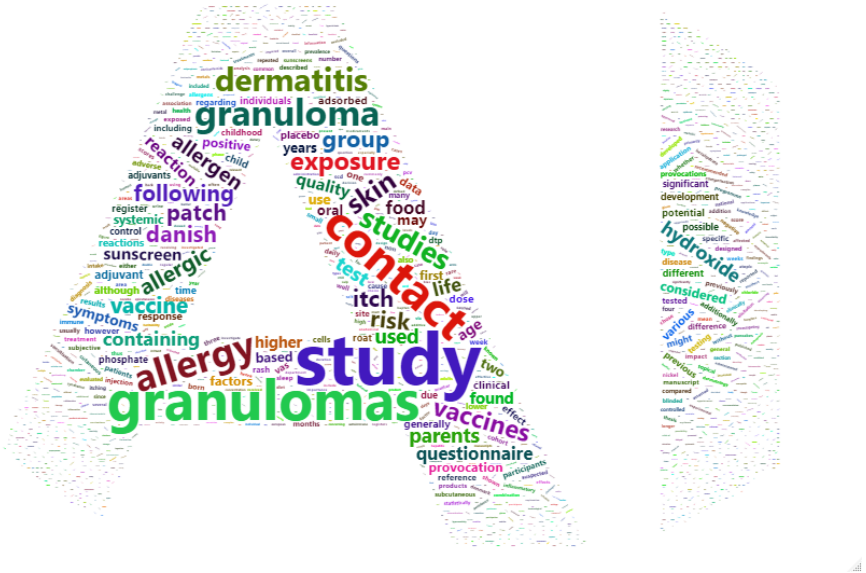
UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

Children with vaccination granulomas and aluminium contact allergy



PhD Thesis

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Principal supervisor: Jeanne Duus Johansen

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Children with vaccination granulomas and aluminium contact allergy

PhD Thesis

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Preface

This thesis is based on scientific work carried out at the National Allergy Research Centre, Department of Dermatology and Allergy, Copenhagen University Hospital – Herlev and Gentofte between 2018 and 2022. The projects received financial support from the Ministry of Environment and Aage Bangs Foundation. The register-based study carried out in collaboration with SSI received financial support from Helsefonden, Læge Sofus Carl Emil Friis og Hustru Olga Doris Friis legat, and The Lundbeck Foundation. All are gratefully acknowledged.

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Stine Hoffmann

Copenhagen, July 2022

Included Studies

This PhD thesis is based on the following four manuscripts:

Manuscript I

Hoffmann SS, Thyssen JP, Elberling J, Hansen KS, Johansen JD.

Children with vaccination granulomas and aluminum contact allergy: Evaluation of predispositions, avoidance behavior, and quality of life.

Contact Dermatitis. 2020 Aug;83(2):99-107

Manuscript II

Hoffmann SS, Elberling J, Thyssen JP, Hansen KS, Johansen JD.

Does aluminium in sunscreens cause dermatitis in children with aluminium contact allergy: A repeated open application test study.

Contact Dermatitis. 2022 Jan;86(1):9-14

Manuscript III

Stine Skovbo Hoffmann, Jesper Elberling, Kirsten Skamstrup Hansen, Jacob P. Thyssen, Charlotte G. Moertz, Rasmus Overgaard Bach, Jeanne Duus Johansen

Adverse reactions after oral provocation with aluminium in children with vaccination granulomas and aluminium contact allergy.

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Manuscript IV

Skovbo Hoffmann S, Thiesson EM, Johansen JD, Hviid A.

Risk factors for granulomas in children following immunisation with aluminium adsorbed vaccines: A Danish population-based cohort study.

Contact Dermatitis. 2022 Jul 2. doi: 10.1111/cod.14180. Epub ahead of print. PMID: 35778959.

Additional publications

Hoffmann SS, Wennervaldt M, Alinaghi F, Simonsen AB, Johansen JD.

Aluminium contact allergy without vaccination granulomas: A systematic review and meta-analysis.

Contact Dermatitis. 2021 Apr 1. doi: 10.1111/cod.13852. Epub ahead of print. PMID: 33797096.

Hoffmann SS, Elberling J.

Vaccination Granuloma Itch Treated with Capsaicin 8% Patches.

Acta Derm Venereol. 2020 Dec 1;100(19)

Abbreviations

ACD	Allergic contact dermatitis
AD	Atopic dermatitis
AIT	Allergen-specific immuno therapy
$Al(OH)_3$	Aluminium hydroxide
$AlPO_4$	Aluminium phosphate
APC	Antigen-presenting cell
CDLQI	Children's dermatology life quality index
CPR	Central person register
DBPCFC	Double-blinded, placebo-controlled food challenge
DPCA	Danish patient compensation association
DTP	Diphtheria, tetanus, pertussis, polio, Haemophilus influenza type b (Hib)
EFSA	European food safety authority
ICP-MS	Inductively coupled plasma mass spectrometry
JECFA	Joint FAO/WHO Expert committee on food additive
LOAEL	Lowest observed adverse event level
NOAEL	No observed adverse event level
PCV	Pneumococcal conjugate vaccine
ROAT	Repeated open application test
RR	Rate ratio
SALP	Sodium aluminium phosphate
SCIT	Subcutaneous immuno therapy
SCD	Systemic contact dermatitis
TWI	Tolerable weekly intake
VAS	Visual analogue scale

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Summary

Aluminium is a ubiquitous metal, commonly used in kitchen utensils, cosmetics, pharmaceuticals and as a food additive. In addition, aluminium salts are the most common adjuvants used in vaccines. Aluminium is generally considered a weak allergen, but as a vaccine adjuvant aluminium may cause vaccination granulomas and concomitant aluminium contact allergy. These granulomas are small, subcutaneous, itching nodules that occur at the injection site. Data regarding the possible adverse effects of using aluminium-containing skin products such as sunscreens, as well as oral intake of aluminium-containing foods, are lacking. In addition, a better understanding of the impact of vaccination granulomas and aluminium contact allergy on quality of life and on lifestyle as well as greater insight into vaccine-related risk factors are needed.

This thesis consists of four studies.

In the first study, we designed a questionnaire to collect data on children with vaccination granulomas regarding their quality of life, exacerbating factors, possible treatments, and avoidance behaviour. We found that children with vaccination granulomas had impaired quality of life, that food and skin products containing aluminium could aggravate granuloma itch, that the available treatments were only effective in a minority of children, and that the parents of more than one-quarter of affected children tended to avoid further vaccination of their child.

Parents reported that 46% of children could not tolerate aluminium-containing sunscreens and that 31% of children exhibited exacerbation of granuloma itch following ingestion of food containing aluminium.

Based on the results of our questionnaire study, we designed two provocation studies. The first study involved a blinded repeated open application test (ROAT), in which a parent applied two sunscreens to small defined areas of skin on the lower back of their child twice daily. One sunscreen contained aluminium, the other did not. One of the 16 participating children developed a pruritic rash on day 2 of the ROAT, but to the aluminium-containing sunscreen only.

The second provocation study was a single-blinded aluminium/placebo food challenge, in which children consumed pancakes both with and without aluminium for 3 consecutive weeks. During the study, the children evaluated different subjective symptoms and granuloma itch on visual analogue

scales (VASs). We also monitored the children's sleep patterns using activity watches. After each provocation week we analysed a urine sample from each child for aluminium excretion.

During aluminium provocation, three children developed a rash that was compatible with systemic contact dermatitis. The VAS scores for granuloma itch and subjective symptoms were generally higher during aluminium provocations, although the differences were small and not statistically significant. There was no correlation between aluminium excretion in the urine and VAS symptom severity, and no differences in sleep patterns.

The final study was a register-based study in collaboration with Statens Serum Institut, investigating risk factors associated with developing vaccination granulomas. We created a cohort consisting of approximately 500,000 children born in Denmark, of whom 1,901 had vaccination granulomas. We found that granuloma formation was more likely to be associated with aluminium hydroxide adjuvants than aluminium phosphate adjuvants, and a total dose of more than 1 mg (compared to less than 1 mg) of aluminium per vaccination appointment increased the risk of developing vaccination granulomas. We also found that having a sibling with a vaccination granuloma was undoubtedly the greatest risk factor for developing vaccination granulomas.

In conclusion, children with allergy to aluminium and vaccination granulomas have impaired quality of life and may develop dermatitis when exposed to aluminium dermally or orally. However, we did not observe a statistically significant difference between the test and control exposures. Changing the type of vaccine adjuvant or decreasing the dose of aluminium in vaccines may help to prevent vaccination granulomas.

Dansk Resumé

Aluminium er et udbredt metal, der almindeligvis anvendes i køkkenredskaber, kosmetik, lægemidler og som fødevaretilsætningsstof. Derudover er aluminiumsalte de mest almindelige adjuvanser der anvendes i vacciner. Som vaccine adjuvans kan aluminium forårsage vaccinationsgranulomer og samtidig aluminium kontaktallergi. Vaccinationsgranulomer er små, subkutane, kløende knuder, der forekommer på injektionsstedet. Viden om mulige bivirkninger ved at bruge aluminiumholdige hudprodukter som solcreme, samt indtagelse af aluminiumholdige fødevarer, har indtil nu manglet og været efterspurgt af både forældre og klinikere. Derudover krævede indvirkningen på livskvalitet samt ikke mindst indsigt i vaccinerelaterede risikofaktorer, yderligere opmærksomhed.

Denne afhandling består af fire studier.

I det første studie karakteriserede vi børn med vaccinationsgranulomer vedrørende livskvalitet, forværrende faktorer, mulige behandlinger og undgåelsesadfærd, ved en spørgeskemaundersøgelse.

Vi konstaterede, at både børn med vaccinationsgranulomer samt deres forældre havde generel nedsat livskvalitet. Forældrene rapporterede derudover, at 46% af børnene ikke kunne tåle aluminiumholdige solcremer, og at 31% af børnene udviste forværring af granulomkløe efter indtagelse af mad indeholdende aluminium. Endelig fandt vi, at forældre til mere end hvert fjerde barn valgte enten at udskyde eller helt undgå yderligere vaccination af deres barn.

Baseret på resultaterne af vores spørgeskemaundersøgelse designede vi to provokationsstudier. Det første af disse studier var en applikationstest, hvor en forælder påførte to solcremer på små definerede hudområder på lænden af deres barn to gange dagligt. Den ene solcreme indeholdt aluminium, den anden gjorde ikke. Et af de 16 deltagende børn udviklede et kløende udslæt på dag 2 af studiet, men udelukkende på det område hvor den aluminiumholdige solcreme var brugt.

Det andet provokationsstudie var et oralt provokationsstudie, hvor børn indtog pandekager både med og uden aluminium, i 3 på hinanden følgende uger. Under studiet vurderede børnene og deres forældre forskellige subjektive symptomer og kløe af vaccinationsgranulomet på VAS-skalaer fra 0-10. Vi overvågede også børnenes søvnmønstre ved hjælp af aktivitetsure. Efter hver provokationsuge analyserede vi en urinprøve fra hvert barn for udskillelse af aluminium.

Under aluminiumprovokationen udviklede tre børn et udslæt, der var foreneligt med systemisk kontaktdermatitis. VAS-scorerne for granulomkløe og subjektive symptomer var generelt højere under aluminiumprovokationerne, selvom forskellene var små og ikke statistisk signifikante. Der var ingen sammenhæng mellem aluminiumudskillelse i urinen og symptomernes sværhedsgrad, og ingen forskel i søvnmønstre.

Det sidste studie var et registerbaseret studie i samarbejde med Statens Serum Institut, der undersøgte risikofaktorer forbundet med udvikling af vaccinationsgranulomer. Vi dannede en kohorte bestående af ca. 500.000 børn født i Danmark, hvoraf 1.901 havde vaccinationsgranulomer. Vi fandt ud af, at granulomdannelse var mere tilbøjelig til at være forbundet med aluminiumhydroxid adjuvanser end aluminiumfosfat adjuvanser, og en samlet dosis på mere end 1 mg (sammenlignet med mindre end 1 mg) aluminium per vaccination øgede risikoen for at udvikle vaccinationsgranulomer. Vi fandt også, at dét at have en søskende med et vaccinationsgranulom utvivlsomt var den største risikofaktor for udvikling af vaccinationsgranulomer.

Konklusionen på denne afhandling er, at børn med vaccinationsgranulomer og aluminium kontaktallergi har en negativ påvirket livskvalitet, og at de kan udvikle hududslæt eller øget kløe af granulomet, når de udsættes for aluminium enten på huden eller via fødevarer. Vi fandt dog ikke en statistisk signifikant forskel mellem test- og kontrolekspo neringerne. Ændring af typen af adjuvans eller nedsættelse af mængden af aluminium per vaccinedosis kan bidrage til at forhindre udviklingen af vaccinationsgranulomer.

1. Introduction

The global introduction of national childhood vaccination programmes has considerably improved children's health, by protecting against debilitating and life-threatening diseases. Vaccines, generally classified as either live or inactivated, are used to safely induce immune responses against particular diseases.

The inactivated vaccines need to be bolstered by adjuvants, to enhance immunogenicity and create a sufficient response.[1] For decades, aluminium has been used as an adjuvant, and it is considered both effective and safe by the Global Advisory Committee for Vaccine Safety, who have reviewed vaccine data since 1999.[2] Although the safety of aluminium adjuvants is indisputable, they may cause small itching nodules at the injection site, known as vaccination granulomas, with concomitant contact allergy to aluminium. This thesis, entitled “Children with vaccination granulomas and aluminium contact allergy”, focuses on risk factors linked to children developing vaccination granulomas, relevant characteristics, and cutaneous and oral aluminium provocations. The following introduction provides background information for the four manuscripts included in this thesis.

1.1 Contact allergy and allergic contact dermatitis

Contact allergy is a delayed hypersensitivity reaction, also known as a type IV allergy. It is an acquired immunological response, with allergic contact dermatitis (ACD) being the clinical manifestation of the disease. Allergens are substances capable of causing an allergic reaction. Haptens are small molecules that are not antigenic themselves, but once they bind to a carrier protein after penetrating the skin, they can elicit an immune response.

ACD involves two phases: a clinically asymptomatic sensitisation phase that generates immunological memory and an elicitation phase with ACD (Fig. 1).[3] During the sensitisation phase, the skin is exposed to haptens, which penetrate the stratum corneum and are taken up by antigen-presenting cells (APCs). APCs are activated by contact with the haptens, and immune cells such as keratinocytes are also activated, leading to the secretion of cytokines. The hapten-carrying APCs reach the draining lymph nodes via afferent lymphatic vessels and present the haptens to naïve T

cells. The naïve T cells are activated and start to proliferate into allergen-specific effector and memory T cells. This whole process takes 10–15 days, depending on factors such as the duration of exposure, the concentration and potency of the hapten, and individual risk factors such as skin barrier defects.[4]

If or when the accumulated skin exposure to a particular hapten surpasses a threshold, a secondary response occurs. This is known as the elicitation phase. When the hapten penetrates the skin, it is taken up by APCs and presented to the T cells that are now allergen-specific. Additionally, keratinocytes and dendritic cells release cytokines and chemokines that attract more allergen-specific T cells. This reaction manifests itself as ACD, usually with clinical symptoms such as pruritus, erythema, vesicle formation, and swelling. The duration between re-exposure to the hapten and clinically visible cutaneous symptoms is usually 24 to 72 hours.[5]

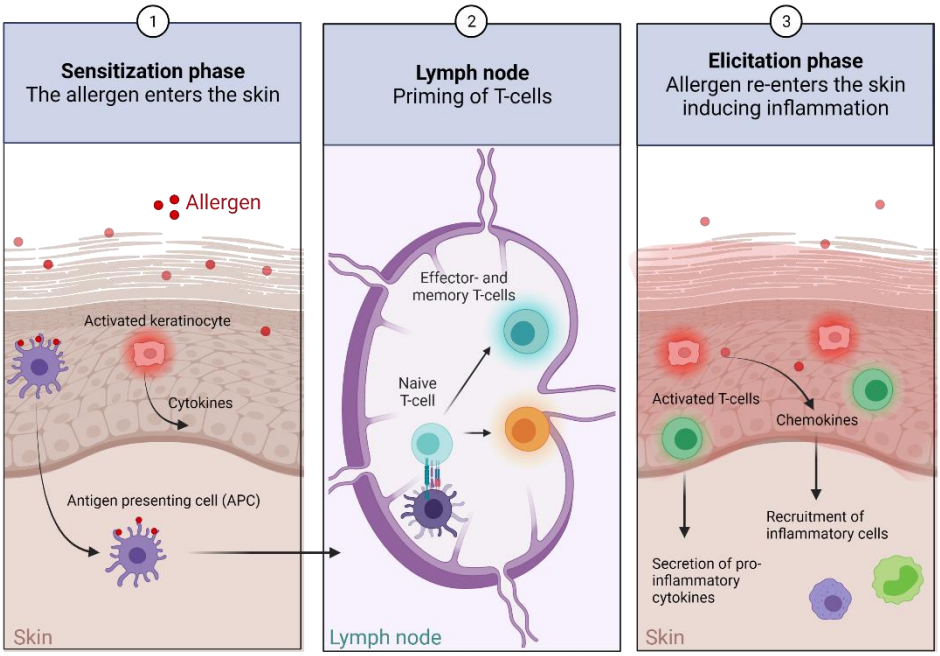


Figure 1. Allergic contact dermatitis is a delayed type IV reaction with a sensitisation phase and an elicitation phase
Created with BioRender.com

Once allergic, subjects may experience skin inflammation (dermatitis) whenever sufficient exposure to a particular hapten occurs.[3] In general, allergy to metals is relatively common. In most countries

worldwide, nickel is the most frequently reported contact allergen, followed by chromium and cobalt.[6] Metal allergies usually develop as described above. However, aluminium allergies usually develop in response to aluminium-adsorbed vaccines, and vaccination granulomas are considered a clinical manifestation of aluminium contact allergy.[7,8]

In the following description, haptens and allergens are both termed "allergens."

1.2 Systemic contact dermatitis

In rare cases, systemic exposure to an allergen may elicit a cutaneous reaction, which is accompanied by various systemic symptoms. This is called systemic contact dermatitis (SCD).[9,10] Systemic exposure can occur via many different routes, including orally, intravenous injections and inhalation.

The most common cause of SCD is medications such as antibiotics, non-steroidal anti-inflammatory drugs and corticosteroids; however, metals such as nickel, gold, mercury, chromium and cobalt may also induce a systemic response following systemic exposure.[10–12] The cutaneous reactions include flare-up reactions in areas that were previously patch tested or had exhibited ACD, flexural exanthema, vesicular hand eczema, or widespread dermatitis.[11,13] "Baboon syndrome" is characterised by eruptions on the buttocks and genital area, and primarily occurs in individuals who are sensitised to mercury and Balsam of Peru.[14] Other systemic allergy symptoms such as diarrhoea and vomiting, nausea and headache may occur in some individuals.[15,16]

The underlying pathogenesis of SCD is complex and apparently involves a type IV hypersensitivity reaction.[17] Jensen *et al.* found that nickel-allergic individuals who developed cutaneous reactions to an oral nickel-challenge, had a decreasing level of memory T cells in the blood 24 hours after oral nickel intake, indication than these T cells migrated to the skin and caused the reaction.[18] The flexural eczematous reactions and non-specific maculopapular rash has been proposed to be caused by non-specific cytokine release.[19]

SCD may be overlooked or misinterpreted due to the prolonged interval (sometimes several days) between exposure and eruptions, or due to its various cutaneous and systemic manifestations. Additionally, SCD may be influenced by the time elapsed since sensitisation, patch test reactions or systemic exposure dose.[11]

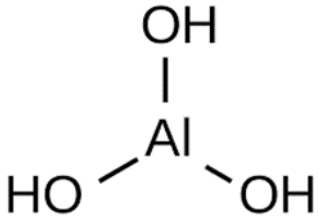
SCD in individuals allergic to aluminium has only been clinically assessed in a single case report, following oral exposure to aluminium-containing toothpaste.[20]

1.3 Aluminium

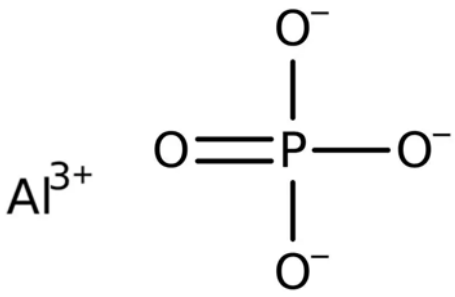
In total, 8% of the Earth's crust consists of aluminium, a ubiquitous metal with the atomic number 13. Aluminium was discovered by the Danish chemist Hans Christian Oersted in 1824, when he managed to separate aluminium from the mineral bauxite. Aluminium has a strong affinity for oxygen and has applications both as a metal and as a salt.[21] As a metal, aluminium has various advantageous properties, being soft, light and non-magnetic, It is used for manufacturing aircraft, foil, and pots and pans. As a salt, aluminium is present in various pharmaceuticals such as antacids, cosmetics and antiperspirants; it is also present in food, either naturally or as an additive. Finally, aluminium is an adjuvant in many vaccines.

1.4 Aluminium adjuvants

Several types of vaccines are routinely given to children, including weakened live viruses (measles, mumps, and rubella), inactivated viruses (polio and hepatitis A), toxoids (diphtheria and tetanus), and conjugates (pneumococcal disease). Live viral vaccines are very similar to the natural infection, although weaker, whereas inactivated vaccines usually need an adjuvant to induce immunity.[1] Aluminium has been used as an adjuvant in vaccines since 1925 when Glenny and colleagues found that the addition of aluminium to a toxoid stimulated a significantly increased immune response.[22] The two major types of aluminium adjuvant most frequently used in human vaccines are aluminium hydroxide, $Al(OH)_3$, and aluminium phosphate, $AlPO_4$.[23]



Aluminium hydroxide $Al(OH)_3$



Aluminium phosphate $AlPO_4$

These two aluminium salts have different properties and diverge in both molecular size and surface charge at physiological pH.[24,25] Aluminium hydroxide activates more immunological pathways and attracts more neutrophils than aluminium phosphate. Of these two salts, the former is retained at the injection site for a longer period than the latter (both intramuscularly and subcutaneously). Therefore, aluminium hydroxide is generally considered the stronger adjuvant and is also the more frequently used.[26,27]

The mechanism by which aluminium adjuvants function is a target of ongoing research. Aluminium adjuvants induce inflammation at injection sites, causing oedema and the recruitment of leukocytes.[26,27] This causes an increase in the level of interstitial fluid, which contains various acids that can chelate metal ions and solubilise the aluminium adjuvants.[28] In addition to its role in vaccines, aluminium is also used as an adjuvant in extracts used for allergen-specific immunotherapy, such as subcutaneous immunotherapy (SCIT).[7,8,29]

Aluminium-adsorbed vaccines available in Denmark from 2008 to 2020 are shown in Table 1.

Table 1. Aluminium-adsorbed vaccines available in Denmark from 2008 to 2020

Commercial name	Aluminium adjuvant	mg Al/dose
Diphtheria, tetanus, pertussis, polio, *Haemophilus influenza* type b (Hib)		
Di-Te-Ki-Pol(/Act-Hib)	Aluminium hydroxide hydrate	1.0
Pentavac	Aluminium hydroxide	0.3
Infanrix Hexa	Aluminium hydroxide Aluminium phosphate	0.5 0.32
Hexyon/Hexacim	Aluminium hydroxide	0.6
Di-Te booster	Aluminium hydroxide hydrate	0.5
Tetravac	Aluminium hydroxide	0.3
Polio vaccine SSI	Aluminium oxide hydrate	1.0
Imovax Polio	Aluminium oxide hydrate	0.5
Tetanus vaccine SSI	Aluminium oxide hydrate	1.0
Pneumococci		
Prevenar 7	Aluminium phosphate	0.5
Prevenar 13	Aluminium phosphate	0.125
Meningococcus group C		
NeisVac-C	Aluminium hydroxide	0.5
Meningococcus group B		
Bexsero	Aluminium hydroxide	0.5
Trumbena	Aluminium phosphate	0.25
Tick-borne encephalitis (TBE)		
Tico Vac	Aluminium hydroxide	0.35
Tico Vac junior	Aluminium hydroxide	0.17
Encepur	Aluminium hydroxide	0.3–0.4
Encepur children	Aluminium hydroxide	0.15–0.2
Human papillomavirus (HPV)		
Gardasil	Aluminium hydroxyphosphate	0.225
Gardasil9	Aluminium hydroxyphosphate	0.225
Cervarix	Aluminium hydroxide hydrate	0.5
Silgard	Aluminium hydroxide phosphate sulphate	0.225
Hepatitis		
Twinrix	Aluminium hydroxide Aluminium phosphate	0.5 0.4
Twinrix paediatric	Aluminium hydroxide Aluminium phosphate	0.25 0.2
Ambirix	Aluminium phosphate	0.4
Havrix	Aluminium hydroxide	0.5
Havrix paediatrix	Aluminium hydroxide	0.25
Vaqta	Aluminium hydroxide phosphate sulphate	0.225
Vaqta paediatric	Aluminium hydroxide phosphate sulphate	0.225
Engerix-B	Aluminium hydroxide	0.5
Engerix-B paediatric	Aluminium hydroxide	0.3
Fendrix	Aluminium phosphate	0.5
HBVaxPRO	Aluminium hydroxide phosphate sulphate	0.5
HBVaxPRO paediatric	Aluminium hydroxide phosphate sulphate	0.25

Modified after information from the Danish childhood vaccination schedule (https://www.ssi.dk/vaccinationer/boernevaccination).

1.5 Vaccination granulomas

Vaccination granulomas are small (approximately 0.5–2 cm in diameter), non-tender, firm, subcutaneous nodules that occur at injection sites during the weeks or months after immunisation with aluminium-adsorbed vaccines. Their existence has been acknowledged for many years,[30,31] but they were previously considered rare and only described on a case-by-case basis until 2003, when a Swedish placebo-controlled vaccine trial of a new aluminium hydroxide-adsorbed vaccine reported granulomas in 645 of 76,000 (0.8%) vaccinated children. 28 children (4%) developed the granuloma after the first vaccine, 117 (18%) after the second and 494 children (77%) developed the granuloma after the third aluminium-adsorbed vaccine.[32] Of these 645 children, 455 were given a patch test for delayed hypersensitivity to aluminium, and 352 (77%) of them had a positive result.[32,33] The main symptom of the granuloma is intense itch, often exacerbated by fever, infections, heat, and subsequent vaccination. In addition to the nodule, the skin above the granuloma is often characterised by eczema, hypertrichosis, and hyper- or hypo-pigmentation (Fig. 2).[34,35]

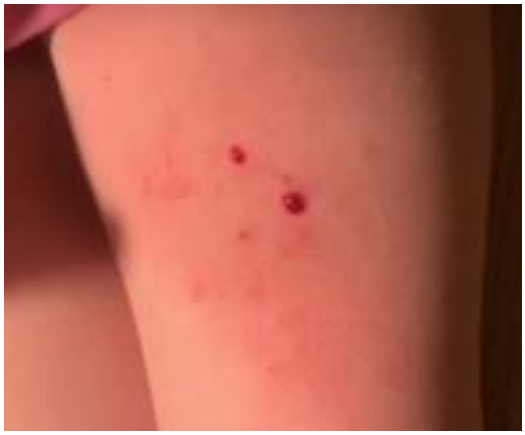


Figure 2. Vaccination granuloma on the left thigh of a 3-year-old girl. The granuloma is extremely itching and there are clear signs of scratching and eczema on the skin above the granuloma. Permission to use the photo has been obtained from both parents and the child.

Several histopathological examinations of vaccination granulomas have been carried out in both humans and animals.[36–39] These granulomas generally exhibit an area of chronic inflammation with aggregates of macrophage-derived cells. This is surrounded by lymphocytes, plasma cells and eosinophils in an infiltrative mix, together with aluminium deposits. However, granulomas may vary among cases.[36,38] Because there may be a long latency period, uncertainty regarding a

granuloma diagnosis is frequently an indication for imaging, with ultrasound being the modality of choice.[40] Typically, hypoechoic avascular nodule(s) may be found deep in the subcutaneous fat, with no vascular malformations, abscesses or foreign bodies (Fig. 3).

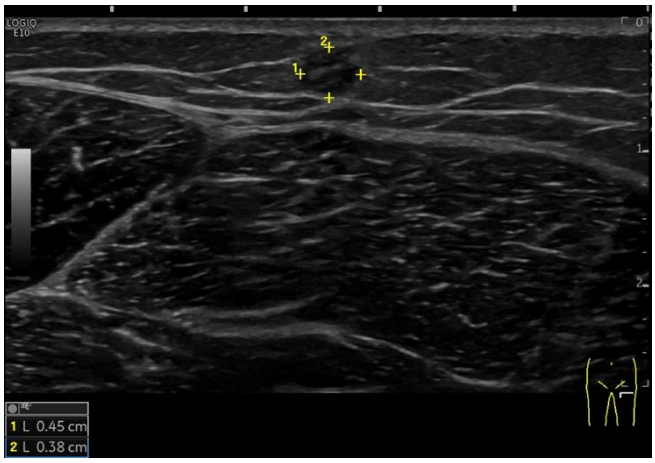


Figure 3. Ultrasonic features of a vaccination granuloma in a 3-year-old child. Ultrasound shows a sharply defined rounded hypoechoic lesion measuring approximately 4 mm in diameter within the subcutaneous adipose tissue. There is no Doppler signal within the lesion and no involvement of the underlying muscle fascia. Permission to use the photo has been obtained from both parents and the child.

Injection technique has previously been proposed as a risk factor for the development of vaccination granulomas, although a study by Bergfors *et al*. described granulomas following both subcutaneous and intramuscular injections.[32] Allergen-specific SCIT may also lead to the development of granulomas in both children and adults.[7,31,41]

1.6 Contact allergy to aluminium

Sensitisation to aluminium usually occurs via subcutaneous or intramuscular exposure to aluminium-adsorbed vaccines. Different sensitisation mechanisms have been proposed. The first aluminium-adsorbed vaccine may induce sensitisation. Alternatively, sensitisation may be due to aluminium deposits at the injection site following vaccination.[42] Aluminium rarely causes contact sensitisation upon epicutaneous exposure,[43] but there have been a few reports of aluminium contact allergies in children and adults following skin exposure to aluminium-containing antiperspirants, topical medications, and repeated contact with metallic aluminium.[44–48]

The relationship between vaccination granulomas and aluminium contact allergies was established by Clemmensen and Knudsen in 1980, when a 13-year-old girl who had previously received hyposensitisation treatment with an aluminium-adsorbed mixture of grass and pollen exhibited a positive patch-test result to six different aluminium allergens.[8]

Aluminium contact allergy is diagnosed by patch testing with an aluminium salt and a metallic aluminium chamber. Patch testing is considered the gold standard when diagnosing contact allergies.[49] Patch testing is described in more detail in the Materials and Methods section.

1.7 Aluminium exposure through the diet

Aluminium is present in most foods. It may occur naturally, as a food additive, or in packaging and cooking utensils. Studies from across the world have estimated the daily dietary intake of aluminium by analysing a multitude of diverse food samples.[50–55] The average dietary exposure to aluminium ranges from 0.18 to 0.36 mg/kg bodyweight/week (mg/kg bw/week) in adults and 0.22 to 0.90 mg/kg bw/week in children, depending on age.[21,53,56] Infants who are bottle fed with formula milk may ingest considerably more aluminium during their first few months, because the concentration of aluminium in infant formula milk may be as much as 40-fold greater than in human breast milk.[57] Food containing aluminium as an additive may have as much as 750 mg aluminium per kg, significantly increasing dietary intake.[58]

The overall bioavailability of ingested aluminium is low, with approximately 0.3% being absorbed from water and 0.1% from food.[59] Unabsorbed aluminium is excreted in the faeces. Aluminium is absorbed by passive diffusion when it forms complexes with various molecules in the body. After absorption, most aluminium is bound to transferrin and rapidly cleared through the renal system.[60]

Various animal studies have investigated the toxicity of aluminium. Studies of behavioural changes and motor disturbances in animals that were administered high levels of intravenous aluminium established a No Observed Adverse Event Level (NOAEL) of 10 mg aluminium/kg bw/day and a Lowest Observed Adverse Event Level (LOAEL) of 50 mg aluminium/kg bw/day. From these animal studies, a tolerable weekly intake (TWI) of aluminium in humans has been defined by the European Food Safety Authority (EFSA): 1 mg Al/kg bw/week.[21] In the United States, the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives (JECFA) permits a TWI of 2 mg Al/kg bw/week.[21]

1.8 Potential adverse effects of aluminium

Because aluminium has no known physical role in the human body, it has been the subject of many toxicity investigations. Bioavailable aluminium is cleared via the kidneys, and patients with impaired renal function, such as preterm infants and patients undergoing dialysis, may occasionally be exposed to greater levels of aluminium, potentially resulting in encephalopathy.[61,62] Bioavailable aluminium can cross the blood–brain barrier. Therefore, aluminium could theoretically accumulate in the brain, and some researchers have postulated links between aluminium toxicity and impaired neurological development, Alzheimer's disease and autism.[62,63] Additionally, the use of aluminium-containing antiperspirants has been linked to the development of breast cancer.[64] Despite this ongoing debate on toxicity, neither oral nor topical aluminium has been shown to cause any of these diseases.[21]

Different autoimmune syndromes have been hypothetically linked with aluminium adjuvants in vaccines and SCIT. Macrophagic myofasciitis (MMF) is a rare inflammatory myopathy characterised by myalgia, arthralgia and muscle weakness, as well as neurological dysfunction such as hypotonia and motor function delay.[65] Muscle biopsies of patients with MMF exhibit inflammatory infiltrations with aluminium deposits, suggesting that MMF may be caused by aluminium adjuvants. Similarly, autoimmune/inflammatory syndrome induced by adjuvants (ASIA), which was first described in 2011, includes various immune-mediated diseases (e.g., sarcoidosis, Sjögren's syndrome, thyroid disease and diabetes) that may be associated with aluminium adjuvants in vaccines.[66] One problem with both MMF and ASIA is that each syndrome is associated with vague symptoms that if linked to vaccines, may lead to numerous individuals qualifying for the diagnoses.[67] A Danish register-based study investigated the association between autoimmune diseases and allergen-specific SCIT compared to the use of conventional allergy treatment (for example nasal steroids) and found that the SCIT group exhibited the lower incidence of autoimmune diseases (hazard ratio, 0.86; 95% confidence interval, 0.74–0.99).[68]

In conclusion, thorough systematic reviews and toxicology reports have evaluated all published studies describing the adverse effects of aluminium and found no evidence of a causal relationship between aluminium exposure in healthy individuals and subsequent disease.[21,69–72]

1.9 Aluminium – Allergen of the Year 2022

Aluminium was recently declared contact allergen of the year by the American Contact Dermatitis Society. This was due to the ubiquity of, and therefore unavoidable exposure to, aluminium in all its forms.[73] Bruze *et al*. highlighted many gaps in our knowledge of aluminium contact allergies, including the potential elicitation of ACD following the use of aluminium-containing consumer products and the potential significance of aluminium in food for the development of dermatitis.

For this PhD project, we designed four different studies to improve our understanding of aluminium allergies and vaccination granulomas, ranging from subjective questionnaire studies on quality of life, to provocation studies that investigate elicitation of ACD, and register-based studies that investigate risk factors associated with the development of aluminium-related vaccination granulomas.

2. Thesis Objectives

Study 1 *Children with vaccination granulomas and aluminium contact allergy: Evaluation of predispositions, avoidance behaviour, and quality of life.*

- To characterise a cohort of children with vaccination granulomas and aluminium contact allergy in terms of their early life conditions, exacerbating factors, avoidance behaviour, treatments, and quality of life.

Study 2 *Does aluminium in sunscreens cause dermatitis in children with aluminium contact allergy - a repeated open application test study.*

- To determine whether contact dermatitis develops following repeated application of aluminium-containing sunscreens in children with aluminium contact allergy and vaccination granulomas.

Study 3 *Adverse reactions after oral provocation with aluminium in children with vaccination granulomas and aluminium contact allergy.*

- To investigate whether a blinded oral aluminium challenge with aluminium pancakes increased the severity of granuloma itch, dermatitis, or subjective symptoms, and whether there is a symptomatic difference between provocations with aluminium and placebo.

Study 4 *Risk factors for granulomas in children vaccinated with aluminium adsorbed vaccines: A Danish population-based cohort study.*

- To investigate vaccine-, child- and maternal-related risk factors for developing vaccination granulomas using various Danish National Health registers.

3. Materials and Methods

Studies 1 and 2 were conducted at the National Allergy Research Centre, Department of Dermatology and Allergy, Herlev and Gentofte Hospital. Study 3 was conducted in collaboration with the Department of Dermatology and Allergy, Odense University Hospital, and study 4 was carried out at Statens Serum Institut, Copenhagen, in collaboration with the Department of Epidemiology Research. *Manuscripts I-IV* provide detailed descriptions of the materials and methods used in the four studies included in this thesis. A summary is provided in this section, with additional descriptions of aspects that are only described briefly in the published/submitted manuscripts.

3.1 Patch testing

Patch testing is an *in vivo* test that reproduces the elicitation phase of ACD following skin exposure to an allergen and it is considered the gold standard method for diagnosing contact allergies. Skin on the subject's back is preferred for patch testing because the flat surface facilitates occlusion and the skin on the back is suitably reactive.[74,75]

Patches are applied on day (D) 0, the subject is exposed to the allergens for 2 days, and the patches are removed on D2. The test area is evaluated by inspection and palpation on D2, D3–4, and D7. In children with suspected aluminium contact allergy, the test area is additionally evaluated by parents on D0 and D1, to avoid unnecessary strong reactions. Reactions are scored according to globally recognised criteria developed by the European Society for Contact Dermatitis (ESCD) and can be weak positive (+) with erythema and infiltration, strong positive (++) with vesicles, or extreme positive (+++) with coalescing vesicles.[49] Reactions may also be classified as doubtful (+?) or irritant (IR). 'No reaction' is classified as a negative reaction.

All children in studies 1–3 were patch tested with 2% aluminium chloride hexahydrate (Allergeaze; Smart Practice, Greven, Germany) applied using a plastic Finn Chamber, an empty aluminium Finn Chamber (Epitest, Tuusula, Finland), and an empty plastic chamber. The test materials were taped to the upper back for 2 days using Scanpor tape (Norgesplaster; Alpharma, Vennesla, Norway).

From March 2021, children older than 8 years of age were tested with 10% aluminium chloride hexahydrate (Allergeaze, Smart Practice).

3.2 Questionnaire construction

For study 1, we constructed a questionnaire with 66 questions. Inspiration was primarily drawn from studies by Bergfors,[34] Netterlid,[7] and Salik,[35] who described skin symptoms and aggravated granuloma itch when children were exposed to various aluminium products. The questions were separated into different sections that covered various aspects including early life conditions, heritability, allergic and chronic diseases, vaccine uptake and overall quality of life for both parents and the afflicted children. Factors that may exacerbate skin symptoms, including various foods and skin products, and other external factors, such as heat, sleep, and infections, were evaluated. Answers were multiple choice, and there was blank space for comments.

Quality of life (QoL) is a term that refers to the wellbeing of a person defined by health and happiness. In dermatology, the Dermatology Life Quality Index is often used to assess quality of life in adults. A similar dermatology life quality instrument, the Children's Dermatology Life Quality Index (CDLQI), has been adapted for children by Lewis-Jones and Finlay,[76] with ten questions focusing on the effect of skin disease on daily life activities during the previous week. Permission to use this tool was provided by Professor Finlay following email correspondence. Each question addresses the degree to which the skin disease affected daily life, with scores from 0–3, resulting in a potential maximum score of 30. The higher the score, the more quality of life was impaired. In addition to the CDLQI, we used four visual analogue scales (VASs) to evaluate impact on life quality in general and when the skin symptoms were worst on an 11-point scale, with 0 being no impact and 10 being the worst possible impact.

To validate the constructed questionnaire, five health care personnel and the parents of six newly referred children with vaccination granulomas completed the questionnaire, making sure relevant topics were covered and the questions could be understood. The questionnaire was then revised and a final version was generated. The questionnaire was sent to the parents/guardians of 245 children, and we also included a reference group of 124 children with various types of dermatitis and contact allergies to other allergens that did not include aluminium.

3.3 Repeated open application test

The repeated open application test (ROAT) is a standardised exposure test, designed in 1986 by Hannuksela and Salo, to mimic the daily use of a specific product containing an allergen of interest.[77] It is used in the clinical setting to verify the relevance of positive or doubtful patch test results to a suspected allergen, and in experimental settings to elicit contact dermatitis following exposure to low doses of the relevant allergen.[78,79]

The original design involves a 7-day study with two daily applications of a particular product. Other studies have since shown that products containing low concentrations of allergen may need longer exposure periods. In experimental settings, 2–4 weeks of exposure is recommended with daily applications.[49] Our study involved two daily applications for 14 days, or until a positive reaction occurred at the test site. Positive ROAT reactions were scored according to a standardised reading scale, which assessed the size of the area of skin that reacts, erythema and infiltration.[80]

3.4 Sunscreen

Many sunscreens contain aluminium salts. Here they function as anticaking agents, improve spreadability and coat the physical UV-filters.[81]

We bought 10 different sunscreens, all available at regular Danish pharmacies and supermarkets (Table 2). We chose five sunscreens that had aluminium listed as a constituent, and five sunscreens that did not contain aluminium. All 10 sunscreens were then sent to ALS Scandinavia (Luleå, Sweden) for inductively coupled plasma mass spectrometry (ICP-MS) analysis. This procedure can detect very low concentrations of metals in liquid samples.[82] We chose sunscreen number 1 as the aluminium sunscreen because this had the highest concentration of aluminium. We also chose a control sunscreen from the same manufacturer that did not contain aluminium.

Table 2. Overview of the sunscreens tested in our repeated open application test study

No.	Aluminium complex	Results mg/kg	Uncertainty mg/kg (±)
1	Aluminium oxide	1,620	279
2	Aluminium starch octenylsuccinate + aluminium hydroxide	1,280	237
3	Aluminium starch octenylsuccinate + aluminium hydroxide	1,140	209
4	Aluminium hydroxide	574	105
5	Aluminium hydroxide	558	102
6	No aluminium	< 4	-
7	No aluminium	< 4	-
8	No aluminium	< 4	-
9	No aluminium	< 4	-
10	No aluminium	< 4	-

3.5 Experimental design of systemic exposure to aluminium

SCD following oral intake of an allergen is rare and has mostly been investigated in individuals with nickel allergy. Here, studies have shown clinically characteristic cutaneous symptoms such as flare-ups in areas that were previously patch tested, eruptions on previously unaffected skin, and subjective symptoms such as headache and malaise.[14–16]

There is no overall consensus on how to assess oral tolerance to contact allergens,[83] and most assessments are conducted as dose–response studies.[13,15]

In individuals with a suspected food allergy, the double-blinded, placebo-controlled food challenge (DBPCFC) is considered the gold standard for diagnosis.[84] The main objective of this procedure is to reproduce symptoms suspected of being triggered by the allergen in question. The procedure involves disguising the suspected food to reduce both patient and observer bias.

Aluminium in food is mainly derived from natural sources, but extensive use of food additives means that these now contribute a significant amount of the aluminium found in food. Previous studies on the food additive sodium aluminium phosphate (SALP), which is designated E541 and commonly used to leaven products such as cheeses and cakes, found that the bioavailability of aluminium was 0.1% and aluminium excretion in the urine was a sensitive marker for absorption.[58,85]

No studies have been conducted on individuals with aluminium allergy, and including children in oral food challenge studies is challenging. With inspiration from DBPCFC studies, we designed a 3-week blinded randomized controlled oral aluminium/placebo provocation, where children consumed pancakes for the first 4 days of each week, followed by 3 days pause before starting the next provocation. The children could ingest SALP pancakes for one week and placebo pancakes for two weeks, or SALP pancakes for two weeks and placebo pancakes for one week. Order of the weeks were randomly designated.

There were no restrictions and the children's regular diets were not monitored.

3.6 Danish Health Service registers

In Denmark we have ideal opportunities for register-based research, with data from national public health registers being available via a unique personal identification number, the Central Person Register (CPR) number. The construction of a nationwide cohort was made possible by the Danish Civil Registration System[86] from which we established a cohort of all children born in Denmark between 1 January 2009 and 31 December 2018; we also identified mothers and older siblings for each child. We added data from the Danish National Health Service Register,[87] which contains vaccination data, including the date of vaccine administration and the CPR number of each recipient.

The primary outcome was claims for vaccination granulomas that were approved by the Danish Patient Compensation Association (DPCA), an independent body dealing with all compensation claims in connection with medical treatment including vaccines. The information collected included date of vaccine administration, date of granuloma appearance and a thorough description of the child's symptoms. The first claim for a vaccination granuloma in the DPCA database was in 2009; therefore, we decided that our study start date should be 1 January 2009.

3.7 Danish childhood vaccination programme

In Denmark, childhood vaccinations are administered by local general practitioners free of charge. The programme consists of eight vaccination appointments that conclude when the child is 12 years of age (Fig. 4). During the first three appointments, a pentavalent vaccine for diphtheria–tetanus–pertussis–polio–Hib (abbreviated DTP in our study) and a conjugate pneumococcal vaccine

(abbreviated PCV) are administered simultaneously. These three DTP plus PCV appointments are scheduled for children aged 3, 5 and 12 months old, and were the focus of our study.

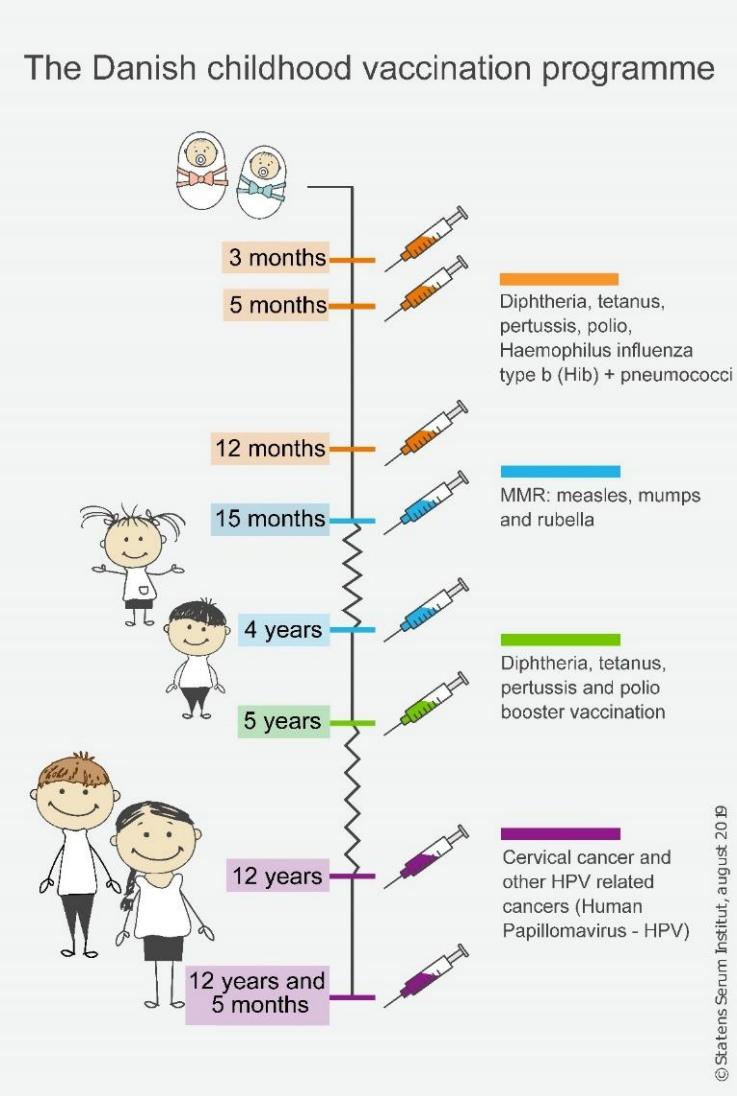


Figure 4. The Danish childhood vaccination programme

Modified after Statens Serum Institut, Det danske børnevaccinationsprogram (https://www.ssi.dk/vaccinationer/boernevaccination).

3.8 Ethical considerations

Vaccination granulomas are primarily observed in small children; hence, our potential study participants would be under 10 years of age. Consequently, we implemented the ethical considerations and precautions necessary for research involving children.[88] Consent was obtained from all parents/guardians. However, when age-appropriate information is available, the child

should also be involved in the decision-making process. In our questionnaire study, most questions could be answered by parents/guardians alone, but we encouraged parents to discuss responses to the CDLQI questionnaire with their children, because this questionnaire is designed for children.[76] In our clinical studies, parents and their children were simultaneously informed about the study procedure, which enabled parents to ensure that children understood the study before agreeing to participate. Additionally, all families had a minimum of 24 hours to consider participation, and they were told that they could withdraw at any time without providing a reason and without jeopardising any future treatment.

Potential risks and harms should never outweigh the benefits of research. Our study procedures did not involve potential risks or harms to the children, except for a possible rash and exacerbation of granuloma itch that may temporarily result in increased irritation. There were no painful procedures; some children found patch testing temporarily irritating. No children withdrew from our studies, and all children benefitted from participation in terms of our investigating the potential for reactions to aluminium in sunscreen and pancakes.

3.9 Ethical approvals

The Danish Data Protection Agency approved storage of data for studies 1, 2 and 3 (VD-2018-137, P-2020-149 and P-2020-950, respectively). Study 4 received approval from the Compliance Department at Statens Serum Institut (journal number 20/09846). Data from the National Database of Contact Allergy were used following approval from the Danish Clinical Quality Program – National Clinical Registers. Permission to contact the patients in study 1 was approved by the Danish Health Data Authority (FSEID-00003682). Studies 2 and 3 were prospectively registered at clinicaltrials.com (NCT04438135 and NCT04921163) and approved by the regional human ethics committee (H-20009217 and H-20060917). The questionnaire study (study 1) and the register study (study 4) did not require ethical approval.

4. Main Results

This section summarises the key findings of each study. The original *manuscripts I-IV* are included at the end of the thesis.

4.1 Study 1

We invited 245 children with vaccination granulomas and a positive patch-test to aluminium to participate in the study. In total, 177 children (72%) completed the questionnaire. The response rate in the reference group was 61/124 (49%).

In the vaccination granuloma group, parents of up to 139 (79%) children reported exacerbation of granuloma itch during infections, following playtime under warm or sweaty conditions, and at bedtime. Other factors that increased the severity of granuloma itch included the use of aluminium-containing sunscreens (81 children, 46%) and aluminium-containing food products (55 children, 31%).

The fear of aggravating factors led to changes in behaviour in 141 families (80%), with avoidance of aluminium-containing sunscreens being the most common change (119 children, 67%). Parents of 73 children (41%) avoided foil-wrapped and canned food or aluminium-containing foods in general (62 children, 35%).

The mean CDLQI scores were 3.10 for the granuloma group and 3.86 for the reference group; however, these scores were not significantly different ($P = 0.92$). Evaluation of quality of life using the VAS scales generally showed that the granuloma group had higher mean scores, and therefore poorer quality of life, than the reference group.

The parents of 47 children (27%) in the granuloma group had chosen to delay or even decline further vaccinations from the Danish childhood vaccination programme, compared with the parents of one child (2%) in the reference group ($P < 0.001$).

4.2 Study 2

We included 16 children aged 2–10 years in the study. All had symptomatic (itching) vaccination granulomas and a positive patch-test result for 2%/10% aluminium chloride hexahydrate. One child developed a positive skin reaction during the ROAT procedure. This reaction appeared on day 2

(after three applications) in the area of skin exposed to aluminium-containing sunscreen. Neither this nor any other child had a reaction to the sunscreen that did not contain aluminium.

The child who exhibited a positive skin reaction was one of the youngest participants. This child had a ++ reaction to 2% aluminium chloride hexahydrate and no history of atopic dermatitis (AD). The child had not been previously exposed to aluminium-containing sunscreen in the test area; therefore, the skin was considered naïve.

Although our results were not statistically significant ($P = 1$, Exact McNemar test), we did observe that one child with vaccination granuloma and aluminium contact allergy developed contact dermatitis following exposure to an aluminium-containing sunscreen.

4.3 Study 3

In total, 15 children with a mean age of 5.7 years (range, 3–9 years) participated in a 3-week provocation study with aluminium and placebo pancakes. Of the participants, parents of three children (20%) expressed a clear suspicion of previous cutaneous reactions to aluminium in food. We evaluated the extent of granuloma itch and subjective symptoms such as headache and stomach ache using VAS scores during each provocation week. We pooled the data from all aluminium and placebo provocations, and used the Wilcoxon signed-rank test for pairwise comparisons.

VAS scores for the granuloma itch were higher during aluminium provocation (mean granuloma itch, 1.6; standard deviation [SD], 1.4) than placebo provocation (mean granuloma itch, 1.4; SD, 1.2), although the difference was not significant ($P = 0.5$). Additionally, VAS scores for subjective symptoms were slightly higher during aluminium provocation: mean VAS of 0.7 (SD 0.7) versus 0.5 (SD 0.7). Here, the difference was significant ($P = 0.028$) but small in terms of mean VAS score severity.

On day 4 of the aluminium provocation week, three children developed maculopapular rashes on either their cheeks or buttocks, with no other obvious explanation. The rashes persisted for 2–4 days and gradually vanished.

There were no significant differences in sleep patterns, and no correlation between aluminium excreted in the urine and VAS scores.

4.4 Study 4

After censoring and exclusions due to emigration, death or deviance from the recommended vaccination schedule, the final cohort consisted of 553,932 children born in Denmark, of whom 1,901 had vaccination granulomas.

Poisson regression analysis was used to calculate rate ratios (RRs). Vaccination appointments were defined as a combination of DTP plus PCV vaccines, because these are administered simultaneously during infancy. We found that the risk of developing vaccination granulomas was lower in children who received the hydroxide plus phosphate adsorbed DTP vaccine than in children who received the hydroxide adsorbed DTP vaccine (RR, 0.58; $P < 0.01$), and a total dose of more than 1 mg (compared to less than 1 mg) of aluminium per vaccination appointment increased the risk of developing vaccination granulomas 1.34-fold ($P < 0.01$).

Girls were at greater risk of developing vaccination granulomas than boys (RR, 1.12; $P = 0.02$), and having a non-Danish-born mother decreased the risk 0.51-fold ($P < 0.01$) compared to having a Danish-born mother.

Undoubtedly, the greatest risk factor for developing vaccination granulomas was having a sibling with a vaccination granuloma when receiving one's first aluminium-adsorbed vaccine (RR, 46.15; $P < 0.01$), suggesting that heritability and/or social factors could be causal.

5. Methodological Considerations

In the following section, strengths and weaknesses of the four studies that are not thoroughly covered in the manuscripts are described.

5.1 Study 1

5.1.1 Study design

Quality of life and the impact of a particular disease may be assessed using a variety of methods, such as interviews, clinical examinations, and questionnaires. Given the number of children eligible to participate in our study, we chose to conduct a questionnaire study. Questionnaire studies are considered cost effective for describing large cohorts of individuals, response times are shorter than for interviews, and questionnaire data can be entered electronically and transformed into formats that are easy to analyse using statistical software. We used a web-based questionnaire, although paper-based questionnaires have sometimes yielded higher response rates in the past.[89] Given the age of our participants, we suspected that parents would have excellent computer skills and easy internet access. The questionnaire was accessible on personal computers, smartphones and tablets to increase the response rate, as studies with higher response rates are considered more representative of a patient population. In questionnaire studies, at least half of a cohort should complete the questionnaire to minimise the risk of selection bias. If individuals who currently have skin symptoms are more likely to participate, then the results of the study may be negatively skewed.[90] Our response rate was 72% from the granuloma group and 49% from the reference group.

5.1.2 Recall and selection bias

The questionnaire was constructed specifically for this study. Therefore, some questions may include bias based on our decisions and assumptions. We based the questions on both published research and clinical experience and sought to diminish the effect of our suppositions by having the questionnaire validated by parents of children with granulomas.

In retrospect, some questions were unsuitable for obtaining the necessary information. This may be particularly true for the questions regarding attempted treatments. We created a list of treatments

based on our clinical knowledge and a study by Salik *et al.*,[35] but we did not ask participants about the instructions they received regarding treatment frequencies and durations. Therefore, we do not know whether lack of effect was due to ineffective medication or lack of adherence.

Because the study was retrospective and some participants had their patch tests several years ago, recall bias is also an unavoidable risk.[91]

5.1.3 Reference group

To avoid having children with undiagnosed granulomas in our reference group, we included only children with no patch-test reaction to metallic aluminium in this group. All children in the reference group had some form of dermatitis (e.g., AD, facial dermatitis, or dermatitis on the feet). Furthermore, only 30% of these children had a positive patch-test reaction to an allergen. Hence, there was wide heterogeneity among this group, and a more homogenous group may have been of greater value. However, if the group had been more homogeneous, we may have missed the opportunity of discovering children with an undiagnosed aluminium contact allergy.

The mean age at patch testing was 3.54 years for the granuloma group but 9 years for the reference group. Expanding the size of the control group by increasing the maximum age at inclusion to 18 years would have meant that the extra participants in the control group would have been 15–18 years old, and quality-of-life comparisons between the groups would have been less relevant.

Finally, all participants were children who were patch tested at the Department of Dermatology and Allergy, Herlev–Gentofte Hospital. This subpopulation may not be representative of all Danish children with vaccination granulomas and aluminium allergies.

5.2 Study 2

This was the first study to investigate the potential elicitation of ACD in children with aluminium contact allergy in a clinical experimental setting.

5.2.1 Anatomical localisation

The forearms are generally used for ROAT studies. However, from a clinical point of view, the best anatomical area to use is probably the most relevant area for each exposure (e.g., the face for cosmetics and the underarms for deodorants). We chose to use the children's lower backs rather than their arms for the ROAT study. This was mainly because we wanted to avoid drawing attention to the test area and to keep the test area shielded from the sun. Using the arms could have exposed the test area to sun, sand, sweat, and scratching, which could have affected our results.

Patch testing is usually performed on the upper back. All our participants were small children and the area between the upper and lower back was small. Therefore, we decided that using the lower back as a test site was feasible. Interestingly, skin reactivity may gradually increase between the upper arm and the neck/facial area.[92] Had we used a more sensitive anatomical site, we may have observed more positive reactions.

5.2.2 Aluminium oxide versus hydroxide

For the test material, we chose the sunscreen with the greatest aluminium content and the salt was aluminium oxide (alumina). Ideally, we could have included another test substance, such as aluminium hydroxide, which besides being more commonly used in sunscreens is an adjuvant used in most aluminium-adsorbed vaccines. Aluminium oxide is less soluble than aluminium hydroxide.[53] Hence, allergic reactions to aluminium oxide may develop more slowly than allergic reactions to aluminium hydroxide. This may have influenced our study and could be a cause of false negative reactions, with only one child having a positive ROAT result.

5.2.3 Sample size and lack of control group

We decided not to use a control group for the study. Perhaps a non-allergic control group would have helped us to assess whether reactions were irritant rather than allergic, especially if we had used the arms as test sites, because the arms are more likely to be exposed to extrinsic factors.

The sample size of 16 children was undoubtedly the biggest disadvantage in our study. In our questionnaire study, we asked parents whether we could contact them regarding participation in further clinical studies and although most agreed, recruitment of children proved to be surprisingly difficult.

5.3 Study 3

This experimental study was the first to address potential systemic effects of aluminium ingestion in aluminium allergic children, in a 3-week blinded randomized controlled oral aluminium/placebo provocation study with pancakes.

5.3.1 Choice of provocation material

Most research on oral intolerance of contact allergens has involved individuals with nickel allergy being given nickel in lactose capsules as part of a blinded placebo-controlled study.[13,16] However, including children in oral food challenges is challenging, and we suspected that small children aged 3 to 9 years would have difficulties ingesting such capsules. We considered using an oral suspension of the anti-reflux medication Gaviscon®, which contains a high dose of aluminium and is approved for children. This would have enabled us to provide a weight-adjusted dose for each child. However, Gaviscon is not particularly palatable and we would need to create a placebo product similar in taste and texture. Furthermore, the children would be required to ingest the product for up to 3 weeks, which would probably result in a very high drop-out rate.

The food additive SALP is used in many baking products and cheeses.[56] Additionally, studies investigating the bioavailability of aluminium use SALP as the tracer. Hence, we chose to conduct our study with SALP pancakes (Fig. 5).

Figure 5. All pancakes, both with and without sodium aluminium phosphate, contained the same amount of milk, oil and egg, and were prepared on a cast iron pan.

5.3.2 Regular diet

A major difficulty involved in investigating the consequences of systemic exposure to an allergen include estimating the "normal" exposure via the diet. Because of the ubiquity of aluminium and the wide range of expected exposure to aluminium, we were unable to estimate the level of additional aluminium exposure via the regular diet of each participant. We did not control for confounding dietary factors, and children were instructed to eat as usual. This could be considered a weakness of the study; however, it does show that the children's regular diets contained insufficient aluminium doses to generate cutaneous reactions.

5.3.3 Urine samples

Because of the long inclusion period, the earliest urine samples were stored in plastic tubes at –20°C for up to 15 months. Fortunately, urinary creatinine levels appear to be stable, although prolonged freezing may result in a decrease in creatinine levels of less than 10%, which is not considered clinically significant.[93] Aluminium in the urine samples was quantified using high resolution Sector Field ICP-SFMS, which is designed to detect very low concentrations of both metals and non-metals. Aluminium levels should not be affected by storage at –20°C and high resolution Sector Field ICP-SFMS can detect aluminium levels of only 5 µg/L.

5.3.4 Garmin Vivofit junior watch

There are various methods for assessing sleep patterns in children. Generally, these methods are better at tracking sleep–wake outcomes than sleep stages.[94] To encourage even the smaller children in our study to wear a device during the night, we used the Vivofit Jr activity watch (Garmin, Olathe, KS, USA) with various Disney themes, to the delight of our participants. The participants wore their watches continually, including in school and kindergarten, becoming familiar with the watches so that these did not disturb their sleep. These wearable devices have previously been used successfully to assess the physical activity of children.[95] To our knowledge, the devices have not been used to track sleep in children, although they are equipped with this feature. Nonetheless, parental observations of the participants' sleep patterns were consistent with the measurements recorded.

5.3.5 Participants

As with the ROAT, we did not to use a control group for this study. Evaluating subjective symptoms in both an allergic and a "healthy" control group would have benefitted the study, but because our primary goal was to investigate SCD, we only included children who were allergic to aluminium and used this group as its own control. Undoubtedly, the greatest weakness of this study was the small sample size of only 15 children. However, although many parents sought reliable information regarding the need to avoid aluminium, very few were willing to include their own child in the study.

5.4 Study 4

This register-based study of more than 500,000 children born in Denmark used statistical analyses to confirm that both the type of aluminium adjuvant and the dose of aluminium per vaccine influence vaccination granuloma development.

5.4.1 Limitations of register-based data

A common method for studying the epidemiology of a disease is to examine register-based data. This enables large cohorts to be analysed and reliable statistical estimates to be obtained. Danish health care registers are generally considered high quality because they include concise and real-time data, all linked via CPR numbers.[86]

One disadvantage of using register-based data is that data analysis is limited to the information in the registers. We know from clinical settings that not all children with vaccination granulomas are registered with the correct diagnosis code, International Classification of Diseases 10th edition (ICD-10) diagnosis code DT881B. Another limitation is that not all parents seek compensation. Therefore, we have probably missed some children with vaccination granulomas. To avoid including children with granulomas in the remaining cohort, we extracted data from the Danish National Patient register and excluded children with the diagnosis code DT881B. Additionally, we retrieved data on vaccination granulomas reported to the Danish Medicines Agency, which monitors adverse reactions to medicinal products in Denmark, and excluded affected children from the cohort. However, children whose parents had sought compensation from the DPCA were not excluded.

5.4.2 Censoring and exclusions

We chose to censor or exclude approximately 55,000 children from the study because their vaccination records differed considerably from recommendations. Consequently, 633 children with vaccination granulomas were excluded, which could have skewed our results. Additionally, we excluded children who did not receive the DTP plus PCV vaccine as their first vaccine. The few children who received the aluminium-adsorbed hepatitis B vaccine at birth were excluded. Whether this additional injection of aluminium adjuvants alters the risk of vaccination granulomas following DTP plus PCV vaccination remains unknown.

5.4.3 Data from the Danish Patient Compensation Association

We know from previous studies that there are more than twice as many granulomas following DTP vaccinations compared with PCV vaccinations (0.66% from Infanrix® alone vs. 0.35% from Prevenar alone), and the frequency of granulomas almost doubled in children who received both aluminium-adsorbed vaccines (1.18%).[42] In the claims data obtained from the DPCA, all parents had registered both a DTP and a PCV vaccine as causes of granuloma formation. Consequently, although both vaccines may cause granulomas, we are unable to assess the separate risk of each vaccine causing granulomas. Therefore, we defined each vaccination appointment as a DTP plus PCV combination.

6. Discussion

6.1 Quality of life

Manuscript I describes how vaccination granulomas and aluminium contact allergy had a negative impact on life quality for both the afflicted children and their families. Studies on children with AD, psoriasis, and vitiligo have also reported negative effects on quality of life. For children, having a skin disease was as detrimental as having various other chronic diseases.[96–98]

In our questionnaire we found a higher prevalence of AD in the reference group than in the granuloma group, a disease known to negatively affect life quality.[99,100] Nevertheless, children in the granuloma group had lower quality of life overall, thus higher VAS scores (4.11 vs. 3.25; P = 0.009). Conversations with parents during our provocation studies revealed that factors which negatively influenced quality of life included the frequently prolonged period between initial symptoms and diagnosis (because many general practitioners are unaware of vaccination granulomas), the conflicting advice that is often provided, sleep disturbance due to scratching and the lack of effective treatments.

6.2 Contact dermatitis following epicutaneous application of aluminium

In *manuscript II*, we reported that one child had a positive ROAT result to an aluminium-containing sunscreen, but no reaction to the control sunscreen. Before the study, parents of 2 of the 16 children (12.5%) who were included reported a previous reaction to aluminium-containing sunscreen, with a small papular pruritic rash occurring in the hours or days after application. The reactions were not clinically assessed at the time and we cannot determine whether they were allergic or irritant, but neither of these two children had a positive ROAT reaction.

Suspected sunscreen allergy has previously been described in children with aluminium allergy. A study by Bergfors *et al*. found that parents of 3 of 19 children (15%) with vaccination granulomas and aluminium allergy self-reported the development of pruritic vesicular dermatitis after using an aluminium-containing sunscreen, although the times of onset and durations were not reported.[101] Other allergens in a sunscreen may cause allergic dermatitis. The aluminium-containing sunscreen we used in our study did contain *Aloe barbadensis* leaf extract, an *Aloe vera* plant-based extract that is used in cosmetics, drinks, lotions, dietary supplements, and food. Despite the widespread use of

Aloe vera, reports on allergic reactions are rare, and a multicentre study by Reider *et al*. that included 702 individuals did not find any positive patch test reactions to three different *Aloe vera* compounds.[102]

A small number of case reports have described aluminium contact allergy developing in individuals after the application of topical medication and antiperspirants containing aluminium, showing that cutaneous reactions in aluminium allergic individuals may happen but are few.[45,46,103–105]

It is not possible to visually distinguish between irritant and allergic contact dermatitis. Water, soap, and hand sanitisers are all common skin irritants. Other relevant environmental factors include friction, sweating and heat. A child who exhibits dermatitis following the application of sunscreen may be considered allergic when, in fact, the skin is reacting to an irritant.

Individuals with aluminium allergy are not told to avoid aluminium-containing products but avoiding aluminium-containing deodorants has been proposed in some individuals as they may contain up to 7.5 % aluminium.[53,105]

6.3 Systemic contact dermatitis from aluminium

In *manuscript I* we investigated the number of children with previous suspected adverse events following oral intake of aluminium. Up to 55 of the 175 parents (31%) indicated that aluminium-containing food such as canned food, dried fruit or even some fresh fruits led to exacerbation of granuloma itch, but this was not clinically assessed.[106]

For *manuscript III*, we designed our oral provocation study to assess any adverse events from aluminium intake, including SCD. Although not statistically significant, 9 of the 15 children in our oral provocation study had higher overall VAS scores for granuloma itch during aluminium provocation compared with placebo. For subjective symptoms, only four children had higher mean VAS scores during aluminium provocation, six children exhibited no difference in the scores, and three children had higher mean VAS scores during placebo provocation. One explanation for the presence of subjective symptoms is the nocebo effect. Oral food challenges, including DBPCFC studies, usually involve the administration of both an allergen and a placebo substance. This type of study may elicit a nocebo effect in allergic individuals, who may have negative expectations of the treatment/test material. The nocebo effect may result in substantial bias but it is difficult to validate, which illustrates the importance of a blinded study design. We tried to assess this potential bias by

having parents guess the aluminium provocation weeks. Overall, 8 of the 15 parents (53%) correctly identified aluminium provocation, but only two of the three parents who had previously suspected their children of having dermatitis or exacerbated granuloma itch caused by aluminium in food guessed correctly ($P = 0.55$).

Interestingly, one of the children in our oral provocation study developed a rash on the buttocks on day 4 of the aluminium provocation, similar to a previous rash that parents suspected could be caused by aluminium. One of the most characteristic cutaneous symptoms of SCD is the "baboon syndrome," which is characterised by confluent erythematous lesions that result in the buttocks resembling the red rumps of baboons.[14] The rash we observed in our study was considerably less intense than that observed in classic baboon syndrome, but nevertheless occurred at the same anatomical site on day 4 of the study and had no other obvious explanation. Two other children also developed rashes on their cheeks during aluminium but not placebo provocation. However, these rashes were less distinctive.

To date, SCD in individuals with aluminium allergy following oral intake of aluminium has only been clinically assessed in a single case report by Veien *et al*., involving three children with vaccination granulomas and aluminium allergy who experienced exacerbated granuloma itch after using an aluminium-containing toothpaste.[20] In two of these children, symptoms could be reproduced in a controlled exposure setting.

A double-blinded placebo-controlled oral nickel challenge by Jensen *et al*. found a definite dose-response dependency in nickel-allergic individuals, with up to 70% reacting with cutaneous reactions, flare-up of previous patch tested areas and/or flare up of previous sites of dermatitis. Up to 50% developed general symptoms (such as headache, nausea and dizziness).[15] Additionally, they found indications of hypersensitive individuals reacting to nickel doses equivalent to the estimated daily exposure through the diet. We did not find a significant association between cutaneous reactions and aluminium excretion, indicating that as with nickel-allergic individuals, aluminium-absorption may vary in aluminium-allergic children. Had we used a higher dose of oral aluminium or designed a study investigating dose-response dependency, we might have seen more children with adverse reactions. Perhaps hypersensitivity could explain why some children had higher VAS scores for subjective symptoms during placebo weeks as a response to their regular diet.

Generally, SCD to metals may be overlooked or disregarded, and whether dietary restrictions to avoid the allergen in question is necessary remains controversial.[10]

6.4 Atopic dermatitis and contact allergy

AD is a chronic, inflammatory and eczematous skin disease, with a complex combination of dysfunctional skin barriers, genetic predisposition, and dysregulation of the immune system as the causative factors.[107] AD is the most common inflammatory skin disease, with a lifetime prevalence of up to 20%.[108] The relationship between contact allergy and AD has been assessed in many studies, with various results. Children with AD may be at greater risk of becoming sensitised to contact allergens, presumably due to the dysfunctional skin barrier and repeated exposure to various topical agents from an early age.[109,110] However, a dysfunctional skin barrier may increase the likelihood of false-positive patch test results, especially for tests involving metals. A register-based study investigating the association between ACD and severe AD found a significant inverse association between the two skin diseases.[111,112]

In our questionnaire study (*manuscript I*), only 12% of children with granulomas had a history of AD, compared with 62% of children in the reference group ($P < 0.001$). This difference is biased because our reference group consisted of children being referred to patch testing due to various types of dermatitis, including AD. In our provocation studies (*manuscripts II and III*), neither the child with a positive ROAT reaction nor two of the three children with rashes during the aluminium-pancake provocation study had a history of AD. Larger studies on the potential association between vaccination granulomas/aluminium contact allergy and AD are warranted and underway.

6.5 Heritability and sociodemographic risk factors

In *manuscript IV*, we report that children of non-Danish-born mothers have a lower risk of developing vaccination granulomas (RR, 0.51) than children of Danish-born mothers ($P < 0.01$). We interpreted these results as potentially due to a combination of heritability and difficulty overcoming the language barrier when seeking information and compensation. However, our results contrast with those of a study by the research group in Gothenburg, who found no apparent differences among ethnic groups, although no statistical calculations were performed.[32] Our register-based study implemented binary categories in which we divided mothers as being born either in Denmark or the rest of the world. This implies that the non-Danish-born mothers spoke poorer Danish than the Danish-born mothers, which is a preconception that we cannot prove.

Girls were at greater risk of developing vaccination granulomas than boys (RR, 1.12; 95% confidence interval, 1.02–1.22; $P = 0.02$). Skin changes such as hypertrichosis may be more likely to go unnoticed in small boys than in small girls. Alternatively, girls may be genetically predisposed to being more susceptible to developing aluminium contact allergy and vaccination granulomas. A recent patch-test study on aluminium contact allergy in which individuals were patch tested with two different aluminium salts did not find any statistically significant difference in prevalence between the sexes,[113] but other experimental studies have identified potential differences in susceptibility to contact allergy between males and females.[114]

Undoubtedly, the greatest risk factor for developing vaccination granulomas was having a sibling with a vaccination granuloma when receiving one's first aluminium-adsorbed vaccine (RR, 46.15; 95% confidence interval, 33.67–63.26; $P < 0.01$). There is no doubt that parental (and general practitioner) knowledge of the condition has an impact on achieving a correct diagnosis. However, as with maternal ethnicity and differences between the sexes, genetic predisposition cannot be ruled out.

6.6 Rationale for vaccination schedules

The rationale for the childhood vaccination administration schedule takes both the maturity of the infant's immune system and the reduction in maternal antibody levels into account. An interval of at least 3 weeks between vaccinations is important to avoid interfering with the primary immune response, and to ensure that the immune response persists.[115]

The Danish Childhood vaccination programme differs slightly from schedules in the rest of Europe and throughout the world. The differences include the recommended vaccines, the number of doses and the child's age at administration.[116,117] In most European countries, children are vaccinated against hepatitis B at birth and receive their first DTP when they are 2 months old. In Denmark, as well as in Sweden and Norway, the first DTP vaccine is administered when the child is 3 months old. In our register-based study we hypothesised that deviating from the recommended schedule could influence the development of vaccination granulomas, especially if a child received their first vaccine before they were 2.5 months old (when the immune system was immature) or if the recommended minimum interval between vaccinations was ignored.[115] We did not find any statistically significant association between a child's age when they received their first vaccine and the development of vaccination granulomas, although our data suggest that children vaccinated both

before they were 2.5 months and after they were 3.5 months old were less likely to develop vaccination granulomas than those who received their first DTP plus PCV vaccination at 2.5 to 3.5 months.

Generally, premature babies should follow the same vaccination programme as full-term babies. We found that gestational age (relative to a full-term gestational age of 37 to 41 weeks) was associated with various statistically significant risk factor levels, with prematurity (being born before full 36 weeks) decreasing the risk (RR, 0.71) and post-maturity increasing the risk (RR, 1.14) of developing vaccination granulomas. Although pre-term babies have an increased risk of severe systemic side effects such as apnoea following immunisation, it seems they have fewer local injection site side effects.[118,119] Post-term babies generally have a higher risk of neonatal morbidities.[120]

Unfortunately, no countries outside of Scandinavia have published systematic statistical analyses of the prevalence of vaccination granulomas. However, from our data, we would not expect the risk of vaccination granulomas to be increased in countries where the immunisation schedule starts when children are 2 months old.

6.7 Adjuvants in vaccines

In *manuscript IV*, we report that the adjuvant aluminium hydroxide was more potent in terms of granuloma formation than aluminium phosphate, and that the dose of aluminium per DTP plus PCV vaccine appointment was also important: a higher dose resulted in a greater risk of developing vaccination granulomas. A link between the type/dose of aluminium and children developing vaccination granulomas was first proposed by Bergfors *et al*. in a vaccine trial study from 2003.[32] The study suggested that alternatives to aluminium adjuvants should be considered.

Fortunately, during the last 10 years, changes in vaccine manufacture in Denmark have resulted in lower doses of aluminium in most child vaccines (Fig. 6). In particular, the DTP vaccine manufacturer has changed, and using a vaccine with 0.3 mg instead of 1.0 mg of aluminium hydroxide since 2019 may decrease the future incidence of vaccination granulomas.

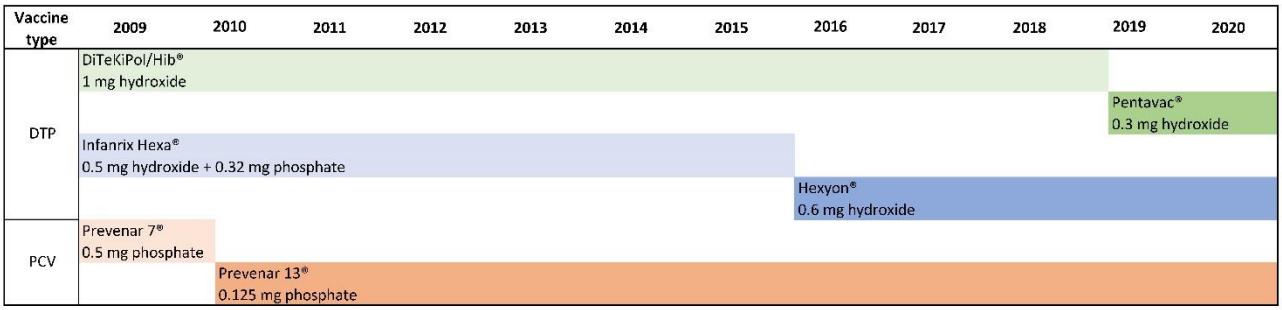


Figure 6. Overview of the DTP and PCV vaccines available for administration during the study period of our register-based study. From. *Risk factors for granulomas in children following immunisation with aluminium adsorbed vaccines: A Danish population-based cohort study.* Hoffmann SS, Thiesson EM, Johansen JD, Hviid A. Contact Dermatitis. 2022 Jul 2.[121]

6.8 Treatment of vaccination granuloma itch

Aggravating itch is undoubtedly the most troublesome facet of vaccination granulomas. Itch is usually clinically challenging, and the spectrum of pruritic conditions is wide ranging and includes AD, urticaria, and psoriasis as well as neuropathic and autoimmune conditions.[122] The underlying pathogenesis of granuloma itch remains poorly understood but it appears to be originating in the skin and not centrally as is the case for neuropathic and neurogenic pruritus.[123] However, because the itch-sensitive C-fibres are generally activated in the stratum granulosum of the epidermis, explaining how a subcutaneous granuloma causes itch is difficult. As described in section 1.5, granulomas consist of inflammatory cells. Therefore, perhaps some of these mediators reach the epidermis and cause the itching sensations.

The most frequently used treatment for granulomas involves applying topical corticosteroids, possibly under occlusion. The first Danish retrospective study on vaccination granulomas by Salik *et al*. described various methods that were used to treat 47 children, with 36 of the children (77%) having been treated with topical corticosteroids.[35] The effects were evaluated by the parents of 29 of these children, with only 2 cases (7%) exhibiting complete relief from itch. In our questionnaire study (*manuscript I*), topical corticosteroids had a satisfactory effect in only 18 of 82 children (22%).

Another potential method of treating granuloma itch is described in our recent case report.[124] An adult woman with multiple granulomas on both upper and lower arms following many years of SCIT was treated with an 8% capsaicin topical patch. Such treatment has previously been used to address neuropathic pain and itch.[125,126] Capsaicin is a transient receptor potential vanilloid-1

(TRPV-1) agonist. When the patch is applied, local cutaneous nociceptors are activated leading to pain and erythema. Following exposure, desensitisation occurs, which relieves the itch. Side effects include application site reactions such as pain, burning sensation and redness, which may be quite severe. The woman in our case reported a significant reduction in itch, from VAS 8 when the treatment was initiated to VAS 1 after 3 years of quarterly treatments. Capsaicin is available as patches (8% w/w) or cream (0.025% w/w), but none of these medications have been systematically tested for use in children under 18 years of age.

One of the children in our ROAT study (not the child with the positive ROAT reaction) experienced severe daily itch that affected the entire anterior surface of the thigh. Topical corticosteroids had little effect and after months of daily crying, off-label treatment with 0.025% capsaicin cream was initiated. This treatment was initially effective, but after a few weeks of daily application the child developed a rebound effect that was very painful and led to the treatment being discontinued.

Surgical excision has been described in case reports, but usually only when the diagnosis is unknown and there is a suspicion of soft tissue malignancy.[38,127] After the nodule is removed, the itch ceases. However, with young children, excision is performed under general anaesthesia, there is a risk of infection during and after the procedure, and there will be a permanent scar. In contrast, when vaccination granulomas resolve, the skin above the granuloma will return to normal and the hyperpigmentation and hypertrichosis will gradually disappear.

6.9 Prevalence of vaccination granulomas and aluminium contact allergy

In *manuscript IV,* we found that 415 of the 1901 vaccination granulomas (22%) developed after the first DTP plus PCV vaccination administered at 3 months, 594 (31%) after the second, and 880 (46%) after the third. The remaining 12 granulomas occurred after the DT-booster at 5 years of age and was not further assessed in our study. Bergfors *et. al.* reported 28/645 (4%) granulomas after the 3-month vaccines,[32] in the study by Salik *et al.* the number was 3/38 children (8%),[35] and in our questionnaire study (*manuscript I*), 33% (58/177) parents reported that the granuloma occurred after the 3-month vaccine.

To investigate the prevalence of aluminium contact allergy from epicutaneous exposure, we designed a systematic review and meta-analysis in addition to studies 1–4. The pooled prevalence

of aluminium contact allergy was 0.36% for adults and 5.61% for children with no history of vaccination granulomas following childhood vaccines or SCIT.[128]

The prevalence of aluminium contact allergy was recently assessed by the Department of Occupational and Environmental Dermatology in Malmö (Sweden) by adding both 10% aluminium chloride hexahydrate and 12% aluminium lactate to the European Baseline Series from 2012 to 2017.[113] Among all patch tested individuals, 0.9% of adults and 5.1% of children were diagnosed with aluminium contact allergy, similar to the findings of our systematic review and meta-analysis.[128] Aluminium is not included in the European Baseline Series, although an empty chamber of metallic aluminium is often used as a control. A study by a research group in Gothenburg group found that approximately 30% of all children with aluminium contact allergy did not react to a metallic aluminium chamber.[129] Thus, testing for aluminium contact allergy using an empty chamber alone is insufficient, and some cases may go unnoticed.[130]

6.10 Vaccination granulomas and aluminium allergy diminish over time

In contrast to other contact allergies, aluminium contact allergy that is associated with vaccination granulomas appears to diminish or disappear over time. In our questionnaire study, the granulomas had disappeared in 49 children (28%) at the time of the study. Of these children, the granulomas persisted for less than 2 years in 11 children (23%), 3–4 years in 28 children (60%), and more than 5 years in 8 children (17%). Parents of two children did not recall the duration of the granuloma.

A 5-year follow-up study on 241 children from the Gothenburg studies showed that the granulomas were no longer symptomatic, and a positive patch test to aluminium could not be reproduced in 186 children (77%).[33] A very recent study found that granulomas might be much more persistent than previously believed. Of the 745 children with vaccination granulomas in the original Gothenburg studies,[32] the median duration of granuloma itch was 6.6 years in the group that had recovered (*n* = 637) and 16.4 years in the group who still had a vaccination granuloma, ranging up to 25 years.[131] In general, skin manifestations normalised after the granulomas had vanished.

It remains unclear why aluminium allergy appears to diminish over time. Bruze *et al*. suggested that false-negative patch-test results could be obtained when re-testing previously allergic individuals with 2% rather than 10% aluminium chloride hexahydrate.[132] Variation in reactivity over time has also been observed in an adult population that exhibited allergy to aluminium.[133] The failure to

include aluminium chloride hexahydrate in the European Baseline Series may have resulted in cases being undetected.

Alternatively, perhaps increased tolerance to aluminium develops due to the presence of aluminium deposits in the vaccination granulomas: when the granulomas disappear, the individual has a higher aluminium tolerance threshold and does not respond to low levels of epicutaneous aluminium contact. This hypothesis has not been confirmed.

6.11 Consequences of vaccination granulomas

In *manuscript I*, we reported that parents of 27% of participating children chose to delay or even decline further vaccinations of their child because of vaccination granulomas. The main reasons included concerns about eliciting further side effects, exacerbating granuloma itch or provoking a new vaccination granuloma. This same tendency has been described in other studies, with up to 38% of parents choosing not to continue the recommended childhood vaccination programme.[34] Choosing not to vaccinate puts children at risk of contracting otherwise preventable serious disease, and parents of children with vaccination granulomas and aluminium contact allergy are not advised to decline further aluminium-adsorbed vaccines.[131]

7. Conclusions and Future Research Perspectives

Aluminium was named allergen of the year 2022, with Bruze and colleagues highlighting many gaps in our knowledge of aluminium contact allergy. In this thesis, many aspects previously not fully investigated has been covered. In the questionnaire study, we found that vaccination granulomas and aluminium contact allergy have a negative impact on the overall life quality of both afflicted children and their families. The available treatments were not effective in most children, and the overall lack of knowledge on the possible effect of aluminium in foods and skin-products, contributed to the negative impact on life quality. Additionally, up to 27% of parents had chosen to delay or even decline further vaccinations from the Danish childhood vaccination programme.

The ROAT study and oral provocation study was designed to investigate the potential adverse effects of aluminium exposure in a controlled setting. Findings from both clinical studies suggest that children with vaccination granulomas and aluminium contact allergy may develop dermatitis and/or exacerbated granuloma itch when exposed to aluminium either dermally or orally. Although there are no general recommendations of avoiding aluminium, it is of importance that both parents and clinicians are aware of the possibility of symptoms occurring.

Studies including more participants are warranted. The ROAT study should be repeated with a sunscreen that contains aluminium hydroxide, a more potent aluminium salt present in both vaccines and sunscreens, with the possibility of more children reacting. Furthermore, our oral challenge study could be repeated with longer provocation periods or higher doses of aluminium (e.g., with participants ingesting aluminium-containing antacids).

In the register-based study, we showed that reducing the dose of aluminium or changing the adjuvant from aluminium hydroxide to aluminium phosphate could decrease the risk of vaccination granulomas developing in children. Additionally, as the risk of vaccination granulomas proved higher regarding both maternal ethnicity and sibling accumulation, further studies on the potential influence of genetic predisposition are warranted and underway.

With granuloma itch being the most bothersome symptom and treatment opportunities are sparse, the pathogenesis underlying the development of granuloma itch should also be investigated. Skin

punch biopsies of granulomas could be examined for inflammatory cells and mediators in the epidermis. A better understanding of the underlying pathogenesis would likely lead to more effective treatments of granuloma itch.

The results from this thesis provide new insight into the effects of cutaneous and systemic aluminium exposure and the importance of both the type of adjuvant and the dose of aluminium present in a vaccine. Our findings have implications for future vaccine development, particularly with respect to the trade-off between optimal immunogenicity and fewer adverse events.

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9. Manuscripts

Manuscript I:

Hoffmann SS, Thyssen JP, Elberling J, Hansen KS, Johansen JD.

Children with vaccination granulomas and aluminum contact allergy: Evaluation of predispositions, avoidance behavior, and quality of life.

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ORIGINAL ARTICLE

CONTACT DERMATITIS WILEY

Children with vaccination granulomas and aluminum contact allergy: Evaluation of predispositions, avoidance behavior, and quality of life

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Abstract

Background: Aluminum contact allergy is mostly seen in children with vaccination granulomas, following immunization with aluminum-adsorbed childhood vaccines.

Objectives: To characterize a cohort of children with vaccination granulomas and aluminum allergy concerning early life conditions, exacerbating factors, avoidance behavior, treatments, and potential impact on quality of life.

Methods: A questionnaire study was conducted among 177 children aged 0 to 15 years with vaccination granulomas and aluminum allergy, and a reference group of 61 children aged 3 to 14 years with various types of dermatitis undergoing patch testing.

Results: All children in the granuloma group were reportedly affected by itch. Infection exacerbated the itch in 59%. Other worsening factors were eating tin-foiled/canned food (31%) and use of aluminum-containing sunscreen (46%). Many parents took precautions to avoid aluminum exposure. Children with granulomas were more likely to be nonadherent to the National Vaccination Program than the reference group (27% vs 2%, $P < .001$). Parents in the granuloma group reported a decreased life quality for both parents and children compared with the reference group.

Conclusions: Itching vaccination granulomas and aluminum allergy have a considerable negative impact on affected children and their families, causing avoidance behavior, reduced adherence to vaccination programs, and a negative effect on the overall life quality.

KEYWORDS

Key-wordsallergic contact dermatitis, aluminum, children, contact allergy, granuloma, patch test, quality of life, questionnaire, vaccine

1 | INTRODUCTION

Aluminum is a common adjuvant in childhood vaccines.[1] Persistent itching subcutaneous nodules after immunization, also known as vaccination granulomas, may occur,[2-4] but until the 1990s, they were considered to be a rare adverse event. Aluminum contact allergy as a cause of granulomas was established in the 1980s.[5-8] The incidence of granulomas in children receiving aluminum-adsorbed childhood vaccines was 0.8% to 0.98%[9] in Swedish studies including up to 76 000 vaccines.[10] A positive test reaction to aluminum is seen in

77% to 95% of children with granulomas.[9,11] The granulomas appear from 2 weeks to 13 months after the injections,[12] and are usually present for an average time of 4.6 years.[10,11] Because of the time gap between vaccinations and the occurrence of the granulomas, they have occasionally been misinterpreted as tumors, leading to unnecessary examinations and fear of malignancy.[13,14] Both granulomas and aluminum allergy are believed to diminish or vanish with time.[6,10,15] Although mostly reported in smaller children, contact allergy to aluminum can also be a side effect to allergen-specific immunotherapy (ASIT) in older children and adults.[3,16-18]

Aluminum is considered a weak allergen and has rarely been reported to cause contact allergy when using skin products containing it or from working in the metal industry.[19,20] Despite this, there have been parental reports on children with aggravating itch and skin rash when using aluminum-containing sunscreen[14,21] or when eating various foods rich in aluminum.[14]

In recent years there has been an increased focus in Denmark on vaccination granulomas. The number of cases reported to the authorities has risen from 120 in 2014 to 417 in 2018,[22] and parents seek medical attention, information, and compensation from governmental insurances. Therefore, more information about potential consequences is needed. In this study, we characterized a cohort of children with vaccination granulomas and confirmed aluminum contact allergy concerning early life conditions, exacerbating factors, treatments, avoidance of future vaccines, and potential impact on quality of life.

2 | METHODS

From January 1, 2010 to September 1, 2018, 283 children 15 years or younger were patch tested at the Department of Dermatology and Allergy, Herlev and Gentofte Hospital, Denmark, due to vaccination granuloma and suspected aluminum contact allergy. Of these, 266 children had a positive patch test reaction to aluminum (94%). Of these children, 245 could be reached and were invited to participate in the questionnaire study. The reference group consisted of 124 children aged 15 years or younger with various types of dermatitis and suspected contact allergy. In total, 30 (24%) of these children had a positive patch test reaction to a contact allergen, mainly nickel or colophonium. Permission to collect personal data was approved by the Danish Data Protection Agency (VD-2018-137, I-Suite 6380), and data from the National Database for Contact Allergy were given by the Regional Clinical Quality Program – National Clinical Registries.

2.1 | Patch test

Children in the granuloma group were patch tested with metallic aluminum (empty Finn Chamber; Epitest, Tuusula, Finland) on Scanpor tape (Norgesplaster; Alpharma, Vennesla, Norway) and aluminum chloride hexahydrate 2% petrolatum (allergEAZE; SmartPractice, Greven, Germany). A plastic chamber from the same producer was used as control. Children in the reference group were tested with a pediatric test series of 33 allergens from the European Baseline series (Chemotechnique Diagnostics, Vellinge, Sweden or allergEAZE, SmartPractice). No children in the reference group reacted to the aluminum disks used for patch testing. The allergens were applied in Finn Chambers, taped to the upper back for 2 days, and the test site was evaluated on day (D) 2 (the day of removal) and D3 and D4. Only children with a negative or doubtful reaction on D2 to D4 was seen again on D7. Positive reactions were classified as +, ++ or +++, based on scoring according to the ESCD recommendations.[23]

2.2 | Questionnaires

We constructed a questionnaire with 66 questions divided into sections. They were sent to the parents by electronic post, asking them to include their child when filling out the questionnaire if possible. Answers were multiple choice, as well as a blank space for comments. The questions covered early life conditions, hereditary factors, allergic and chronic diseases, vaccines, and life quality. Symptoms and characteristics regarding the granuloma and aluminum contact allergy; worsening factors, including various foods and skin products; and other external factors, such as heat, sleep, and infections, were evaluated. We asked about treatment attempts and the effect of these, and if parents deliberately avoided contact to aluminum.

The reference group received a shorter version of the questionnaire, excluding the specific questions related to the vaccination granuloma and aluminum contact allergy. We used the Children's Dermatology Life Quality Index, CDLQI,[24] a questionnaire with 10 questions focusing on various aspects of the skin, covering scratching, social behavior, playing, sleeping, and treatment during the last week. Results are a score between 0 and 30, with the higher the score, the more quality of life is impaired (Table SS1). We anticipated that some children no longer had symptoms due to a long follow-up time and added four visual analog scale (VASs) covering life quality in general and when the skin symptoms were worst.

Parents of six newly referred children with vaccination granulomas validated the questionnaire, making sure relevant topics were covered and the questions were understandable. The questionnaires were implemented in Research Electronic Data Capture, REDCap,[25] and were in Danish.

2.3 | Statistical analysis

Comparisons between groups were mainly made using the chi-square test, except when sample size was $n \leq 5$ in which case the Fischer exact test was used. Odds ratios with 95% confidence intervals were additionally supplied. Data were tested for normal distribution before analysis. The CDLQI and VAS score both had a nonparametrical distribution, and the Mann-Whitney *U* test was thus used to compare the mean scores and determine whether there were any significant differences in the mean score. The CDLQI-score was calculated according to published instructions.[24] A *P*-value <. 05 was considered

TABLE 1 Early life conditions and dispositions

Variable	Granuloma % (n/N of total)	References % (n/N of total)	Odds ratio (95% confidence interval)	*P*-value
Baseline characteristics				
Sex (male)	47 (83/177)	48 (29/61)	0.97 (0.54-1.75)	.93
Age at test, y (SD)	3.54 (1.79)	9 (3.22)	–	**<.001**
Follow-up, y (SD)	3.18 (1.39)	2.51 (0.99)	–	**.001**
Atopic dermatitis (AD) based on MOAHLFA	12 (21/177)	62 (38/61)	0.08 (0.04-0.16)	**<.001**
Early life conditions and dispositions				
Chronic disease	6 (10/177)	32 (18/61)	0.13 (0.06-0.31)	**<.001**
Diabetes	–	17 (10/61)	–	–
Asthma	12 (21/177)	22 (13/61)	0.46 (0.21-0.99)	.053
Mother AD	10 (18/177)	18 (11/61)	0.52 (0.29-1.16)	.12
Father AD	3 (5/177)	12 (7/61)	0.22 (0.07-0.74)	**.014**
Siblings AD	12 (22/177)	15 (9/61)	0.82 (0.36-1.9)	.66
Gestational age			-	.65
Extremely premature	1 (2/175)	–		
Very premature	1 (2/175)	–		
Moderately premature	12 (20/175)	18 (10/56)		
Term	86 (150/175)	82 (46/56)		
Birthweight in gram (SD)	3513 (535)	3533 (648)	–	.60
Nutrition during the first 6 mo of life:			–	.75
Only breastfeeding	45 (79/174)	48 (27/56)		
Primarily breastfeeding, formula supplement	23 (40/174)	27 (15/56)		
Equally breastfeeding and formula	16 (28/174)	11 (6/56)		
Only formula	16 (27/174)	14 (8/56)		
Any vaccines missing	27 (47/177)	<1 (1/61)	21.33 (2.87-158.32)	**<.001**
Reason for missing vaccines				
Fear of side effects including granulomas	83 (39/47)	100 (1/1)	16.96 (2.28-126.29)	**<.001**
Infections	2 (1/47)	–		
Forgetfulness	13 (6/47)	–		
Refuse further vaccinations	15 (26/177)	–		

Note: chi-square-test, or Fischer exact test applied if n ≤ 5.
Notes: Independent *t* test used for comparing means, with Mann-Whitney *U* test for skewed data.
Notes: Significant *P*-values (<.05) shown in bold.
Abbreviation: AD, atopic dermatitis; MOAHLFA, Male, Occupational dermatitis, Atopic dermatitis, Hand involement, Leg involvement, Facial involvement, Age<40 years; SD, standard deviation.

statistically significant. Data were analyzed with IBM SPSS Statistics (SPSS, Chicago, Illinois) for Windows (release version 25.0).

3 | RESULTS

The overall response in the granuloma group was 72% (177/245) and 49% (61/124) in the reference group ($P < .001$). Baseline demographic characteristics showed no significant differences when comparing responders with nonresponders in each group, regarding sex, follow-up time, presence of atopic dermatitis (AD), and number of patch test-positive children. In the reference group, nonresponders had a slightly older age than responders (10.5 years vs 9 years, $P = .009$; Table S2).

The reference group had a significantly higher mean age and a higher prevalence of AD and chronic diseases, predominantly type 1 diabetes and asthma compared with the granuloma group ($P < .001$; Table 1). Most children in both groups were born mature with a birth weight appropriate for gestational age. Breastfeeding was the primary source of nutrition during the first 6 months of life in both groups.

3.1 | Avoidance behavior

A highly significant difference regarding future vaccines was found, with 47 (27%) children in the granuloma group already missing vaccines they should have received according to the Danish Childhood

Vaccination Program at the given time, compared with only one in the reference group ($P < .001$). The main reason was fear of side effects including another granuloma (Table 1).

Of the 47 children currently missing vaccines, 26 parents (15% of the total granuloma group) refused to further vaccinate their child. As seen in Figure 1, avoidance behaviors in the granuloma group overlap, and parents avoiding either aluminum-containing sunscreen or foods were also more likely to avoid vaccines, with 27 (15%) avoiding all three.

3.2 | Granuloma: Onset, worsening factors, avoidable behavior, and treatment

The characteristics of children in the granuloma group are summarized in Tables 1 and 2. In total, 61 (35%) children only had one granuloma, 77 (45%) two, and 35 (20%) three or more granulomas. Most children, 137/177 (77%), had a ++ patch test reaction to aluminum. The reactions mainly occurred on D2. A total of 100 (57%) went to their general practitioner at least once, 22 (13%) were seen by a pediatrician, and 61 (35%) by a practicing dermatologist before being referred to Herlev and Gentofte Hospital. Prior to referral, 10 (6%) children had an ultrasound examination, two (1%) had blood tests, and in one child (0.5%) a biopsy was performed.

Establishing the diagnosis of vaccination granuloma took less than 3 months in 99 (56%) children. Forty-one (23%) received the diagnosis 4 to 12 months after the granuloma was noted. In 16 (9%) children it took 1 to 4 years, and finally one child (0.6%) waited between 5 and 10 years for the diagnosis.

In most children, the vaccine associated with the discovery of the (first) granuloma was either the 3rd, 5th, or 12th month vaccination (i.e. the first, second, or third dose of diphtheria, tetanus, pertussis, polio, *Hemophilus influenzae* type b [DiTeKiPol/Hib], and invasive pneumococcal disease), respectively, in 58 (33%), 29 (16%), and

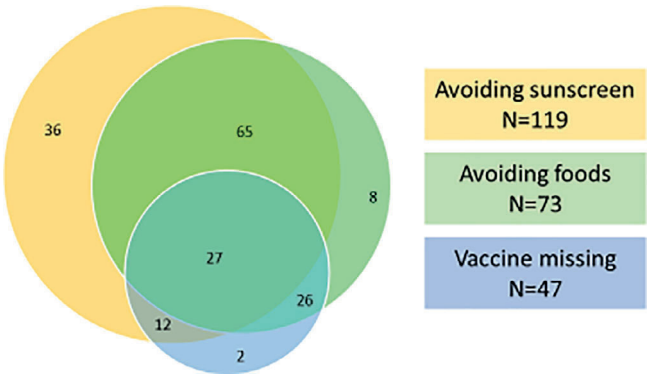

FIGURE 1 Venn-diagram illustrating the avoidable behavior of the granuloma group. Venn diagram showing the number of families stating avoidable behavior, with 65 (37%) avoiding both sunscreen and foods, 12 (7%) avoiding both sunscreen and vaccines, and 26 (15%) avoiding foods and vaccines. A total of 27 (15%) parents show avoidable behavior of all these three mentioned

TABLE 2 Characteristics related to the granuloma

Variable	Number of children, % (n/N of total)
Granuloma still present	72 (128/177)
Itch	100 (177/177)
Patch test reaction	
+	20 (36/177)
++	77 (137/177)
+++	3 (4/177)
Number of granulomas	
1	35 (61/173)
≥ 2	65 (112/173)
Elapsed time from debut to diagnosis	
1-6 months	71 (125/177)
7-12 months	8 (15/177)
13 months to 2 years	6 (11/177)
3-4 years	3 (5/177)
5-10 years	0.5 (1/177)
Do not remember	11 (20/177)
Examinations and procedures before diagnosis	
Ultrasound	6 (10/176)
Blood test	1 (2/176)
Biopsy	0.5 (1/176)
Vaccine in the Danish Vaccination Program associated with the discovery of the (first) granuloma	
3 months	33 (58/177)
5 months	16 (29/177)
12 months	26 (46/177)
15 months	10 (17/177)
4 years	0.5 (1/177)
5 years	1 (2/177)
Do not remember	14 (24/177)
Exacerbating foods	
Canned food	31 (55/175)
Dried fruit (raisins, figs)	28 (49/175)
Fresh fruit/vegetables	15 (27/175)
Exacerbating skin products	
Sunscreen with aluminum	46 (81/177)
Lotion	14 (25/177)
Aluminum-free sunscreen	9 (16/177)
Other exacerbating factors	
Infections	59 (104/175)
Heat/sweat	45 (80/175)
Bedtime	34 (60/175)
Avoiding behavior	
Use aluminum-free sunscreen	67 (119/177)
Avoid tinfoil/canned food	41 (73/177)
Avoid aluminum -containing food	35 (62/177)

46 (26%) children. Seventeen (10%) stated that the 15th month vaccine (measles, mumps, and rubella vaccine not containing aluminum) was associated with development of the granuloma. Only two children (1%) developed the granuloma after the 5-year DiTeKiPol booster vaccine.

Parents reported worsening factors in 139 (79%) cases, mostly infections (n = 102, 58%) and heat/sweats during playtime (n = 80, 45%) and during bedtime (n = 60, 34%). Skin products were reported to exacerbate itch, especially aluminum-containing sunscreen (n = 80, 45%) and regular moisturizers (n = 25, 14%). Various food items were also reported to exacerbate itch, mostly tin-foiled or canned food (n = 55, 31%) and dried fruit, especially raisins and figs (n = 49, 28%).

Worsening factors led to changes in behavior in 141 (80%) families. The most common was avoiding the use of aluminum-containing sunscreens (n = 119, 67%). Sixty-six (37%) even stated that they chose to avoid the use of aluminum-containing sunscreen, although reportedly never having had a reaction to this type of product. Avoiding tin-foiled and canned food (n = 73, 40%) and aluminum-containing foods in general (n = 62, 35%) was also common.

Treatment of symptoms had been tried by 108/177 (61%) in various ways, shown in Figure 2. The main treatment offered by dermatologists in Denmark is topical corticosteroids, tried by 82 with a good effect in only 18 (22%). Injected corticosteroids and surgical removal were not reported by any responders.

In 49 (28%) cases, the granuloma had disappeared at the time of this questionnaire study. In these children, the duration of the granulomas was less than 2 years in 11 (23%), 3 to 4 years in 28 (60%), and for more than 5 years in eight (17%). The tendency of avoidance behavior and reported worsening factors did not differ statistically between the two groups of children with present vs vanished granulomas.

3.3 | Quality of life

The mean CDLQI score (Table 3) was 3.10 for the granuloma group but higher for the reference group with a score of 3.86, though no significant difference was found (P = .92). Only one of the subquestions in the CDLQI had a significantly different P-value of .001. This was the question "Over the last week, how much of a problem has the treatment for your skin been?," with the reference group having a higher score (mean 0.52 vs 0.26 for the granuloma group). In all four VASs (Table 3), the granuloma group had a higher score, indicating a higher negative effect on life quality, with all but "affecting the child in general" being statistically significant compared with the reference group.

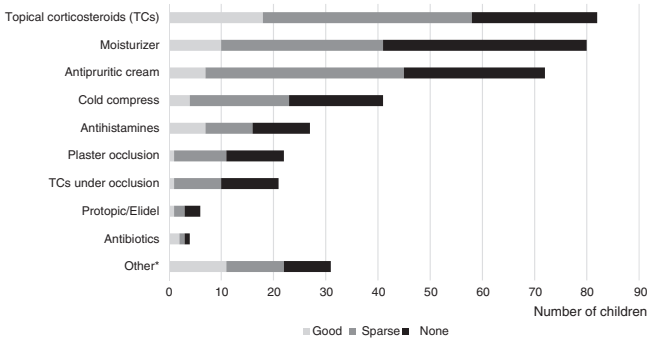

FIGURE 2 Attempted treatments. Attempted treatments of the granulomas and self-evaluated effect hereof. Other*: massage, drinking FIJI water or SILICIUM water, reflexology, sleeping with tight pajamas. Surgical removal of the granuloma was also an option in the questionnaire, but not chosen by any of the participants

4 | DISCUSSION

Over a 9-year period (2010-2018) 283 children were referred because of vaccination granulomas. A positive patch test reaction to aluminum was found in 266 (94%). In a similar study from another Danish department of dermatology, patch test data were collected from approximately 70 dermatologists in private practice and other dermatology departments in Denmark. Over a 10-year period (2003-2013) 47 children with vaccination granulomas were registered,[14] of whom 92% reacted to aluminum. Although there is an overlap in the two periods of reporting, there is no overlap in patients and the

TABLE 3 Self-reported evaluation of quality of life, measured using Children's Dermatology Life Quality Index (CDLQI) and a visual analog scale (VAS) from 0 to 10, with 0 being no impact on life quality

	Granuloma N = 172	References N = 58	*P*-value
CDLQI total			
Mean (SD)	3.10 (3.21)	3.86 (5.20)	.92
Range	0-17	0-27	
CDLQI 10			
Over the last week, how much of a problem has the treatment for your skin been?			
Mean (SD)	0.26 (0.52)	0.52 (0.73)	**.009**
Range	0-2	0-3	
VAS: Affecting the child in general			
Mean (SD)	4.11 (2.97)	3.25 (2.23)	.061
Range	0-10	0-9	
VAS: Affecting the family in general			
Mean (SD)	3.98 (2.84)	3.0 (2.60)	**.017**
Range	0-10	0-9	
VAS: Affecting the child when symptoms were worst			
Mean (SD)	6.65 (2.82)	5.25 (3.34)	**.007**
Range	0-10	0-10	
VAS: Affecting the family when symptoms were worst			
Mean (SD)	6.67 (2.69)	5.55 (3.29)	**.046**
Range	0-10	0-10	

Notes: Mann-Whitney *U* test, nonparametric distribution.
Notes: Significant *P*-values (<.05) shown in bold. SD, standard deviation.

considerable larger group of children with vaccination granulomas seen in our department probably reflects an increased awareness among parents due to increased public attention on vaccines and their side effects.

4.1 | Vaccines and aluminum load

Since 2005 there have been changes in the Danish Childhood Vaccination Program concerning vaccine production companies. The amount of aluminum in the DiTeKiPol vaccines has generally been 1 mg aluminum per dose, but from January 2014 to March 2018, two vaccines with 0.625 mg aluminum were also available. In this study it was not possible to distinguish between the vaccines that had been administered, but reports of adverse effects from the Danish Medicines Agency showed that most cases of granulomas were in children vaccinated in 2015 and 2016.[22]

Concerning continued vaccinations, Salik et al described that one-third of parents in their study had decided to omit or postpone further vaccination of their child,[14] and a similar behavior was described by Bergfors and Trollfors.[11] We observed that 47 (27%) of the parents had delayed further vaccinations, with 26 (15%) even refusing further vaccinations. The main reason was fear of another granuloma or exacerbating symptoms of an existing one. This emphasizes the need for careful advice to these families regarding vaccination.

The induction of granulomas could be associated with the accumulated aluminum load received through vaccines.[14] In our study 75 (42%) children developed granulomas after the second or third vaccine (administered at 5 and 12 months, respectively), supporting this hypothesis; however, in 58 (33%) the granulomas appeared after the first vaccine. The risk of additional granulomas following future vaccinations has until now only been investigated in a study of 25 children, where two (8%) developed new granulomas.[11] We found that 63% reported to have more than one granuloma. This is solely based on the questionnaires and not clinically confirmed.

4.2 | Avoidance behavior

A high proportion (up to 67%) of families were trying to avoid contact to aluminum. This is in contrast to the very few known case reports of aluminum contact allergy following external exposure. Axillary eczema following the use of antiperspirants has been described in older children and adults following ASIT,[16,26,27] and in studies by Bergfors et al and Salik et al, three of 19 and four of 39, respectively, reported a worsening in the skin condition of the child when using an aluminum-containing sunscreen.[14,21] In our study, reactions to aluminum-containing sunscreens were reported by 81 (46%). These reports are of course subjective and might be biased by the parents' knowledge of the positive test reaction to aluminum during patch testing. Reactions following the use of antiperspirants were reported by 16 (9%). This could be due to the young age of our study population, by whom this type of products is not yet used. In addition, the dermal penetration of aluminum through normal skin is reported to be low. In a study measuring the transdermal penetration of aluminum chlorohydrate, an absorption of less than 0.07% was shown. A similar result was recently found in a study by the cosmetic industry.[28,29]

4.3 | Systemic allergic dermatitis

Many patients with contact allergy/dermatitis speculate on the influence of food intake on symptoms. Aluminum is naturally present in both plants and animals. It is also used as a food additive, and especially dried products such as fruit and tea leaves contain a high amount of aluminum. The same is true for foodstuff prepared or stored in aluminum utensils.[30,31] In addition, the concentration of aluminum in various milk- and soy-based formulas is much higher than in human breast milk.[32,33]

Systemic allergic dermatitis is well known, but a rare entity, where a person sensitized by skin contact develops symptoms from systemic exposure (eg orally). The symptoms vary from a local flare of previous dermatitis to the so-called baboon syndrome.[34] However, gastrointestinal bioavailability of aluminum in general is considered low[35-37] and at least in some cases, a more general flare with characteristic symptoms of systemic allergic dermatitis would be expected if certain food items were involved. This has not (yet) been reported. In nickel-allergic individuals previous studies have indicated that oral nickel contact via dental braces leads to a reduced frequency of nickel contact allergy.[38] Early nutritional exposure to aluminum could potentially create tolerance, causing the children who were bottle-fed to be less prone to develop the granulomas. However, we found no difference between the groups regarding nutrition during the first 6 months of life (P = .75).

4.4 | Impact on quality of life

Looking at the CDLQI data, we found that the reference group had a slightly higher score compared with the granuloma group (5.44 vs 4.25), although not significantly so (P = 0.21). Studies on children with psoriasis showed a similar score with a mean 5.05 (SD ± 5.0),[39] but a higher score in children with AD (7.1 [SD ± 4.4]).[40] Although the CDLQI focuses on the skin, chronic disease is a confounder and might contribute to the higher score in the reference group, along with the significantly higher prevalence of AD. As the granulomas tend to disappear over time, another explanation of the lower score in the granuloma group could be the follow-up time. On the VAS, the granuloma group had a significantly higher score than the reference group. This difference in VAS and CDLQI could be because CDLQI questions are quite specific and concern only the child, whereas the VAS we created addressed both the children and their parents. The mean VAS score when symptoms were worst in the granuloma group was similar in the child and the families (6.7), indicating that the parental subjective feeling of the impact of everyday life is very much affected, with all probability due to the changes and precautions they feel they must take.

4.5 | Strengths and limitations

To our knowledge, this the most extensive questionnaire study regarding vaccination granulomas and aluminum contact allergy, and the first published one with a reference group for at least partial comparisons. Overall, our response in the granuloma group was 72%, similar to the response in other printed questionnaire studies.[41] Response in the control group was 49%, which might be because only 24% of these children had a positive patch test and the parents therefore did not find it relevant to participate. Another weakness is the subjective nature of answers in a questionnaire, especially regarding worsening factors and the number of granulomas. A clinical aspect instead of only subjective description would have strengthened our results.

There is a significant age difference between the groups, which makes the comparison difficult, especially regarding quality of life. Older children tend to be more self-conscious concerning the perception on how a disease affects their life. There is also a significant difference in the follow-up period, with more time passed since patch testing of the reference group. This could be another reason for the lower response, as the symptoms might have vanished over time, just as it might be a cause of recall bias in both groups. The reference group itself is heterogeneous concerning diagnoses, but instead of choosing healthy children, we decided to use a group of children with various forms of dermatitis. This allowed us to compare quality of life based on skin conditions.

The study design is limited by its retrospective nature. Only children referred due to granulomas are tested for aluminum allergy routinely in our clinic. The frequency of granulomas and aluminum allergy in the reference group are expected to be at the level as generally noted among children, around 1%, which is less than one person in our control sample. In addition, none in the control group reacted to the metal disk (Finn Chambers). Even though studies have shown that not all positive children react to both the salt and the metal,[42,43] the risk of them having granulomas and aluminum allergy was considered low and therefore we decided to use these children as the reference group.

The prevalence of chronic diseases was higher in the reference group, especially diabetes. This correlates with our chosen reference group suffering from various forms of dermatitis and suspected contact allergy, and the tendency of contact allergy to the medical devices used by diabetes patients (eg, insulin pumps), which is increasingly becoming a problem.[44]

In the reference group questionnaire, it was not possible to include questions regarding aluminum-containing products and the specific questions concerning the granuloma. This means we are unable to tell if the reported reactions in the granuloma group to, for example, sunscreens are specific for the granuloma group or more generally seen in children.

It is important to emphasize that the granuloma group in this questionnaire is a selected group, and given the easy access to online information, it is likely that many parents have sought information regarding aluminum allergy. During consultations with dermatologists at Herlev and Gentofte hospital following patch tests, parents are informed that generally no precautions are needed, except if the child has a reaction to a certain product. The information and advice parents obtain by using the Internet might not always have a scientific background and could be a risk factor of further avoidance behavior.

In our material it was not possible to evaluate the number of undiagnosed children with vaccination granulomas, who might be unaware of the possible aluminum contact allergy and therefore have no expectations regarding exacerbating factors and avoidance behavior. Because this study was questionnaire based, we have not been able to repeat patch tests on children in whom the granulomas have disappeared; therefore, no conclusions can be drawn on the course of their aluminum allergy.

5 | CONCLUSION

This study showed that although the granulomas and aluminum allergy are benign, the condition has a considerable impact on more than half of the group. They lead to avoidance of further vaccines, precautions in use of sunscreen, and/or dietary restrictions, which are perhaps unnecessary and negatively affect overall quality of life. Omission of vaccines should be taken seriously, as it puts the child at risk and lowers the vaccination coverage in the population. Based on the findings in this study, experimental provocation studies will be designed.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Stine Hoffmann: Formal analysis; investigation; methodology; writing-original draft; writing-review and editing. **Jacob Thyssen:** Methodology; supervision; writing-original draft; writing-review and editing. **Jesper Elberling:** Methodology; supervision; writing-original draft; writing-review and editing. **Kirsten Hansen:** Methodology; supervision; writing-original draft; writing-review and editing. **Jeanne Duus Johansen:** Conceptualization; funding acquisition; methodology; supervision; writing-original draft; writing-review and editing.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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ORIGINAL ARTICLE

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Does aluminium in sunscreens cause dermatitis in children with aluminium contact allergy: A repeated open application test study

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Abstract

Background: Parents report that children with aluminium contact allergy and vaccination granulomas may react to aluminium-containing sunscreen following application.

Objectives: To evaluate whether contact dermatitis develops following repeated application of aluminium-containing sunscreens in children with aluminium sensitization and vaccination granulomas.

Methods: Sixteen children aged 2-9 years (mean age 5 years) with vaccination granulomas and a positive patch test reaction to aluminium chloride hexahydrate 2%/10% petrolatum completed a blinded repeated open application test (ROAT) with two daily applications of two sunscreens for 14 days. One cream contained aluminium and the other did not. The children served as their own controls.

Results: Sixteen children completed the study. Only one child (6%) had a positive skin reaction during ROAT on day 2 to the sunscreen with aluminium. None reacted to the sunscreen without aluminium.

Conclusions: Use of aluminium-containing sunscreens may on a case basis lead to allergic contact dermatitis in aluminium allergic children.

KEYWORDS
allergy, aluminium, children, dermatitis, granuloma, patch test, ROAT, vaccine

1 | INTRODUCTION

Aluminium contact allergy is mainly seen in children following immunization with aluminium-adsorbed vaccines. Aluminium is used as an adjuvant in many vaccines, including vaccines against diphtheria, tetanus, and pertussis and pneumococcal infections, to stimulate the immune system. Adjuvants in relation to vaccines are substances added to enhance the immune response, with aluminium hydroxide and aluminium phosphate being the two most commonly used.[1] Aluminium-adsorbed vaccines can cause long-lasting itching nodules at the injection site, known as vaccination granulomas. These granulomas occur in almost 1% of all vaccinated children, with up to 96% of the children also being sensitized to aluminium.[2] The granulomas usually appear weeks to months after vaccination, and may last for many years.[3] Swelling and exacerbated itch are often seen when the children have common viral infections or receive subsequent vaccines.[4,5]

Aluminium is in general considered a weak allergen,[6] and aluminium contact dermatitis following cutaneous exposure is only on a case basis described in the literature.[7-12] There are surprisingly frequent parental reports on exacerbated granuloma itch and eczematous skin reactions, after aluminium-sensitized children are exposed to skin products such as sunscreen and lotions containing aluminium.[4,5,13]

Many different aluminium compounds are used in cosmetics, antiperspirants, toothpaste, and skin products. The most extensively used aluminium compound in cosmetics is aluminium chlorohydrate in antiperspirants. Other similar insoluble compounds often used in cosmetics are aluminium chloride, aluminium chlorohydrate, and zirconium–aluminium compounds.[14] In sunscreens, alumina (aluminium oxide) is added to improve spreadability and function as a pigment carrier of the physical UV filter titanium dioxide, as well as to function as an absorbent, anticaking, and bulking agent.[15]

In this study, we investigated whether exposure to aluminium as alumina in sunscreens could elicit allergic contact dermatitis in aluminium-sensitized children, by using the repeated open application test (ROAT),[16] a standardized exposure test mimicking daily use of a specific skin product.[17,18]

2 | MATERIALS AND METHODS

2.1 | Study design

The study was a double blind, randomized, controlled study. Primary outcome was the ROAT reaction, while enhanced granuloma itch and flare up of previous patch tested areas were secondary outcomes.

2.2 | Participants

All participants were children aged 2-10, referred to the Department of Dermatology and Allergy, Gentofte Hospital due to itching granulomas following immunization with aluminium-adsorbed vaccines. All children were patch tested with aluminium chloride hexahydrate 2% petrolatum (pet.), (allergEAZE; SmartPractice, Greven, Germany) from 2016 to 2021. Children older than 8 years were from 2021 tested with aluminium chloride hexahydrate 10% pet. according to renewed recommendations.[2,19] Children with a positive patch test and a vaccination granuloma with current granuloma itch were invited to participate. Exclusion criteria were a negative patch test reaction to aluminium, systemic immunosuppressant treatment, or a vaccination granuloma that was no longer symptomatic.

Sixteen children fulfilled the criteria and agreed to participate; parental consent was given in writing and one or both parents participated in all consultations. We did not include a separate control group, as the children functioned as their own controls.

The ROAT was initiated on day 2 (D2) or D7 of their patch test in 9/16 (56%) children, within 12 months of the patch test in 1/16 children (6%), and 6/16 (38%) children were included in the ROAT 1-4 years after their initial patch test.

On the first day of the study, all participants had a clinical examination of their skin including palpation of the granulomas. Any eczema, redness, or rash was photo documented prior to beginning of the study. During the consultation we obtained a medical history regarding vaccination, allergy, use of aluminium-containing products, and overall medical condition of the children, parents, and eventual siblings.

2.3 | Sunscreens

We acquired 10 different sunscreens for children available in regular Danish supermarkets and pharmacies, both national and international brands. No duplicates were collected. According to the content declaration, five sunscreens contained aluminium compounds and five did not contain aluminium. All sunscreen samples were shipped to ALS Scandinavia (Luleå, Sweden) for inductively coupled plasma mass spectrometry analysis. This type of mass spectrometry is known for its ability to detect metals in liquid samples, even in low concentrations of minimum 4 mg/kg. The five sunscreens declaring aluminium had a content of 558-1620 mg/kg, the ones without did not have a content over 4 mg/kg.

We used the sunscreen with the highest amount of aluminium—1620 mg aluminium/kg (0.16%)—as the aluminium-containing product for this study, Derma sun lotion SPF 30 Baby (DermaPharm A/S, Faarup, Denmark). The compound was alumina. The placebo was a sunscreen from same manufacturer, Änglamark sun lotion SPF 30 (DermaPharm A/S, Faarup, Denmark), with an aluminium content not exceeding 4 mg/kg. Sunscreens contain either physical or chemical sun filters with aluminium functioning as a physical filter. Consequently, the ingredients differed between the two sunscreens, but both sunscreens were free from perfume and parabens and recommended by Asthma Allergy Nordic and the Nordic Swan Ecolabel.

2.4 | Patch testing

Aluminium chloride hexahydrate 2% (allergEAZE; SmartPractice, Greven, Germany) was used as test substance. From March 2021 children older than 8 years were tested with aluminium chloride hexahydrate 10% (allergEAZE; SmartPractice, Greven, Germany). Further, an empty aluminium Finn Chamber (Epitest, Tuusula, Finland) was applied and an empty plastic chamber from the same producer was used as control. The allergen was applied in plastic Finn Chambers. The test materials were taped to the upper back for 2 days using a Scanpor tape (Norgesplaster; Alpharma, Vennesla, Norway). Parents were instructed to check the test sites the following day to avoid unnecessarily extreme reactions. The test site was then evaluated on D2 (the day of removal), and on D3-4 and D7. Only children with a negative or doubtful reaction on D2 were seen on D3-4. Reactions were classified as negative (0) or irritant, doubtful (+?), positive (+), strong positive (++), or extremely strong positive (+++), based on scoring according to the ESCD recommendations.[20]

2.5 | Repeated open application test procedure

The ROAT was performed on the children's lower back in order to avoid contact with previous patch tested area as well as minimizing risk of contamination by itch. We cut out two identical holes in a see-through piece of plastic as a template and placed it at level L4-L5 on all children. We marked two circles in red and blue, respectively, using the plastic template. Each circle measured 10.2 cm^2 (diameter 3.6 cm).

Each patient had two sets of cream, one with aluminium (1620 mg/kg) and one without. The creams were randomly allocated in a red or blue 1-mL syringe (BD Plastipak, Madrid, Spain) by a project nurse, with both project leader and patient/parents being blinded. In both areas, 0.02-mL cream was applied two times daily, equivalent of 2 mg/cm^2 corresponding to the recommendations on the use of sunscreen.[21] In the area exposed to aluminium-containing sunscreen, this would result in an overall exposure of aluminium of 32.4 μg per application. The creams were applied in the centre of the test areas and gently distributed using one specific clean fingertip to avoid contamination and was left to dry for 2-3 minutes. This procedure was carried out twice a day. If too much cream was applied, parents were instructed to remove excess test material by gently wiping the area with a paper towel, and to inform SH about the incidence.

During the ROAT, children were allowed to bathe as usual, but no skin product on the test area was allowed.

All participants were exposed twice daily for up to 14 days and evaluated on D7 and D14, earlier in case of a positive reaction.

Reactions were scored according to published guidelines, with a positive ROAT reaction defined as 5 or more points with erythema covering at least 25% of the exposed area and papules or homogenous infiltration, as proposed by Johansen et al.[22] All readings were performed by SH and were documented by photography. If reading was performed on other days due to dermatitis, reading day and score were noted and the ROAT study terminated.

3 | STATISTICAL ANALYSIS

Statistical analysis was performed using R version 3.6.1. The exact McNemar test for a pair design was used to detect difference between cutaneous reactions to the two sunscreens.

4 | ETHICS

This study was approved by the Regional Ethics Committee in Denmark (H-20009217), the Danish Data Protection Agency and conducted according to the Declaration of Helsinki. The study was prospectively registered at www.clinicaltrial.gov (NCT04438135). Participation required signed consent forms from both parents, and all children agreed to participate in the possible extent given their age.

5 | RESULTS

5.1 | Characteristics

Characteristics of the participants are presented in Table 1. Mean age of the children was 5 years at inclusion. Most children (n = 14) had a history of strong reactions (++) to aluminium chloride hexahydrate 2%, one had an extreme reaction (+++), and one child a weak reaction (+). Ten children (63%) also had positive reactions to the empty aluminium Finn Chamber.

TABLE 1 Characteristics of the participants

Participant number	Sex	Age (years)	Age at granuloma debut in months	Most recent positive patch test year	Patch test result 2%/10%[a] petrolatum	Empty Finn chamber reaction	Atopic dermatitis	Previous self-reported sunscreen reaction
1	F	9	12	2017	+[b]	++	No	No
2	F	5	12	2020	++	+?	No	No
3	M	5	12	2019	++	+?	No	No
4	F	7	12	2020	++	+?	Yes	No
5	F	3	15	2020	++	+	No	No
6	F	7	5	2020	++	+	No	No
7	F	4	24	2020	++	+	No	No
8	F	3	12	2020	++	++	No	No
9	M	5	12	2020	++	+?	Yes	No
10	M	8	3	2017	++[b]	++	No	Yes
11	F	3	5	2020	+++	++	No	No
12	F	6	34	2021	++	++	No	No
13	F	4	12	2021	++	+?	No	No
14	M	8	12	2021	++[a]	++	No	No
15	M	4	12	2021	++	+?	No	Yes
16	M	2	12	2021	++	++	No	No

[a]Patch test result 10% pet.

[b]Original patch test performed respectively 3 and 4 years prior to study inclusion. Retest was negative in participant number 1 (tested with aluminium chloride hexahydrate 2% petrolatum) and not performed in participant number 10.

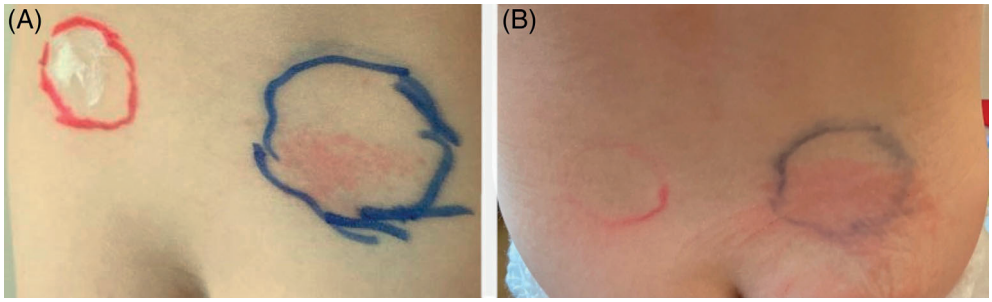

FIGURE 1 Repeated open application test (ROAT) reaction on day (D) 2 and D3 in participant number 16. (**A**) A positive reaction in the aluminium-exposed area on the evening of D2, after three applications. (**B**) The morning on D3 with clear signs of scratching and dermographism

Parents of participant numbers 10 and 15 reported that their child had previously developed a cutaneous reaction following aluminium-containing sunscreen use, with a universal erythematous reaction appearing within a few days following application.

In all children, DiTeKiPol was the vaccine suspected of causing the granulomas; either the first dose at 3 months (participant number 10), the second at 5 months (participants numbers 6 and 11), or the third dose administered at 12 months (the remaining 13 participants).

Six children were offered a new patch test as the first patch test was done more than 12 months before. One child declined (participant number 10), and another child, 9-years of age (participant number 1) had a negative second patch test to aluminium chloride hexahydrate 2% pet.

5.2 | ROAT results

All 16 children completed the study. One child, participant number 16 (1/16 = 6%) had a skin reaction to the aluminium-exposed area just before the fourth application (D2; Figure 1), with erythema and papules covering approximately 50% of the test area (ROAT score 7/18). No children, including the child with a reaction to the aluminium-exposed area, reacted to the control sunscreen. This difference was nonsignificant ($P > 0.99$) by the exact McNemar test. No children had flare-up of previous patch tested areas. In seven children (44%; participant numbers 3, 4, 5, 8, 9, 12, and 16) parents reported enhanced granuloma itch with no other given risk factors, such as infections or subsequent vaccines, also known to boost granuloma itch.

6 | DISCUSSION

In this study, we investigated whether daily use of sunscreen containing aluminium could cause contact dermatitis in aluminium-sensitized children in a blinded and randomized design, where the children served as their own controls. One child out of 16 (6%) developed a skin reaction after 2 days of exposure to the aluminium-containing sunscreen. No reaction was observed in ROAT areas exposed to the aluminium-free sunscreen in this child nor in any other children in the study. Even though it by definition is not possible by a ROAT to tell if a cutaneous reaction is of allergic origin,[23] the result indicates that some aluminium-sensitized smaller children may have a clinically relevant contact allergy, and not solely itching granulomas.

In general, the risk of elicitation depends on exposure concentration and penetration of the allergen as well as the sensitivity of the individual. In this study we chose the sunscreen on the market with the highest amount of aluminium (0.16%) according to our investigation. As no restrictions are given for the use of aluminium compounds in sunscreens in the European Union, an increased concentration may potentially cause reactions in more children. A few cases have been published concerning reactions to aluminium-containing deodorants in aluminium-sensitized teenagers and adults,[24-26] as generally the concentration of aluminium is higher in deodorants/antiperspirants and additionally aluminium chloride is more bioavailable than alumina.[27]

Robust data on aluminium absorption through the skin are lacking. An in vivo study on the dermal absorption of aluminium from antiperspirants indicated an absorption rate of 0.0014%.[28] A study on five human skin biopsies showed an average penetration of up to 2% of the epicutaneous dose through intact skin and 10.7% in damaged (tape-stripped) skin.[27,29] A recent study investigating the systemic load of aluminium following daily use of antiperspirants found no measurable contribution in the urine after an exposure time of 14 days.[30] In total, there is no consensus on the dermal absorption of aluminium, which may also depend on the specific compound. In our study aluminium oxide (alumina) was present in the sunscreen. It is a nanoparticle with an average mass of 101.96 Da. Most common allergens have a molecular weight under 500 Da; therefore, alumina should be able to penetrate the skin.[31] The skin penetration of alumina nanoparticles 24 hours following exposure has recently been investigated, which indicated a significantly higher amount of aluminium only in damaged skin, but not in intact skin.[32] Alumina might take longer to cause an allergic reaction than other aluminium salts, and using another aluminium compound could influence the results of our study.

Atopic dermatitis (AD) is the most common inflammatory skin disease in childhood, affecting up to 15%-30% of children.[33] Patients with AD have a dysfunctional skin barrier, and although the relationship between AD and contact allergy is not fully understood, individuals with milder forms of AD seem to be at greater risk of sensitization to some allergens.[34] In our study, 2/16 (12%) children had AD, but the one child reacting positive to the ROAT did not have AD.

Regarding sensitivity, the only child with a positive reaction to the aluminium sunscreen was also the youngest participant (only 2 years old). It seems that reactivity to aluminium decreases with age.[3] The patch test showed a ++ reaction, but this was also seen in most of the other children, even +++ in a child with a negative ROAT. The child with the positive ROAT was patch tested just prior to start of ROAT; however, this was also done in five other children—all with negative responses. Had all children participated in the ROAT days after their patch test, there might have been more positive ROAT reactions.

Previous experimental studies indicate that skin reactivity depend on the anatomical region, increasing from the upper arm and neck.[35] Although the upper arms are most frequently used in ROAT studies, other skin sites are also sensitive and products should be tested in a manner that resembles the natural use.[36] We chose the lower back due to various reasons; because the participants were children, we wanted to diminish their attention to the test areas in order to prevent scratching and touching of the skin, avoid application with other creams during the test period, and avoid sun exposure of the skin, potentially interfering with the ROAT results. Although the area is not the typical location of the ROAT, it was close to the patch test areas where aluminium-specific T cells could induce a response. Additionally, a recent mouse study showed that upon exposure of naïve skin to the allergen in question, inflammation was mediated by circulating T cells 48-96 hours following exposure.[37]

Moreover, many children participated in the ROAT study only days after their patch test, where suspected aluminium-specific T cells still persist at the inflammation site; however, the ROAT was performed on a näive test site at the lower back. Still, only one child had a positive reaction, and no flare-up reaction was seen at the patch tested areas in any of the participants.

Some limitations do apply for this study. It could also be argued that "real life" exposure of sun, sweat, and swimming would affect the ROAT skin areas, making them more or less susceptible to allergic reactions. Except for participant numbers 10 and 15, the children in our study had not previously developed a rash following use of sunscreen. Participant number 1 had a positive patch test in 2017 but a negative patch test when she was retested in 2020 following participation in the study, despite her granuloma still being active (itching). However, she was 9 years old and retested with aluminium chloride hexahydrate 2% pet., and recent guidelines have suggested testing children older than 8 years old with aluminium chloride hexahydrate 10% pet. instead of 2% pet. to avoid false-negative results.[2,19] Several different aluminium compounds are used in cremes and cosmetics, and the dermal penetration could vary among the combinations. Histological evaluation of a punch biopsy could have added important information about the skin reaction.

7 | CONCLUSION

According to our findings, aluminium in sunscreen may on a case basis be a source of contact dermatitis in sensitized children.

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AUTHOR CONTRIBUTIONS

Stine Hoffmann: Conceptualization (equal); formal analysis (equal); funding acquisition (supporting); investigation (lead); methodology (equal); project administration (lead); writing – original draft (lead). **Jesper Elberling:** Conceptualization (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Jacob Thyssen:** Conceptualization (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Kirsten Hansen:** Conceptualization (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Jeanne Duus Johansen:** Conceptualization (equal); funding acquisition (lead); methodology (equal); supervision (lead); writing – review and editing (equal).

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Manuscript III

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Adverse reactions after oral provocation with aluminium in children with vaccination granulomas and aluminium contact allergy.

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Adverse reactions after oral provocation with aluminium in children with vaccination granulomas and aluminium contact allergy.

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Abstract

Background: According to their parents, some children with aluminium contact allergy and vaccination granulomas may react to aluminium-containing foods with rash, granuloma itch and subjective symptoms.

Objectives: To evaluate whether oral intake of aluminium-containing pancakes can cause adverse events and/or systemic contact dermatitis in aluminium-allergic children.

Patients/Methods: 15 children aged 3-9 years (mean age 5 years) with vaccination granulomas and a positive patch test reaction to aluminium chloride hexahydrate 2%/10% pet. completed a 3-week blinded randomized controlled oral aluminium/placebo provocation study with pancakes. Dermatitis, granuloma itch, and subjective symptoms we evaluated daily on a visual analogue scale. Sleep patterns were tracked with an electronic device. Aluminium bioavailability was assessed by the urinary aluminium excretion. The children served as their own controls.

Results: 15 children completed the study. The mean VAS scores were slightly higher during aluminium provocations for both granuloma itch (mean VAS 1.6 vs 1.4, *P*=0.5) and subjective symptoms (0.7 vs 0.5, *P*=0.028). There were no differences in sleep patterns and no correlation between urine aluminium excretion and symptom severity. Three children developed a rash on face or buttocks on day 4 of the aluminium provocation. 8/15 (53%) of parents correctly identified the aluminium provocations.

Conclusions: Oral provocation with aluminium may in a minority of aluminium-allergic children be associated with the development of contact dermatitis and exacerbated granuloma itch.

Introduction

Aluminium is a ubiquitous metal, with numerous industrial and domestic applications. Many different sources contribute to aluminium exposure in humans, including food, antacids, deodorants, and vaccines.[1,2] As an allergen, aluminium is generally considered weak, and aluminium contact allergy is predominantly seen in children who develop small itching subcutaneous nodules following immunisation with aluminium-adsorbed vaccines, known as vaccination granulomas. These granulomas occur in up to 1% of vaccinated children, usually appear weeks to months after vaccination, and may last for several years.[3–6] Rarely, aluminium-allergic individuals may develop dermatitis or exacerbated granuloma itch when exposed to dermal aluminium products such as sunscreens and deodorants.[7–10]

Systemic contact dermatitis (SCD) is a skin condition where an individual sensitized through skin exposure reacts with a rash to that same allergen via the systemic route. It has been described in individuals allergic to both metals, medications and foods.[11,12] There is only one published study on systemic contact dermatitis in aluminium allergic children, with aluminium-containing toothpaste causing granuloma itch in 3 children.[13] Nevertheless, parents of aluminium allergic children have on several occasions reported that their children may react with granuloma itch, rash/dermatitis as well as subjective symptoms such as headaches, abdominal pain and agitation, when consuming aluminium-containing foods.[5,14,15]

The food additive sodium aluminium phosphate (SALP) has been used in studies investigating aluminium bioavailability.[16,17] SALP is an authorised food additive categorised as "additives other than colours and sweeteners" with the number E541, used in many bake-off products including pancakes as a leavening acid to react with baking soda.[18]

Our study aimed to investigate if ingested aluminium in daily food-exposure doses could induce a systemic response such in aluminium allergic children using a blinded randomized controlled oral provocation with SALP pancakes and aluminium-free (placebo) pancakes.

Further, we wanted to examine if there was an association between doses of oral aluminium challenge, symptoms and aluminium excreted in the urine.

Materials and methods

Study design

The study consisted of a 3-week blinded randomized controlled oral aluminium provocation, where participants consumed pancakes for the first 4 days of each week, followed by 3 days wash-out before starting the next provocation. Urine samples were made on day 4. Children could ingest SALP pancakes for one or two weeks, and placebo pancakes for one or two weeks. (Fig. 1).

There were no restrictions or monitoring of their regular food intake during the study.

Participants

A-priori assessment showed that 23 participants were needed to obtain a power of 80% and a level of significance 0.05, if 31% of the participants would react as described in a previous questionnaire study.[14] All efforts were made to obtain this, but after a year and a half, we had to close the study. In total, 15 children aged 3-9 years, referred to the Department of Dermatology and Allergy, Gentofte Hospital or Department of Dermatology and the Allergy Centre, Odense University Hospital, due to itching granulomas following immunisation with aluminium-adsorbed vaccines, were included. Exclusion criteria were allergy to any ingredients in the pancakes other than aluminium (egg, wheat, milk), kidney- or bone disease, systemic immunosuppressant treatment, vaccination within the last week, use of antacid within the last week, illness during the study period or a vaccination granuloma that was no longer itching.
Inclusion took place from February 2021 to May 2022.

Patch testing

All children were patch tested with aluminium chloride hexahydrate 2% pet., (allergEAZE; SmartPractice, Greven, Germany) and an empty aluminium Finn Chamber (Epitest, Tuusula, Finland). Children older than 8 years were from 2021 tested with aluminium chloride hexahydrate 10% pet. according to new recommendations.[4,19] The allergen was applied in a plastic Finn Chamber, and an empty plastic chamber was used as control. All chambers were taped to the upper back for two days using Scanpor tape (Norgesplaster, Alpharma, Vennesla, Norway). Parents were instructed to check the test sites both on the application day and the following day, to avoid extreme reactions. The test site was then evaluated on either day (D)2 or D3-4, depending on which department performing the test, and D7. Reactions were classified as negative (0) or irritant (IR), doubtful (+?), positive (+), strong positive (++) or extremely strong positive (+++), based on scoring according to the ESCD recommendations.[20]

Pancakes

Two different types of pancake mix were used, one with SALP as an additive and one with out aluminium, similar in texture and taste. The mixes were analysed for aluminium content at ALS Scandinavia, Luleå, Sweden, showing 1640 mg aluminium/kg mix (± 224 mg/kg) in the SALP pancakes and <5 mg/kg mix in the placebo pancakes. All pancakes were cooked in a cast-iron skillet and packed in plastic bags and stored at -20°C until distribution to the participants.

From various studies on oral toxicity of aluminium risk assessments of aluminium exposure exists, and the tolerable weekly intake (TWI) of aluminium, defined by the European Food Safety Authority (EFSA), is 1 mg Al/kg bw/week.[18] The US equivalent, Joint FAO/WHO Expert Committee on Food Additives (JECFA) allows the double amount, 2 mg Al/kg bw/week.[18]
We aimed for the children to consume aluminium pancakes equivalent to 3-4 mg Al/kg bw/week during SALP-pancake ingestion, thus children with a bodyweight of 20 kg or less should eat 2 pancakes, and children weighing more than 20 kg should eat 3.

Symptom assessment

From a previous questionnaire study and parental reports,[14,15,21] we created a diary with a list of subjective symptoms to be evaluated during the three-week study. The symptoms included headache, irritability/agitation, stomach ache, and tiredness, and in addition, parents had the opportunity to choose symptoms not listed in the diary.[14] Parents choose up to three symptoms they wished to evaluate, and each was scored on a Visual Analogue Scale ranging from 0-10.
All children were given a Garmin Vivofit Junior activity watch (Garmin Ltd, Olathe, Kansas), and were instructed to wear it during nighttime, tracking minutes of total sleep and awakenings. Parents downloaded the matching app on their smartphones.
Additionally, cutaneous reactions were subdivided into the following categories:

- Flare-up reactions in the previous patch tested areas.
- Any large- or small-scale clinical skin eruptions on previously affected and non-affected skin.
- Granuloma itch.

After finishing all three weeks, parents were asked to identify the aluminium week (s) based on their child´s symptoms.

Urine samples

The bioavailability of aluminium from the diet is low, and the average oral absorption is estimated to be 0.1%.[22] The absorbed aluminium is excreted in the urine within days, and the amount of aluminium content in the urine is a sensitive marker of the general absorption of aluminium.[16,17,23] To estimate the possible increased uptake of aluminium during the SALP pancake week, and to further evaluate the possibility of a correlation between excreted aluminium and VAS symptom scores, all participants made a urine sample after each of the three provocations. The urine samples were analysed for aluminium by ALS Scandinavia

(Luleå, Sweden), with inductively coupled plasma mass spectrometry, known to detect even small levels of aluminium in liquid samples.

Statistical analysis

Patient characteristics were described as frequencies with percentages (for categorical variables), and mean with standard deviation (SD, for continuous variables).

Distinguishing between the 3 provocation weeks, the mean VAS scores for symptoms 1-3 combined and granuloma itch were calculated for each participant. Cutaneous flare-up reactions and parental guesses (correct or incorrect guess of aluminium period) were analysed as binary categories (yes or no). Binary variables were analysed with the Chi-Square test for independence or Fischer's exact test for counts less than 5. Non-parametric statistical methods were applied for analyses of the VAS scores, with the Friedman test comparing the three weeks' reactions. The Wilcoxon signed rank test was used for pairwise comparisons between pooled aluminium and placebo provocations.

The correlation between VAS scores and urine aluminium excretion was assessed by Spearman´s rho.

P-values < 0.05 was considered statistically significant.

Data were analysed with IBM SPSS Statistics (SPSS, Chicago, Illinois) for Windows (release 25.0).

Ethics

This study was approved by the Danish Data Protection Agency and the regional ethics committee in Denmark (H-20060917) and conducted according to the declaration of Helsinki. The study was prospectively registered at www.clinicaltrial.gov (NCT04921163).

Results

Characteristics of the participants are shown in Table 1. In total, 15 children with a mean age of 5.7 years (SD 1.8)) participated the study. Of the participants, 8/15 children (57%) were girls, and in 3/15 children (20%) parents had a clear suspicion of previous cutaneous reactions to aluminium in food.

Granuloma itch, VAS scores and oral exposure

We evaluated the granuloma itch and symptomatic VAS scores during each of the three provocation weeks with use of Friedman's test, yielding non-significant $P = 0.86$ and $P = 0.23$ for granuloma itch and subjective symptoms, respectively.

Next, we pooled data from the aluminium provocations and placebo provocations making two groups for comparison and used the Wilcoxon Signed Ranks Test for these pairwise comparisons (Table 2).
VAS score for the mean granuloma itch during aluminium provocations was 1.6 (SD 1.4), and lower during the placebo provocations (1.4, SD 1.2). This slight difference was not statistically significant with $P = 0.5$.
Mean VAS scores for subjective symptoms during aluminium provocations were 0.7 (SD 0.7) vs. 0.5 (SD 0.7) for placebo provocations, with a significant $P = 0.028$ although as with granuloma itch, in terms of VAS score severity the difference was sparse. The distribution of each child´s VAS scores in the aluminium and placebo weeks are shown in Supplementary Fig. 1, with oral aluminium intake defined as mg/kg bw/week illustrated in Supplementary Fig. 2.
The ingested pancakes provided a dose of aluminium equivalent of 3.7 (SD 0.5) mg/kg bw/we ek (Table 2).
The relationship between oral aluminium intake and symptom scores is shown in Fig. 2, with Spearman´s Rho finding a non-significant $P = 0.23$ for subjective symptoms and $P = 0.69$ for granuloma itch.
Three children developed a rash, two in the face and one on the buttocks on day 4 in the aluminium week, similar to the previous rashes suspected by parents of being triggered by aluminium in food (Fig. 3). The rashes were slightly palpable, non-fluctuant and itchy, and gradually disappeared again after 3 to 5 days. All affected children continued the study and followed the protocol.
No children had flare-up reactions of previous patch tested areas.

Aluminium concentration in urine samples and the relationship with symptom scores

We were not able to stratify according to the sum of aluminium children received through their regular diet, which could potentially contribute to oral aluminium intake. Instead, we investigated the correlation between aluminium excretion in the urine as a proxy and the VAS scores for both granuloma itch and other subjective symptoms. As shown in Table 2, the mean urinary aluminium excretion was 12.7 µg/L (SD 8.3) during aluminium provocations and 6.7 µg/L (SD 2.1) during placebo provocations (P=0.006). Mean creatinine excretion was 7.5 mmol/L (SD 2.7) vs 6.3 mmol/L (SD 2.6) for aluminium and placebo, respectively (P=0.041), all children had measurements of creatinine within the normal range.[24]
The three children with rash during aluminium provocations had aluminium excretion levels of 14.4, 8.39 and 5 µg/L.
We investigated the relationship between aluminium bioavailability during aluminium provocations and VAS score severity (Fig. 4) and found no correlation between urine aluminium excretion and VAS scores (Spearman's Rho, $P = 0.63$ for granuloma itch and $P = 0.66$ for subjective symptoms).
Adjusting for creatinine in the urine did not change the results.

Change in sleep patterns

With the rationale that an itching granuloma would affect sleep patterns, we analysed the difference in both the average amount of sleep per week and the percentage of awakenings per week, from data collected via the Garmin Vivofit Junior watches. Results are shown in Table 2, with the total amount of sleep being higher during the aluminium provocation than the placebo (9:08 versus 8:59 hours, respectively), although not significant ($P = 0.59$). The percentage calculated awakenings of the total sleep were the same: 1.7% of total sleep ($P = 0.68$).

Parental guess

We constructed binary variables regarding previous suspected aluminium reaction (yes/no; 3/12) and correct identification of aluminium provocation (yes/no; 8/7), as shown in Table 2. Parents to two of the three children with previous cutaneous reactions correctly identified the aluminium provocations (P=0.55).

Discussion

In this study, we aimed to investigate if aluminium allergic children could react when systemically exposed to aluminium in food. We evaluated outbreaks of dermatitis, exacerbation of granuloma itch and subjective symptoms in a single-blinded and controlled design. We found a statistically significant higher excretion of aluminium during the aluminium provocations, but no significant differences between symptoms scored by parents and bioavailable aluminium measured as excretion in urine, neither between weeks with aluminium nor placebo.

Metals have previously been shown to cause systemic contact dermatitis.[11,12] Nickel allergic patients have experienced flare-up of previous patch tested areas following oral challenge,[25] as well as dermatitis, both flare up and *de novo*, after going through a high-nickel content diet.[26] Other cutaneous eruptions include the "Baboon syndrome", symmetrically patches of erythema on the nates, and vesicular hand eczema.[25–27] Additionally, patients might experience general symptoms such as headache, malaise and abdominalia.[28] Three participants developed a rash during the aluminium provocation, either on the buttock or facial, which cannot be explained by any other obvious exposure. Two of these 3 children had a history of atopic dermatitis but no flare-up within the last year, and in all three cases, parents had before this study suspected aluminium-rich food as a cause of skin symptoms. In another study, we showed skin exposure to aluminium-containing sunscreen in a child with aluminium contact allergy caused a rash on the site of application, with no rash seen with a placebo sunscreen.[7]

Interestingly, we did not see any flare-up of previous patch tested areas, not even in children with dermatitis and a recent patch test. In nickel allergic patients, a previous strong (+2 or +3) patch test reaction was correlated to flare-up reactions during oral nickel challenge.[25]

Other studies have shown that dose-and time elapsed since patch testing both are important factors in the risk of possible SCD reactions.[25,26] It might be that the dose we used was too low and the exposure time too short to provoke symptoms and reach statistical significance.

However, measurement of the daily exposure to the given metal is quite difficult, and many variables must be taken into accounts, such as bioavailability, individual sensitivity and the type of administration.[11,29] Because of the ubiquity of aluminium and the wide range of expected aluminium exposure, it has not been possible to estimate the additional aluminium exposure through regular diet in the participants.

Bioavailability of aluminium is complex and very dependent on the route of exposure.[30] The average oral absorption from food is 0.1%, increased by lactate and fluoride but decreased by silicates. We chose to use SALP, as this approved food additive has been used in other studies investigating bioavailability and urinary excretion of aluminium.[16,17] Other aluminium salts could alter the outcome.

Another limitation of our study is our study group was small as only 15 children wished to participate, limiting the statistical power. Secondly, we could have designed our study with increasing aluminium doses, to evaluate any dose-dependency in VAS scores and cutaneous eruptions. We could have provided the children with a very high dose of aluminium, for example by using antacids, to see if SCD could be provoked in all children. Aluminium antacids are available over the counter in many countries. These antacids are generally considered safe, and the recommended dose of 10 mL would result in an intake of approximately 150 mg aluminium hydroxide. This dose may be repeated several times daily. In a previous study, healthy adults were provoked with a daily dose of 1.8 g aluminium/day, with no influence on the immune system.[23] We chose to imitate a realistic dose of aluminium and not to drastically exceed the well-defined levels of tolerable weekly intake.

Conclusion

No statically significant difference was found between increased oral exposure to aluminium and symptoms such as granuloma itch and duration of sleep in this single-blinded controlled design. 3 children developed a rash on the buttocks and/or face only during aluminium provocations, which indicates that although not statistically significant, cutaneous reactions may occur in a minority of children.

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Fig. 1. Flowchart of study

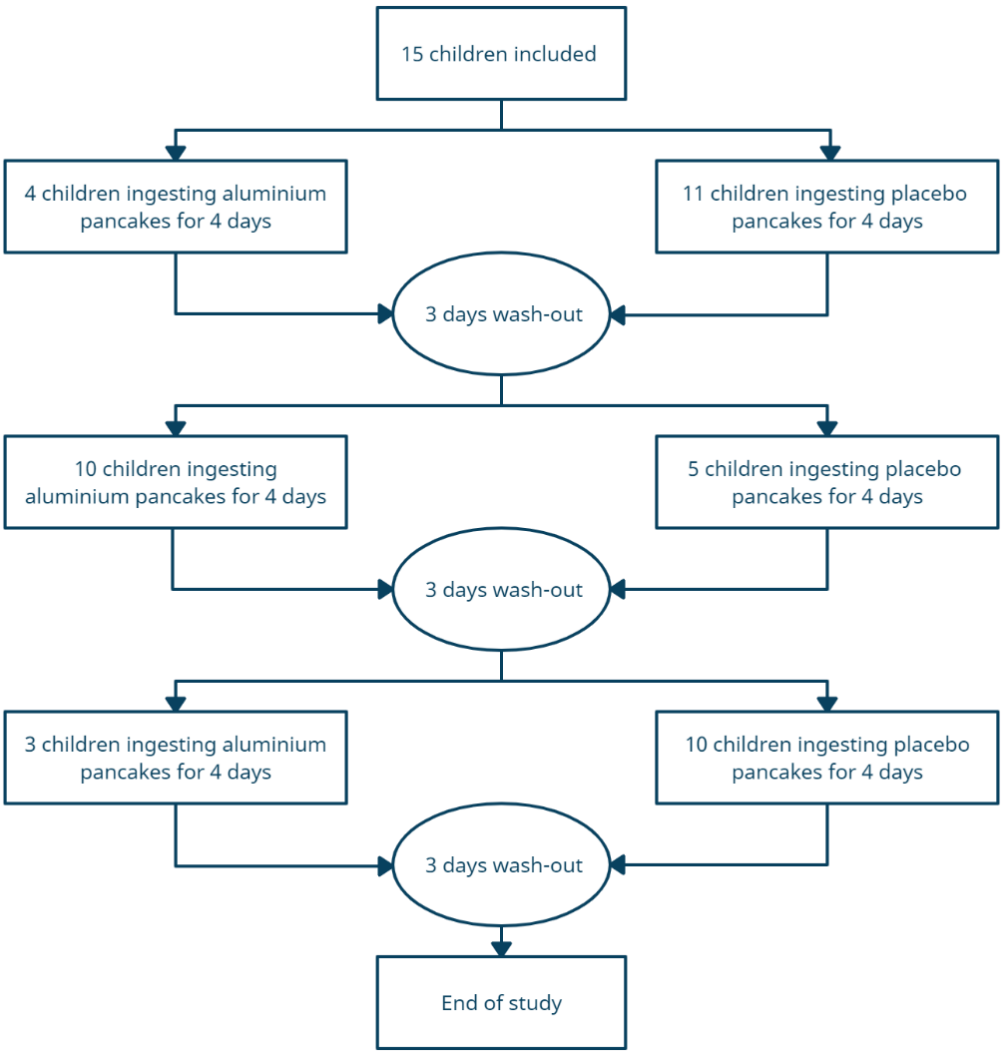
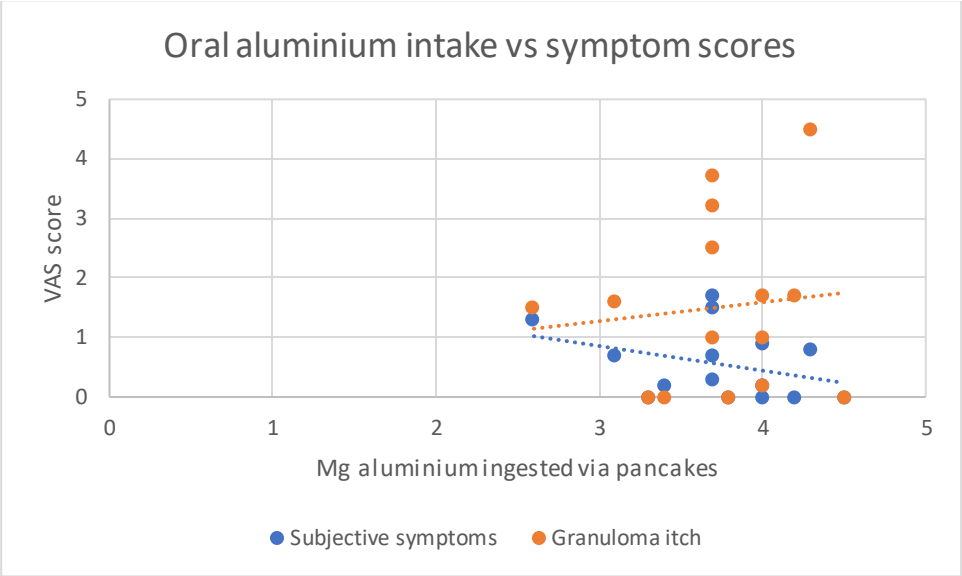


Fig. 2. Oral aluminium intake via pancake ingestion vs VAS scores of subjective symptoms and granuloma itch.



We used Spearman´s Rho to investigate any correlation between aluminium-pancake ingestion and VAS scores, yielding non-significant $P = 0.23$ for subjective symptoms and $P = 0.69$ for granuloma itch.

Fig. 3. Infiltrated, itching, non-fluctuant rash on the nates and face of three participants, occurring on day 4 in the aluminium provocation week and gradually disappearing within 3-5 days.

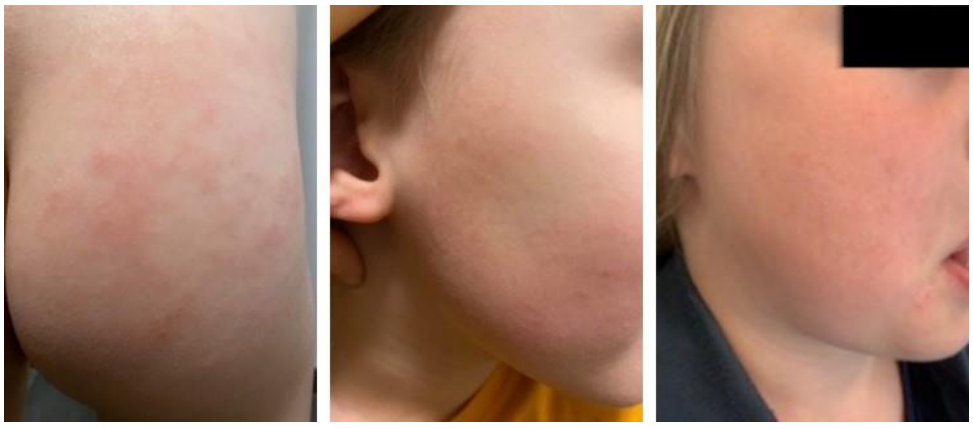
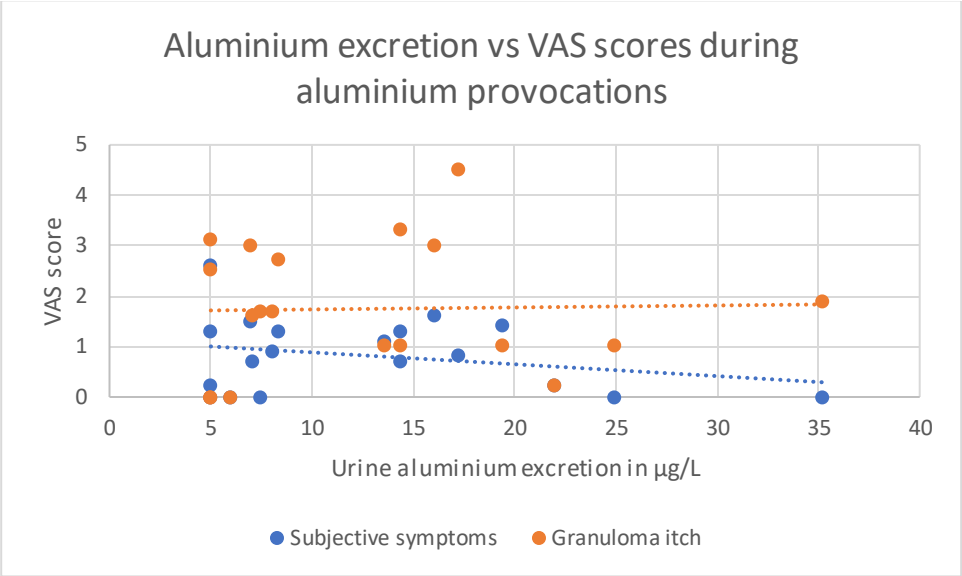


Fig. 4. Aluminium bioavailability vs VAS scores of subjective symptoms and granuloma itch during the aluminium provocations.



Detection level of aluminium in urine samples are 5, all samples with the result of <5 are defined as 5 in this plot.

There was no correlation between urine aluminium excretion and VAS scores (Spearman's Rho, $P = 0.63$ for granuloma itch and $P = 0.66$ for subjective symptoms).

Table 1. Characteristics of the participants.

Participants	**Total n=15**
Girls	8/15 (53%)
Mean age in years (SD)	5.7 (1.8) years
Mean weight in kg (SD)	21.4 (4.7) kg
Atopic dermatitis	7/15 (47%)
Pos. patch test reaction to aluminium chloride hexahydrate 2/10% pet	15/15 (100%)
Pos. patch test reaction to empty aluminium chamber	8/15 (53%)
Maximum patch test reaction (pet or chamber)	
+1	1/15 (7%)
+2	13/15 (86%)
+3	1/15 (7%)
Previous suspected cutaneous reaction to aluminium in food	3/15 (20%)

Table 2. Aluminium digestion, excretion, and symptom assessment for aluminium and placebo provocations.

	Aluminium provocation(s)	**Placebo provocation(s)**	***P*-value**
Oral aluminium mg/kg bw, mean (SD)	3.7 (0.5)	-	-
Urine aluminium excretion µg/L, mean (SD)	12.7 (8.3)	6.7 (2.1)	*0.006**
Urine creatinine excretion mmol/L, mean (SD)	7.5 (2.7)	6.3 (2.6)	*0.041**
Symptom assessment			
Granuloma itch, VAS mean (SD)	1.6 (1.4)	1.4 (1.2)	0.5
Subjective symptoms, VAS mean (SD)	0.7 (0.7)	0.5 (0.7)	*0.028**
Dermatitis (% of total)	3 (20%)	0 (0)	-
Flare-up of patch test reaction (% of total)	0 (0)	0 (0)	-
Sleep pattern			
Total sleep (hours:minutes (SD))	8:59 (0:26)	09:08 (0:39)	0.59
Awakenings (% (SD) of total sleep)	1.7 (2.2)	1.7 (2.2)	0.68
Parental assessment			
Previous cutaneous reaction (% of total) †	3 (20%)	-	-
Correct parental identification (% of total) ‡	8 (53%)	-	-
Previous reaction vs correct identification (% of total)	2 (13%)		0.55

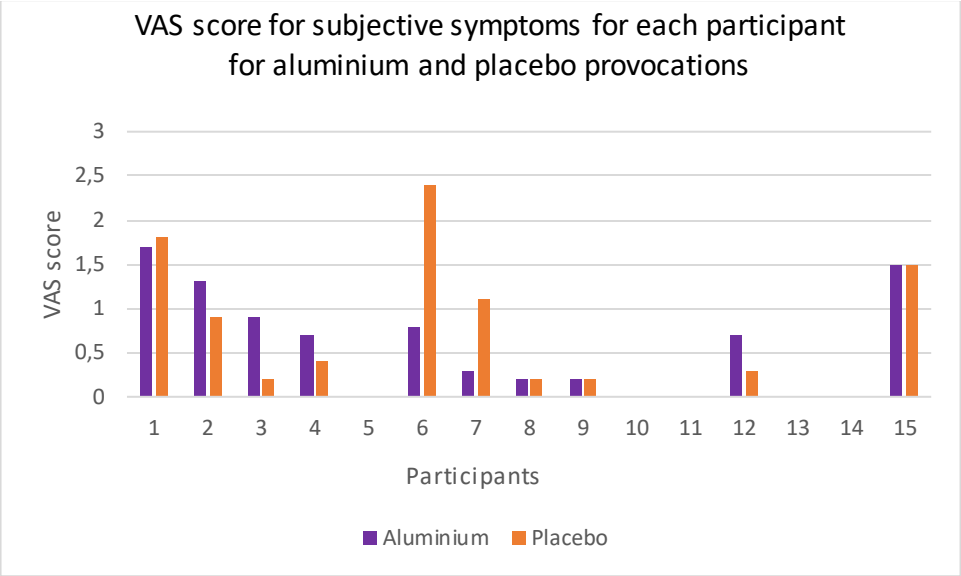
Difference between mean VAS scores, urine excretion, and sleep patterns between aluminium and placebo provocations were assessed with Wilcoxon Signed Ranks Test for non-parametric paired data. Fischer´s exact test was used to evaluate the association between previous suspected reaction to aluminium in food and correct parental identification og the aluminium week(s).

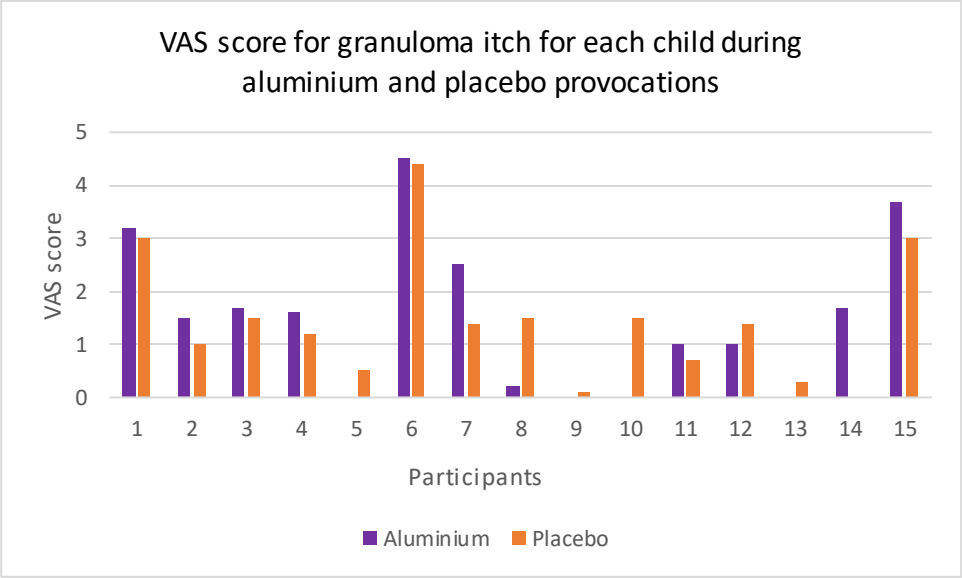
* Statistically significant *P*-value

† Parents have suspected previous reaction to aluminium-rich food

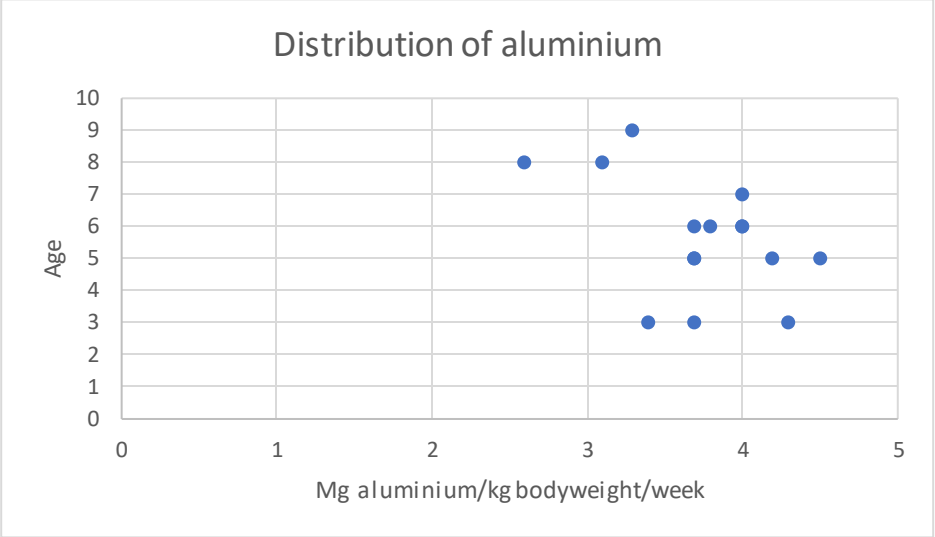
‡ correct identification of the week of aluminium provocation.

Supplementary Figure 1. VAS scores for each child (both subjective symptoms and granuloma itch evaluated by parents), for aluminium and placebo provocations, respectively.





Supplementary Figure 2. Distribution of oral aluminium expressed as mg per kilogram bodyweight per week during aluminium provocation per age in years of the participants.



Manuscript IV

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Risk factors for granulomas in children following immunisation with aluminium adsorbed vaccines: A Danish population-based cohort study.

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Title: Risk factors for granulomas in children following immunisation with aluminium adsorbed vaccines: A Danish population-based cohort study

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Abstract

Background*:* Aluminium adsorbed vaccines may in some children cause severely itching nodules at the injection site, known as vaccination granulomas.

Objective: To investigate vaccine-, child- and maternal level risk factors for the development of vaccination granulomas following immunisation with aluminium adsorbed vaccines.

Methods: A Danish population-based cohort study with 553 932 children born in Denmark from 1 January 2009 to 31 December 2018, vaccinated with an aluminium adsorbed vaccine during the first year of life, followed until 31 December 2020. Poisson regression was used to estimate granuloma rate ratios according to type of adjuvant, accumulated dose of aluminium, timing of vaccination appointments, sex, gestational age, having siblings with granulomas, maternal age, and maternal ethnicity.

Results: We identified 1 901 vaccination granuloma cases (absolute risk, 0.34%). Among vaccine level factors, revaccination (third vs first vaccination appointment, adjusted rate ratio [RR] 1.26, 95% confidence interval [CI] 1.03-1.55), the specific adjuvant used (aluminium phosphate vs hydroxide, RR 0.58, 95% CI 0.48-0.70) and dosage (≥1.0 mg vs <1.0 mg, RR 1.34, 95% CI 1.19-1.52) were associated with risk of granulomas; the timing of vaccination appointments was not. Among child level factors, female sex (vs males, RR 1.12, 95% CI, 1.02-1.22), prematurity (vs term birth, RR 0.71, 95% CI, 0.54-0.93) and having sibling(s) with granulomas (vs no siblings with granulomas, RR 46.15, 95% CI, 33.67-63.26) were associated with risk of granulomas. Among maternal level factors, non-Danish ethnicity (vs. Danish, RR 0.51, 95% CI, 0.42-0.63) and young maternal age (<20 yrs. vs 20-39 yrs., RR 0.46, 95% CI 0.25-0.83) were associated with risk of granulomas.

Conclusions: Several risk factors for vaccination granulomas at both the vaccine, child, and maternal level, was identified. Reducing the dose of aluminium or replacing aluminium hydroxide with aluminium

phosphate could reduce the risk of granulomas. However, this must be balanced against the potential for reduced immunogenicity.

1 | Introduction

A wide range of vaccines used in national childhood vaccination programmes employ aluminium adjuvants to enhance the immune response.[1] Adjuvants increase the adaptive immune response via activation of innate immune cells that, via a cascade of signals, activates the lymphocytes, although the exact mechanism remains a target of ongoing research.[2] For decades it has been apparent that aluminium-adjuvants may cause severely itching nodules at the injection site, known as vaccination granulomas, appearing weeks or even months following immunisation.[3–7] There are also reports on development of granulomas following subcutaneous injections with allergen-specific immunotherapy, ASIT.[8] Granulomas are associated with the development of contact sensitisation to aluminium, an otherwise rare contact allergy.[9,10] Vaccination granulomas were considered rare until 2003, where a Swedish placebo-controlled vaccine trial of a new aluminium hydroxide-adsorbed vaccine reported granulomas in 645 out of 76,000 (0.8%) vaccinated children. The high frequency was speculated to be caused by both the change in adjuvant from phosphate to hydroxide and the aluminium content in the vaccines increasing from 0.5 mg to 1 mg per dose,[5] however this was never evaluated in a study. Two of the most commonly used aluminium based adjuvants are aluminium hydroxide, $Al(OH)_3$ and aluminium phosphate, $AlPO_4$, which differ both in molecular size, properties and surface charge at physiological pH.[11,12] It is theoretically plausible that one type of adjuvant may enhance the risk of developing a granuloma compared to the other, as they possess different pharmacokinetic properties.[13] Other potential risk factors such as genetics, age at vaccination and dose interval, has not yet been systematically assessed.

Although granulomas are not life-threatening, they may be long-lasting, intensively itching and cause significant distress for the afflicted child and family.[14–17] In Denmark, vaccination granulomas may entitle individuals to compensation from the Danish Patient Compensation Association, being adverse reactions to

vaccines in the Danish Childhood Immunisation Programme. They are still poorly understood, and early recognition in primary health care would avoid unnecessary examinations and reduce general vaccine mistrust.[18] Up to 34% of children with vaccine granulomas postpone or omit further vaccines in the Danish Childhood Vaccination Program.[14,19]

Aluminium has recently been designated Allergen of the year 2022, highlighting the urgent need for further research.[20] To provide insights into this understudied adverse event and to inform on the potential for prevention, we conducted the first large nationwide cohort study of vaccination granuloma risk factors, at the vaccine-, maternal- and child level.

2 | Methods

2.1 | Study cohort

Since 1968, all individuals living in Denmark have been assigned a unique personal 10-digit identification number in the Danish Civil Registration System,[21] which enables accurate linkage between the multiple national registers containing extensive health-care information on an individual level.[22] From these national registers, we established a cohort of all children born in Denmark between January 1 2009 and December 31 2018, followed until December 31 2020. The study was approved by the Danish Data Protection Agency (internal compliance number 20/09846). Approval from an Ethical Committee is not required for register-based research in Denmark.

2.2 | Vaccines

The Danish Childhood Vaccination Programme includes vaccines against diphtheria, tetanus, pertussis, polio, and *Haemophilus influenzae* type b (DTaP-IPV-HiB, in this article abbreviated DTP) either alone or in combination, as well as pneumococcal conjugate vaccines (abbreviated PCV). These vaccines are offered in three doses at 3, 5 and 12 months of age, as well as a booster dose at 5 years (Table 1). Importantly, DTP and PCV are almost always administered at the same appointments during infancy; this is termed a DTP+PCV appointment in the following. Depending on the vaccine manufacturer, a child following the recommended Danish Childhood Vaccination Programme during 2008 to 2020 will have received a total dose of 0.425 mg to 4.5 mg aluminium from vaccines administered during the first year of life (Figure 1). In Denmark, general practitioners carry out all childhood programme immunisations. They are reimbursed after reporting each vaccination to the Danish National Board of Health.[23] Here, all vaccines are assigned a 4-digit unique code, allowing for the identification of the manufacturer, type of aluminium adjuvant and the amount of aluminium in each vaccine.

2.3 | Aluminium adjuvants

During our study period, DTP vaccines were either adsorbed to hydroxide alone or a combination of hydroxide/phosphate, whereas PCV vaccines solely contained phosphate. Hence, we divided adjuvants into two main groups: hydroxide + phosphate or hydroxide/phosphate + phosphate for each DTP+PCV appointment. The dose of aluminium in each of the vaccines in our study ranged from 0.125 to 1 mg per vaccine (Figure 1). For each DTP+PCV appointment, the total amount of aluminium injected could vary between 0.425 and 1.5 mg depending on the vaccine combination: hence we defined the total amount of aluminium for each of the first three vaccination appointments with DTP+PCV vaccines as either high (≥1.0 mg) or low (<1.0 mg).

2.4 | Vaccination granulomas

Vaccination granulomas are long-lasting subcutaneous nodules occurring at the vaccine injection site, usually appearing weeks or even months after vaccination. They are characterised by an intense itch and local skin changes, such as hypertrichosis, hyperpigmentation, and eczema. Granulomas are associated with aluminium contact allergy, and may persist for several years.[5,7,24]

The cases in our study were identified from approved claims of vaccination granulomas via the Danish Patient Compensation Association, an independent body dealing with all compensation claims in connection with medical treatment. The first claim for vaccination granulomas identified in this database was in 2009.

2.5 | Child and maternal level risk factors

Gestational age (≤36 weeks, 37-41 weeks and ≥42 weeks) was obtained from the Danish Medical Birth Register.[25] We defined a sibling with a vaccination granuloma as an older sibling with an approved claim of a vaccination granuloma at the time the index child received her/his first dose of DTP+PCV.

Maternal ethnicity was defined by maternal country of birth (Denmark or the rest of the world), and maternal age at the time of child´s birth was considered a categorical variable (younger than 20 years, 20 to 39 years, and 40 years or older).

Missing values were only present for gestational week (1.1%); in our statistical analyses, missing values were considered a separate category.

2.6 | Statistical analysis

We followed each child in the cohort from the first administration of DTP+PCV vaccines and until end of follow-up on December 31, 2020, emigration, death, or until the occurrence of a vaccination granuloma, whichever event came first.

The resulting incidence rates were analysed with Poisson regression, a log-linear regression analysis on the incidences with the logarithm of follow-up time as offset. This yielded rate ratios according to potential risk factors. Rate ratios with 95% confidence intervals not crossing 1.0 were considered statistically significant.

Risk factors were divided into three descriptive levels: the vaccine level (dose number, the adjuvant combination, dose of aluminium, age at first DTP+PCV appointment, and interval between vaccines), the child level (sex, gestational age and sibling with granuloma) and the maternal level (ethnicity and age at child´s birth). Potentially relevant sets of confounders were pre-defined separately for each potential risk factor of interest (supplementary table 1).

In the analyses of vaccine level risk factors, we considered completed appointments as a time-varying variable where each child contributed follow-up after the latest completed appointment according to the characteristics of the vaccines received (dose number, adjuvant combination, dose of aluminium and dose interval) until the next appointment.

Calendar periods were divided into 2-year intervals and age was divided at 0.5, 1, 2, and 5 years. In Denmark, the recommended age for receiving the first dose of DTP+PCV is 3 months of age, and we constructed a categorical “age at first vaccination” variable (before 2.5 months of age, 2.5 to 3.4 months of

age and after 3.5 months of age). Based on the recommended dose interval as presented in Table 1, we constructed a categorical "dose interval" variable as either "recommended", with a minimum interval of 2 months between the first and second DTP+PCV or "less than recommended", if the interval between doses was shorter than 2 months. Between the second and third DTP+PCV, the recommended interval was minimum 6 months, with less than 6 months between doses being "less than recommended".

P-values for tests of association between cohort characteristics and granuloma status were calculated using Chi-Squared tests.

P-values for tests of homogeneity of effect across different risk factor levels were calculated using Analysis of Variance (ANOVA) tests.

R statistical software version 4.1.0 (R Project for Statistical Computing) was used for data management and statistical analyses. We used the Epi package to construct follow-up intervals and the stats package for Poisson regression analyses. Data analysis took place from September 2021 to February 2022.

3 | Results

3.1 | Study cohort

We identified 594 787 children born in Denmark January 1 2009 to December 31 2018, with 2 751 children having a recorded vaccination granuloma during the January 1 2009 to December 31 2020 period. Exclusions and censorings are shown in Figure 2. The resulting cohort consisted of 553 932 children with 1 901 (0.34 %) vaccination granulomas.

Cumulative incidence curves according to child's age for each of the three DTP+PCV appointments are shown in Figure 3. The cumulative incidence after the 3rd appointment (1.07%) was the largest compared to the 1st (0.42%) and 2nd (0.42%) appointments. Most granulomas occurred before 2 years of age (96.11.%).

Characteristics of the cohort children are presented in Table 2. The median age at first DTP+PCV vaccine appointment was 3.1 months for children both with and without vaccination granulomas (IQR 3-3.4 and 3-3.5, respectively). The median (IQR) age at granuloma onset was 12.2 (5.5-12.9) months. The majority of children in both groups (73.2% with vaccination granulomas and 74.0% without) were born at gestational week 37-41, of Danish-born mothers (94.8% with vaccination granulomas and 88.4% without), and of mothers aged 20-39 years (95.9% with vaccination granulomas and 95.2% without). The calendar period 2015-2016 contributed the greatest proportion of vaccination granulomas to the cohort (32.0%).

3.2 | Vaccine level risk factors

For all three DTP+PCV appointments combined, the risk of vaccination granulomas significantly decreased with the adjuvant combination of hydroxide/phosphate (DTP) + phosphate (PCV), compared to the hydroxide (DTP) + phosphate (PCV) combination (adjusted RR 0.58, 95% CI 0.48-0.70) (Table 3). Vaccines with a high dose of aluminium yielded a significantly higher risk compared to vaccines with lower doses of aluminium, adjusted RR 1.34 (95% CI 1.19-1.52).

There were no statistically significant differences in the risk of vaccination granulomas between children following or deviating from the recommended interval between vaccination appointments.

3.3 | Child level risk factors

Girls were at a statistically significant higher risk of having a vaccination granuloma, adjusted RR 1.12 (95% CI 1.02-1.22) (Table 3). Premature birth significantly decreased the risk of vaccination granulomas compared to full term birth (adjusted RR 0.71, 95% CI 0.54-0.93).

Among children who had a sibling with a vaccination granuloma at the time of receiving their own first DTP+PCV vaccines, the risk increased remarkably (46.15, 95% CI 33.67-63.26), when compared to children who did not have a sibling with a vaccination granuloma.

3.4 | Maternal level risk factors

Mothers were primarily Danish-born; compared with Danish-born mothers, the risk was reduced (0.51, 95% CI 0.42-0.63) for mothers born outside Denmark.

Children born of mothers under the age of 20 were at lower risk of getting a vaccination granuloma (0.46, 95% CI 0.25-0.83), compared with mothers aged 20 to 39 years.

3.5 | Prevalence of vaccination granulomas at 2 years of age

The prevalence of granulomas at 2 years of age among selected subgroups of cohort children with 3 completed vaccination appointments was as follows: Girls with ≥ 1 sibling with a granuloma, born to term of mothers with any ethnicity, and vaccinated with hydroxide (DTP) + phosphate (PCV) adsorbed vaccines, had the highest prevalence (5.00% (2.65% to 9.23%)) for the high dose of aluminium, and 4.26% (1.67% to 10.44%) for the low dose of aluminium, respectively). In contrast, the prevalence was 0.34% (0.33% to 0.36%) among children with no sibling with a granuloma and with any combination of maternal ethnicity, sex, gestational age, aluminum dose and adjuvant used. Results are shown in supplementary table 2.

4 | Discussion

4.1 | Principal findings

In this nationwide cohort study of 553 928 Danish-born children, we found that both the type of aluminium adjuvant, and the dose of aluminium per DTP+PCV appointment in the Danish Childhood Vaccination Programme significantly increased the risk of developing vaccination granulomas.

PCV vaccines are only phosphate adsorbed, whereas DTP vaccines may contain both adjuvants, but are usually hydroxide adsorbed. Both aluminium salts are able to induce granulomas.[26] Hydroxide is retained longer than phosphate at the injection site.[27] This retainment could be a contributing factor to the development of granulomas and increased risk of sensitisation to aluminium.

All DTP and PCV vaccines available for administration during our study is shown in Figure 1. We observed a significantly lower incidence of granulomas following vaccination with the Infanrix Hexa vaccine, containing both adjuvants (0.5 mg hydroxide +0.32 mg phosphate), than with the DiTeKiPol/Act-Hib, containing 1.0 mg hydroxide. Another important risk factor is the dose of aluminium. Looking solely at the amount of hydroxide, there is twice as much of this specific adjuvant in the DiTeKiPol/Act-Hib than in the Infanrix Hexa. This could imply that although we show that a high level of aluminium increases the risk of granulomas, the type of adjuvant could be of greater importance.

Only a minority develop vaccination granulomas, and thus predisposing factors at the individual-level likely exist. In this study we found indications of higher risk in girls, similar to what has been described earlier.[5] Vaccination granulomas are almost always associated with contact allergy to aluminium, which is considered the main aetiology. It is well known that more women than men develop contact allergy, which may be due to differences in exposure,[28,29] although a recent study did not find a statistically significant difference between females and males in regard to aluminium contact allergy.[30] Some experimental evidence support that there is a difference in susceptibility between sexes.[31]

While the aggregation of vaccination granulomas among siblings does hint at the possibility of genetic susceptibility, we speculate that parents with prior experience of granulomas are more likely to pursue

compensation and that this may at least in part explain the association.[5] This speculation is also reflected in the calendar period trends; from 2014 the number of reported cases to the Danish Patient Compensation Association rose drastically with a peak during 2015-2016 where the condition was discussed in national newspapers.[32]

We observed a lower rate of granulomas among children born preterm compared to term. Preterm infants have been shown to have an overall lower incidence of local injection site side effects following DTP vaccination.[33]

We found that international mothers have children who are at lower risk of getting a granuloma when compared to children with Danish-born mothers. The studies by Bergfors et. al. did not find ethnicity to be decisive but did mention the possibility of heredity.[5] Our finding are most likely a reflection of accessibility/awareness to information and compensation for internationals who do not speak Danish, but the possibility of predisposing or protective genetic factors cannot be ruled out and requires further investigation.

4.2 | Comparison with other countries

Childhood vaccination schedules vary amongst countries, both regarding recommended vaccines, number of doses and age of administration.[34] Thus, a child following the UK recommended schedule will receive up to 4.335 mg aluminium through vaccines within the first two years of life. In other countries throughout the world, including the United States, immunisation start at birth with the first of three vaccinations against Hepatitis B, also aluminium adsorbed. A child following the US schedule will thus be exposed to between 1.68 and 6.0 mg aluminium from birth and up to 2 years of age.[35] Until now, the largest study on incidence rates originates from the Swedish vaccine trial study by Bergfors et.al.[5] There is only one study on incidence rates outside Scandinavia, an Australian study reporting a total of 49 granulomas over a decade, [36] but vaccination granulomas are regularly described on a case-basis in many countries, [37] and consequently our

results are of relevance to all children receiving aluminium adsorbed vaccines, although schedules may vary.

4.3 | Strengths and limitations

Our study is the largest to date of vaccination granulomas, and we based our results on high quality individual health records available for research in Denmark, eliminating potential concerns regarding selection- and recall bias. Some limitations do apply to our study. We know from clinical experience that many vaccination granulomas go unnoticed, and a concern is that the total number of granulomas in our study is likely underestimated. Thus, ascertainment bias, whereby some families are more likely to pursue and receive a diagnosis and subsequently receive compensation, is a possibility in our study and should be considered when interpreting our results.

The claims from the Danish Patient Compensation Association does not provide information as to what site the children developed granulomas. DTP and PCV vaccines are administered at the same appointment but in each thigh. In the claims, parents had listed both vaccines as causal, hence our decision to combine the vaccines into appointments instead of looking at them individually, as we know from previous studies that children may have more than one granuloma.[14,26] The associations with granuloma siblings and non-Danish born mothers may be prone to ascertainment bias.

4.4 | Policy implications, future research, and conclusions

Vaccine safety in general is often scientifically reviewed by expert panels, and although the safety of aluminium adjuvants is strongly reassuring,[38] diminishing the dose of aluminium or changing the type of aluminium adjuvant from hydroxide to phosphate could be desirable in order to reduce the risk of

vaccination granulomas. This should be carefully weighed against the possibility of reduced immunogenicity.[12]

The World Health Organization (WHO) estimates that 2 to 3 million children are saved each year due to the current immunisation programmes, but despite this, vaccine hesitancy is a rising challenge.[18] Anecdotal reports and small animal studies suggesting spurious associations between aluminium and various severe diseases such as autism and autoimmune-related diseases are regularly being published,[39,40] and although none of these claims have been verified in well-controlled studies, it leads to parental concern and vaccine hesitancy.[41–43]

In our study, by far the biggest risk factor was having a sibling with a vaccination granuloma, and this increased risk among siblings require attention. Further studies on the genetics of granulomas, as well as studies on the long-term prognosis of children with vaccination granulomas, are warranted.

Contributors

All the authors designed the study. JDJ and AH supervised the study and obtained funding. SH and AH obtained the data. SH and AH directed the analysis, which was carried out by EMT. All authors participated in interpretation of the results. SH wrote the initial draft, and all authors revised the manuscript, approved the final version and met the ICMJE criteria for authorship. SH, EMT and AH had full access to all data and takes responsibility for the accuracy of the data analysis.

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Ethical approval

The study was conducted using administrative register data. According to Danish law, ethical approval is not required for such research.

Data availability statement

The datasets analysed during the current study are not freely available owing to national regulations, but similar data are accessible to authorized researchers after application to the Danish Health Data Authority. The lead author (SH) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned (and, if relevant, registered) have been explained.

Dissemination to participants and related patient and public communities

The results of the study will be disseminated through social media postings, press releases, and interviews explaining the result to news media and to the general public.

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Table 1. The Danish Childhood Vaccination Programme since 2007 including the recommended minimum and maximum intervals between doses.

Age	Vaccination against	Recommended interval	Min.	Max.
3 months	Diphtheria-Tetanus-Pertussis-Polio-Hib (DTP) 1 and Pneumococcal conjugate *vaccine* (PCV) 1	Between 1. and 2. injection	2 months for DTP 1 month for PCV	None
5 months	Diphtheria-Tetanus-Pertussis-Polio-Hib 2 and PCV-2	Between 2. and 3. injection	6 months for DTP 2 months for PCV	None
12 months	Diphtheria-Tetanus-Pertussis-Polio-Hib 3 and PCV-3			
15 months	Measles, Mumps, Rubella (MMR) 1	Between 1. and 2. injection	1 month	None
4 years	MMR 2			None
5 years	Diphtheria-Tetanus-Pertussis-Polio booster	From last DTP vaccine	3 years	None
12 years†	MMR 2 – if the child has not yet received 2 MMR-vaccines			None
12 years‡	Human Papilloma Virus HPV 1 and 2	Between 1. and 2. injection	5 months	13 months

† MMR vaccination of 12-year old children was ceased in 2016
‡ HPV vaccination was introduced in 2009

Table 2. Characteristics of 553 932 cohort children born in Denmark 2009 to 2018 by granuloma status during study follow-up.

	Children with granulomas N (%)	Children without granulomas N (%)	*P*-value*
Age at first DTP+PCV vaccine appointment (months), median (IQR)	3.1 (3-3.4)	3.1 (3-3.5)	
Age at granuloma debut (months), median (IQR)	12.2 (5.4-12.9)	-	
Sex			.02
Male	923 (48.6%)	283187 (51.3%)	
Female	978 (51.4%)	268844 (48.7%)	
Sibling(s) with granuloma			<.01
0	1861 (97.9%)	551400 (99.9%)	
≥1	40 (2.1%)	631 (0.1%)	
Granulomas by calendar period			
2009-2010	44 (2.3%)	-	
2011-2012	265 (13.9%)	-	
2013-2014	494 (26.0%)	-	
2015-2016	608 (32.0%)	-	
2017-2018	426 (22.4%)	-	
2019-2020	64 (3.4%)	-	
Gestational week			.01
≤36	53 (2.8%)	22707 (4.1%)	
37-41	1391 (73.2%)	408342 (74.0%)	
≥42	434 (22.8%)	114760 (20.8%)	
Missing	23 (1.2%)	6222 (1.1%)	
Maternal ethnicity			<.01
Denmark	1802 (94.8%)	488082 (88.4%)	
Other	99 (5.2%)	63949 (11.6%)	
Maternal age at child´s birth			.08
<20 years	11 (0.6%)	6187 (1.1%)	
20-39 years	1823 (95.9%)	525590 (95.2%)	
≥40 years	67 (3.5%)	20254 (3.7%)	

* P-values calculated using Chi-squared tests for association.

Table 3. Risk of vaccination granulomas among 553 932 children born in Denmark 2009 to 2018 by vaccine-, child- and maternal level risk factors.

	Risk of vaccination granuloma per vaccine appointment			
	Cases	Person-years	RR# (95% CI)	*P*-value*
Appointments				<.01
Appointment 1	415	162242	1 (ref)	
Appointment 2	594	541127	1.48 (1.25-1.74)	
Appointment 3	880	1849199	1.26 (1.03-1.55)	
	Risk of vaccination granulomas for the three DTP+PCV appointments			
	Cases	Person-years	RR (95% CI)	*P*-value*
Vaccine level risk factors				
Adjuvant				<.01
Hydroxide (DTP) and phosphate (PCV)	1762	2365702	1 (ref)	
Hydroxide + phosphate (DTP) and phosphate (PCV)	127	186865	0.58 (0.48-0.70)	
Mg aluminium				<.01
<1.0 mg	338	1253200	1 (ref)	
≥1.0 mg	1563	2125297	1.34 (1.19-1.52)	
Age at first DTP+PCV				.02
2.5 to 3.4 months	1544	2582949	1 (ref)	
<2.5 months	6	14893	0.66 (0.30-1.48)	
≥3.5 months	351	780655	0.86 (0.76-0.96)	
Interval†				.34
As recommended	1572	3105461	1 (ref)	
Less than recommended	329	273036	1.06 (0.94-1.20)	
Child level risk factors				
Sex				.02
Male	923	1731450	1 (ref)	
Female	978	1647047	1.12 (1.02-1.22)	
Gestational week				<.01
37 to 41	1391	2500404	1 (ref)	
≤36	53	140044	0.71 (0.54-0.93)	
≥42	434	699111	1.11 (0.99-1.23)	
Missing	23	38937	1.14 (0.76-1.72)	
Sibling with granuloma‡				<.01
0	1861	3376053	1 (ref)	
≥1	40	2444	46.15 (33.67-63.26)	
Maternal level risk factors				
Maternal ethnicity				<.01
Denmark	1802	3040430	1 (ref)	
Other	99	338067	0.51 (0.42-0.63)	
Maternal age at child´s birth				.01
20 to 39 years	1823	3217490	1 (ref)	
<20 years	11	40687	0.46 (0.25-0.83)	

≥40 years	67	120319	1.02 (0.80-1.30)	

†As recommended by the Danish National Board of Health (Table 1).
‡A sibling with a granuloma at time of first DTP+PCV vaccination.
#Covariate adjustment sets for each potential risk factor is given in supplementary table 1.
**P*-values calculated using Analysis of Variance (ANOVA) tests. *P*-values <.05 indicates a statistically significant difference.

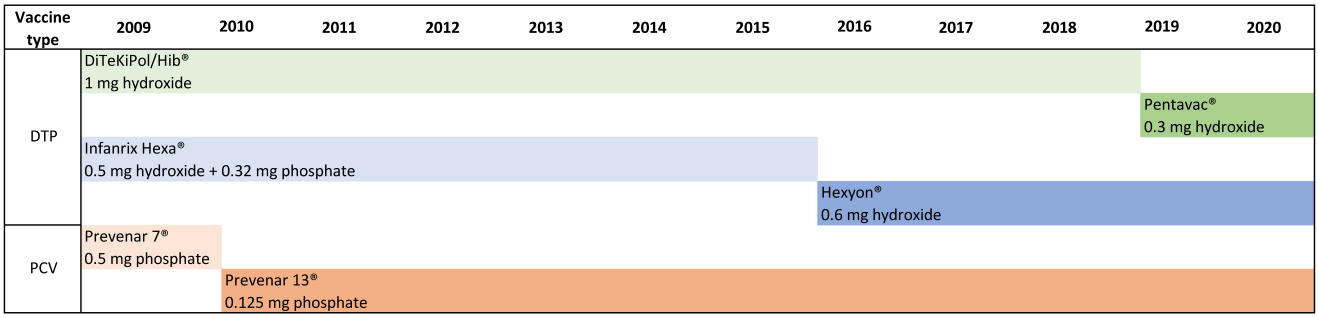
Figure legends

Figure 1. Overview of the vaccines available during our study period, including the amount of aluminium per dose and the type of aluminium adjuvant.

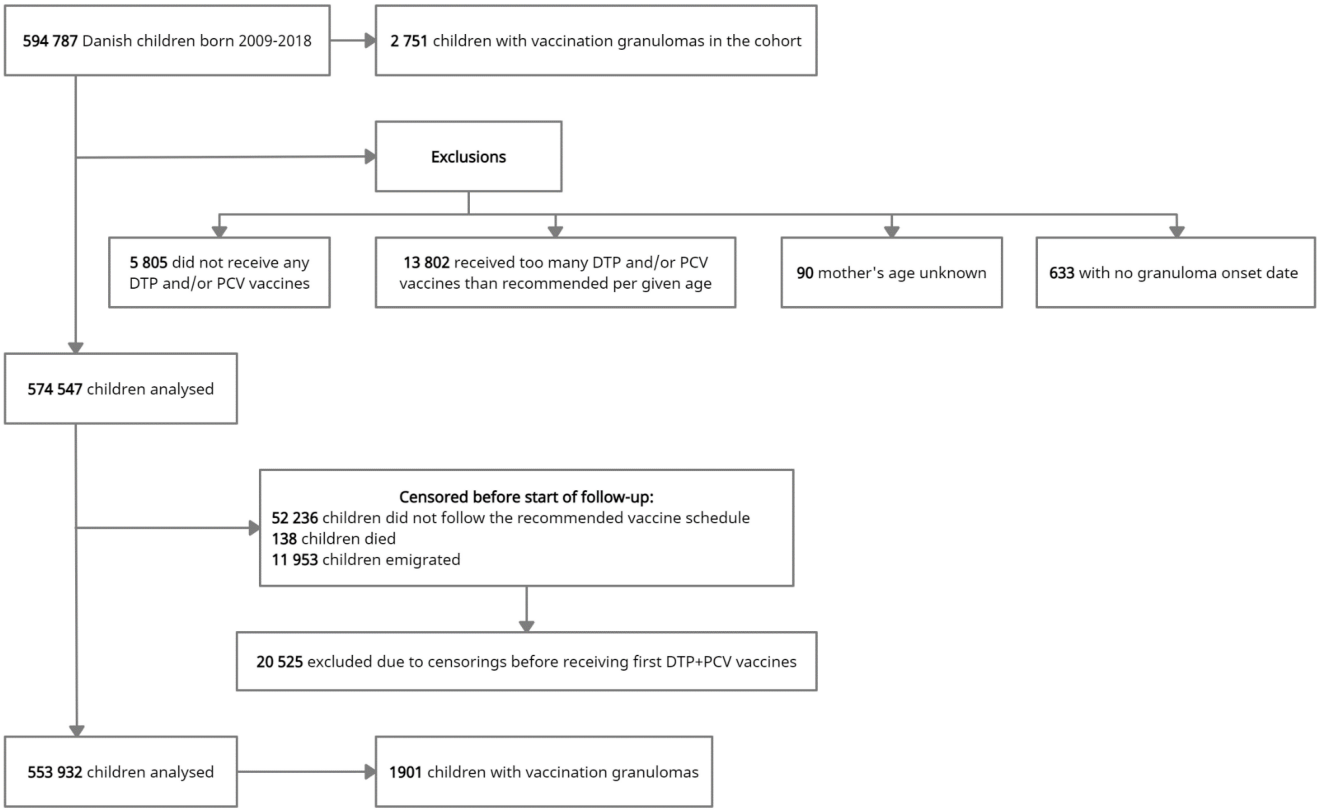
Figure 2. Study flowchart.

Figure 3. Cumulative incidence curves for each of the three DTP+PCV appointments.

The cumulative incidence after the 3rd appointment (1.07%) was the largest compared to the 1st (0.42%) and 2nd (0.42%) appointments. Most granulomas occurred before 2 years of age (96.11%). Shaded areas indicate 95% confidence bands.



COD_14180_Figure 1.jpg



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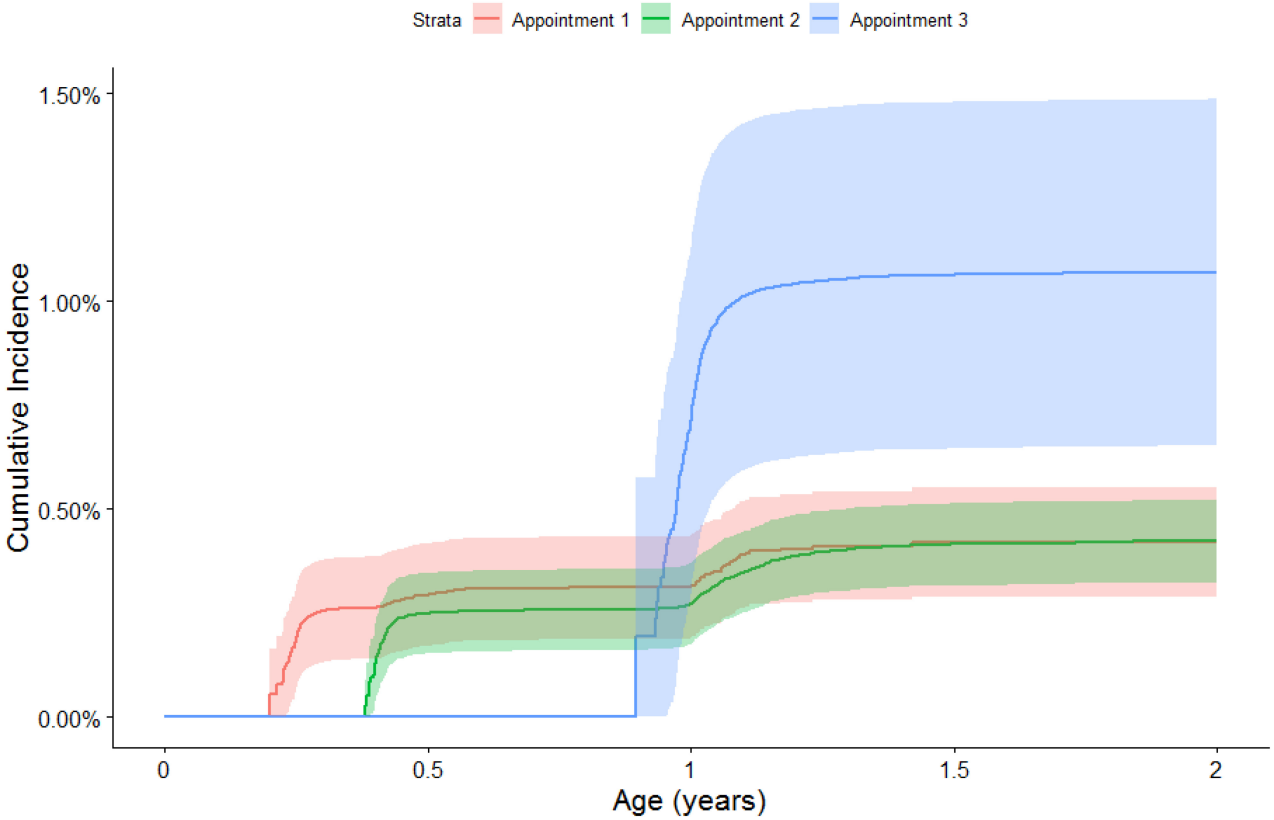

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