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INVESTIGATIVE REPORT

Does Allergen-specific Immunotherapy Induce Contact Allergy to Aluminium?

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Persistent, itching nodules have been reported to appear at the injection site after allergen-specific immunotherapy with aluminium-precipitated antigen extract, occasionally in conjunction with contact allergy to aluminium. This study aimed to quantify the development of contact allergy to aluminium during allergen-specific immunotherapy. A randomized, controlled, single-blind multicentre study of children and adults entering allergen-specific immunotherapy was performed using questionnaires and patch-testing. A total of 205 individuals completed the study. In the 3 study groups all subjects tested negative to aluminium before allergen-specific immunotherapy and 4 tested positive after therapy. In the control group 4 participants tested positive to aluminium. Six out of 8 who tested positive also had atopic dermatitis. Positive test results were found in 5/78 children and 3/127 adults. Allergen-specific immunotherapy was not shown to be a risk factor for contact allergy to aluminium. Among those who did develop aluminium allergy, children and those with atopic dermatitis were more highly represented. ***Key words: aluminium allergy; atopic dermatitis; allergen-specific immunotherapy; itching nodules; adjuvant.***

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The development of persistent itching nodules at the injection site after allergen-specific immunotherapy (ASIT) with aluminium-precipitated antigen extract has been described in several reports (1–5), as it has after vaccination with aluminium-adsorbed vaccines (6–13). However, it is considered a rare event. Contact allergy to aluminium in individuals with persistent itching nodules after ASIT has been reported since 1980 (14), and after immunization since 1985 (8). In a report from Gothenburg, Sweden, persistent itching subcutaneous nodules at the injection site were described in children vaccinated with aluminium hydroxide-adsorbed acellular monocomponent pertussis vaccine (aP), after combined diphtheria-tetanus-acellular pertussis vaccine (DTaP), and after diphtheria-tetanus toxoid-vaccine (DT). These vaccines were all manufactured by Statens Serum Institut (SSI), Copenhagen, Denmark. Contact allergy to aluminium was demonstrated in 77% of 645 children with itching nodules (15). The lesions were first observed after subcutaneous injection, but also occurred after intramuscular injection. An additional 19 cases of persistent pruritic nodules and contact allergy to aluminium after injection of commonly used aluminium-adsorbed vaccines have been reported from Sweden (16).

Vaccines containing aluminium hydroxide have been implicated in a rare muscular disease, macrophagic myofasciitis, characterized by diffuse myalgia and aluminium-containing cytoplasmic inclusions in macrophages in the deltoid muscle (17). The observation suggested that aluminium may remain at the site of injection for a considerable time, but the connection between these findings and the disease has been deemed uncertain, and in 2008 the World Health Organization (WHO) Global Advisory Committee on Vaccine Safety stated that there was no reason to conclude that there is an association between macrophagic myofasciitis and aluminium-containing vaccines (18).

A cross-sectional study on children who had undergone ASIT at a paediatric unit at Halmstad County Hospital, Sweden, reported contact allergy to aluminium in 8/37 patients (19). Among those 8, 6 were found to have persistent itching nodules. In a group not exposed to ASIT no cases with aluminium allergy were detected. The conclusion from this study was that there probably is an association between contact allergy to aluminium and persistent subcutaneous nodules.

The aims of the present study were: (*i*) to investigate whether ASIT with allergen preparations containing aluminium hydroxide induces contact allergy to aluminium; (*ii*) to investigate whether development of aluminium allergy is linked to persistent itching nodules; (*iii*) to determine whether aluminium allergy can be detected in children and adults with an underlying allergic disease who have not been treated with ASIT; and (*iv*) to explore other possible risk factors for the development of contact allergy to aluminium.

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MATERIALS AND METHODS

Design

This epidemiological investigation uses data from a randomized, controlled, single-blind multicentre study of children and adults who started treatment with ASIT in routine care during 2007 and 2008. The participants starting ASIT in the autumn of 2007 were randomly assigned sequence numbers in the database in order to facilitate blind assessment, and were divided into 3 randomly generated groups with different schedules of patch-testing (see below), all labelled "exposed". All individuals in the 3 groups were patch-tested with aluminium immediately before the start of ASIT. The test was read by the participant and the result was registered in a protocol that was placed in a sealed envelope. During the ASIT, the participants were patch-tested with aluminium at different intervals, depending on which randomization group they belonged to, with the last testing for all of them being made after one year (Fig. 1). The reading dermatologist was blind to these results. After one year of ASIT, at the study termination, all groups were in addition to aluminium also patch-tested with allergen extracts used for ASIT and a baseline series routinely used to detect contact allergy.

The investigator examined the limbs with respect to itching nodules at the study start and after one year of ASIT, immediately before the patch-test.

To exclude that a possible contact allergy to aluminium in the exposed individuals could be explained by aluminium sensitization through the patch-test, the participants in groups 1–3 were patch-tested with aluminium 1, 2 or 3 times during one year of ASIT (Fig. 1). After one year, a control group, which had not yet started ASIT, was included to exclude altered or increased aluminium exposure in the environment as an explanation for a possible development of contact allergy to aluminium.

The control group, labelled "unexposed", consisted of children and adults, who were intended to start ASIT in the autumn of 2008. These atopic patients were asked to take part in the study before their treatment started. The control group, like the exposed groups (groups 1–3), were randomly assigned sequence numbers in the database in order to facilitate blind assessment. The controls were patch-tested with the same baseline series supplemented with aluminium and allergen extracts used for ASIT, as the exposed participants. On all patch-testing and reading days, both exposed and unexposed individuals were included in a randomized way. The patch-test readers were blind to whether the patch-tested individuals had undergone ASIT for one year or had not yet started ASIT (Fig. 1).

The study protocol also included a statistical power calculation based on a retrospective Swedish study in patients undergoing ASIT and in unexposed atopic control patients where contact allergy to aluminium was found in 22% of the exposed individuals and in 0% of the unexposed individuals (19). Assuming 5% contact allergy to aluminium in the exposed individuals and 0.1% in the unexposed, 500 individuals (250 each of exposed and unexposed) would give a statistical power of 97% to detect this difference between exposed and unexposed atopic individuals.

Study population/participants

A total of 202 children and 349 adults with allergic rhinoconjunctivitis and/or asthma and/or allergy to insect venoms who were scheduled to start treatment with ASIT during 2007 and 2008 at 14 medical units in southern Sweden were asked to participate in the study. Medical contraindications for participation were limited to anaphylaxis at previous skin testing. Before inclusion and after oral information about the study procedure as well as inclusion and exclusion criteria, a written informed consent was obtained from adult participants, children ≥ 15 years of age and parents/guardians to children below 15 years of age.

Questionnaire

At the study start all participants answered a questionnaire containing questions regarding atopic diseases, metal sensitivity, piercing, use of antiperspirants, aluminium-containing medication, vaccinations and other sources of non-occupational and occupational aluminium exposure.

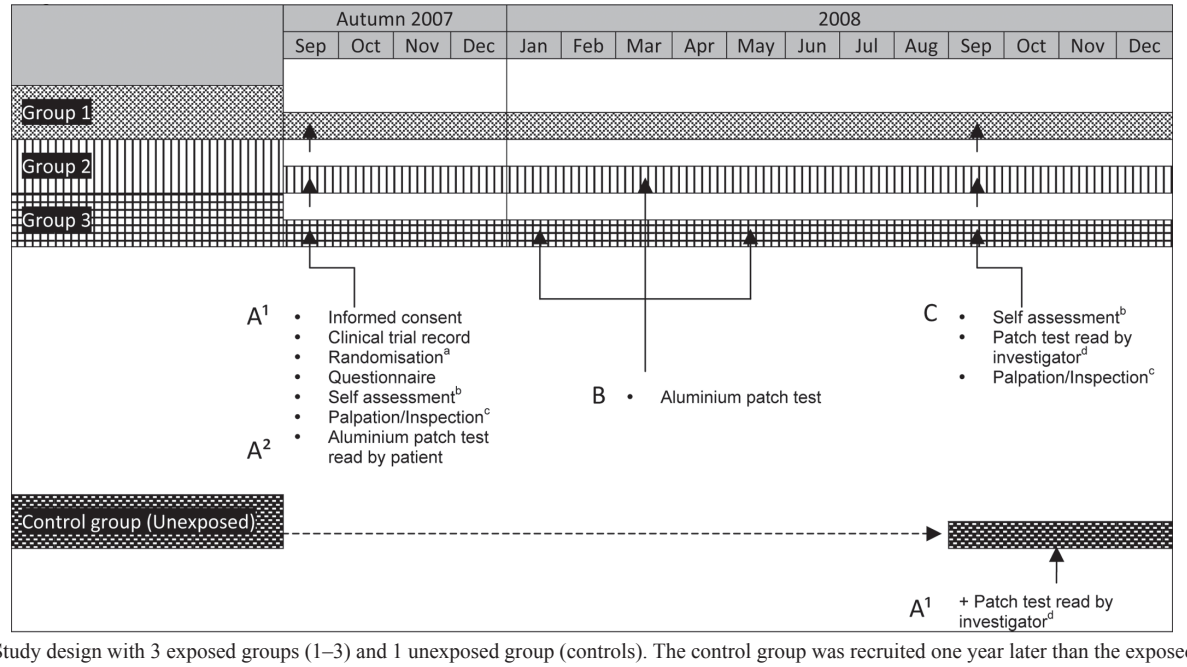

Fig. 1. Study design with 3 exposed groups (1–3) and 1 unexposed group (controls). The control group was recruited one year later than the exposed group. [a]Randomly assigned database sequence numbers in order to facilitate blind assessment and randomized group belonging. [b]The subject's assessment of itching. [c]Palpation of possible nodules and inspection of signs of pruritus (scratch mark) by the investigator. [d]Patch-test with the European baseline series supplemented with aluminium preparations and antigen extracts.

Physical examination

A physical examination with visual inspection and palpation of the injection sites, i.e. both upper arms, was performed before the study start and for the exposed groups also before the final patch-test session.

Definition of persistent nodule with or without itching

A nodule at the injection site was defined as the presence of a discrete or well-demarcated soft tissue mass or lump that was firm *and* was located at the injection site (for the purpose of this investigation the upper arms) but with no abscess formation, erythema or warmth (20).

Self-assessment of the presence of pruritus

Both exposed and unexposed individuals or their parents/guardians, were asked about the presence of pruritus before treatment with ASIT and, for the exposed group, also after one year of treatment with ASIT before the application of the patch-test.

Patch-testing

Patch-testing was performed with the European baseline test series (21), slightly modified and supplemented with aluminium preparations and allergen extracts used for ASIT. The allergen extracts were 3-trees (alder, birch, hazel), 5-grass pollen mixture, mugwort (*Artemisia vulgaris*), cat dander, dog dander, 2-Dermatophagoides mites, honeybee venom and yellow jacket venom, all being Alutard SQ 100 000 SQ-E/ml manufactured by ALK-Abelló A/S, Hørsholm, Denmark. Each of these preparations contains aluminium hydroxide, 3.3 mg/ml. The allergens were applied onto IQ chambers on a non-woven adhesive tape (Chemotechnique Diagnostics, Vellinge, Sweden). The tests were applied to the upper part of the back and left for 48 h. 25 µl of liquid test preparations were micropipetted onto the IQ chamber. For petrolatum preparations the amount of test preparation applied was approximately 30 mg. Aluminium chloride hexahydrate (MP Biomedicals, Inc. Eschwege, Germany) in white petrolatum (Pharmacy, Skåne University Hospital, Malmö, Sweden) at 2.0% w/w, 10.0% w/w and 20.0% w/w prepared at our laboratory and an empty Finn Chamber (diameter 8 mm; Epitest Ltd Oy, Tuusula, Finland) were used to trace aluminium allergy. Everyone was tested with an empty Finn Chamber and aluminium chloride hexahydrate at 2.0% and 10.0%. The 20.0% aluminium chloride hexahydrate preparation was tested only on those who had a doubtful reaction to any aluminium preparation on the first reading occasion.

This paper presents the patch-test results with aluminium preparations and allergen extracts.

Patch-test reading

To evaluate the patch-test reactions in an unbiased manner, test patients and controls were read randomly; thus the reading dermatologist did not know whether the individual to be read was exposed or unexposed. Furthermore, the reading dermatologist was also blinded to all data collected and to the results of the physical examination. The tests were read according to ICDRG guidelines (22) on days 3 (D3) and 7 (D7) The retest with 20.0% aluminium chloride hexahydrate was only read once on D7, i.e. 4 days after the application on D3.

Statistical analysis

The non-parametric Mann–Whitney *U* test was used when comparing ordinal and continuous outcome variables between 2 independent groups, for example exposed vs. controls. When comparing binary outcome variables, for example frequencies of Al^+ and Al^-,Fisher's exact test was used. McNemar's test was used to compare 2 proportions estimated from paired observations, for example baseline vs. follow-up observation. Power calculation in the design phase was conducted in StatXact-6 (Cytel Software Corp., Cambridge, MA, USA). All data analyses were performed using SPSS 17.0 for Windows (SPSS Inc., SPSS IBM Corp., Armonk, NY, USA). Exact methods for *p*-value calculations were used throughout. *p*-values < 0.05 were considered statistically significant.

Ethics

The Regional Ethical Review Board in Lund, Sweden, approved the study on 26 June 2007 (Dnr 277/2007). Written informed consent was obtained from adult participants, children 15 years of age and above, and parents/guardians.

The trial has been registered in the ISRCTN database (www.isrctn.org) number: ISRCTN57796160.

RESULTS

In total 551 individuals at 14 medical units in southern Sweden were invited to participate in the study. A total of 248 (45%) individuals participated; 86 children (35%) and 162 adults (65%). In total 83% (78 children and 127 adults) took part in the final patch-testing with the baseline series supplemented with aluminium and allergen extracts used for ASIT. The numbers in the various groups are given in Fig. 2.

There were, in general, no differences between the groups when comparing the answers in the questionnaire (Table SI; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1409) nor were there any relevant differences between the groups when comparing the information collected in the Case Report Form (CRF) by the investigator (Table SII; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1409).

When comparing baseline values with respect to prevalence of contact allergy to aluminium, there was a significant difference between the exposed and unexposed groups (0/133 vs. 4/72; $p = 0.01$). During follow-up 4/133 (3.0%) participants developed contact allergy to aluminium in the exposed group ($p = 0.12$ for comparison of Al+ prevalence at baseline and at follow-up). In total, contact allergy to aluminium was found in 8 participants (8/205; 3.9%), 4 in the exposed groups after one year of ASIT and 4 in the unexposed group (4/133 vs. 4/72, $p > 0.3$). The median age in the group with contact allergy to aluminium was 15.5 (9–31) years and in the group without contact allergy to aluminium 27.0 (5–74) years with no significant difference between the groups ($p = 0.11$). Contact allergy to aluminium was numerically, but not statistically, overrepresented in females (6 positive among 102 females, 2 positive among 103 males; $p = 0.17$). The patch-test reactions to aluminium chloride hexahydrate in petrolatum and to an empty Finn Chamber in the group testing posi-

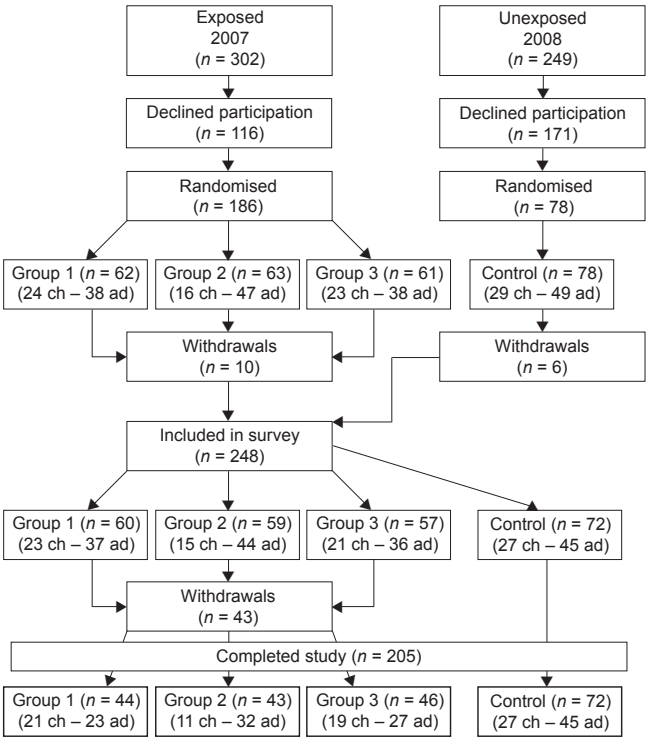

Fig. 2. Study participants. ch: children; ad: adults.

tively to aluminium are given in Table SIII (available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1409). There were no positive reactions to the empty Finn Chamber. Out of the 8 aluminium-hypersensitive patients, 7 tested positively to aluminium chloride hexahydrate at 10%, while 5 tested positively to 2%. To diagnose contact allergy to aluminium, a second reading after 7 days was necessary for 3 patients, of which 2 had a doubtful reaction on day 3.

When comparing the randomized groups there was no statistical difference in development of contact allergy to aluminium between those who had been patch-tested 1, 2 or 3 times (2/44, 1/43, 1/46; $p > 0.3$).

Six of the 8 participants with contact allergy to aluminium had or had previously had atopic dermatitis, and 4 of those 6 were in the exposed group. In the exposed individuals contact allergy to aluminium was overrepresented in individuals with atopic dermatitis (4 of 50 vs. 0 of 79; $p = 0.021$), but not in unexposed individuals (2 of 31 vs. 2 of 37; $p > 0.3$) (Table SIV; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1409). In the control group 2 of the 4 aluminium-hypersensitive individuals reported that they had experienced a rash from deodorants (Table SIII).

Table SIV gives information on baseline values in both unexposed and exposed individuals as well as values after treatment for one year on nodules and pruritus in the exposed group. Assessment by the physician before ASIT and after one year of treatment revealed a significant increase in nodules (0 of 130 vs. 23 of 130; $p < 0.001$). The same degree of significance was obtained when the number of nodules in unexposed atopic individuals at baseline was compared with the number of nodules in exposed atopic individuals after treatment for one year (0 of 72 vs. 23 of 130; $p < 0.001$).

There was no association between atopic dermatitis and the development of nodules in the exposed individuals (9 of 48 vs. 13 of 78; $p > 0.30$).

In the exposed group significantly more individuals reported pruritus and significantly more individuals were judged by the investigator to have scratch marks on at least one arm after one year of ASIT than before the start of the treatment (56/94 vs. 12/94; $p < 0.001$ and 10/98 vs. 2/98; $p = 0.039$). When unexposed and exposed subjects were compared concerning the investigators' evaluation of pruritus there was no difference regarding the baseline values (0/68 vs. 2/98; $p > 0.30$). A difference was, however, shown when unexposed individuals at baseline were compared with the exposed group having received treatment for one year (0/68 vs. 10/98; $p = 0.006$). When pruritus on the arms was assessed by the individuals themselves the corresponding comparisons between unexposed and exposed individuals showed no significance between baseline values, but there was a difference between unexposed individuals at baseline compared with the exposed group having received treatment for one year (4/50 vs. 12/94; $p > 0.30$ and 4/50 vs. 56/94; $p < 0.001$, respectively). There was a numerical, but not statistically, significant association between the subjectively assessed pruritus on at least one arm after treatment for 1 year, and the presence of atopic dermatitis (27/36 vs. 37/65; $p = 0.087$).

In the exposed group, there was no statistically significant association between the presence of nodules investigator-assessed and self-reported pruritus after treatment for one year, but there was a statistically significant association between investigator-assessed nodules and pruritus after treatment for one year ($p > 0.30$ and $p < 0.001$, respectively).

Contact allergy to allergen extracts used for ASIT was demonstrated in one exposed patient without contact allergy to aluminium but with a positive prick test to cat allergen and a positive patch-test reaction to cat dander allergen extract. This patient was treated with ASIT but with allergen extracts other than cat dander.

DISCUSSION

The main aim of this study was to investigate whether ASIT with allergen preparations containing aluminium hydroxide would induce contact allergy to aluminium. The finding that contact allergy to aluminium developed in 4 exposed individuals is indeed based on a small number, but could nevertheless indicate that the aluminium allergy had been induced by the ASIT, particularly as there were no patch-test reactions to aluminium when the individuals were tested with aluminium chloride

hexahydrate at 10% immediately before the start of the therapy. This test was read by the exposed individuals themselves for practical reasons. It is easy to read a negative patch-test, but education and experience are required to read any kind of positive tests (irritant, allergic, and doubtful). In addition to induction of aluminium allergy during ASIT, the aluminium allergy in the exposed individuals could, at least theoretically, be explained by active sensitization through the patch-test procedure with aluminium test preparations or induction of aluminium allergy during the year when the ASIT took place due to exposure to an aluminium source not related to ASIT. In order to explore the possibility of active sensitization at patch-testing, the design was made with randomizing of the exposed individuals into 3 groups, patch-tested 2, 3 or 4 times with aluminium. Nothing indicated that the patch-testing with aluminium was significant for the induction of contact allergy to aluminium.

The control group was recruited to enable a blind patch-test reading and to explore whether a source of environmental aluminium exposure other than the allergen exposure could have induced aluminium allergy. Indeed, contact allergy to aluminium was also detected among the controls.

It should be noted that the main comparison of the present investigation is based on groups (i.e. exposed and controls) that are not randomized. Therefore, systematic differences between the groups with respect to environmental aluminium exposure, such as vaccination with aluminium-containing vaccines, cannot be excluded. This design does not allow us to study how aluminium allergy develops over time in the control group. However, this design was the only possible way with respect to ethics for each patient to be offered the best available treatment, i.e. ASIT.

The prerequisites used to test the hypothesis that ASIT induces contact allergy to aluminium under the assumptions made were not met, as only approximately half the required number of participants was recruited. We were able to include 248 patients out of 551 who started treatment with ASIT during 2007–2008 at 14 medical units in Sweden. There were 205 patients taking part in the final patch-test with aluminium chloride hexahydrate and the baseline series. The participation rates differ notably between the exposed and the unexposed groups. Generally, however, no clear differences in demographics or clinical characteristics were noted between the 2 groups. According to the investigators at the medical units the main reason for not participating was lack of time as the patients were studying or working and also that participants at some clinics had to travel to another clinic for the final patch-test. Furthermore, neither the participating clinics nor patients obtained any financial support.

Nevertheless, this study demonstrated a high proportion of contact allergy to aluminium (8 of 205; 3.9%) in atopic patients with allergic rhinitis and asthma, but it did not demonstrate, nor exclude, that ASIT is a risk factor for induction of aluminium allergy.

So far, contact allergy to aluminium has mainly been reported after vaccination (7–9, 11, 15, 16, 23–28) and after ASIT (2–5, 14, 19, 23). Whether the development of contact allergy found in our study is due to ASIT, previous vaccination, a new exposure in the environment or has another explanation, can only be speculated on. One possible reason could be differences in the vaccines that had been given at different ages as there was a tendency towards a lower age in the aluminium positive group. In 1996 the pertussis vaccines containing aluminium hydroxide as an adjuvant were reintroduced in the Swedish vaccination programme in combination with diphtheria and tetanus vaccine (29). Before 1996, except for large pertussis vaccine trials in 1991–1994 (30–32), all children in Sweden were offered a combined diphtheria and tetanus vaccine using aluminium phosphate as adjuvant. In the report from the Gothenburg area all 352 children with contact allergy to aluminium had received vaccination with aluminium hydroxide adsorbed vaccines (15). In 2002 the booster dose of the diphtheria-tetanus vaccine using aluminium phosphate was replaced by a vaccine using aluminium hydroxide as adjuvant (13). The use of other vaccines, such as hepatitis B vaccine and human papilloma virus (HPV) vaccine, containing adjuvants based on aluminium hydroxide has increased in the last few years. For example, one of the unexposed individuals who tested positively to aluminium had recently received 3 doses of HPV vaccine and another unexposed patient with contact allergy to aluminium had received 3 doses of hepatitis B vaccine. In our previous study (19) on aluminium allergy in patients undergoing ASIT treatment we found a higher frequency of aluminium allergy (22%) compared with the frequency in the present study. Possible explanations for this difference are more injections and higher total doses of aluminium injected as well as later follow-up with regard to the start with the ASIT treatment. This is supported by the finding of at least 3 children that have contracted contact allergy to aluminium after the termination of our study.

As expected, nodules appeared during the ASIT treatment at the injection sites on the upper arms (2–4, 19, 23, 33, 34). However, during the treatment a statistically significant number of treated individuals developed nodules (0/130 vs. 23/130; $p < 0.001$). Theoretically, the development of nodules could be ascribed to either the ASIT or an environmental factor not related to ASIT. However, the unexposed group investigated at the same time in a blinded manner did not demonstrate any nodules, strongly suggesting that ASIT caused the nodules (Table SV; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1409). The development of nodules was not associated with the presence of atopic dermatitis.

The baseline values of pruritus, as assessed indirectly by signs of scratching registered by the investigator, were present in a few individuals in both the exposed and unexposed groups. During the treatment a statistically significant number of exposed individuals developed signs of scratching on the upper arms ($p = 0.039$). Obviously, these indirect signs of pruritus are only manifested when the pruritus is severe. Therefore, when looking at the pruritus reported by the patients themselves a higher number of individuals in the exposed as well as in the unexposed group reported pruritus at the baseline level without any statistical significance between the groups ($p > 0.3$). Again, the self-assessed pruritus demonstrated a significant increase in individuals who were treated with ASIT for one year ($p < 0.001$). Once more, the increase in individuals with pruritus during treatment could theoretically be explained by ASIT or something in the environment not related to the therapy. However, the comparison at the same time regarding the presence of pruritus between the exposed individuals treated for one year and the unexposed individuals just before their start of treatment gave statistically significant differences, which indicated that it was ASIT that caused the pruritus. Furthermore, there was some indication that development of self-reported pruritus, but not the investigator-assessed pruritus, on the upper arms was associated with atopic dermatitis ($p = 0.087$).

In conclusion, this study demonstrated a high proportion (3.9%) of contact allergy to aluminium in atopic individuals with allergic disease, but the result does not necessarily imply that ASIT is a risk factor for induction of contact allergy to aluminium. Aluminium allergy was overrepresented in young individuals and in those with atopic dermatitis. There is a significant development of nodules and pruritus in exposed atopic patients during ASIT. There was some, albeit weak, evidence that the development of pruritus, but not nodules, is associated with atopic dermatitis. Future studies on a relationship between aluminium allergy and ASIT should include a longer treatment period and possibility to adjust for previous vaccinations with regard to total doses of aluminium obtained and type of aluminium compound used as adjuvant in the vaccines. The significance of atopic dermatitis for aluminium allergy should also be explored as well as the general clinical relevance of the demonstrated contact allergy to aluminium.

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The authors declare no conflicts of interest.

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