

Variation in aluminium patch test reactivity over time

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doi:10.1111/cod.12836

Summary

Background. Contact allergy to aluminium has been reported more frequently in recent years. It has been pointed out that positive patch test reactions to aluminium may not be reproducible on retesting.

Objectives. To investigate possible variations in patch test reactivity to aluminium over time.

Methods. Twenty-one adults, who had previously reacted positively to aluminium, were patch tested with equimolar dilution series in pet. of aluminium chloride hexahydrate and aluminium lactate, four times over a period of 8 months.

Results. Thirty-six of 84 (43%) serial dilution tests with aluminium chloride hexahydrate and 49 of 84 (58%) serial dilution tests with aluminium lactate gave negative results. The range of reactivity varied between a negative reaction to aluminium chloride hexahydrate at 10% and/or to aluminium lactate at 12%, and a positive reaction to aluminium chloride hexahydrate at 0.1% and/or to aluminium lactate at 0.12%. The highest individual difference in test reactivity noticed was 320-fold when the two most divergent minimal eliciting concentrations were compared.

Conclusions. The patch test reactivity to aluminium varies over time. Aluminium-allergic individuals may have false-negative reactions. Therefore, retesting with aluminium should be considered when there is a strong suspicion of aluminium contact allergy.

Key words: allergic contact dermatitis; aluminium; delayed hypersensitivity; patch testing.

Patch testing has been the gold standard for diagnosing contact allergy since Jadassohn introduced his 'application method' in 1895 (1–3). Many studies were performed in order to optimize the patch test procedure, and today it is a well-standardized method (1, 2, 4, 5). However, the topic of false-negative or false-positive patch test results has always been a matter of controversy (6).

Trials based on patch tests with duplicates of allergens applied symmetrically to the left and right sides of the patients' upper backs have shown limited concordance in obtaining double-positive or double-negative results (7–10). A test reaction that was positive on only one side was regarded as non-reproducible. The observed variation in patch test reactivity has been explained by methodological and immunological factors, for example uneven distribution of allergens in the vehicles, different amounts and poor adherence to the skin of applied test preparations, regional variations in skin absorption and responsiveness, and, for certain allergens such as nickel, also hormonal factors (11–15).

The loss of positive patch test reactions over time is also a known phenomenon. A number of longitudinal studies have analysed the reproducibility and variation

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Conflicts of interest: The authors have no conflicts of interest to declare.

Accepted for publication 2 May 2017

in patch test reactivity to metal salts such as nickel, cobalt and gold based on routine test procedures (14, 16–19). Varying test reactivity over time, including negative reactions, has been reported after repeated patch testing of nickel-allergic and palladium-allergic women (14, 20). Contact allergy to aluminium was not reproducible in 186 of 241 retested children (77%) several years after they had been diagnosed with aluminium allergy (21, 22).

At our department, we patch tested aluminium-allergic individuals with different aluminium test preparations in order to find an optimal aluminium compound and an optimal test concentration (3). In that previous study, 2 of 21 participants did not show any skin reaction. Five of 8 participants who had been diagnosed with contact allergy to aluminium by a positive reaction to aluminium chloride hexahydrate 2% pet. had negative or doubtful reactions to it in that study. One possible explanation for this phenomenon may be individual variation in test reactivity to aluminium. In the same study, patch testing with aluminium lactate in pet. showed positive reactions to a concentration of 7.7% in 13 of 21 (62%) participants. We found significantly more positive reactions to the test concentration of 2.4% than to aluminium chloride hexahydrate 2% pet. ($p = 0.03$). Therefore, aluminium lactate has been added to the baseline series at our department in Malmö.

The main aim of this study was to investigate possible variation over time in patch test reactivity to aluminium chloride hexahydrate and to aluminium lactate by repeated testing of aluminium-allergic individuals. We also aimed to investigate whether there was a difference in patch test reactivity between various subgroups, for example women versus men, atopic versus non-atopic individuals, or individuals treated with allergen-specific immunotherapy (ASIT) versus those not treated with ASIT, and, finally, whether there is a correlation between test reactivity to aluminium chloride and test reactivity to aluminium lactate.

Materials and Methods

Study design

The study was performed as described by Hindsén et al. (14). All participants of the present study, diagnosed with contact allergy to aluminium, were patch tested on four test occasions (TA, TB, TC, and TD) with equimolar dilution series of aluminium chloride hexahydrate and aluminium lactate over a period of 8 months, with an interval of 2.5 months between test occasions. All participants completed a self-administered questionnaire

before each test occasion. The patch tests were applied by the same personnel on all occasions from November to June. The two dermatologists reading all test reactions were calibrated in their patch test reading through long experience in joint patch test reading. Both were blinded regarding the order of the places on which the dilution series were applied on the participants' backs. The Regional Ethical Review Board in Lund approved the study. Written informed consent was obtained from each participant.

Questionnaire

At study start and before each patch test, all participants completed a questionnaire regarding atopic diseases such as type I allergies and/or having had childhood eczema, with regard to which they were classified as atopics and non-atopics, and also regarding metal sensitivity, and the use of antiperspirants, immunomodulating medication, and additional vaccinations; women also indicated their stage of the menstrual cycle.

Study population

Twenty-one volunteers (14 females and 7 males; mean age 49 years; range 29–72 years) were enrolled, all of whom had previously been diagnosed with contact allergy to aluminium. Only 5 of 21 subjects had also participated in a previous trial (3). Information about the study participants and the aluminium patch test concentrations used when the aluminium allergy was established is shown in Table 1. Fifteen of 21 participants had been previously patch tested at the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö for suspected allergic contact dermatitis, and 6 of 21 had been previously patch tested at the Department of Dermatology and Venereology, Skåne University Hospital, Lund for suspected contact allergy to aluminium.

Patch testing

Two trivalent aluminium salts, namely aluminium chloride hexahydrate 99% ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), named aluminium chloride, and aluminium lactate [aluminium L-lactate 97%; $\text{Al}(\text{C}_3\text{H}_5\text{O}_3)_3$], were obtained from Sigma Aldrich (Steinheim, Germany). At the Department of Occupational and Environmental Dermatology, Malmö, two equimolar dilution series of aluminium chloride and aluminium lactate, both in pet., were prepared (Table 2). IQ Chambers[®], made of additive-free polyethylene plastic and applied on a non-woven adhesive tape (Chemotechnique Diagnostics, Vellinge, Sweden), were used for patch

Table 1. Information on study participants

Person number	Sex	Age (years)	Time since diagnosis of aluminium allergy (months)	Patch test preparation in % pet. when diagnosed with aluminium allergy					
				AlCl ₃ 6H ₂ O				Al(C ₃ H ₅ O ₃) ₃	
				20	15	10	2	12	2.4
1	F	60	51	++	NT	NT	–	NT	NT
2	F	38	26	NT	NT	NT	+	NT	NT
3	M	58	8	NT	NT	(+)	+	NT	NT
4	M	42	20	NT	NT	++	++	++	++
5	F	68	80	+	+	NT	NT	NT	NT
6	F	51	70	+	+	NT	NT	NT	NT
7	M	72	226	NT	NT	NT	+++	NT	NT
8	F	31	13	NT	NT	+	+	–	–
9	F	70	69	NT	NT	NT	+	NT	NT
10	F	41	44	++	NT	NT	++	NT	NT
11	M	29	48	+	NT	NT	–	NT	NT
12	F	52	178	NT	NT	NT	+++	NT	NT
13	F	38	13	NT	NT	+	+	NT	NT
14	F	43	21	NT	NT	++	++	++	–
15	F	23	1	NT	NT	++	NT	++	++
16	F	51	36	+	NT	NT	+	NT	NT
17	M	53	51	++	NT	NT	+	NT	NT
18	M	53	77	+++	–	NT	NT	NT	NT
19	F	35	70	++	++	NT	NT	NT	NT
20	M	62	91	+	NT	NT	NT	NT	NT
21	F	68	214	NT	NT	NT	+	NT	NT

AlCl₃ 6H₂O, aluminium chloride hexahydrate; Al(C₃H₅O₃)₃, aluminium lactate; F, female; M, male; NT, not tested.

testing in all participants. Pet. was chosen as vehicle because of the insolubility of aluminium lactate in water. An amount of 30 mg (37 mg/cm²) (5) of each of the test preparations was placed in each chamber. The chambers with both dilution series were placed as two separate rows of decreasing concentration; that is, the highest concentration was always placed on top, and the lowest at the bottom. On each test occasion, the two patches were applied on different areas of the participants' upper backs in a randomized way according to a latin square table (14). Thus, none of the participants was tested at the same location twice. Within the test area, the order of application of the two patches with serial dilution tests of aluminium chloride and aluminium lactate was also randomized on each test occasion, and was not known by the reading dermatologist. The patch tests were removed after 48 h by the participants themselves, and the reactions were read on day (D) 3/4 and on D7. The strongest reaction on D3/4 or D7 was used for the statistical calculation. The patch test reactions were scored according to ICDRG guidelines (23). Additionally, strong + and ++ reactions were graded +(+) and ++(+), respectively (14).

Table 2. Equimolar dilution series of aluminium chloride hexahydrate (AlCl₃ 6H₂O) and aluminium lactate [Al(C₃H₅O₃)₃]

Aluminium concentration (% wt/wt)	AlCl ₃ 6H ₂ O pet. (% wt/wt)	Al(C ₃ H ₅ O ₃) ₃ pet. (% wt/wt)
3.5	(32) ^a	(38) ^a
1.1	10	12
0.35	3.2	3.8
0.11	1	1.2
0.035	0.32	0.38
0.011	0.1	0.12
0.0035	0.032	0.038
0.0011	0.01	0.012

^aHypothetical minimal eliciting test concentration in participants with contact allergy to aluminium but showing negative reactions in this study.

Statistical analysis

For statistical analysis, the various scores were assigned the following values: negative = 0; ?+ = 0.5; + = 1; +(+) = 1.5; ++ = 2; ++(+) = 2.5; and +++ = 3. The patch test results were considered in two ways: (i) the lowest concentration eliciting at least a + reaction was registered as the minimal eliciting concentration (MEC);

and (ii) the scores for all skin reactions were summed and registered as the summarized test score (STS). The positive test reactions shown by a participant were not always continuous. When the number of negative and/or doubtful reactions were followed by the same number or more of positive reactions, the concentration eliciting the lowest positive reaction was registered as the MEC. In all other situations, the concentration above that eliciting the first negative or doubtful reaction was registered as the MEC. If no positive reactions to the dilutions of the tested salt were observed, the MEC was estimated to be the theoretically next higher patch test concentration, following the increasing steps of the dilutions. A Friedman test was performed to determine whether there were any significant differences between MEC and STS values for aluminium chloride and aluminium lactate from TA to TD. The Mann–Whitney *U*-test was used to compare means of MEC and STS values between different subgroups on each test occasion, for example women versus men, atopsics versus non-atopsics, individuals treated with ASIT versus those not treated with ASIT, and individuals with additional versus no additional vaccinations. Fisher's exact test was used for contingency tables. Spearman's rank-order correlation coefficient, r_s , was calculated to evaluate the covariation of test reactivity and the concomitant reactivity to both aluminium salts on TA–TD. The covariation of both salts was analysed as correlations between the changes of the MEC values from TA to TB, from TB to TC, and from TC to TD, and in the same manner between the changes of the STS values. The concomitant reactivity to both salts was analysed by comparing MEC and STS values for each participants on each test occasion, as well as mean MEC and mean STS values. Differences were considered to be significant at $p < 0.05$. In view of the exploratory nature of the data analysis, no alpha-adjustment techniques were employed.

Results

The results regarding the participants' answers to the questionnaires on each test occasion are shown in Table S1.

Test reactivity in all participants on four test occasions

Twenty-one participants finished the study. MEC and STS values as calculated for all test reactions to aluminium chloride and to aluminium lactate on each test occasion are shown in Table S2. Four of 21 participants did not show any positive reactions throughout this study, but 17 of 21 reacted positively to at least one test salt on at least one test occasion (Table S2; Fig. 1). Eleven of 21 participants did not react to aluminium chloride on at least one

occasion up to a maximum of three occasions: 6 of 11 did not react on one occasion, 1 of 11 did not react on two occasions; and 4 of 11 did not react on three occasions. Fifteen of 21 participants did not react to aluminium lactate on one up to all test occasions: 5 of 15 did not react on one occasion; 6 of 15 did not react on two occasions; and 4 of 15 did not react on any of the four occasions. In 6 of 21 participants, contact-allergic reactions to aluminium chloride and/or aluminium lactate were seen on all four test occasions; 2 of 6 reacted positively to both aluminium chloride and aluminium lactate on all four test occasions. For no. 12, we calculated the same MEC of aluminium lactate and for no. 17 we calculated the same MEC of aluminium chloride on all occasions (Table S2; Fig. 1).

Differences in patch test reactivity

Twenty-one participants underwent four serial dilution tests with both aluminium chloride and aluminium lactate on four test occasions; that is, there were 84 serial dilution tests with each test salt. Thirty-six of 84 (43%) serial dilution tests with aluminium chloride and 49 of 84 (58%) with aluminium lactate gave negative results. The range of reactivity varied between negative reactions to aluminium chloride 10% pet. and/or to aluminium lactate 12% pet., and positive reactions to aluminium chloride 0.1% pet. and/or to aluminium lactate 0.12% pet. (Fig. 1). The highest individual difference noted in test reactivity to aluminium chloride/aluminium lactate was 320-fold when the two most divergent MECs (no. 14/nos. 10 and 15) were compared. There was no statistically significant difference between the distribution of MEC and STS values on the four test occasions, and nor did we see any significant difference when we compared test reactivity estimated as mean MEC and mean STS for both aluminium salts on the four test occasions. The results of the comparison between various study subgroups concerning MEC and STS values for both test salts, each separately, are shown in Table S3. Lower MEC values and, at the same time, higher STS values in one subgroup indicate stronger test reactivity than in the opposite subgroup: numerically, but not statistically, all MEC values for aluminium chloride and for aluminium lactate were lower and all STS values for both salts were higher in atopsics than in non-atopsics. The minimum MEC of aluminium chloride ($p = 0.03$) and that of aluminium lactate ($p = 0.03$), and the MEC values of aluminium lactate on TA ($p = 0.045$) and TB ($p = 0.05$), were (statistically significantly) lower in atopsics than in non-atopsics. The STS values for aluminium chloride on TB ($p = 0.04$) and TC ($p = 0.045$), the STS for aluminium lactate on TB ($p = 0.01$) and the sum of STS values for both salts ($p = 0.02$; $p = 0.01$) were

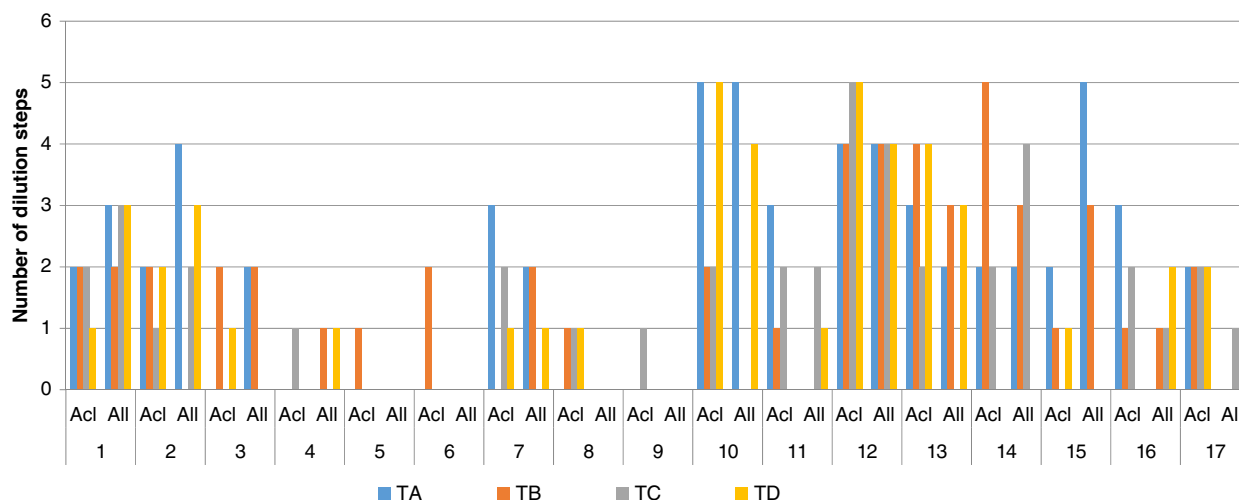


Fig. 1. Variation of aluminium patch test reactivity in 17 aluminium-allergic participants showing at least one positive reaction on four test occasions. The minimal eliciting concentration is expressed as a scale of dilution steps of equimolar concentrations of aluminium chloride hexahydrate (Acl) and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

statistically higher in atopics than in non-atopics (Table S3). In participants who had been previously treated with ASIT, we found mainly numerically but, not statistically, lower MEC values and, at the same time, higher STS values for both salts than in those who had not been previously treated with ASIT (Table S3). There were no statistical differences in patch test reactivity, for either MEC or STS values, for both test salts between the subgroups women/men, individuals with/without additional vaccination, atopics treated with ASIT/not treated with ASIT, and individuals using antiperspirant causing a rash/not causing a rash. No association was found between MEC and STS values for any salt and the stage of women's menstrual cycle during patch testing over time.

Covariation of test reactivity to aluminium chloride and aluminium lactate

The pairwise comparison between the variation of patch test reactivity to both test salts over time, assessed by calculating the change in MEC values and the change in STS values from TA to TB, TB to TC and TC to TD showed a statistically significant correlation only regarding the change in STS values between TA and TB ($r_s = 0.55$; $p = 0.01$).

Concomitant reactivity to aluminium chloride and aluminium lactate

On pairwise comparison of the MEC values and the STS values of both test salts on each test occasion, statistically

significant strong correlations were found. Figure 2 shows these between the test reactivity to aluminium chloride and that to aluminium lactate according to the means of MEC values ($r_s = 0.86$; $p < 0.0001$) and the means of STS values ($r_s = 0.92$; $p < 0.0001$), each averaged over the four tests.

Discussion

The main aim of this study was to investigate possible variation over a relatively short time in patch test reactivity to aluminium chloride and to aluminium lactate by repeated patch testing of aluminium-allergic individuals. In general, we noted many negative reactions when we patch tested all 21 aluminium-allergic study participants with serial dilutions of both test salts (Table S2; Fig. 1), pointing to weak patch test reproducibility. In only 6 of 21 participants could a contact-allergic reaction to at least one test salt be reproduced on all four test occasions, namely, to aluminium chloride in 6 of 6 and to aluminium lactate in 2 of 6. Fifteen of 21 participants did not react to aluminium chloride and 19 of 21 did not react to aluminium lactate on at least one occasion (Table S2; Fig. 1). Is there any explanation for this difference in test reactivity and non-reproducibility?

Hindsén et al. tested 30 nickel-allergic women and Rosholm Comstedt et al. tested 15 palladium-allergic women on four different occasions. Two of 30 (6.7%) nickel-allergic and 1 of 15 palladium-allergic women did not react on one occasion (14, 20). Other trials investigating the reproducibility of contact allergy to various

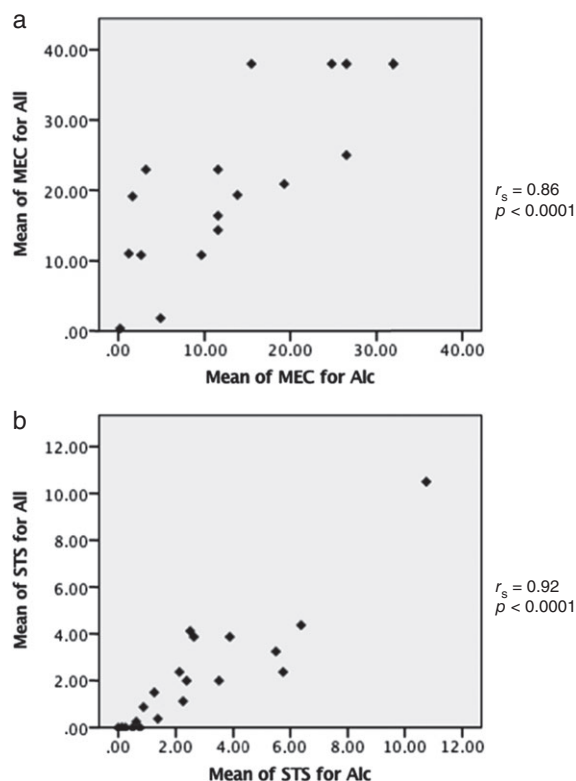


Fig. 2. (a, b) Concomitant patch test reactivity calculated as correlation between the means of the minimal eliciting concentration (MEC) and the summarized test score (STS) for aluminium chloride hexahydrate (Alc) and aluminium lactate (All) for each study participant, averaged over all four tests.

metals reported negative test results when metal-allergic individuals were retested. In 2001, Lee and Maibach (24) published a review on patch test follow-ups years after the initial test. Concerning contact allergy to metals, the authors reported on gold sodium thiosulfate, nickel, and cobalt (17–19, 25). The number of retested individuals varied from 10 to 104 in the different studies. However, the participants were retested only once, and the proportion who had become negative ranged from 4% to 42%. Björk et al. (13) retested individuals with contact allergy to gold ($n = 19$) and to nickel ($n = 12$) with triplicate applications of serial dilutions. In all gold-allergic and in 11 of 12 nickel-allergic subjects, contact allergy could be reproduced, but the degree of reactivity varied. The authors found that reproducibility decreased in a dose-dependent fashion when the previous reactivity had been low, for example a 1+ reaction (13).

Regarding contact allergy to aluminium, there have been only a few trials on reproducibility (3, 21, 26). Gente Lidholm et al. performed a follow-up study of

children sensitized to aluminium after vaccination with an aluminium hydroxide-adsorbed vaccine. The authors showed that aluminium allergy decreased significantly when aluminium-allergic children were retested after several years with aluminium chloride 2% pet. Aluminium allergy was no longer found in 186 of 241 (77%) retested children. The authors observed that the chance of having a negative retest result became higher with increasing age and a longer time interval between the first vaccination and the second patch test.

Interestingly, we found a similar tendency in the present study. In those 4 participants (nos. 18–21) who only showed negative reactions, >60 months since the diagnosis of aluminium allergy had passed. Among the 17 participants (nos. 1–17) showing at least one positive reaction, there were only 5 who had been diagnosed with aluminium allergy >60 months ago, 12 having been diagnosed with the allergy <60 months ago (4/0 versus 5/12; $p = 0.02$) (Table 1). This result supports the notion that the time since a previous patch test has an impact on its reproducibility.

In the present study, 13 of 21 participants had previously reacted positively to aluminium chloride 2% (Table 1). Nine of these 13 participants reacted positively on TA with an MEC of aluminium chloride of $\leq 3.2\%$, which is a concentration close to 2%. If these had been the only tested concentrations and if there had been only one retest, the aluminium allergy would have ‘disappeared’ in 4 of 13 participants. Among the 9 participants who reacted positively on TA, there were 4 who reacted positively to aluminium chloride 3.2% and 5 who reacted positively to concentrations of <3.2%. Thus, the proportion of ‘loss of allergy’ would have been 69% or 61%, respectively, if retesting had been performed on only one test occasion with a test concentration of 3.2% or <3.2%, a result that is similar to that of Gente Lidholm et al., as far as comparisons are meaningful in view of a limited sample size in the present study. On all four test occasions, 10 of 13 participants reacted positively to aluminium chloride $\leq 3.2\%$, and 3 of 13 did not; 7 of 13 reacted positively to aluminium chloride <3.2%, and 6 of 13 did not. Hence, the reproducibility of aluminium allergy increased with repeated patch testing in this study.

Seven of 21 participants had previously been diagnosed with contact allergy to aluminium by a positive reaction to aluminium chloride 20% pet. and/or 15% pet. (Table 1). These are quite high test concentrations as compared with those recommended for patch testing with aluminium (3, 27–29). Individuals reacting positively only to concentrations of 20% and/or 15% may have a weak aluminium allergy, which, as expected, would be difficult

to reproduce on retesting. It is also tempting to believe that these original reactions may have been false-positive, that is, irritant reactions. However, 4 of those 7 individuals diagnosed with aluminium allergy by a positive reaction to aluminium chloride 20% and/or 15% reacted down to the MEC of aluminium chloride at 1% (no. 11), at 3.2% (nos. 1 and 6) and at 10% (no. 5) in the present study (Table S2; Fig. 1), indicating that all prestudy test reactions may have been true-positive reactions. Ideally, we should have patch tested all participants with the same test concentration as in the prestudy patch test.

Aluminium is a metal that is widely used in everyday life. It can be found, for example, in antiperspirants, sunscreens, dental cements, food, and medical preparations (30). Sensitization to aluminium is mainly reported after vaccination with aluminium-adsorbed vaccines and after ASIT with aluminium-adsorbed antigen extract (31). Considering the extent of exposure, contact allergy to aluminium is rare, which is why it is deemed to be a weak allergen (30). Another reason for not being able to detect aluminium allergy may be that there are false-negative reactions. A false-negative reaction can be caused by patch testing with a test concentration that is too low to elicit an allergic skin reaction. Recently, patch testing with aluminium chloride 10% pet. was recommended to detect aluminium allergy (3, 27–29), instead of the routinely used concentration of 2% pet. Another cause of false-negative results when individuals strongly suspected of having aluminium allergy are patch tested may be the second patch test reading not being performed around D7, as recommended according to ESCD guidelines (5). Netterlid et al. showed that contact allergy to aluminium could have been missed if the second reading had not been performed on D7 (29, 31). In the present study, all readings were performed twice, on D3/4 and on D7. Five of 161 (3%) positive reactions to aluminium chloride and 73 of 128 (10%) positive reactions to aluminium lactate would have been missed in this study if test readings had been performed only on D3/4.

Finally, a false-negative reaction can be explained by individual variation in test reactivity, as has been shown in both nickel-allergic and palladium-allergic women (14, 20, 32). The variation in individual test reactivity to both aluminium salts in 17 participants is shown in Fig. 1.

Thirty-six of 84 (43%) serial dilution tests with aluminium chloride and 49 of 84 (58%) serial dilution tests with aluminium lactate gave negative results. The highest intraindividual difference assessed as the MEC for both test salts was 320-fold. This intraindividual variation is in the same order of magnitude as the 250-fold found in nickel-allergic women by Hindsén et al. (14).

Several reasons for variation in test reactivity, for example methodological and immunological factors, have been discussed in the literature (13–15, 20, 32). Our study was designed to reduce or eliminate these factors. The same experienced personnel prepared and applied all patch tests, and the same experienced dermatologists read all tests in a blinded way. Regional differences have been shown in duplicate patch test studies (11, 12). We chose symmetrical parts on the patients' upper back as the test region, as it is the preferred site. The serial dilution tests were applied in a randomized order according to a latin square, which made blinded reading possible. Recently, Björk et al. (13) confirmed prior findings that there is high reproducibility between the right and left sides of the upper back (33).

A more crucial factor that may have influenced test reactivity is the vehicle pet. used in this study. Aluminium lactate, which is a new aluminium test salt for patch testing, is insoluble in water at a concentration of 12.4%, and has therefore been incorporated in pet. (3). Therefore, pet. was used as a vehicle for the aluminium patch tests in the present study. As polar substances such as metal salts are insoluble in pet., uneven distribution of the salts may have occurred (20). Consequently, concentration gradients within the test preparations may have affected further dilutions, and thereby also variation in test reactivity. However, we consider it unlikely that this possible factor accounted for more than a minor part of the variation in test reactivity shown in this study.

It is known that patch testing during the summer can result in weaker test reactions, which means that a strong patch test reaction in the winter may become weaker in the summer (34, 35). We started this trial in autumn, but, because of the methodological set-up, we also had to patch test the participants during late spring. Statistical evaluation did not show any significant differences in test reactivity assessed as the MEC and STS for both salts during the study period.

On comparison of patch test reactivity between various subgroups, our results indicate stronger aluminium allergy, that is, lower MEC and higher STS values, in atopic individuals than in non-atopic individuals (Table S3). None of the participants was suffering from severe eczema during patch testing. However, atopics may have an abnormal skin barrier, which probably facilitates the penetration of the allergen, especially of metals, and this may be one explanation for a stronger allergic reaction (36). A similar result was found when subjects treated with ASIT were compared with those not treated with ASIT (Table S3). Individuals treated with ASIT suffer from severe type I allergies, and thus also from severe atopic disease. Expectedly, as they are atopics, they have

stronger allergy than the no-ASIT subgroup, consisting of non-atopics ($n = 8$) and atopics without ASIT ($n = 7$), who probably have weaker atopic disease than those treated with ASIT.

Statistical analysis of patch test reactivity between the other subgroups did not show any significant differences, and nor did we find any difference in patch test reactivity between those participants who had initially been patch tested because of a suspicion that they had allergic contact dermatitis, and those suspected of having aluminium allergy. As mentioned before, significantly more individuals reacted positively to aluminium lactate 2.4% pet. than to the equimolar concentration of aluminium chloride (2.0% pet.) in a previous study ($p = 0.03$) (3). Aluminium lactate therefore seemed to be more suitable for patch testing, although this could not be confirmed in the present study. However, we found a strong correlation between aluminium chloride and aluminium lactate when we analysed the concomitant test reactivity by comparing both MEC values, STS values and mean responses of the participants investigated on each test occasion (Fig. 2). These results suggest that we were testing with the same hapten in both salts, and that aluminium lactate is as reliable as aluminium chloride. However, the results of the present study are not suitable for making any new recommendations for patch testing with aluminium test preparations.

We could not identify single factors explaining variation in patch test reactivity when repeated patch tests were performed in aluminium-allergic individuals. It may be that the problem of the 'silent back syndrome', that is, the problem of false-negative test results, is underestimated, as Gollhausen argued in 1989 (6). It can also be hypothesized that the astringent effect that aluminium salts are known for may influence penetration through the skin.

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Conclusions

Aluminium-allergic individuals may have false-negative reactions, which is why retesting with aluminium should be considered when there is a strong suspicion of allergic contact dermatitis caused by aluminium. Aluminium lactate may be as reliable a test salt as aluminium chloride when a person who is strongly suspected of having allergic contact dermatitis caused by aluminium is patch tested. However, on the basis of the results of the present study, no new recommendations can be given regarding patch testing with aluminium test preparations, and aluminium chloride 10% pet. is recommended for patch testing.

Acknowledgements

Financial support from the Skane County Council's research and development foundation, from the Edvard Welander Foundation and from the Finsen Foundation is gratefully acknowledged.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Information about study participants according to questionnaire results.

Table S2. Patch test reactivity in all study participants. MEC (minimal eliciting concentration) in % and STS (summarized test score) on test occasions A–D, strongest on day 3 or 7, for dilution series of aluminium chloride hexahydrate and aluminium lactate.

Table S3. Differences in MEC (minimal eliciting concentration) and STS (summarized test score) for aluminium chloride hexahydrate (Alc) and aluminium lactate (All) between subgroups.

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Table S1. Information about study participants according to questionnaire results

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

^{a)} Study person participant number; ^{b)}Atopy: having had childhood eczema and/or having typ 1 allergi; ^{c)}mild to moderate skin symptoms as eczema, rosacea, itch;

^{d)}ASIT Allergen-specific immunotherapy; ^{e)}TA, TB, TC, TD Test occasion A-D; ^{f)} immuno-modulating medication; ^{g)}no answer; ^{h)}postmenopausal; ⁱ⁾Un Unknown;

^{j)}intrauterine device;

Table S2. Patch test reactivity in all study participants MEC^{a)} (%) and STS^{b)} on test occasions A-D, strongest on day 3 or 7, for dilution series of

Nr ^{c)}	Aluminium chloride hexahydrate								Aluminium lactate							
	TA ^{d)} _MEC ^{a)}	TA ^{d)} _STS ^{b)}	TB ^{e)} _MEC ^{a)}	TB ^{e)} _STS ^{b)}	TC ^{f)} _MEC ^{a)}	TC ^{f)} _STS ^{b)}	TD ^{g)} _MEC ^{a)}	TD ^{g)} _STS ^{b)}	TA ^{d)} _MEC ^{a)}	TA ^{d)} _STS ^{b)}	TB ^{e)} _MEC ^{a)}	TB ^{e)} _STS ^{b)}	TC ^{f)} _MEC ^{a)}	TC ^{f)} _STS ^{b)}	TD ^{g)} _MEC ^{a)}	TD ^{g)} _STS ^{b)}
1	3.2	2.5	3.2	3.5	3.2	2	10	2	1.2	7	3.8	2.5	1.2	5	1.2	2
2	3.2	4.5	3.2	2.5	1	5	3.2	3.5	0.38	7.0	>12	1.5	3.8	3	1.2	4.0
3	>10	1	3.2	2	>10	1	10	1	3.8	1.5	3.8	2.0	>12	1	>12	1.5
4	>10	1	>10	0	10	2	>10	0.5	>12	0	12	1.5	>12	0.5	12	1.5
5	>10	0	10	1	>10	0.5	>10	0.5	>12	0	>12	0	>12	0	>12	0
6	>10	0	3.2	2.5	>10	0.5	>10	0	>12	0	>12	0	>12	0	>12	0
7	1	3.5	>10	1	3.2	2.5	10	1.5	3.8	4.0	3.8	2.5	>12	1.5	12	1.5
8	>10	0.5	10	1	10	1.5	10	2.5	>12	0	>12	0	>12	1.5	>12	0
9	>10	1	>10	0.5	10	1	>10	0	>12	1	>12	0	>12	0	>12	0
10	0.1	11.5	3.2	4	3.2	3	0.1	7	0.12	9.5	>12	1.5	>12	1	0.38	5.5
11	1	5	10	1.5	3.2	2	>10	0.5	>12	1	>12	0	3.8	2	12	1.5
12	0.32	10	0.32	9.5	0.1	10.5	0.1	13	0.38	10.5	0.38	8.5	0.38	11.5	0.38	11.5
13	1	2.5	0.32	8.5	3.2	2.5	0.32	9.5	3.8	2.5	1.2	4	>12	0	1.2	3
14	3.2	4.5	0.1	12	3.2	5.5	>10	0	3.8	2	1.2	5.5	0.38	5.5	>12	0
15	3.2	6.5	10	2	>10	0.5	10	1.5	0.12	10.5	1.2	3	>12	1.5	>12	0.5
16	1	4	10	2.5	3.2	2.5	>10	0.5	>12	1	12	2.5	12	2	3.8	2.5
17	3.2	3	3.2	2.5	3.2	4.0	3.2	4.5	>12	3.0	>12	1	12	1.5	3.8	2.5
18	>10	1	>10	0	>10	0	>10	0	>12	0	>12	0	>12	0	>12	0
19	>10	0.5	>10	0	>10	0	>10	0	>12	0	>12	0	>12	0	>12	0
20	>10	0	>10	0	>10	0	>10	0	>12	0	>12	0	>12	0	>12	0
21	>10	0.5	>10	0	>10	0	>10	0	>12	0	>12	0	>12	0	>12	0

^{a)}MEC Minimal eliciting concentration; ^{b)}STS Summarized test score;^{c)}Study ~~person~~ participant number; ^{d)-g)}Test occasions A,B,C,D

Table S3. Differences in MEC (minimal eliciting concentration) and STS (summarized test score) for aluminium chloride hexahydrate (Alc) and aluminium lactate (All) between subgroups.

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

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

^{a)}p<0.05 is statistically significant; ^{b)}n number of study persons; MMEC Minimum of MEC during TA to TD; SSTS Sum of STS during TA to TD