



Elevated aluminum excretion in patients by long-term subcutaneous immunotherapy – A cross-sectional case-control study

Julia Hiller^{a,*}, Thomas Göen^a, Hans Drexler^a, Carola Berking^b, Nicola Wagner^b

^a Institute and Outpatient Clinic of Occupational, Social, and Environmental Medicine, Friedrich-Alexander-Universität Erlangen-Nürnberg, Henkestraße 9–11, 91054, Erlangen, Germany

^b Department of Dermatology, Uniklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Deutsches Zentrum Immuntherapie (DZI), Ulmenweg 18, 91054, Erlangen, Germany

ARTICLE INFO

Keywords:

Adjuvant
Aluminum
Biomonitoring
Immunologic desensitization
Risk assessment
Venom hypersensitivity

ABSTRACT

Background: Aluminum (Al) adjuvants have been used in vaccines and subcutaneous immunotherapy (SCIT) for decades. Despite indisputable neurotoxic properties of Al, there is no clear evidence of a causal relationship between their use and any neurotoxic side effects. However, recent rat studies have shown an accumulation of Al from adjuvants in tissues, especially in bones.

Objectives: Since the human toxicokinetics of Al-adjuvants are poorly understood, this study aimed to evaluate whether up-dosed or long-term SCIT with Al-coupled extracts leads to increased Al load in humans.

Methods: This observational cross-sectional case-control study explored Al excretion in hymenoptera venom allergy patients recruited in 2020 before initiation (n = 10) and during ongoing (n = 12) SCIT with Al-based preparations. Urine samples were collected before and 24 h after the SCIT injections and analyzed for aluminum content by using atomic absorption spectrometry. The cumulative administered Al dose was extracted from patient records. Patients receiving long-term immunotherapy were treated between 2.8 and 13.6 years (mean 7.1). Other potential sources of Al exposure were surveyed.

Results: Patients who had received Al-coupled immunotherapy for several years showed significantly (p < 0.001) higher Al excretion than the controls at initiation of immunotherapy (mean 18.2 µg/gC vs. 7.9 µg/gC) and predominantly (73%) were above the 95th percentile of the general populations' exposure (>15 µg/gC), however, without reaching levels of toxicological concern (>50 µg/gC). Taking both groups together excreted Al levels correlated with the cumulative administered Al dose from SCIT (linear regression: $Al_{urine} = 8.258 + 0.133 \cdot Al_{cum}$; p = 0.001).

Discussion: These results suggest a relevant iatrogenic contribution of long-term SCIT to human internal Al burden and potential accumulation. Considering the medical benefits of Al-adjuvants and SCIT a differentiated risk-benefit analysis is needed. For certain scenarios of potential toxicological concern in clinical practice biomonitoring might be advisable.

1. Introduction

Allergies can occur against a wide variety of agents from our environment and are often accompanied by extensive consequences and health problems for the affected patients. Allergen immunotherapy (AIT) is a well-established treatment option for allergy patients which draws on causative, disease-modifying aspects (Pfaar et al., 2019). Therapy allergens are usually administered repeatedly over a longer period of time either sublingually or subcutaneously (SCIT =

subcutaneous immunotherapy). Several actions, such as standardization of allergen extracts, sublingual application and using allergoids or adjuvants, were taken to ensure the efficacy and safety of such AIT applications (Jensen-Jarolim et al., 2020, 2021). Regarding SCIT, such measures may include utilizing adjuvants, which have been used in human vaccines for decades. Adjuvanted extracts for SCIT are especially widespread in Europe, with aluminum hydroxide being the most commonly used adjuvant (Alvaro-Lozano et al., 2020; Jensen-Jarolim et al., 2020, 2021).

Several overviews (Alvaro-Lozano et al., 2020; Hogenesch, 2012;

* Corresponding author.

E-mail addresses: Julia.hiller@fau.de (J. Hiller), Thomas.goen@fau.de (T. Göen), hans.drexler@fau.de (H. Drexler), carola.berking@uk-erlangen.de (C. Berking), nicola.wagner@uk-erlangen.de (N. Wagner).

<https://doi.org/10.1016/j.ijheh.2024.114337>

Received 31 October 2023; Received in revised form 19 January 2024; Accepted 6 February 2024

Available online 10 March 2024

1438-4639/© 2024 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Nomenclature

AIT	Allergen immunotherapy
Al	Aluminum
Al-adjuvants	Aluminum-containing adjuvant
Al _{cum}	Cumulative aluminum dose from immunotherapy injections
AUC	Area under the curve
EFSA	European Food Safety Authority
µg/gC	Mikrogramm per gram creatinine
SCIT	Subcutaneous immunotherapy
TWI	Tolerable weekly intake

Jensen-Jarolim et al., 2020, 2021; Masson et al., 2018) have addressed the benefits and shortcomings of aluminum (Al) adjuvants, mostly with respect to the immunological actions and mechanisms elicited by those adjuvants. While the use of adjuvants improves the immunological response to the extract and ameliorates tolerance of allergen injection due to precipitation at the injection site (Alvaro-Lozano et al., 2020; Jensen-Jarolim et al., 2020, 2021), acute and long-term toxicological effects are also a concern (Alvaro-Lozano et al., 2020; Jensen-Jarolim et al., 2020). Especially the known neurotoxic effects of systemic Al exposure have been extensively discussed in this context and in the light of environmental Al exposures (Alvaro-Lozano et al., 2020; Jensen-Jarolim et al., 2020; Klotz et al., 2017). So far, “there have been no pharmacovigilance signals for acute toxicity linked to AIT” (Jensen-Jarolim et al., 2020). And by citing several older evaluations (Institute of Medicine, 2001, 2004; WHO, 2012) an experimental intervention study in rats (Weisser et al., 2019) indicated that “there is no scientific evidence for a causal relationship between Al containing vaccinations and acute or chronic neurological impairment”. Concerns about vaccine safety, for example fears of a weakened immune system or the causation of autism or developmental delays in children, are debated in context with Al adjuvants or the sheer number of vaccine shots administered (Geoghegan et al., 2020). Within this assumption, the more numerous injections during SCIT would be even more troublesome and constitute a high exposure scenario. The scientific evidence, however, has so far refuted such concerns about vaccine safety (Geoghegan et al., 2020). Nonetheless, there is an unmet need to gain further knowledge on Al clearance from Al-adjuvants.

There are abundant exposure possibilities to Al from the human environment, such as soil, plants, water, air, consumer products and many pharmaceuticals, and Al is the most common metal on earth (ATSDR, 2008). However, the general fate of Al and the body's homeostatic mechanisms are not fully understood yet. It has long been clear, that most Al is eliminated by urinary excretion within days or months after it enters the bloodstream, while some Al is retained in the body, especially the skeleton (Priest, 1993, 2004). Such bone recovery of Al was also found from adjuvants in recent animal studies after 80 days (Weisser et al., 2019, 2020), but without showing any measurable effects on the Al plasma concentrations in between. Al might also be released from such longterm body deposits in the course of time and increase the systemically available Al again before its excretion. Recent physiology-based modeling for Al toxicokinetics in the most important tissues described a multi-compartment distribution of Al with varying elimination kinetics in different tissues. Overall, the modeling indicates that Al excretion in urine is a good reflection of the total body burden (Hethy et al., 2021), as the slow urinary kinetics correlate well with the modeled fraction in bone (as a deep storage compartment). Therefore, urinary Al can be used as a surrogate for the currently systemically available Al load.

The current knowledge on Al-adjuvant in humans – especially for SCIT – is also limited and does not allow definitive conclusions; rather,

further monitoring is required (Jensen-Jarolim et al., 2020; Masson et al., 2018). Based on recent experimental animal data, it was postulated that single injections of Al-adjuvants do not cause measurable changes to Al load in human adults (Weisser et al., 2019, 2020). However, after several years of SCIT, a substantial deposit of Al in bones can be expected (Jensen-Jarolim et al., 2020; Weisser et al., 2020). Regarding the use of multiple or long-lasting AIT treatments – such as in multi-allergic individuals or hymenoptera venom allergy, where an up-dosed or lifelong AIT is indicated in special cases (Golden et al., 1996, 1998; Keating et al., 1991; Przybilla and Rüegg, 2010; Reisman, 1993; Sturm et al., 2018) – a substantial and potentially worrisome accumulation of Al might occur.

Furthermore, observations from our own clinical practice (unpublished data) provided further reason for concern: due to medical indications and inquiries, three patients, all of whom received an up-dosed SCIT for treatment of hymenoptera venom allergy, were screened for their respective Al excretions during their treatment to address concerns of their Al exposure. Urinary Al concentrations (measured at 12.3, 18.1 and 39.4 µg/gC (=µg/g creatinine)) exceeded the reference value of 15 µg/gC (Klotz et al., 2020) in two cases; one measurement even exceeded the reference value more than threefold (39.4 µg/gC). Their respective treatment duration was 1 year, >3 and >8 years. Therefore, among these three patients, the cases with a longer duration of Al-based SCIT also had a higher Al excretion, which may hint at a noticeable contribution of Al-based SCIT over time to the overall Al burden in humans.

With respect to these findings, it is important to generate further knowledge on the Al burden and elimination with regard to SCIT Al-adjuvants in humans. Therefore, a cross-sectional case-control study for the proof of principle was conceptualized in order to evaluate whether up-dosed or long-lasting SCIT treatment with Al-adjuvanted extracts leads to short-term or chronically increased Al excretion in humans. Hereby its complex toxicokinetics involving different compartments have to be considered and if Al excretion is raised, to detect the magnitude of such effect.

2. Material and methods

2.1. Study design and collective

This cross-sectional case-control study explored Al excretion in humans before initiation (controls) and during ongoing SCIT with Al-adjuvanted hymenoptera venom (cases). The study was performed in accordance with the ethical standards of the institutional research committee as well as with the Helsinki declaration of 1964 and its later amendments. The study design, including handling of patient data, was reviewed and approved by the local ethics committee (Friedrich-Alexander-Universität Erlangen-Nürnberg (Vote No. 445.19 B)). All study participants gave written, informed consent for participation and scientific data usage before inclusion.

All patients were recruited in 2020 during their presentation in the Allergy Unit of the University Hospital's Department of Dermatology. Inclusion criteria were legal age (≥18 years) and a history of hymenoptera venom allergy.

Consecutively, patients with an ongoing hymenoptera venom SCIT with an Al-based extract were asked to participate if the cumulative administered Al dose from SCIT was ≥30 mg. Two patients declined and 12 patients were included in the study as Group 1 (previously exposed). The SCIT extracts used were all from the same producer (bee or wasp venom adjuvanted to Al hydroxide) and contained 1.13 mg aluminum per 100 µL venom.

Twelve control patients were recruited consecutively as Group 2 (no previous exposure); they had been advised to start an Al-based hymenoptera venom SCIT and had no known history of a former Al-adjuvanted SCIT. Two patients had to be excluded after sample analysis, as one did not provide a sample 24 h after SCIT injection and the other one provided excessively diluted urine samples with creatinine

concentrations of less than 0.3 g/L (outside the validated analytical range). As a result, Group 2 for data analysis consisted of $n = 10$ patients.

Due to a limited availability of patients, and with no age- or sex-differentiated reference values for Al excretion established to this date, no specific emphasis was placed on matching those parameters among groups but rather on the underlying condition of a hymenoptera allergy.

2.2. Sample collection and analysis

Spot urine samples were collected in acid-washed, sterile polypropylene cups twice per patient: directly before the next (Group 1) or the first (Group 2) injection of the Al-adjuvanted allergen extract (between 8:00 and 10:00 a.m.), as well as on the following morning (about 24 h later) to additionally screen for short-term effects on Al excretion post-injection. For all other evaluations, the first urine samples – collected before the SCIT injection – were used (data set available in supplemental material (Table S1)). For Group 1, samples were taken as part of the next scheduled injection of the ongoing treatment. The interval between the last injection and the first urine sample was between 26 and 57 days (mean $\pm$ SD: 42 ± 10 days). In all controls (Group 2), the induction of immunotherapy was performed with a lyophilized preparation up to ten days in advance of the study sampling (during the first Al-adjuvanted injection).

Urinary Al was analyzed upon arrival in the toxicological laboratory of the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (located in Germany) using a quality-controlled and validated atomic absorption spectrometry procedure as described (Letzel et al., 2020). Concentrations in $\mu\text{g/L}$ were normalized to creatinine ($\mu\text{g/gC}$) to account for varying urinary dilutions in spot urine samples and for comparison with reference values. Urinary creatinine concentration was determined photometrically per Jaffe's method (Larsen, 1972). Samples with creatinine concentrations outside the acceptable range (0.3–3.0 g/L urine) (WHO, 1996) were excluded.

Biological reference values are oriented towards the population's background exposure, generally equating to the 95th percentile of the general population without occupational exposure. A preliminary reference value of 15 $\mu\text{g/L}$ Al in urine has long been established (UBA, 1998) and was recently supported by a thorough literature review, whereby a reference value of $\leq 15 \mu\text{g/gC}$ was established (Klotz et al., 2020).

2.3. Further data collection

Current age and cumulative administered Al dose (Al_{cum}) from hymenoptera venom SCIT were determined from patient records. The SCIT extracts contained 1.13 mg aluminum per 100 μL venom; the amount of each venom injection administered was added up to calculate Al_{cum} . All prior injections, but not the current one were included in the Al_{cum} calculations. In one case, the available records did not cover the entire treatment period (due to external initiation going back as far as 2007 and a change of physician). This case was excluded from evaluation of an association between Al_{cum} and Al excretion. In a tentative approach, for this patient Al_{cum} of the unaccounted period (2007 to October 2013) was estimated by assuming injections every 6 weeks (with 100 μL venom until 2009 and 200 μL venom from 2010 onwards) and added to the Al_{cum} extracted from the records.

Furthermore, all patients completed a questionnaire (translated version included in supplemental material, study questionnaire) on personal and medical factors (e.g. smoking habits, current medications, pre-existing conditions), as well as other potential environmental sources of Al exposure. These questions were based on exposures which have either been critically discussed in the past with regard to their potential Al burden for humans, such as Al-containing deodorants or kitchenware or Al-burdened foods, or where a relevant Al content is known for certain products (vaccines, heartburn medication such as antacids or

mineral clay).

2.4. Data and statistical analysis

OriginLab Origin® 2019 and SPSS Statistics Version 28 were used for statistical analysis and graphics. Standard distribution of data was tested with Shapiro-Wilk. For the descriptive statistics means and standard deviation (SD) are reported for normally distributed data, the median is reported when data are not normally distributed. Comparison of age and Al excretion between Groups 1 and 2 was performed with a two-tailed t-test (normally distributed data). Two-tailed Wilcoxon tests were used to investigate concentration differences in paired urine samples before and after the injection (acute injection effect; data not normally distributed). An association between urine concentration and Al_{cum} was explored by linear regression. Due to the sample size, exact p-values are reported, whereby $p < 0.05$ is considered significant.

3. Results

3.1. Medical data and collective characteristics

The overall collectives for data analysis consisted of 12 and 10 patients in Groups 1 and 2, respectively. Aggregated patient characteristics and other potential sources for Al exposure (surveyed by questionnaire)

Table 1
Aggregated characteristics of patients ($n = 22$) receiving subcutaneous immunotherapy (SCIT) for hymenoptera venom allergy by group.

	Group 1 ($n = 12$)	Group 2 ($n = 10$)
Sex [N (%)]		
- Female	5 (42)	8 (80)
- Male	7 (58)	2 (20)
Age ^a [years]	Mean $\pm$ SD: 63.1 ± 15.3 Min.-Max.: 41–87	Mean $\pm$ SD: 54.2 ± 17.9 Min.-Max.: 18–72
Smoking [N (%)]		
-Current smoker	1 (8)	2 (20)
-Ex-smoker	3 (25)	2 (20)
-Non-smoker	8 (67)	6 (60)
Duration of ongoing Al-based SCIT [years]	Mean $\pm$ SD: 7.1 ± 2.9 Min.-Max.: 2.8–13.6	Start of treatment
Cumulative Al dose (Al_{cum}) from SCIT [mg]	Mean $\pm$ SD: 73.8 ± 24.4^b Min.-Max.: 45.0–120.9 ^b	0
Other former SCIT treatments	• Former SCIT with Al-free hymenoptera venom ($n = 1$) • Preseasonal SCIT against pollen for 3 years (around the year 2000) ($n = 1$)	• Former SCIT with Al-free hymenoptera venom ($n = 1$)
Other current medical Al exposures	Occasional oral medication with Al-containing antacids (500 mg aluminum-magnesium-silicate (1:2:3) per tablet, corresponding to $\sim 56 \text{ mg Al}$) ($n = 1$)	One vaccination within the last 12 month containing aluminum (total content: 0.5 mg Al^{3+}) ($n = 1$)
Oral ingestion of healing clay	Daily ($n = 1$)	Occasionally ($n = 1$)
Use of Al foil for cooking ($\geq 1 \times$ per month) [N (%)]	3 (25)	6 (60)
Consumption of lye pastry ($\geq 1 \times$ per week) [N (%)]	2 (17)	2 (20)
Use of Al-containing antiperspirant [N (%)]	3 (25)	4 (40)
Use of facial masks for cosmetic treatments [N (%)]	0 (0)	2 (20)

^a T-test for differences between groups: $p = 0.22$.

^b Data for $n = 11$ (excluding one case with only estimated Al_{cum} of $\sim 203 \text{ mg}$).

are reported by group in Table 1. Men were more prevalent in Group 1. Age did not differ significantly among groups.

No patient reported renal failure (possibly influencing excretion parameters), received topical treatment with Al hydroxide for hyperhidrosis, or obtained a tattoo within the preceding 12 months.

Regarding possible other environmental and iatrogenic Al exposures, a thorough evaluation of their potential influence on the Al excretion data was performed. In summary, a relevant Al exposure beyond the hymenoptera venom SCIT treatment could not be excluded for one patient of Group 1 due to daily ingestion of healing clay. Despite a repeated sample collection after stopping the supplementation, persisting confounding effects could not be ruled out for this patient and ultimately led to the exclusion from this study's further analyses. However, the patient's data from the repeated sampling are shown in the figures marked with an asterisk for descriptive purposes. All other potential environmental or iatrogenic exposures were considered negligible or of secondary importance compared to the Al doses from SCIT (for rationale see supplemental material, B. Other Al exposures). Therefore, the final Groups 1 and 2 included 11 and 10 patients, respectively.

3.2. Short-term effects

To evaluate possible acute, short-term effects of single SCIT injections with Al-adjuvanted extracts, the Al concentrations in urine before and 24 h after SCIT injection were compared (Fig. 1). The post-injection concentration was higher than pre-injection in 12 (57 %) patients and lower in nine (43 %) patients. In 48% of patients, the lower determined level was within 80–100% of the higher level and within the range of analytical reliability. Descriptive statistics were comparable (Table 2). Furthermore, testing for differences showed no significant variation in Al excretion before and after injection, neither when all data (n = 21) were considered together (Wilcoxon: p = 0.44) nor when separated into groups (Wilcoxon: Group 1: p = 0.70; Group 2: p = 0.18)).

3.3. Comparison with background levels and among groups

Nine samples showed Al concentrations above the reference value (15 µg/gC), indicating elevated exposure. Group 1 showed a distinct

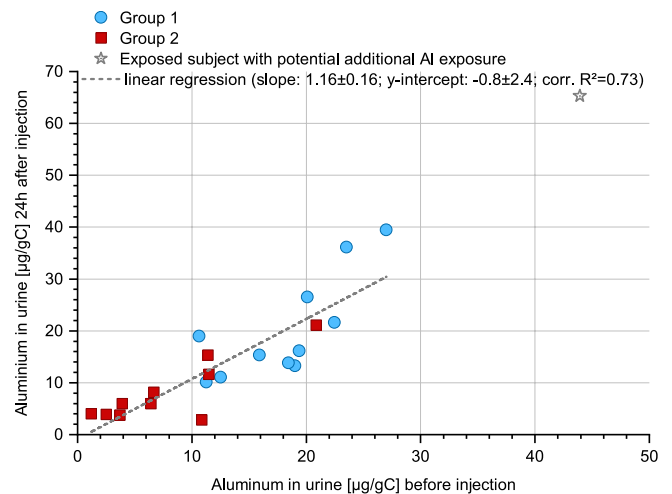


Fig. 1. Scatter plot of aluminum excretion before and 24 h after the next (Group 1, blue/circles, n = 11) or first (Group 2, red/squares, n = 10) subcutaneous immunotherapy injection with hymenoptera venom (with aluminum hydroxide adjuvant) in allergy patients with linear regression. One special case (originally from group 1), in which additional aluminum exposure with influence on the current excretion could not be excluded, is depicted by a star. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Aluminum excretion before and 24 h after injection of one dose of aluminum hydroxide adjuvanted hymenopteran venom during subcutaneous immunotherapy (SCIT) in allergy patients (n = 21).

	Aluminum concentration in urine [µg/gC]	
	Directly before injection	24 h after injection
Min.-Max.	1.2–27.0	2.8–39.5
Median	11.5	13.3

deviation from background levels of Al excretion (Min.-Max.: 10.6–27.0 µg/gC; mean ± SD: 18.2 ± 5.2 µg/gC). The reference value of 15 µg/gC was exceeded in eight patients (73%) and the Al-excretion levels were significantly (t-test: p < 0.001) higher compared to Group 2 (Fig. 2). In contrast, in patients without prior SCIT (Group 2), Al excretion was within the expected range prior to treatment (Min.-Max.: 1.2–20.9 µg/gC; mean ± SD: 7.9 ± 5.9). Only one patient (10%) exceeded the reference value.

3.4. Association between Al excretion and administered Al dose

Al excretion was plotted against Al_{cum} (Fig. 3) to evaluate a possible association between excretion and cumulative Al dose from SCIT injections. For Group 2, Al_{cum} was assumed to be zero. As described above, the Al_{cum} of one case could not be conclusively determined but rather estimated due to missing data. The final data basis for this analysis therefore consisted of n = 20 data points. A linear regression was performed with these data, assuming a direct association. The overall regression model was found to be highly significant (p = 0.001) and with a fair goodness-of-fit (corr. R² = 0.426). Both the slope (0.133, SE ± 0.034, p = 0.001) and the intercept (8.258, SE ± 1.726, p < 0.001) proved to be significant, yielding a regression model of Al_{urine} [µg/g] = 8.258 + 0.133*Al_{cum} [mg].

A second tentative analysis was conducted to further test and validate the regression model. Besides the indisputable cases (n = 20) used for the aforementioned modeling, some less robust data from five more cases existed (further specification in Table 3). This additional analysis proved consistent with the primary regression model.

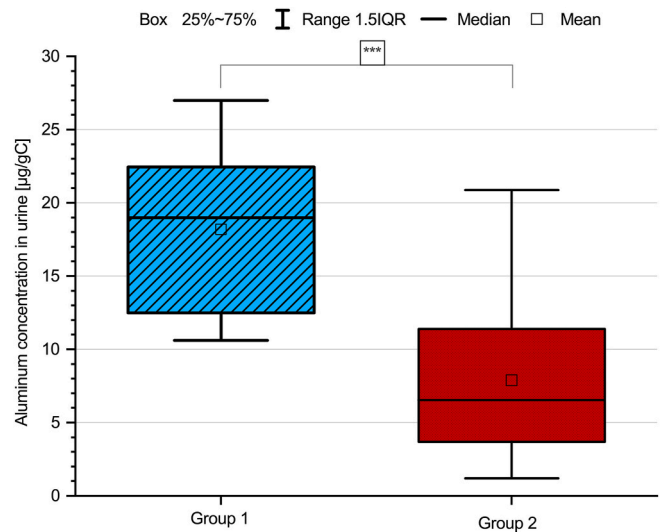


Fig. 2. Comparison of aluminum excretion in µg per gram creatinine (µg/gC) between Group 1 (blue/diagonally striped, hymenoptera venom allergy patients with ongoing aluminum-based subcutaneous immunotherapy, n = 11) and Group 2 (red/dotted, patients before start of aluminum-based subcutaneous immunotherapy, n = 10). Highly significant difference: ***p < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

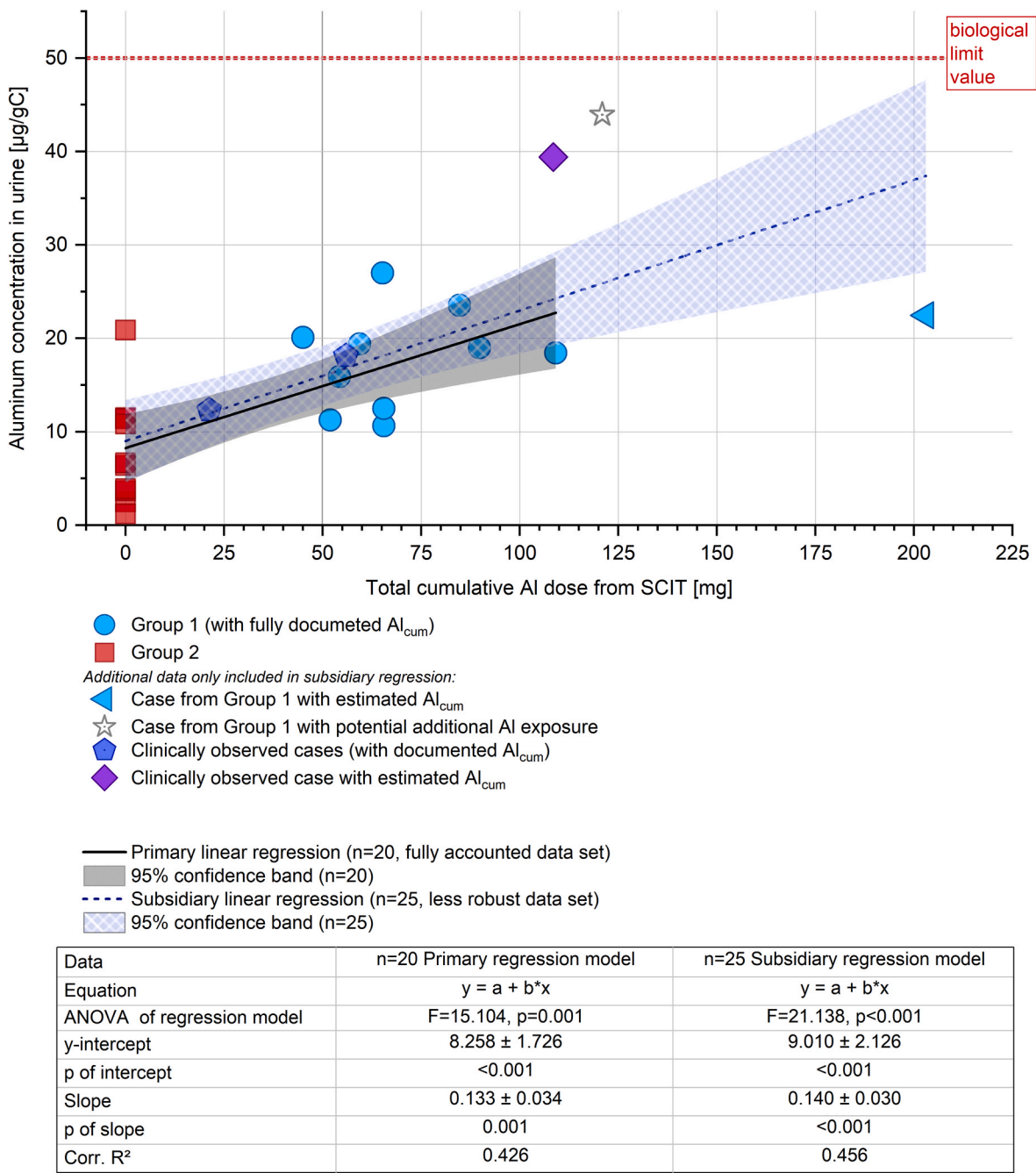


Fig. 3. Scatter plot of aluminum excretion in µg per gram creatinine (µg/gC) and cumulative aluminum dose administered through subcutaneous immunotherapy for hymenoptera venom allergy. Linear-regression models with 95% confidence bands were fitted for a primary model (black/grey/continuous line, n = 20, p = 0.001) and a subsidiary second model (blue/broken line, n = 25, p < 0.001). Both models include n = 10 patients from Group 1 with ongoing therapy, for whom the cumulative Al dose from SCIT was fully documented, and all patients from Group 2 before treatment initiation (n = 10). The subsidiary model also includes five additional, less robust data points from clinical cases or patients in Group 1 without fully accounted cumulative dosage data or other potential conflicting exposure sources. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

In our study, we observed, that patients who had received Al-coupled immunotherapy for several years showed significantly higher Al excretion than the controls at initiation of immunotherapy and predominantly exceeded the reference value of the general population. Furthermore, excreted Al levels correlated with the cumulative administered Al dose from SCIT.

To our knowledge, the biomonitoring results hereby reported are the first valid human data to specifically evaluate the effects of long-term Al-

adjuvanted SCIT on systemic Al exposure in allergy patients. As stated above, the numerous injections during SCIT and especially in the herein examined cases (with increased doses or prolonged treatment) constitute a worst-case scenario.

Recent toxicokinetic modeling illustrates that urinary Al accurately reflects total body burden (Hetthey et al., 2021). Al excretion can therefore be seen as a surrogate for systemically available Al exposure. Moreover, established reference values can be used to compare the results with the background levels of the general population (Klotz et al., 2020), as well as with health-derived limits for occupational exposure

Table 3
Specification and limitations of five additional cases used in second analysis to further test the original linear-regression model (Fig. 3).

Data origin	Reason for exclusion in initial regression model (Figure 3)
Group 1 No. 11 from study data set: originally excluded from data for initial regression model	Cumulative aluminum dose Al_{cum} from subcutaneous immunotherapy partially estimated
Group 1 No. 12 from study dataset: originally excluded from all statistical analyses	Potentially relevant, additional Al exposures from ingestion of healing clay
Clinically observed Case 1 (described in introduction)	Not formally included in study data set, data only obtained from clinical practice (before study conceptualization)
Clinically observed Case 2 (described in introduction)	Not formally included in study dataset, data only obtained in clinical practice (before study conceptualization)
Clinically observed Case 3 (described in introduction)	Not formally included in study dataset, data only obtained in clinical practice (before study conceptualization); additionally cumulative aluminum dose Al_{cum} from subcutaneous immunotherapy partially estimated

(Klotz et al., 2017, 2019).

While no distinguishable acute effect on Al excretion could be observed from a single SCIT injection in our results, patients with an ongoing Al-adjuvanted SCIT excreted significantly more Al than patients with the same medical condition prior to treatment. The former also had clearly elevated Al excretion levels compared to the expected levels from the general population. With urinary Al being a good reflection of the total body burden (Hethey et al., 2021) and significant fractions of single Al-adjutant injections being found in bone after 80 days (Weisser et al., 2019, 2020), this finding is a clear indication of an increased internal Al load from long-term SCIT treatment and suggests a relevant accumulation in the body over time. Of course, it should be noted, that the increased urinary excretion cannot conclusively prove such accumulation in bone (or other compartments) as it does not measure the Al content there directly, however the synopsis of the literature and our data very much suggest an accumulation. A linear-regression model of the Al excretion and Al_{cum} from SCIT showed a fair fit consistent with this assumption. While such increased total Al body load is likely to be distributed over multiple compartments (Hethey et al., 2021; Priest, 2004), it manifests itself primarily via the chronically increased excretion.

On the other hand, the lack of a difference in Al excretion before and 24 h after injection is plausible and fully in line with the reported slow absorption of Al from insoluble salts after subcutaneous or intramuscular injection from recent experimental animal studies (Weisser et al., 2019, 2020). The results therefore support the conclusions of Weisser et al. (2019, 2020) that a single subcutaneous or intramuscular injection of an Al-adjutant dose would be also indistinguishable from baseline levels in humans. Still, a relevant amount of Al from marketed SCIT adjuvants was found in rat bones 80 days after a single subcutaneous injection; an increase in plasma levels ($AUC_{0-80\text{ d}}$ (= Area under the curve)) was also seen for one Al-adjutant extract (Weisser et al., 2020). Therefore, when the Al concentrations in rat bones were extrapolated to a three-year perennial SCIT course in humans (36 doses, each containing 1.25 mg Al), a substantial increase of Al in bone was expected by referring the extrapolated increase to normal bone Al content (Weisser et al., 2020).

Although most of our exposed patients had an even higher Al_{cum} (between 45.0 and 120.9 mg; mean 73.8 mg) than Weisser et al. (2020) calculated for their accumulation scenario ($36 \times 1.25\text{ mg} = 45\text{ mg}$), our data largely confirm these assumptions in principle. We saw frequent exceedances of the reference value for Al in Group 1, potentially pointing to an accumulation in typical body storage compartments. According to literature data such accumulation would be expected to

affect especially bones and brain (Hethey et al., 2021; Priest, 2004; Weisser et al., 2020).

However, levels above the reference value do not necessarily constitute a health risk. For such evaluation of potential health risks only references to biological limit values, which are formally just established for long-term occupational exposure, can be discussed. Among all evaluated toxicities of Al, neurotoxicity is considered the most relevant adverse event (ATSDR, 2008; EFSA, 2008; Klotz et al., 2017; Klotz et al., 2019). Critical evaluation of available studies of occupational exposure concluded that neurotoxic effects are unlikely for urinary excretion of $<50\text{ }\mu\text{g/gC}$, even following long-term exposure (Klotz et al., 2017, 2019). This biological limit value was not surpassed by any individual of Group 1, with the exception of one case with a potential, competing lifestyle-related source of Al exposure which showed an Al excretion above $50\text{ }\mu\text{g/gC}$ 24 h after the SCIT injection. Therefore, no definite health concerns arose from the investigated SCIT treatments. Still, all Group 1 patients receiving an Al-based specific immunotherapy – due to higher risk concerns of their venom allergy – for a period longer than five years were advised to switch to an Al-free formula of the venom, consistent with medical recommendations.

The linear-regression model predicts that patients receiving a cumulative Al dose of about 51 mg subcutaneously through SCIT are likely to exceed the established reference value ($15\text{ }\mu\text{g/gC}$). Depending on the length of the SCIT injection intervals a cumulative Al dose of 51 mg could be reached after just under four years (4-week intervals) or more than seven years (8-week intervals). However, to exceed the biological limit value (with reference to potential health risk) of $50\text{ }\mu\text{g/gC}$, a cumulative Al dose of about 314 mg is required according to the regression model. Even with monthly injections and the highest allowed Al content for parenteral vaccines and allergen extracts of 1.25 mg per dose (European Pharmacopoeia, 2017), such exposure would require more than 20 years of SCIT, i.e., a total of 251 injections. In this context, it is important to consider that this evaluation is only explorative and orientating, as its reliability is limited by the rather small sample size which restricts the calculations of Al doses for exceedances of the limit value.

Some authorities and scientific opinions have recommended to keep Al intake as low as possible, considering unavoidable everyday environmental contact (EFSA, 2008; BfR, 2019; Tietz et al., 2019). Health-based guidance values for general consumer exposures, e.g. by the European Food Safety Authority (EFSA, 2008), however, extrapolate from dietary animal studies and derive a tolerable weekly intake (TWI) (Affourtit et al., 2020). Notwithstanding, such dietary guidance values fall short in light of parenteral exposure and without reference to necessary medical procedures.

In contrast to purely environmental Al exposures, the risk-benefit assessment in clinical practice must include desired medical effects. Al has been used as a potent adjuvant since the first half of the 20th century. Due to its efficacy, tolerability, and safety reports, it still supersedes the use of other adjuvants (Alvaro-Lozano et al., 2020; Jensen-Jarolim, 2015; Petrovsky, 2015; Petrovsky and Aguilar, 2004; Terhune and Deth, 2018; Willhite et al., 2014; Zubeldia et al., 2019). In many vaccines, its use is the very factor that enables an effective vaccination in the first place (Jensen-Jarolim et al., 2020). Furthermore, there is currently no evidence for a causal relationship between the use of Al-adjuvants and potential toxic effects despite long-term usage (Jensen-Jarolim et al., 2020; Weisser et al., 2019). Our exploratory results do, however, suggest that a potential accumulation might become relevant in special cases (multiple, up-dosed, or long-lasting SCIT) and should be assessed.

For the thorough appraisal of the study results, some limitations must be addressed. First, the sample size of the collective is rather small and should initiate confirmation by further investigations with larger collectives. Second, the cross-sectional design limits the monitoring of Al excretion over the entire course of SCIT treatment. Such longitudinal data would be desirable in the future; however, the cross-sectional

design allows a less time-consuming and more resources efficient initial exploration. Considering the explorative proof-of-principle approach the study design was still very valuable, especially as Al_{cum} distribution among Group 1 still comprised a range with a triplicate increase. The age- and sex-composition among groups may present another limitation. As no subdivided reference values have been established (UBA, 1998; Klotz et al., 2017, 2020), no focus was placed on matching Groups 1 and 2 in this regard, causing women to be notably more prevalent in Group 2 and the average age of Group 2 to be slightly younger. Referring to two large studies on urinary Al levels in the general population which had been included to establish a biological reference value (Klotz et al., 2020), there are indications for relevant differences between sexes but inconsistent results concerning age. Both studies (Hoet et al., 2013; Nisse et al., 2017) reported a significantly higher creatinine-adjusted Al excretion in women. However, only Nisse et al. (2017) reported an additional increase of the creatinine-adjusted Al level with age, whereas Hoet et al. (2013) did not report an age effect for Al. As such, while a potential sex-based bias on average Al excretion cannot be excluded, general conclusions would not change because Group 2 included more women, which would only mask an even lower excretion in a more sex-balanced control collective.

On the other hand, several strengths of our study support its high relevance and provide a valuable basis for future research. The data reported here constitute the first valid human biomonitoring data to specifically evaluate the effects of Al-adjuvanted SCIT on systemic Al load in a real-life setting. Urine samples were collected and prepared, and Al analysis was performed by a laboratory highly experienced in Al analytics (Hiller et al., 2023; Letzel et al., 2020) using quality-proven analysis (Göen et al., 2012), taking special precautions to avoid contamination, an important factor for Al determination due to numerous potential sources of environmental pollution. Due to the wide range of Al_{cum} included in the investigated cases, it was also possible to include an initial, tentative prediction on SCIT exposures that would lead to an exceedance of the reference value and possible toxicological health concerns. Also the heterogeneity of Al_{cum} and SCIT duration in Group 1 could be viewed as a pseudo-longitudinal observation and might be very helpful for conceptualizing future long-term monitoring studies to follow-up the Al exposure over the course of SCIT treatment.

5. Conclusion

The exploratory results indicate a relevant iatrogenic contribution of Al-adjuvanted SCIT to the human internal Al burden during multi-annual SCIT, exceeding a typical period of 3–5 years (Pfaar et al., 2022). Given the medical benefits of SCIT and the higher immunogenicity of Al-adjuvanted extracts, a differentiated risk-benefit analysis for medical applications should be taken into account. The authors want to point out that they do not question the general benefits of vaccines and of therapeutic allergens in principle. They explicitly reject any ongoing anti-vaccine discussion and misinformation. It is recommended to carefully monitor emerging data on Al-adjuvants and initiate further studies on the systemic fate of subcutaneously administered Al in humans during and after recurring SCIT injections. Cohort studies monitoring Al excretion longitudinally over the course of SCIT treatment and degradation kinetics after treatment cessation would be beneficial. Especially multiple or long-lasting SCIT applications with Al-adjuvanted extracts – such as in multi-allergic individuals or individuals allergic to hymenoptera venom – constitute a scenario of potential concern. Likewise, particularly susceptible population groups such as children or people with underlying conditions hindering Al excretion, such as impaired kidney function, might require special consideration. Therapy accompanied by repeated biomonitoring might be advisable in such cases in the clinical practice to consider toxicological concerns.

Funding

This research received no specific grant or material support from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability statement

All data relevant to this study are included in the article or uploaded in the supplemental online material; no further raw data are available for reasons of medical confidentiality.

CRediT authorship contribution statement

Julia Hiller: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Thomas Göen:** Conceptualization, Investigation, Methodology, Resources, Visualization, Writing – review & editing. **Hans Drexler:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **Carola Berking:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **Nicola Wagner:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nicola Wagner reports a relationship with ALK-Abellö, Novartis Pharma GmbH, Allergopharma GmbH & Co KG, Sanofi-Aventis Deutschland GmbH, Shire/Takeda, Blueprint, AbbVie Deutschland GmbH & Co KG and with Biocryst that includes: consulting or advisory, funding grants, and speaking and lecture fees.

Acknowledgements

The authors thank Linda McCargo for language editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2024.114337>.

References

- Affourtit, F., Bakker, M., Pronk, M., 2020. Human Health Risk Assessment of Aluminium. RIVM Report 2020-0001. National Institute for Public Health and the Environment, RIVM. <https://doi.org/10.21945/RIVM-2020-0001>. Published by: <https://www.rivm.nl/bibliotheek/rapporten/2020-0001.pdf>. (Accessed 22 May 2023).
- Agency for Toxic Substances and Disease Registry (ATSDR), 2008. Toxicological Profile for Aluminum. U.S. Department of Health and Human Services, Public Health Services, Atlanta, GA. <https://www.atsdr.cdc.gov/ToxProfiles/tp22.pdf>. (Accessed 9 January 2024).
- Alvaro-Lozano, M., Akdis, C.A., Akdis, M., Alviani, C., Angier, E., Arasi, S., Arzt-Gradwohl, L., Barber, D., Bazire, R., Cavkaytar, O., Comberlati, P., Dramburg, S., Durham, S.R., Eifan, A.O., Forchert, L., Halken, S., Kirtland, M., Kucuksezer, U.C., Layhadi, J.A., Matricardi, P.M., Muraro, A., Ozdemir, C., Pajno, G.B., Pfaar, O., Potapova, E., Riggioni, C., Roberts, G., Rodríguez del Río, P., Shamji, M.H., Sturm, G. J., Vazquez-Ortiz, M., 2020. EAACI allergen immunotherapy user's guide. *Pediatr. Allerg. Immunol. (Leipz.)* 31 (Suppl. 25), 75–81. <https://doi.org/10.1111/pai.13189>, 1–101 (Chapter 13).
- Bundesinstitut für Risikobewertung (BfR), 2019. Reducing aluminium intake can minimise potential health risks. Opinion No. 45/2019 BfR. <https://doi.org/10.17590/20191213-112240>. <https://www.bfr.bund.de/cm/349/reducing-aluminium-intake-can-minimise-potential-health-risks.pdf>. (Accessed 22 May 2023).
- European Food Safety Authority (EFSA), 2008. Safety of aluminium from dietary intake - scientific opinion of the panel on food additives, flavourings, processing aids and food contact materials (AFC). *EFSA J.* 754, 1–34. <https://doi.org/10.2903/j.efsa.2008.754>.
- European Pharmacopoeia (Ph Eur) 9.0, 2017 (1). Allergen Products, 1063, pp. 811–812.

- Geoghegan, S., O'Callaghan, K.P., Offit, P.A., 2020. Vaccine safety: myths and misinformation. *Front. Microbiol.* 11, 372. <https://doi.org/10.3389/fmicb.2020.00372>.
- Göen, T., Schaller, K.H., Drexler, H., 2012. External quality assessment of human biomonitoring in the range of environmental exposure levels. *Int. J. Hyg Environ. Health* 215 (2), 229–232. <https://doi.org/10.1016/j.ijheh.2021.113711>.
- Golden, D.B., Kwiterovich, K.A., Kagey-Sobotka, A., Valentine, M.D., Lichtenstein, L.M., 1996. Discontinuing venom immunotherapy: outcome after five years. *J. Allergy Clin. Immunol.* 97 (2), 579–587. [https://doi.org/10.1016/S0091-6749\(96\)70302-0](https://doi.org/10.1016/S0091-6749(96)70302-0).
- Golden, D.B., Kwiterovich, K.A., Kagey-Sobotka, A., Lichtenstein, L.M., 1998. Discontinuing venom immunotherapy: extended observations. *J. Allergy Clin. Immunol.* 101 (3), 298–305. [https://doi.org/10.1016/S0091-6749\(98\)70239-8](https://doi.org/10.1016/S0091-6749(98)70239-8).
- Hethy, C., Hartung, N., Wangorsch, G., Weisser, K., Huisinga, W., 2021. Physiology-based toxicokinetic modelling of aluminium in rat and man. *Arch. Toxicol.* 95 (9), 2977–3000. <https://doi.org/10.1007/s00204-021-03107-y>.
- Hiller, J., Göen, T., Seibold-Wulf, N., Meyer, S., Drexler, H., 2023. Effect of an aluminum foil-processed diet on internal human aluminum burden. *Environ. Int.* 177, 108000. <https://doi.org/10.1016/j.envint.2023.108000>. Epub 2023 May 29.
- Hoet, P., Jacquery, C., Deumer, G., Lison, D., Haufroid, V., 2013. Reference values and upper reference limits for 26 trace elements in the urine of adults living in Belgium. *Clin. Chem. Lab. Med.* 51 (4), 839–849. <https://doi.org/10.1515/cclm-2012-0688>.
- Hogensch, H., 2012. Mechanism of immunopotentiality and safety of aluminum adjuvants. *Front. Immunol.* 3, 406. <https://doi.org/10.3389/fimmu.2012.00406>.
- Institute of Medicine, 2001. Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders. The National Academies Press (US, Washington, DC). <https://doi.org/10.17226/10208>.
- Institute of Medicine, 2004. Immunization Safety Review: Vaccines and Autism. The National Academies Press (US, Washington, DC). <https://doi.org/10.17226/10997>.
- Jensen-Jarolim, E., 2015. Aluminium in allergies and allergen immunotherapy. *World Allergy Organ. J.* 8 (1), 7. <https://doi.org/10.1186/s40413-015-0060-5>.
- Jensen-Jarolim, E., Bachmann, M.F., Bonini, S., Jacobsen, L., Jutel, M., Klimek, L., Mahler, V., Mösges, R., Moingeon, P., O'Hehir, R.E., Palomares, O., Pfaar, O., Renz, H., Rhyner, C., Roth-Walter, F., Rudenko, M., Savolainen, J., Schmidt-Weber, C.B., Traidl-Hoffmann, C., Kündig, T., 2020. State-of-the-art in marketed adjuvants and formulations in allergen immunotherapy: a position paper of the European academy of allergy and clinical immunology (EAACI). *Allergy* 75 (4), 746–760. <https://doi.org/10.1111/all.14134>.
- Jensen-Jarolim, E., Roth-Walter, F., Jordakieva, G., Pali-Schöll, I., 2021. Allergens and adjuvants in allergen immunotherapy for immune activation, tolerance, and resilience. *J. Allergy Clin. Immunol. Pract.* 9 (5), 1780–1789. <https://doi.org/10.1016/j.jaip.2020.12.008>.
- Keating, M.U., Kagey-Sobotka, A., Hamilton, R.G., Yunginger, J.W., 1991. Clinical and immunologic follow-up of patients who stop venom immunotherapy. *J. Allergy Clin. Immunol.* 88 (3 Pt 1), 339–348. [https://doi.org/10.1016/0091-6749\(91\)90095-6](https://doi.org/10.1016/0091-6749(91)90095-6).
- Klotz, K., Drexler, H., Hartwig, A., MAK-Commission, 2020. Aluminium - addendum for evaluation of a BAR. Assessment values in biological material - translation of the German version from 2019. MAK Collect. *Occup. Health Saf.* 5 (1), Doc014. <https://doi.org/10.34865/bb742990e5.1>.
- Klotz, K., Meyer-Baron, M., van Thriel, C., Pallapies, D., Nasterlack, M., Letzel, S., Roßbach, B., Triebig, G., Weistenhöfer, W., Drexler, H., Hartwig, A., MAK-Commission, 2019. Addendum to Aluminium. BAT value documentation, 2019. MAK Collect. *Occup. Health Saf.* 4 (4), 2279–2311. <https://doi.org/10.1002/3527600418.bb742990e2419>.
- Klotz, K., Weistenhöfer, W., Neff, F., Hartwig, A., van Thriel, C., Drexler, H., 2017. The health effects of aluminum exposure. *Dtsch. Arztebl. Int.* 114 (39), 653–659. <https://doi.org/10.3238/arztebl.2017.0653>.
- Larsen, K., 1972. Creatinine assay by a reaction-kinetic principle. *Clin. Chim. Acta* 41, 209–217. [https://doi.org/10.1016/0009-8981\(72\)90513-x](https://doi.org/10.1016/0009-8981(72)90513-x).
- Letzel, M., Drexler, H., Göen, T., Hiller, J., 2020. Impact of daily antiperspirant use on the systemic aluminum exposure: an experimental intervention study. *Skin Pharmacol. Physiol.* 33 (1), 1–8. <https://doi.org/10.1159/000502239>.
- Masson, J.D., Crépeaux, G., Authier, F.J., Exley, C., Gherardi, R.K., 2018. Critical analysis of reference studies on the toxicokinetics of aluminum-based adjuvants. *J. Inorg. Biochem.* 181, 87–95. <https://doi.org/10.1016/j.jinorgbio.2017.12.015>.
- Nisse, C., Tagne-Fotso, R., Howsam, M., Richeval, C., Labat, L., Leroyer, A., 2017. Blood and urinary levels of metals and metalloids in the general adult population of Northern France: the IMEPOGE study, 2008–2010. *Int. J. Hyg Environ. Health* 220 (2 Pt B), 341–363. <https://doi.org/10.1016/j.ijheh.2016.09.020>.
- Petrovsky, N., 2015. Comparative safety of vaccine adjuvants: a summary of current evidence and future needs. *Drug Saf.* 38 (11), 1059–1074. <https://doi.org/10.1007/s40264-015-0350-4>.
- Petrovsky, N., Aguilar, J.C., 2004. Vaccine adjuvants: current state and future trends. *Immunol. Cell Biol.* 82 (5), 488–496. <https://doi.org/10.1111/j.0818-9641.2004.01272.x>.
- Pfaar, O., Agache, I., de Blay, F., Bonini, S., Chaker, A.M., Durham, S.R., Gawlik, R., Hellings, P.W., Jutel, M., Kleine-Tebbe, J., Klimek, L., Kopp, M.V., Nandy, A., Rabin, R.L., van Ree, R., Renz, H., Roberts, G., Salapatek, A.M., Schmidt-Weber, C.B., Shamji, M.H., Sturm, G.J., Virchow, J.C., Wahn, U., Willers, C., Ziegler, M., Akdis, C.A., 2019. Perspectives in allergen immunotherapy: 2019 and beyond. *Allergy* 74 (Suppl. 108), 3–25. <https://doi.org/10.1111/all.14077>.
- Pfaar, O., Ankeremann, T., Augustin, M., Bubel, P., Böing, S., Brehler, R., Eng, P.A., Fischer, P.J., Gerstlauer, M., Hamelmann, E., Jakob, T., Kleine-Tebbe, J., Kopp, M.V., Lau, S., Mülleneisen, N., Müller, C., Nemat, K., Pfützner, W., Saloga, J., Strömer, K., Schmid-Grendelmeier, P., Schuster, A., Sturm, G.J., Taube, C., Szépfalusi, Z., Vogelberg, C., Wagenmann, M., Wehrmann, W., Werfel, T., Wöhr, S., Worm, M., Wedi, B., 2022. Guideline on allergen immunotherapy in IgE-mediated allergic diseases: S2K guideline of the German society of allergology and clinical immunology (DGAKI), society of pediatric allergology and environmental medicine (GPA), medical association of German allergologists (AeDA), Austrian society of allergology and immunology (ÖGAI), Swiss society for allergology and immunology (SSAI), German dermatological society (DDG), German society of oto-rhino-laryngology, head and neck surgery (DGHN-KHC), German society of pediatrics and adolescent medicine (DGKJ), society of pediatric pulmonology (GPP), German respiratory society (DGP), German professional association of otolaryngologists (BVHNO), German association of paediatric and adolescent care specialists (BVKJ), federal association of pneumologists, sleep and respiratory physicians (BdP), professional association of German dermatologists (BVDD). *Allergol. Select.* 6, 167–232. <https://doi.org/10.5414/ALX02331E>.
- Priest, N.D., 1993. The bioavailability and metabolism of aluminium compounds in man. *Proc. Nutr. Soc.* 52 (1), 231–240. <https://doi.org/10.1079/pns19930055>.
- Priest, N.D., 2004. The biological behaviour and bioavailability of aluminium in man, with special reference to studies employing aluminium-26 as a tracer: review and study update. *J. Environ. Monit.* 6 (5), 375–403. <https://doi.org/10.1039/b314329p>.
- Przybyla, B., Ruëff, F., 2010. Hymenoptera venom allergy. *J. Dtsch. Dermatol. Ges.* 8 (2), 114–127. <https://doi.org/10.1111/j.1610-0387.2009.07125.x> quiz 28–30.
- Reisman, R.E., 1993. Duration of venom immunotherapy: relationship to the severity of symptoms of initial insect sting anaphylaxis. *J. Allergy Clin. Immunol.* 92 (6), 831–836. [https://doi.org/10.1016/S0091-6749\(93\)90060-s](https://doi.org/10.1016/S0091-6749(93)90060-s).
- Sturm, G.J., Varga, E.M., Roberts, G., Mosbech, H., Bilö, M.B., Akdis, C.A., Antolín-Amérigo, D., Cichocka-Jarosz, E., Gawlik, R., Jakob, T., Kosnik, M., Lange, J., Mingomataj, E., Mitsias, D.I., Ollert, M., Oude Elberink, J.N.G., Pfaar, O., Pitsios, C., Pravettoni, V., Ruëff, F., Sin, B.A., Agache, I., Angier, E., Arasi, S., Calderón, M.A., Fernandez-Rivas, M., Halken, S., Jutel, M., Lau, S., Pajno, G.B., van Ree, R., Ryan, D., Spranger, O., van Wijk, R.G., Dhimi, S., Zaman, H., Sheikh, A., Muraro, A., 2018. EAACI guidelines on allergen immunotherapy: hymenoptera venom allergy. *Allergy* 73 (4), 744–764. <https://doi.org/10.1111/all.13262>.
- Terhune, T.D., Deth, R.C., 2018. Aluminum adjuvant-containing vaccines in the context of the hygiene hypothesis: a risk factor for eosinophilia and allergy in a genetically susceptible subpopulation? *Int. J. Environ. Res. Publ. Health* 15 (5). <https://doi.org/10.3390/ijerph15050901>.
- Tietz, T., Lenzner, A., Kolbaum, A.E., Zellmer, S., Riebeling, C., Gürtler, R., Jung, C., Kappenstein, O., Tentschert, J., Giubudagian, M., Merkel, S., Pirow, R., Lindtner, O., Tralau, T., Schäfer, B., Laux, P., Greiner, M., Lampen, A., Luch, A., Wittkowski, R., Hensel, A., 2019. Aggregated aluminium exposure: risk assessment for the general population. *Arch. Toxicol.* 93 (12), 3503–3521. <https://doi.org/10.1007/s00204-019-02599-z>.
- Umweltbundesamt (UBA), 1998. Stellungnahme der Kommission "Human-Biomonitoring" des Umweltbundesamtes. Aluminium. *Bundesgesundheitsblatt* 41 (6), 271.
- Weisser, K., Göen, T., Oduro, J.D., Wangorsch, G., Hanschmann, K.O., Keller-Stanislowski, B., 2019. Aluminium in plasma and tissues after intramuscular injection of adjuvanted human vaccines in rats. *Arch. Toxicol.* 93 (10), 2787–2796. <https://doi.org/10.1007/s00204-019-02561-z>.
- Weisser, K., Göen, T., Oduro, J.D., Wangorsch, G., Hanschmann, K.O., Keller-Stanislowski, B., 2020. Aluminium from adjuvanted subcutaneous allergen immunotherapeutics in rats is mainly detected in bone. *Allergy* 75 (1), 215–217. <https://doi.org/10.1111/all.13982>.
- Willhite, C.C., Karyakina, N.A., Yokel, R.A., Yenugadhati, N., Wisniewski, T.M., Arnold, I.M., Momoli, F., Krewski, D., 2014. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. *Crit. Rev. Toxicol.* 44 (Suppl. 4), 1–80. <https://doi.org/10.3109/10408444.2014.934439>.
- World Health Organization (WHO), 1996. In: Office of Occupational Health. *Biological Monitoring of Chemical Exposure in the Workplace: Guidelines*. World Health Organization; 1996, Geneva, 1WHO/HPR/OCH/96.1.
- World Health Organization (WHO), 2012. Global Advisory Committee for Vaccine Safety (GACVS), Report of Meeting Held 6-7 June 2012. WHO Weekly Epidemiological Record.
- Zubeldia, J.M., Ferrer, M., Dávila, I., Justicia, J.L., 2019. Adjuvants in allergen-specific immunotherapy: modulating and enhancing the immune response. *J. Invest. Allergol. Clin. Immunol.* 29 (2), 103–111. <https://doi.org/10.18176/jiaci.0349>.