



Prospective comparison of a nonmodified and a modified mite extract for immunotherapy in children and adolescents

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Aim: This prospective study compares nonmodified and modified house dust mite extracts for allergen immunotherapy (AIT) in pediatric patients with allergic asthma. **Materials & methods:** Total 95 patients underwent bronchial allergen provocation (BAP). AIT was recommended to 62 patients. Complete datasets of 54 subjects were obtained. Primary aim was the comparison of treatment success defined by BAP between two extracts after 1 year. Secondary parameters were laboratory parameters and clinical symptoms. **Results:** Significant improvement ($p < 0.001$) was measured by BAP in both treatment groups. No change was seen in the controls. Both extracts exerted comparable effects on all parameters. **Conclusion:** After 1 year of AIT, the extracts were equally efficient, with significant improvements in 70.0% (nonmodified) and 72.2% (modified) of patients.

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Bronchial asthma is the most common chronic childhood disease in Germany [1] and its global prevalence is steadily increasing. Asthma is often caused by an allergen-driven airway inflammation. A potential curative therapy for allergies is allergen immunotherapy (AIT) [2]. AIT was developed more than 100 years ago [3], and its efficacy was confirmed by the WHO approximately 20 years ago [4]. Besides allergen avoidance, AIT is the only disease-modifying therapy that has been proven to change the natural course of allergic diseases [4–6]. The positive effects of AIT have been extensively reported [7–13] where it induces immunological tolerance and decreases the need for pharmacotherapy such as inhaled corticosteroids (ICS) for asthma [9,14]. Furthermore, AIT increases the quality of life [14,15] and decreases the specific bronchial hyperreactivity (BHR) of patients [14]. In addition to the immediate effects, AIT also exerts a long-term protective effect by decreasing the incidence of new sensitizations and preventing the progression of allergic disease [7,13,16].

Before initiating subcutaneous immunotherapy (SCIT), patients must be diagnosed with an IgE-mediated allergic disease. While positive skin-prick test (SPT) and radioallergen sorbent test (RAST) reveal allergic sensitization, the diagnosis must be confirmed by patient's history or allergen provocation tests [17]. For perennial allergies, such as house dust mite (HDM) allergy, the German guidelines suggest that, if possible, an allergen provocation test should be performed initiation of AIT [17]. In the patients with allergic asthma, a bronchial allergen provocation (BAP) test can determine the causality between allergic sensitization and asthma symptoms [18].

HDM immunotherapy is usually a treatment that is administered continuously over the course of 3 years. Although new forms of administration have been developed, SCIT is still the predominant mode used for children in Germany [19]. SCIT has been well studied, and its efficacy and tolerability have been proven in children and adolescents in numerous studies [7–11,20].

To date, the wide variety of mite extracts can be divided into two major groups: nonmodified mite extracts and chemically modified mite extracts, for example allergoids. Advantages and disadvantages of both types have been reported in the current literature. On the one hand, nonmodified mite extracts have been reported to be more effective than modified mite extracts [13]. Furthermore, the nonmodified mite extracts are supposed to contain all relevant epitopes [6]. On the other hand, modified mite extracts are presumed to contain fewer B-cell epitopes [13] and therefore display a lower IgE-binding capacity [20]. Through this modification, allergoids may exhibit a low risk of adverse events while preserving the immunological reactivity [21,22]. Interestingly, a large retrospective cohort study of AIT by Schmidt *et al.* [13] showed a stronger preventive effect of nonmodified mite extracts compared with modified mite extracts. This finding was recently confirmed by another large cohort study, where nonmodified mite extracts performed better than four different modified mite extracts [23]. Also, studies of the differences between nonmodified and modified mite extracts have been reported. To our knowledge, no studies have been conducted in children and adolescents [22,24].

The aim of this study was to determine whether nonmodified and modified mite extracts differ significantly in terms of efficacy and tolerability after 1 year of AIT in pediatric patients with asthma and HDM allergy. Primary parameter was the change in the severity of early asthmatic reaction (EAR) after 1 year of AIT, measured as maximum fall in FEV1 (max. FEV1 fall) by BAP [8,25,26]. Secondary parameters were clinical symptoms and titers of total IgE, specific IgE, IgG and IgG4 antibodies against HDM before and after AIT.

Materials & methods

Study design

All included patients were recruited from the Department for Allergology, Pneumology and Cystic Fibrosis at the Children's Hospital of the University Hospital Frankfurt, Germany. In this randomized prospective study, we collected the data on first (between October 2012 and January 2014) and second (1 year later) visits to our outpatient clinic. The investigation was a nonsponsored, investigator-initiated study. In our outpatient clinic, clinical history was obtained from all the patients. If signs of allergic disease or asthma presented, an SPT was conducted. Additionally, blood was obtained to measure the total-IgE and specific IgE, IgG and IgG4 against HDM. All patients with a positive SPT for HDM and asthma symptoms were invited for a BAP test in order to determine the clinical relevance of allergic sensitization to asthma symptoms. In case of consistent results with previous tests and clinical history, an AIT was recommended. In terms of BAP test, a max. FEV1 fall of 20% or greater from baseline was considered as 'positive' test result and therefore an indication for AIT. The BAP tests were performed twice a week (on Tuesdays and Thursdays). Treatment of patients was allocated according to the day of investigation. All patients tested on Tuesdays were treated with the modified mite extract and all patients tested on Thursdays were treated with the nonmodified mite extract. According to the German AMG (*Arzneimittelgesetz*, law for development of medications) a precise randomization procedure is only possible in a pharmaceutical study. If so, the medication had to be delivered for free by both companies. Since both companies denied sponsoring our trial, a precise randomization procedure was not possible. Therefore, we choose an unscientific, real-life allocation to get well-balanced patient groups. The following parameters were obtained at each visit: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), max. FEV1 fall in early (EAR) and late (LAR) asthmatic reactions, Tiffeneau index (FEV1/FVC), PD20FEV1-Mite, total IgE, specific IgE, IgG and IgG4 antibody titers. Additionally, the exhaled NO (eNO) was measured before each BAP, a medication history was recorded and a standardized questionnaire was completed [27].

The study was approved by the Frankfurt Ethics Committee, performed in accordance with the declarations of Good Clinical Practice, and registered at ClinicalTrials.gov NCT01134757. All parents provided written informed consent for the analysis of their children's data.

Patients

Total 95 patients (aged 8.5 ± 2.8 years) diagnosed with asthma and sensitization to HDM have undergone a first BAP test. Asthma was diagnosed by clinical history, spirometry, body plethysmography and bronchial reversibility testing. Furthermore, all patients had a clinical history indicative of HDM allergy. Sensitization to HDM was confirmed through positive SPT in all 95 patients. RAST results for an additional verification by serum specific IgE against mite were obtained from 89 patients (93.7%). At the time of investigation, all patients had well-controlled asthma. Of those patients, 37 (38.9%) were treated with a short-acting β_2 -agonist (SABA) on demand (Global Initiative for Asthma [GINA] 1), 29 (30.5%) were treated with regular low-dose ICS or leukotriene receptor

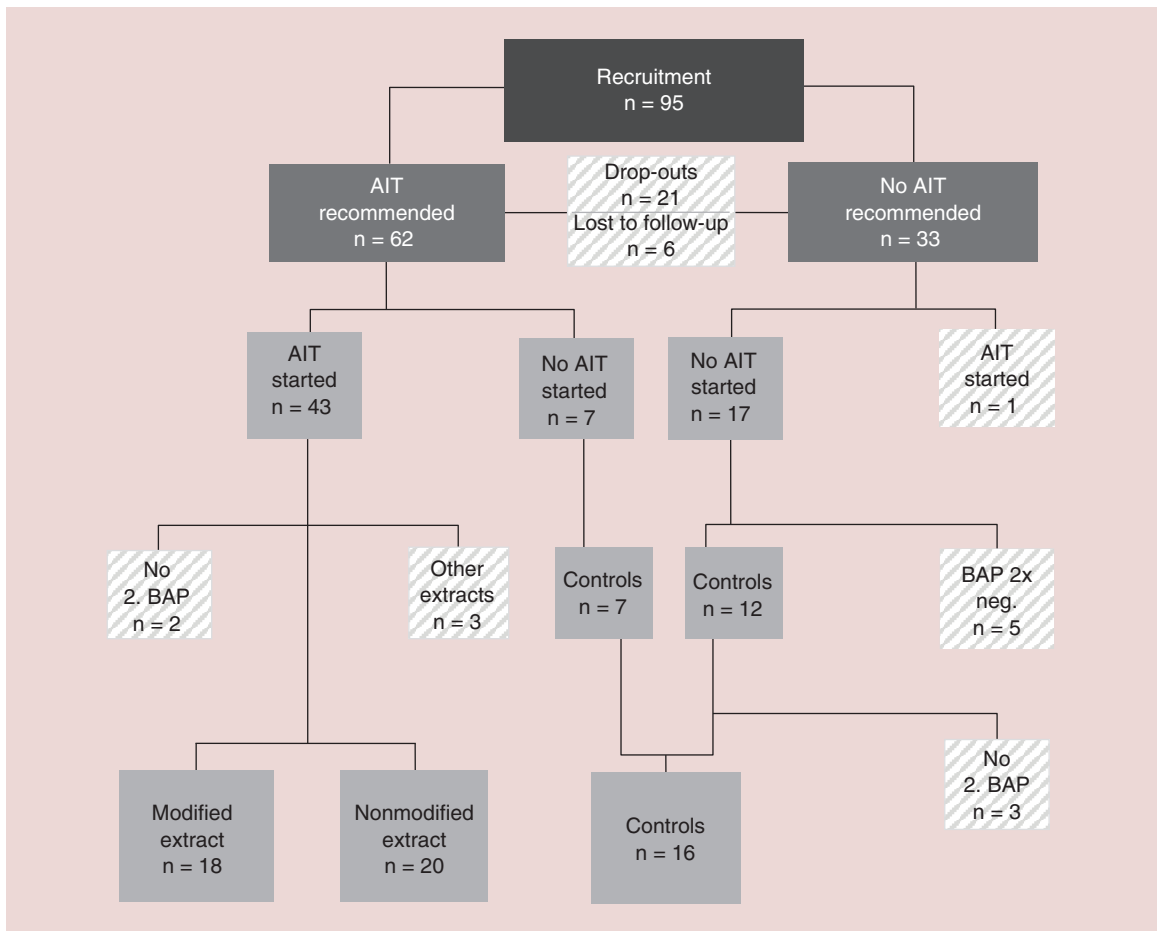


Figure 1. Study flow chart. At the first visit, 95 patients underwent a BAP, 62 were recommended to start AIT, 33 were recommended to wait with AIT. Six patients were lost to follow-up and 21 patients dropped out before the second BAP. At the second visit, 18 patients had started AIT with the modified mite extract and 20 with the nonmodified mite extract, 16 patients who did not start AIT served as controls.
AIT: Allergen immunotherapy; BAP: Bronchial allergen provocation.

antagonist and as-needed SABA (GINA 2), and 29 (30.5%) patients were treated with a regular combination of ICS and long-acting β_2 -agonists (LABA) and as-needed SABA (GINA 3). An AIT was recommended for 62 patients (65.3%), who had a positive BAP test (max. FEV1 fall $\geq 20\%$). The other 33 patients (34.7%) had a negative BAP test, and therefore, AIT was not recommended at the time of first BAP test. After 1 year, six patients (6.3%) were lost to follow-up, and 21 patients (22.1%) had to drop out of the investigation due to personal reasons. The remaining 68 patients (71.6%) underwent a second BAP test. Of these 68 patients, 14 (20.6%) were excluded from further analysis: three patients were treated with other AIT extracts, five patients had a negative BAP result both times, five patients did not undergo a second BAP test and one patient started SCIT after a negative BAP test.

Of the 14 excluded patients, five patients were not able to undergo a second BAP. One of them was treated with the nonmodified mite extract, one with the modified mite extract and three were controls. The BAP could not be started because of a significant fall of FEV1 after saline inhalation. This could be indicative for an increased bronchial hyperreactivity and therefore be important for our results. For that reason, we did a sensitivity test by reanalyzing the collected data including those five patients.

Overall, 24 (35.3%) of the 68 patients with two visits did not start an AIT; hence, they served as the control group. In the control group, 17 patients (70.8%) showed no significant reaction in BAP, despite a significant sensitization for HDM revealed by SPT and RAST and typical allergic symptoms by clinical history. They were recommended to wait with AIT and revisit for a follow-up 1 year later. Five patients (20.8%) still had a negative BAP testing in the 1-year follow-up; therefore, they were excluded from further analysis, as shown in Figure 1.

Table 1. Characteristics of the three groups at the time of 1 and 2 bronchial allergen provocation.

Gender	Nonmodified extract		Modified extract		Controls	
	1. BAP	2. BAP	1. BAP	2. BAP	1. BAP	2. BAP
	n = 20		n = 18		n = 16	
	m = 11 w = 9		m = 7 w = 11		m = 11 w = 5	
Age (years)	8.8 ± 2.5	9.8 ± 2.6	8.1 ± 2.9	9.2 ± 2.9	8.6 ± 2.6	9.6 ± 2.6
FEV1 (%)	97.9 ± 14.0	98.2 ± 13.5	99.4 ± 14.4	99.3 ± 14.1	98.8 ± 20.5	95.3 ± 15.1
Total-IgE (lu/ml)	288.0 (25.5–1465.0)	454.0 (40.1–2580.0)	220.0 (63.7–6913.0)	342.0 (45.5–6948.0)	402.0 (36.6–5032.0)	364.0 (57.4–7097.0)
Spec. IgE D1 (KU/l)	59.2 (7.6–795.0)	49.0 (9.9–246.0)	88.7 (1.2–298.0)	45.3 (2.39–1339.0)	65.7 (0.7–415.0)	25.3 (3.6–428.0)
Spec. IgE D2 (KU/l)	57.5 (23.9–292.0)	49.8 (10.1–173.0)	64.2 (0.6–271.0)	43.1 (1.27–1363.0)	69.7 (0.4–438.0)	50.3 (2.0–522.0)
Spec. IgG D1 (mg/l)	12.0 (6.1–31.3)	34.5 (13.3–54.7)	22.5 (6.9–65.4)	57.0 (18.2–128.0)	18.3 (5.4–81.0)	16.3 (7.4–100.0)
Spec. IgG D2 (mg/l)	7.3 (5.9–18.9)	25.1 (12.0–86.2)	15.2 (6.7–115.0)	40.9 (12.3–148.0)	14.5 (3.6–34.1)	16.0 (4.7–45.3)
EAR (max. FEV1 fall; %)	28.6 ± 8.5	15.5 ± 10.8	28.6 ± 7.4	16.7 ± 12.6	24.6 ± 12.8	27.4 ± 11.6
LAR (max. FEV1 fall; %)	35.6 ± 11.9	27.8 ± 12.3	30.4 ± 21.0	26.8 ± 18.5	34.7 ± 17.2	20.8 ± 16.5

Means ± standard deviation or medians and ranges are reported.
BAP: Bronchial allergen provocation; EAR: Early asthmatic reaction; LAR: Late asthmatic reaction.

Three patients (12.5%), who had a negative BAP at first visit, showed a severe response to saline inhalation and therefore did not complete the second BAP. Seven patients did not start AIT, despite initial recommendation and detailed explanation of the benefits of AIT. The following reasons were given by the parents: AIT was not necessary, scared by the risk of side effects, organizational issues such as living too far away from our outpatient clinic. Overall, 16 patients were included in the analysis as controls. Ten (62.5%) control patients had their first BAP on Tuesday and six (37.5%) on Thursday.

Collectively, the data of 54 patients were analyzed in this study. The study flowchart is shown in [Figure 1](#). About 20 (37.0%) of 54 patients received the nonmodified mite extract, 18 (33.3%) received the modified mite extract, and 16 (29.6%) AIT-untreated patients, served as controls.

The characteristics of the three groups are shown in [Table 1](#). Comparison via analysis of variance (ANOVA) and Kruskal–Wallis test showed no significant differences among the three groups before AIT, except for the specific IgG D2 titer, which was lower (Kruskal–Wallis test; $p < 0.005$) before SIT in the nonmodified mite extract group compared with the modified mite extract group, but both groups were not significantly different from the control group. However, a combined analysis of specific IgG D1 and D2 did not show any significant differences among the three groups.

AIT with nonmodified & modified mite extracts

The mite extracts used for AIT were both well-established HDM extracts for SCIT with a recommended continuous treatment over the course of 3 years. As many studies have evaluated the treatment success (side effects and reduction of steroids) after a shorter duration, we chose to assess and compare the extracts after 1 year of treatment.

The nonmodified mite extract used was Avanz[®] from ALK-Abelló Arzneimittel GmbH, Hamburg, Germany. The preparation included equal parts of *D. pteronyssinus* and *D. farinae*. The other constituents of the product were aluminum hydroxide, sodium chloride, sodium bicarbonate, phenol and water for injection purposes. The up-dosing and maintenance of the treatment was done according to the manufacturer's recommendations. The maintenance injections were given at approximately 6-week intervals. At second BAP, patients were treated for 12 ± 2 months, comprising an average cumulative allergen dose of median 157,400 SQ-U (range 131,775–208,650 SQ-U).

In regard to modified mite extract, the preparation Acaroid[®] from Allergopharma GmbH & Co. KG; Rheinbeck, Germany was used. The preparation was a modified high-dose hypoallergenic HDM preparation (allergoid) containing equal parts of *D. pteronyssinus* and *D. farinae* that have been chemically modified using formaldehyde. Further constituents were aluminum monohydroxide, phenol, sodium chloride, sodium bicarbonate and water for injection purposes. The up-dosing and maintenance treatment was done according to the manufacturer's recommendations. At maintenance, a dose of 0.6 ml of 10,000 U/ml was regularly injected at 6-week intervals. At second BAP, patients were treated for 12 ± 2 months, comprising an average cumulative allergen dose of median 58,300 AE (range 47,300–80,300 AE).

Pulmonary function test

Three pulmonary function tests were performed before each BAP test in order to document the baseline lung function of each patient. The recommendations of the American Thoracic Society and European Respiratory Society (ERS) were followed for pulmonary function tests [28]. The following measurements were obtained: FVC, FEV1, max. FEV1 fall in EAR and LAR, FEV1/FVC, and PD20FEV1-Mite. The last FEV1 measurement was used as a baseline value in the BAP test. When signs of an acute obstruction (FEV1 <75%) were present, the BAP test was postponed.

Bronchial allergen provocation

All patients were instructed not to take any asthma medication at least 24 h before each BAP test. All provocation tests were done in the morning, between 8:00 am and 12:30 pm. The Aerosol Provocation System (VIASYS Healthcare GmbH, Höchberg, Germany), which is a flow controlled nebulizer system [26], was used for every BAP test. This technique allowed an exact measurement of the allergen and calculation of the dose and concentration. The nebulization was conducted by a Medic Aid nebulizer with a constant output of 160 mg/min, resulted in particles of 3.2 µm in size [26]. The amount of aerosol inhaled varied depending on multiple factors, such as functional residual capacity, inspiratory vital capacity, inspiratory velocity and duration of inspiration. To ensure ideal conditions, an inhalation time longer than 5 s and aerosol flow below 0.5 l/s was required. Continuous monitoring of the patient's breathing by Aerosol Provocation System during the provocation ensured an inhalation of a precise amount of allergen. For this purpose, an integrated flow sensor continuously measured the inspiratory flow and inspiration time [26], enabling the device to adapt the duration of inhalation to the amount of allergen to be inhaled. For the allergen solution, lyophilized HDM allergen (*D. pteronyssinus*, Dpt. 725 and 706, Allergopharma GmbH & Co. KG; Rheinbeck, Germany) was dissolved in 5 ml of a 0.9% saline solution, resulted in an allergen solution of 5000 SBU/ml. After the determination of baseline FEV1, the patient inhaled a 0.9% saline solution without allergen. About 2 min later, the FEV1 was measured. In the case of a >10% fall in FEV1, BAP test was postponed due to likelihood of an acutely increased unspecific hypersensitivity. Otherwise, the BAP test was conducted by stepwise inhalation of increasing amounts of allergen solution. About 8 min after each step, spirometry was performed. The first step was inhalation of 10 SBU allergen solution. Afterward, the dose was doubled until a ≥20% fall in FEV1 was reached or a cumulative dose of 310 SBU was administered. In the 1-year follow-up visit, the BAP test was performed using the same procedure, but it was only continued until a cumulative dose that was tolerated in the first BAP was reached or until FEV1 declined ≥20%. Subsequently, the change in max. FEV1 fall when challenged with the same cumulative allergen dose was compared before and after 1 year with or without AIT, respectively. At the end of each BAP test, every patient inhaled a rapid-acting β₂-agonist (RABA) so that the FEV1 reached at least 80% of the baseline value. Responders were defined by negative BAP (FEV1 fall of <20%) in the test 1 year after AIT.

To measure the LAR, FEV1 was measured and recorded by the patients and their parents with the asthma monitor AM1[®] (VIASYS Healthcare GmbH, Höchberg, Germany) for up to 12 h after the BAP. LAR was defined as a max. FEV1 fall ≥20%. Patients were instructed to use a RABA as rescue medication if LAR occurs.

Skin-prick test

An SPT using standard allergens of *D. pteronyssinus* and *farinae* (Allergopharma, Rheinbeck, Germany) was performed before the first BAP. In the prepared allergen solution, the allergens were dissolved in a 0.9% saline solution and phenol for conservation, resulting in a concentration of 50.000 BU/ml. With a pipette, one drop of the test solution was placed on the previously marked skin area. Afterward, with a prick needle, the skin was lightly perforated through the drop in a vertical direction. The response was recorded as the wheal diameter in millimeter. It was considered positive only if the diameter was at least 3 mm greater than the saline control.

Exhaled NO

The eNO level was measured before each BAP using a Chemiluminescence analyzer (NIOX[®] Nitric Oxide Monitoring System, Aerocirne, Sweden), according to the American Thoracic Society and ERS recommendations [29].

Clinical & medication history

A general clinical history, and a standardized medical history were taken from the patients and their parents before each BAP test. Additionally, all patients were categorized into treatment steps according to GINA [27].

Questionnaire

A questionnaire modified from the ISAAC questionnaire [30] was completed by all patients and their parents before each BAP. Every patient and his or her parents answered the questions about his or her asthma symptoms. An asthma score (ranging from 0 to 16 points) was calculated using this data. After 1 year of AIT, all treated patients and their parents additionally answered questions regarding treatment outcomes, adverse events and evaluations.

Laboratory measurements

Blood samples were collected from all patients after each BAP test. A full blood count was routinely done to determine the eosinophilic count. Furthermore, levels of total-IgE antibodies, specific IgE antibodies and specific IgG antibodies for *D. pteronyssinus* and *farinae* were analyzed in our laboratory using a two-sided chemiluminescent assay (Immulite 2500, Siemens AG, Eschborn, Germany), according to the manufacturer's instructions.

Furthermore, specific IgG4 levels were determined using ELISA (Diagnostic Systems & Technologies GmbH, Schwerin, Germany). The analysis was performed according to the manufacturer's instructions.

Statistical analyses

According to a previous power calculation of our study group, a statistically significant change in EAR can be observed in the case of successful AIT, when 14 subjects are included in each treatment arm [8]. We recruited more patients and increased the sample sizes in the nonmodified mite extract arm, the modified mite extract arm and the control arm to $n = 20$, $n = 18$ and $n = 16$, respectively.

Primary statistical aim was the comparison of the primary parameter, in other words, the treatment success defined by BAP (change in EAR) after 1 year of AIT between the treatment arms. Secondary parameters were laboratory parameters and clinical symptoms.

To compare two parameters, all normally distributed data were analyzed using a paired *t*-test, and the means $\pm$ standard deviation (SD) were reported. The data that did not display a normal distribution were analyzed using a Mann–Whitney U Test, and the medians and ranges are reported. To compare all three groups, all normally distributed data were analyzed using a one-way ANOVA. The data that did not display a normal distribution were analyzed using a Kruskal–Wallis test. Consecutively, we conducted paired *post hoc* test in all subgroups. To compare the changes among the three groups after 1 year, we performed the previously described tests on the absolute change (Δ) of each parameter, additionally, we compared the relative change ($\Delta\%$) of the primary parameters. Categorical data were analyzed using Fisher's exact test.

Five patients had to be excluded, as a second BAP test could not be performed, we did a sensitivity test by reanalyzing the collected data including those five patients using a Wilcoxon test. This analysis confirmed the results of our statistical analysis without the five patients; therefore, we concluded a good sensitivity of our previously mentioned tests and sample. We continued the further analysis only with the patients with complete datasets.

The p-value was calculated for every result, and $p < 0.05$ was considered statistically significant.

Results

Bronchial allergen provocation

The primary aim was the change in severity of EAR measured by BAP. As shown in Figure 2, after 1 year of AIT, the max. FEV1 fall in EAR was significantly reduced in both treatment groups. Significant improvements in the nonmodified mite extract group from a max. FEV1 fall of $28.6 \pm 8.6\%$ to $15.5 \pm 10.8\%$ ($p < 0.001$) and in the modified group from $28.6 \pm 7.4\%$ to $16.6 \pm 12.7\%$ ($p < 0.001$) were observed. No change in max. FEV1 fall after 1 year without AIT was noted in the control group ($24.6 \pm 12.8\%$ to $27.4 \pm 11.6\%$; $p = 0.4$). A one-way ANOVA showed a significant difference among the three groups ($p < 0.005$) and the *post hoc* comparison showed a significant difference between each treatment group and control group, but no difference between the two treatment groups.

A patient with negative BAP test after 1 year of AIT was considered as a 'responder'. After 1 year of AIT, the BAP test was negative (max. FEV1 fall $< 20\%$) in 70.0% ($n = 14/20$) of patients in the nonmodified mite extract group, 72.2% ($n = 13/18$) of patients in the modified mite extract group and 18.8% ($n = 3/16$) of patients in the control group. Fisher's exact test comparing the three groups showed a significant difference ($p = 0.002$).

According to the study protocol, all patients should be challenged with the same cumulative allergen dose in both BAP tests, as long as there was no severe reaction (FEV1 fall $\geq 20\%$) at the previous step with a lower allergen dose. In 43 of 54 patients (79.6%), the same allergen dose could be administered in both BAP tests. Two patients in

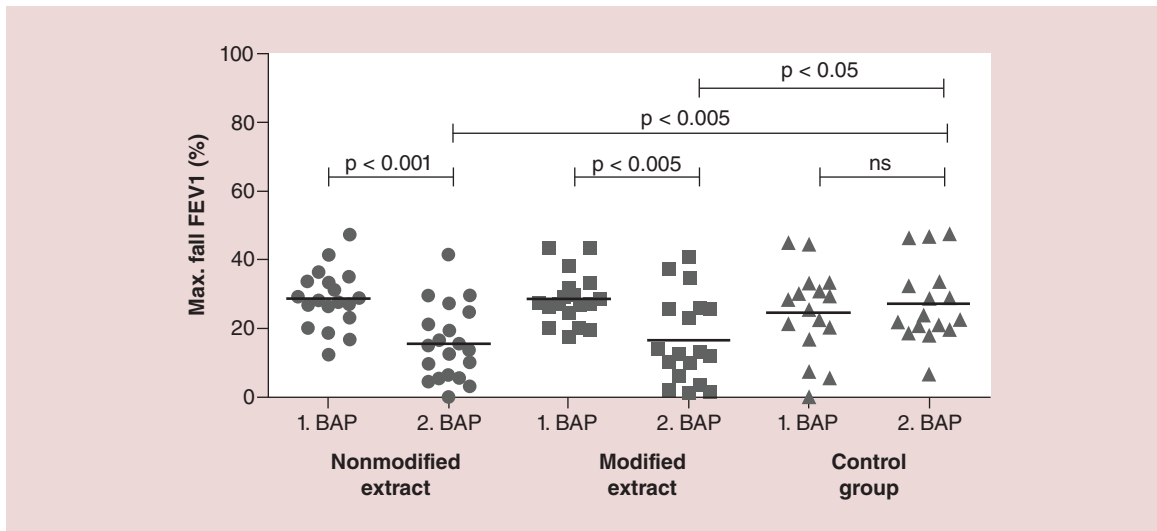


Figure 2. Scattered blot of early asthmatic reaction at 1 and 2. Bronchial allergen provocation in all three groups. The max. FEV1 fall was defined by the bronchial allergen provocation results. Means $\pm$ standard deviation are reported. A p-value <0.05 was considered statistically significant.

the nonmodified mite extract group, one patient in the modified mite extract group and eight control patients were not able to tolerate the same allergen dose. This finding was not significantly different between the two treatment groups ($p = 0.6$). But significantly more patients in the control group were not able to tolerate the same allergen dose in the second BAP test compared with the treated patients (nonmodified mite extract: $p < 0.01$; modified mite extract: $p < 0.005$).

No significant improvement in LAR was observed in the treatment groups after 1 year of therapy. The mean max. FEV1 fall in the LAR before and after therapy was $35.6 \pm 11.5\%$ and $27.8 \pm 12.3\%$ ($p = 0.08$) in the nonmodified group, $30.4 \pm 21.0\%$ and $26.8 \pm 18.5\%$ ($p = 0.6$) in the modified mite extract group and $34.7 \pm 17.2\%$ and $20.8 \pm 16.5\%$ ($p < 0.05$) in the control group. One-way ANOVA showed no difference among the three groups ($p = 0.79$).

Subsequently, the possible influences of main confounders were analyzed even though such an analysis is limited by sample size. Over the course of the year, a decrease in the need for ICS was observed in both treatment groups. Four of 11 (36.4%) patients in the nonmodified mite extract group and three of ten (30.0%) patients in the modified mite extract group were able to stop ICS treatment. A comparison with Fisher's exact test showed no significant difference between the two treatment groups ($p = 1.0$). In the control group, the need for ICS therapy did not change before ($n = 9/16$) and after 1 year ($n = 8/16$) of observation. We compared the patients treated with and without ICS therapy before initiation of AIT treatment, to determine the Influence of ICS use on BAP test. In terms of EAR, no difference in the max. FEV1 fall was observed between those groups ($26.5 \pm 1.7\%$ vs $27.1 \pm 2.2\%$; $p = 0.83$). However, in the LAR, a significant difference in the max. FEV1 fall was observed in patients treated with and without ICS ($28.9 \pm 2.4\%$ vs $39.5 \pm 4.2\%$; $p < 0.05$). Moreover, 66.7% responders were female. In a subsequent comparison of the max. FEV1 fall, we observed a significant difference in this parameter between male and female patients after 1 year of AIT ($20.8 \pm 2.8\%$ vs $12.2 \pm 2.2\%$; $p < 0.005$) with no difference between the two groups in any other measured parameter.

Sensitization

Concerning sensitization, we found that 35.0% (7/20) patients in the nonmodified group and 44.4% (8/18) in the modified mite extract group were monosensitized. In the control group, 18.8% (3/16) were monosensitized. Overall, 33.3% (18/54) patients were monosensitized. All other patients were polysensitized. A Fisher's exact test comparing the three groups showed no significant difference ($p = 0.30$).

After AIT, we identified equal numbers of mono- and polysensitized patients in the group of responders (48.2 vs 51.9%). Nevertheless, 81.8% nonresponders were polysensitized, and 18.2% were monosensitized.

Exhaled NO

After 1 year of treatment, the eNO levels did not change significantly in the analyzed subjects. The medians before and after therapy in nonmodified and modified mite extract groups were 24.0 and 21.0 ppb ($p = 0.91$) and 17.0 and 26.0 ppb ($p = 0.26$), respectively. The medians in the control group were 39.0 and 33.0 ppb ($p = 0.35$). Kruskal–Wallis test showed no difference among the three groups ($p = 0.27$).

Medication history

There was a decrease in need for corticosteroids in both treatment groups. The use of ICS changed from 55.0% ($n = 11/20$) before therapy to 35.0% ($n = 7/20$) after therapy in the nonmodified mite extract group. In the modified mite extract group, 55.6% ($n = 10/18$) used ICS before SIT and 38.9% ($n = 7/18$) after 1 year of SIT. In the control group, the need for ICS therapy did not change (56% [$n = 9/16$] to 50.0% [$n = 8/16$]).

In the nonmodified mite extract group, the percentage of patients treated with SABA on demand (GINA 1) changed from 35.0 to 60.0%, and in the modified mite extract group from 33.3 to 61.1%. In the control group, there was also a slight increase (37.5 vs 50.0%). In the nonmodified mite extract group, before AIT, 60.0% of patients and after AIT 35.0% of patients were treated with regular low doses of ICS or leukotriene receptor antagonist and as-needed SABA (GINA 2). In the modified mite extract group, there was a change from 61.1 to 27.8%. There was a smaller decrease in the control group (50.0 vs 43.8%). In the nonmodified mite extract group, the number of patients treated with a regular combination of ICS and LABA and as-needed SABA (GINA 3) did not change (5.0 vs 5.0%) and the modified mite extract group showed a slight change from 5.6 to 11.1%. In the control group, the number of patients treated with GINA step 3 decreased slightly from 12.5 to 6.3%.

Questionnaire

After 1 year of therapy, the asthma score changed significantly from 7.2 ± 0.7 points to 4.7 ± 0.5 points ($p < 0.02$) in the nonmodified mite extract group and from 8.0 ± 0.7 points to 3.3 ± 0.6 points ($p < 0.005$) in the modified mite extract group. In the control group, a smaller change from 6.3 ± 0.6 points to 4.3 ± 0.5 points ($p < 0.05$) was noted.

The tolerability was rated positively by 95% ($n = 19/20$) patients in the nonmodified mite extract group and 94% ($n = 17/18$) patients in the modified mite extract group. Furthermore, the treatment outcome was considered positive by 90% ($n = 18/20$) patients and their parents in the nonmodified mite extract group, and by 89% ($n = 16/18$) in the modified extract group. Furthermore, 100% ($n = 20/20$) and 94% ($n = 16/17$) patients in the nonmodified and modified mite extract groups would recommend this therapy to other patients.

Laboratory measurements

Eosinophil count

The percentage of eosinophils did not change significantly in any group. It was 7.50 and 7.45% ($p = 0.62$) in the nonmodified mite extract group before and after therapy, respectively. In the modified mite extract group before and after therapy, it was 10.15 and 8.70% ($p = 0.52$), respectively. In the control group, the medians before and after 1 year without SIT were 6.60 and 6.95% ($p = 0.79$), respectively. Kruskal–Wallis test showed no difference among the three groups ($p = 0.17$).

Total IgE

No significant change in the total-IgE antibodies after 1 year of AIT was observed in the nonmodified mite extract (288.0 vs 454.0 IU/ml; $p = 0.21$), modified mite extract (220.0 vs 324.0 IU/ml; $p = 0.65$) and control group (402.0 vs 402.0 IU/l; $p = 0.85$). Kruskal–Wallis test showed no difference among the three groups ($p = 0.46$).

Specific IgE

There was no significant change in the specific IgE antibodies in either group. The median antibody levels before and after AIT in the nonmodified mite extract group were 81.6 and 44.8 KU/l ($p = 0.15$), respectively. In the modified mite extract group, it was 58.7 KU/l before therapy and 49.8 KU/L after 1 year of AIT ($p = 0.62$). No significant change was observed in the control group (71.8 vs 62.3 KU/l; $p = 0.76$). Kruskal–Wallis test showed no difference among the three groups ($p = 0.24$).

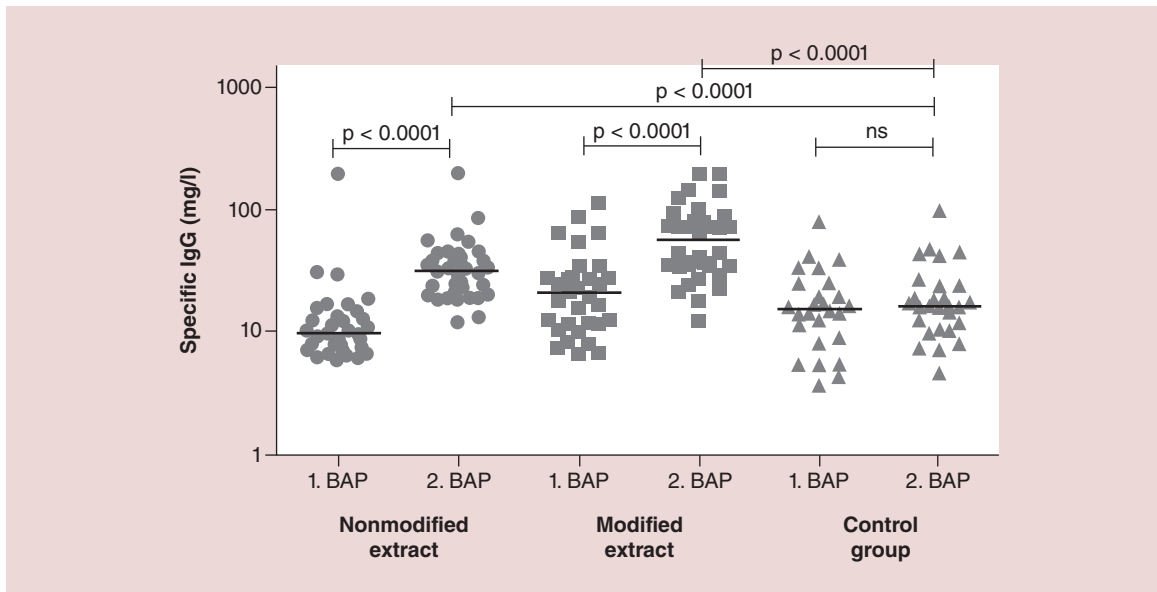


Figure 3. Scattered blot of specific IgG levels (IgG D1 and D2) at 1 and 2. Bronchial allergen provocation in all three groups. Medians and ranges are reported. A p-value <0.05 was considered statistically significant.

Specific IgG

As shown in Figure 3, after 1 year of therapy, a significant increase in the median specific IgG levels was observed in both treatment groups. In the nonmodified mite extract group, the antibody levels before and after AIT were 9.7 and 32.0 mg/l ($p < 0.0001$), respectively. Furthermore, in the modified mite extract group, the specific IgG levels before and after therapy were 20.8 and 57.0 mg/l ($p < 0.0001$), respectively. No significant change was observed in the control group (15.5 vs 16.4 mg/l; $p = 0.49$). Kruskal–Wallis test showed a significant difference among the three groups ($p < 0.0001$) and the *post hoc* comparison showed a significant difference among each treatment group and control group, but no difference between the two treatment groups.

Specific IgG4

As shown in Figure 4, after 1 year of therapy, a significant increase in the median specific IgG4 levels was observed in both treatment groups. In the nonmodified mite extract group, the specific IgG4 levels before and after AIT were 61.8 and 343.3 U/l ($p < 0.005$), respectively. Furthermore, in the modified mite extract group, the levels before and after therapy were 139.5 and 1270.7 U/l ($p < 0.0001$), respectively. No significant change was observed in the control group (126.9 vs 256.2 mg/l; $p = 0.15$). A Kruskal–Wallis test showed a significant difference among the three groups ($p < 0.0001$) and the *post hoc* comparison showed a significant difference among each treatment group and the control group, but no difference among the two treatment groups.

Discussion

Apart from the allergen avoidance, AIT is the only curative therapy for IgE-mediated allergic diseases [5]. For the children with HDM allergy, AIT is a very well-established therapy, and its efficacy has been proven in numerous studies [2,8,10,12,31]. According to current guidelines, standardized allergen extracts should be used in either nonmodified or modified (e.g., allergoids) forms with well-defined biological activity [17]. Recent data suggested that nonmodified extracts could be more effective than modified extracts [23]. To our knowledge, the present study is the first to assess and prospectively compare the efficacy and tolerability of nonmodified and modified mite extracts in children and adolescents using BAP test. After 1 year of treatment, nonmodified and modified mite extracts were equally effective, as defined by the BAP test results. In addition, the mite extracts were equally effective on all secondary parameters, including symptom scores, medication use and immunological laboratory markers.

While interpreting the results, it has to be taken into account that the allergen concentrations of two different extracts may be entirely different since no international allergen standardization exists; thus, the allergen units

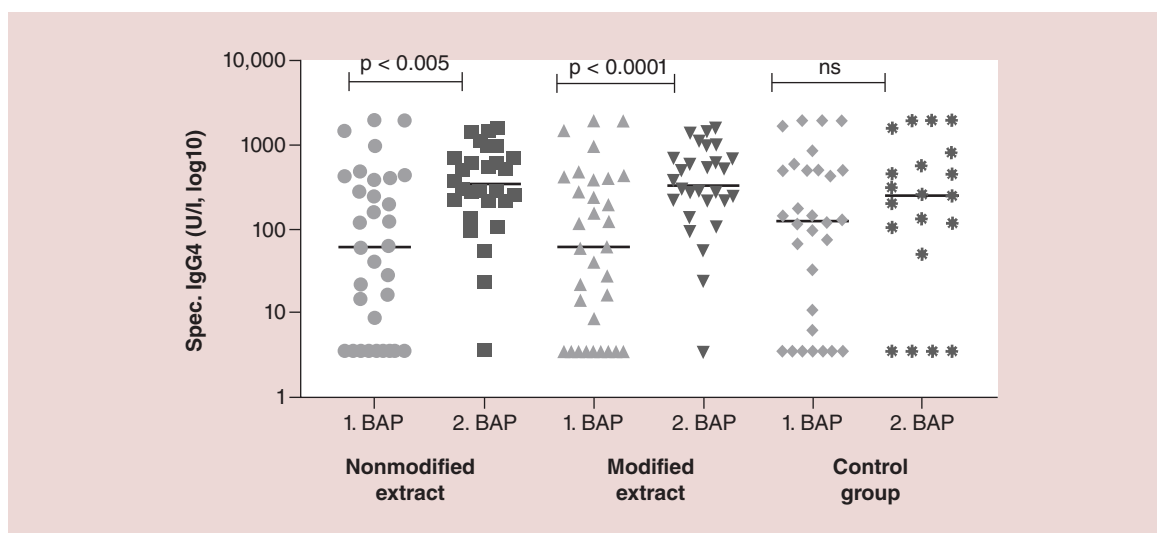


Figure 4. Scattered blot of specific IgG 4 levels (IgG 4 D1 and D2) at 1 and 2. Bronchial allergen provocation in all three groups. Medians and ranges are reported. A p-value <0.05 was considered statistically significant.

of one extract by one manufacturer are not the equivalent to those of an extract by another manufacturer. In addition, it is well known that manufacturers are typically not interested in comparing their extracts head to head in prospective trials. This dilemma was the reason, why we used a real-life approach to compare the two different extracts in the current study. Primary statistical aim was the comparison of the primary parameter, in other words, the response in the BAP after 1 year of treatment. The BAP is a well-established tool for the evaluation of new antiobstructive drugs [32,33] and for estimating the effectiveness of AIT [14]. Since we used this objective measure before and after treatment, we were able to neglect the allergen content of the extracts and evaluate the treatment success in a real-life setting.

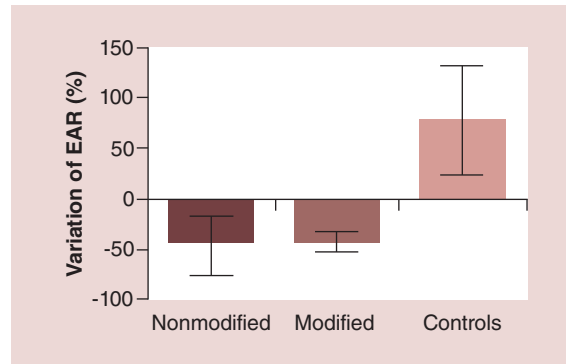
While 3 years is the usual duration of AIT according to current guidelines, treatment success is often studied after a shorter duration. Especially with new fast up-dosing schedules of new extracts and almost all Phase II studies choose 1 year, or one cycle period of treatment to draw significant conclusions. In concurrence with this, we truly believe that a 1-year period of treatment is sufficient to compare a treatment effect of two different extracts.

BAP test is a well-established method to determine the effectiveness of AIT in patients with allergic asthma [17,18]. Using this test as a follow-up parameter, the overall responder rate was 71.1%, which was considered a good result after the first year of HDM AIT. Our findings are consistent with several studies using BAP testing to measure the efficacy of AIT. After 1 year of AIT with a modified mite extract, in our previous study, the responder rate was 60.7% [8], which was comparable with rate of 66.7% reported by other researchers [34]. Equivalent results were obtained by Blumberga *et al.* [10] in adults treated with a nonmodified mite extract. A methodological difference was that in the present study, the same cumulative allergen dose was used in the BAP tests performed before and after 1 year of treatment and the change in max. FEV1 fall was measured, whereas other studies using BAP calculated the increase of allergen dose inducing a 20% fall in FEV1 after AIT [8,25].

During the 1-year follow-up visit, some patients were not able to inhale the same cumulative allergen dose during BAP as in the first visit. As expected this occurred significantly more often in the control group compared with the patients treated with nonmodified or modified mite extract. As shown in Figure 5, an impressive reduction of maximum FEV1 fall of EAR ($\Delta\%$) was recorded after 1 year of AIT, whereas controls showed a significant increase in this parameter after 1 year without AIT. Thus, it seems very likely that allergen-specific BHR increased in the control group, consistent with the result from a previous study [35]. One of the agents responsible for this phenomenon might be IL-10. Braza *et al.* showed in their study that bronchial inflammation is supported by IL-10 deficiency asthmatic patients [36]. Furthermore, Bohm *et al.* found that AIT increases the production of IL-10, which in turn decreases inflammation, remodeling and BHR [37]. This could be one of the protective mechanisms through which AIT prevents an increase in BHR in the treatment groups.

Interestingly, in contrast to previous studies, we were not able to confirm a significant effect of AIT on the LAR. This finding is surprising and might be due to the use of ICS in patients in our real-life study. We analyzed the

Figure 5. Box plot of change ($\Delta\%$) of max. FEV1 fall in the EAR over the course of 1 year of AIT (nonmodified and modified mite extract) or without AIT (controls), respectively. Means $\pm$ standard deviation are reported. $p < 0.05$ was considered statistically significant. ALT: Allergen immunotherapy; EAR: Early asthmatic reaction.



influence of ICS use as a possible confounder during the first BAP test and noticed a significant difference in the max. FEV1 fall between patients treated with and without ICS. The ICS treatment protects the bronchial tissue from inflammation and therefore reduces the LAR. Thus, LAR recorded before and after 1 year might not be comparable if an ICS treatment was started or changed in the meantime. This was a major limitation in our real-life study, as treatment was adjusted and tapered when patients were well controlled. We observed that after 1 year of AIT, fewer patients needed ICS, whereas the use of ICS in the control group did not change. Interestingly, we did not observe an influence of ICS on EAR, potentially indicating that the mechanisms responsible for EAR might be influenced by ICS to a lesser extent. Additionally, in our previous study, it was observed that ICS treatment did not influence the value of specific IgE antibody levels in predicting an EAR [38]. Based on these findings, EAR might be a more reliable parameter for assessing the efficacy of AIT in real-life studies.

In earlier studies, reduced need for medication after initiation of AIT was noted [14]. Consistently, we found a shift of long-term medication in the treatment groups from GINA 2 to GINA 1. No change was seen in the GINA 3 patients; this might be due to the small number of patients in this subgroup. Previously, our group and other researchers have described a steroid-sparing effect of AIT in children and adolescents [9,14,39,40]. In coherence to those studies, we found that lesser patients needed long-term therapy after 1 year of AIT. In terms of ICS therapy, we noted a relative reduction of ICS use of 36.4% in the nonmodified mite extract group and of 30.0% in the modified mite extract group. In comparison, the use of ICS in the control group did not change significantly. The decreased need for asthmatic medication after 1 year of SIT suggests a better asthma control and therefore supports a therapeutic success.

As shown by many studies, asthma symptoms decreased significantly after AIT [41,42]. This finding could be confirmed in our treatment groups. There was a significant decrease in the asthma symptom score after 1 year of AIT. In addition, approximately 90% parents and patients treated with either mite extract considered the treatment outcome positive. Again, both mite extracts were equally effective.

Previous studies analyzed the effect of AIT on immunological laboratory parameters [43–46]. We also compared such parameters before and after 1 year of AIT. Based on the current knowledge of the mechanism of action of AIT, a decrease in the total-IgE level would be expected, but we observed a slight increase in both treatment groups and no change in the control group. This trend has also been observed in other studies [47,48]. It is likely that after AIT initiation, the amount of total-IgE increases initially but may decrease over the long term [47]. Another immunological parameter that was investigated in earlier studies is the eosinophil count [46,49]. However, we found no significant change in the eosinophil count in both treatment groups. This might be due to the small size of our study cohort. Regarding the other measured laboratory parameters, we did not detect a change in the specific IgE levels, but a significant change in the specific IgG levels. This finding corresponds with the results from previous studies [50]. The changes in all parameters presented as $\Delta\%$ can be found in Table 2.

Although there was a significant improvement in the BAP results and symptoms with both mite extracts, approximately 30% patients did not benefit from the AIT in the first year, they were ‘nonresponders’. As described in previous studies, risk factors of nonresponder status include older age, polysensitization, vitamin D deficiency and genetic factors [46,51–53]. Recently, it was observed that gender could be a significant predictor of the patients’ response to AIT [46]. The analysis of treated-patient characteristics showed a significant improvement in the max. FEV1 fall in EAR after 1 year of AIT in female patients compared with male patients. All other analyzed

Table 2. Comparison of change (Δ %) of major parameters over the course of 1 year of allergen immunotherapy (nonmodified and modified mite extract) or without allergen immunotherapy (control group), respectively.

	Nonmodified extract, n = 20	Modified extract, n = 18	Control group, n = 16
Total-IgE (Δ %)	46.9 (-19.3–360.6)	-1.3 (-54.6–234.9)	19.2 (-53.5–137.6)
Spec. IgE D1 + D2 (Δ %)	-41.3 (-98.5–5082.5)	-39.5 (-74.8–1263.0)	18.1 (-56.5–1164.4)
Spec. IgG D1 + D2 (Δ %)	179.6 (0.0–872.8)	145.6 (-21.13–2390.7)	13.0 (-26.9–45.5)
Spec. IgG4 D1 + D2 (Δ %)	123.5 (-871.5–993.6)	335.5 (-96.1–1935.8)	0.0 (-302.7–38.1)
EAR (Δ %)	-43.4 $\pm$ 37.5	-42.7 $\pm$ 42.5	77.84 $\pm$ 226.3
LAR (Δ %)	-24.1 $\pm$ 36.0	-6.8 $\pm$ 68.6	-25.1 $\pm$ 71.3

Mean $\pm$ standard deviation or medians and ranges are reported.
EAR: Early asthmatic reaction; LAR: Late asthmatic reaction.

parameters, including age, specific IgE levels, and treatments were similar in female and male patients. Further studies are necessary to determine if this finding will be confirmed in larger cohorts.

Some studies concluded that modified extracts are better tolerated than native extracts [22,34]. However, we did not observe a significant difference in the occurrence or severity of side effects between nonmodified and modified mite extracts, although this was not the main focus of our study. In our cohort, all side effects were local reactions, and no patient reported any systemic or severe adverse events. Moreover, the patients and their parents evaluated the tolerability as equally good in both treatment groups.

Our study has several limitations. First, the study had a real-life design but not a placebo-controlled design. Second, results may be influenced by the ICS treatment as patients were not obligated to stop their long-term medication before participating in this study. Third, we could not confirm the effect of AIT on LAR, but those results might not be reliable, as the datasets were incomplete and analyzed subgroups were too small.

Conclusion

Overall, the nonmodified and modified mite extracts investigated in this study were not significantly different in the measured parameters. The comparison of the change in EAR, measured by the max. FEV1 fall, showed no difference between the treatment groups. There was no change in the LAR after 1 year of AIT in either treatment group, possibly due to the influence of ICS therapy. The evaluation of asthma symptom improvement showed an equal improvement in both treatment groups. Furthermore, the tolerability was evaluated equivalent for the nonmodified and modified mite extract. Moreover, analysis of immunological parameters revealed no difference among the two groups. There was a significant change in specific IgG in both treatment groups.

In conclusion, in regards of the parameters measured in this study, nonmodified mite extract and modified mite extracts can be considered equally efficient and tolerable. After 1 year of AIT, 70.0% (nonmodified mite extract) and 72.2% (modified mite extract) treated patients exhibited significant improvements, as defined by the BAP results.

Author contributions

D Hartmann played a major role in planning and conducting the study, as well as the statistical analysis and is the main author of the manuscript. A Fischl provided constant help in interpreting and reviewing results, as well as a thorough review of manuscript. E Herrmann shared her expertise in statistical analysis, power calculation and reviewed the manuscript thoroughly. J Schulze was involved in the planning and conducting of the study, he was a constant help in interpreting and reviewing results, conducted a thorough review of the manuscript and shared his clinical expertise. R Schubert granted constant help in interpreting and reviewing laboratory results, conducted a thorough review of the manuscript and shared his laboratory expertise. S Zielen provided and organized the study setting, played a major role in planning and conducting the study, provided constant help in interpreting and reviewing results, conducted a thorough review of the manuscript and shared his clinical and research expertise.

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A Fischl reports fees from Aimmune Therapeutics outside the submitted work. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Ethical conduct of research

The study was approved by the Frankfurt Ethics Committee and performed in accordance with the declarations of Good Clinical Practice (GCP) and registered at ClinicalTrials.gov NCT01134757. All parents provided written informed consent for the analysis of their children's data.

Informed consent disclosure

The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

Summary points

- According to our results, the nonmodified mite extract and the modified mite extract can be considered equally beneficial.
- After 1 year of house dust mite allergen immunotherapy, 70.0% (nonmodified mite extract) and 72.2% (modified mite extract) of the patients exhibited significant improvements, as defined by the bronchial allergen provocation results.
- Asthmatic symptoms improved equally in both treatment groups.
- The need for asthma medication, especially inhaled corticosteroids, was reduced in both treatment groups after one year of allergen immunotherapy.
- Among others, predictors for nonresponder status could be male gender and polysensitization.
- There was a significant change in specific IgG and IgG4 in both mite extract groups and no change in the control group.
- No significant difference in immunological parameters was noted between the two treatment groups.
- Tolerability was evaluated equivalent for the modified and the nonmodified mite extract.

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