

A 1-year, placebo-controlled, double-blind house-dust-mite immunotherapy study in asthmatic adults

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Thirty-one adult patients with asthma caused by house-dust mites (HDM) were included in this placebo-controlled, double-blind study to evaluate the efficacy and safety of specific immunotherapy (SIT) with biologically standardized extracts of HDM. The specific diagnosis was confirmed by skin prick tests, specific IgE, and bronchial provocation tests with HDM allergens. The patients were randomized to receive active treatment with extracts of either *Dermatophagoides pteronyssinus* (Dpt) or *D. farinae* (Dfa) (Alutard® SQ, ALK, Denmark) or placebo injections. Twenty-three patients completed the study. After 1 year of treatment, we found a clinically important and significant reduction in both asthma medicine consumption (inhaled steroids 38% and β_2 -agonists 46%) and symptom score (57%) in the actively treated group, but not the placebo group. These findings were confirmed by a significant decrease in skin and bronchial sensitivity to HDM in the active group. Additionally, there was a significant difference in the patients' scores for effect in favor of the actively treated group. Total IgE and specific IgE to HDM showed no significant changes before and after treatment for either group. Spirometric lung-function measurements showed a significant increase in forced expiratory volume in 1 s (FEV₁) from 85% before to 89% of predicted values after treatment for the actively treated group. Peak-flow measurements at home showed no significant changes during the study. It is concluded that allergen SIT is an effective treatment in adult patients suffering from asthma due to HDM.

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House-dust mites (HDM) are a common allergen source (1), and allergic symptoms due to HDM affect approximately 1% of the total population of Denmark (2). Effective HDM allergen elimination seems to be difficult (3), and even when a partial reduction in allergen exposure is achieved, asthmatic symptoms may be unaffected (4). Since HDM allergy is a serious condition, causing rhinoconjunctivitis and often severe cases of asthma, other allergen-specific treatment methods should be considered. Allergen specific immunotherapy (SIT) is recommended, especially in pollen allergies (5, 6). A number of trials of SIT with HDM extracts in asthmatics have previously been performed. A few of these have been double-blind, placebo-

controlled studies (7), and these have had varying outcomes, probably due to the different quality of extracts used (8) and the selection of patients. Another study showed a clear dose-response effect, but was performed with an open control group (9). Today, standardized extracts of HDM are available, and placebo-controlled studies to investigate the clinical efficacy of these extracts are needed – to comprehend not only the immunologic and paraclinical factors, but also the relevant clinical data such as medicine consumption and symptom score.

Using clinical as well as paraclinical data, this study aimed to evaluate the safety and efficacy of allergen SIT in patients suffering from bronchial asthma due to HDM.

Material and methods

Patients

Thirty-one asthmatic patients of both sexes, aged 18–64 years (demographic data given in Table 1), were selected by the following criteria:

- 1) a clinical history indicating asthma due to HDM; i.e., exacerbation of the perennial, asthmatic symptoms in the autumn, and wheezing after dust exposure as in cleaning, and with a daily need for symptomatic medication
- 2) a positive skin prick test with allergen extracts of HDM (Soluprick® SQ, ALK, Denmark)
- 3) significant levels of specific IgE antibodies to Dpt and Dfa in sera (Magic Lite® SQ, ALK, Denmark)
- 4) a positive bronchial provocation test with Dpt and Dfa ($PC_{20} < 100\,000$ SQ-U/ml) (Aquagen® SQ, ALK, Denmark)
- 5) a dust sample from the patient's bed, positive to HDM allergen (>500 ng Der p 1 and Der f 1/g dust).

Patients with major medical diseases, patients with low compliance, patients who had immunotherapy (IT) with extracts of HDM within 5 years of the project starting, and patients who received treatment with systemic corticosteroids were excluded. Patients who were pregnant or were planning pregnancy were also excluded. Patients with other perennial allergies were also excluded if they were exposed to the specific allergen to which they were allergic at the time of the study (e.g., dog and cat). Skin prick tests with standard inhalant allergens were performed before and after treatment, to investigate whether any of the patients developed new perennial allergies during the study. During the study, the patients' homes, especially their bedrooms, were not allowed to be altered with respect to the indoor environment.

Design

The study was randomized and double-blinded; i.e., the extracts were blinded by the manufacturer during the entire treatment period. Extracts were labeled for named patients. The code was not known by any of the treatment team and was stored in the clinic in sealed envelopes to be opened only in an emergency. During a 4-week run-in period, the patients recorded symptoms and medication score. Thereafter, they were stratified by age, sex, and severity of asthma and divided into three treatment groups by a computer-generated randomization code. Group I received SIT with extracts of Dpt (Alutard® SQ) and group II with extracts of Dfa (Alutard SQ), while group III

Table 1. Demographic data

	Age (years) Mean (range)	Weight (kg) Mean (range)	Height (cm) Mean (range)
Active group (n=17)	32.0 (18–56)	72.9 (52–89)	179.1 (158–191)
Placebo group (n=6)	40.7 (22–64)	73.2 (63–88)	175.0 (163–190)

received placebo injections (histamine dihydrochloride). During the treatment period, inhaled steroids were gradually withdrawn, after evaluation of symptoms and use of rescue medication, where possible. After 1 year, the code was revealed with regard to whether the patients received active or placebo treatment. Those receiving placebo were then randomized to receive either Dpt or Dfa under double-blind conditions. All patients treated with allergen extracts continued their treatment unchanged. The code for this part of the study has still not been revealed.

Immunotherapy

The two actively treated groups received SIT with biologically standardized, aluminum hydroxide-adsorbed allergen extracts of Dpt or Dfa (Alutard SQ), respectively, as single-dose up dosing with weekly injections from dose-increase vials containing 100 SQ-U/ml, 1000 SQ-U/ml, 10 000 SQ-U/ml, and 100 000 SQ-U/ml. The dosage schedule is shown in Table 2. A maintenance dose of 100 000 SQ-U (≈ 7 µg Der p 1 or ≈ 10 µg Der f 1) was reached after 15 weeks. The next maintenance dose

Table 2. Immunotherapy dosage schedule

Dose no.	Allergen concentration (SQ-U/ml)	Volume (ml)	Dose (SQ-U)	Interval between injections (weeks)
1	100	0.1	10	1
2	100	0.5	50	1
3	1000	0.1	100	1
4	1000	0.4	400	1
5	1000	0.7	700	1
6	10 000	0.1	1000	1
7	10 000	0.3	3000	1
8	10 000	0.5	5000	1
9	10 000	0.8	8000	1
10	100 000	0.1	10 000	1
11	100 000	0.2	20 000	1
12	100 000	0.4	40 000	1
13	100 000	0.6	60 000	1
14	100 000	0.8	80 000	1
15	100 000	1.0	100 000	1
16	100 000	1.0	100 000	3
17 and so on	100 000	1.0	100 000	6

was given after 3 weeks, and thereafter dosing continued at 6-week (± 1 week) intervals.

The placebo group received injections of histamine dihydrochloride in aluminum hydroxide solution. Updosing was performed with weekly injections from four dose-increase vials (0.000 01, 0.0001, 0.001, and 0.01 mg/ml histamine dihydrochloride), which were identical in appearance to the vials containing allergens. Thus, the updosing and maintenance schedule was equal for all three treatment groups.

A clinical evaluation of the patients was made with respect to suitability of receiving treatment before injection at each visit. After extract administration, the patients were closely observed for 30 min with a special focus on systemic reactions. Before the patients left the clinic, a repeated clinical evaluation took place, including recording the size of the local reaction at the injection site.

Skin prick tests

At inclusion and after 1 year, skin prick test screening with a standard panel of inhalant allergens, including *Betula verrucosa*; *Phleum pratense*; *Artemisia vulgaris*; horse, cat, and dog dander; Dpt; Dfa; *Alternaria alternata*; and *Cladosporium herbarum*, was performed. Diluents with and without histamine dihydrochloride (10 mg/ml) were used as positive and negative controls, respectively. The extracts for these tests were glycerinated (Soluprick SQ). Skin prick tests were performed with double estimations, and were evaluated according to the Nordic Guidelines (10).

Titration skin prick tests with aqueous extracts of Dpt and Dfa (Aquagen SQ), respectively, were performed at the inclusion and after 12 months, using a series of 10 twofold dilutions of extracts from 100 000 SQ-U/ml to 195 SQ-U/ml. Diluents with or without histamine dihydrochloride (10 mg/ml) were used as positive and negative controls, respectively. The sensitivity of the skin was estimated by the size of the wheal, calculated by the following formula: size = (DA+dA)/2, where DA = largest diameter of allergen wheal, and dA = midorthogonal diameter of allergen wheal.

Lung-function measurements

Lung function was measured on a dry spirometer (Vitalograph®, Buckingham, UK), and FEV₁ and forced vital capacity (FVC) were recorded at the inclusion and after 1 year of treatment. The values were compared to normal values (11). The percentages of the predicted normal values were used for the calculations.

Diary cards

For 1 month before treatment and 1 month after treatment, the patients kept diary cards, recording the following:

- 1) morning and evening peak flows, best of three measurements (Mini-Wright® peak flow meter, Airmed, Harlow, UK)
- 2) symptom score on a scale from 0 (no symptoms) to 4 (very severe symptoms)
- 3) daily medicine consumption.

Bronchial allergen challenges

Bronchial allergen challenges were performed according to the method described by Chai et al. (12) with extracts of HDM (Aquagen SQ) in twofold concentrations ranging from 195 SQ-U/ml to 100 000 SQ-U/ml. Challenges with the two extracts were performed with at least 14-day intervals, at inclusion, and after 1 year of treatment. The patients abstained from taking their inhaled short-acting β_2 -agonists for at least 6 h before the tests, and abstained from long-acting and peroral β_2 -agonists as well as theophylline for at least 24 h before the tests. Inhaled steroids were not withheld. Wright Jet Nebulizer® (Aerosol Products, UK), output 0.13 mg per min, was used for nebulization. The nebulization time was 2 min, during which the patients inhaled at normal rate and tidal volume. The patients' lung function was measured immediately and at 5 and 10 min after allergen inhalation. The challenges continued in increasing strengths, until a 20% decrease in FEV₁ (PC₂₀) was reached. Thereafter, the patients were observed in the clinic until their lung functions returned to normal and were stable.

Patient evaluation of treatment

After 1 year of treatment, before the code was revealed, all patients were asked to grade the treatment effect into the following classes: 0 (no effect), 1 (slight effect), 2 (good effect), and 3 (very good effect).

In vitro tests

Sera collected at the time of inclusion and after 12 months of treatment were analyzed for the level of total and specific IgE to Dpt and Dfa (Magic Lite SQ).

Ethical considerations

The protocol was approved by the local ethics committee and the Danish National Board of Health.

The patients received both verbal and written information about the project. Informed consent was obtained from all patients.

Analysis of data

All statistical tests were performed in SPSS/PC+ as two-tailed tests, either the Mann-Whitney U-test and two-sample *t*-tests between groups, or the Wilcoxon matched-pairs signed rank tests and paired samples *t*-tests within the groups. The chi-square test of independence was used to compare the number of patients.

Results

Thirty-one patients were included, and 21 patients were allocated to receive active treatment with Dpt or Dfa. Ten patients were allocated to placebo treatment. Four patients (40%) in the placebo group and four patients (19%) in the active group dropped out before the maintenance dose was reached. Two patients moved out of the area, and six patients dropped out because of unexpected poor compliance. None of the patients dropped out because of side-effects. In evaluation, 23 patients completed the study, all reaching the maintenance dose as scheduled. No significant difference between the groups according to age, height, or weight was observed. Demographic data are shown in Table 1.

Side-effects

None of the patients developed systemic side-effects during up dosing or maintenance dosing. Local swellings and redness at the site of the injection were observed, but considered an inevitable effect rather than an adverse reaction.

Symptom score

Symptom scores before and after treatment showed a significant decrease for the actively treated group ($P<0.001$), where all patients had a reduction in symptoms. Of the placebo-treated group, two-thirds of the patients had either increased or unchanged symptoms. There were no significant changes in symptom score (Fig. 1).

Medicine consumption

In the active group, a decrease in the use of inhaled β_2 -agonists (46%) (Fig. 2) and inhaled steroids (38%) (Fig. 3) was observed after SIT, and these findings were statistically significant ($P<0.05$). No statistically significant changes before and after

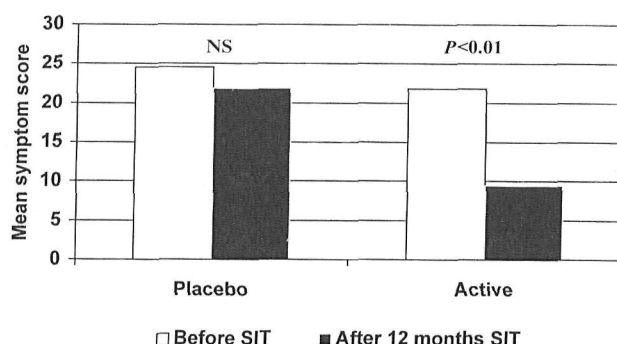


Fig. 1. Mean symptom score per week in allergen-treated and placebo groups before and after treatment. NS=nonsignificant.

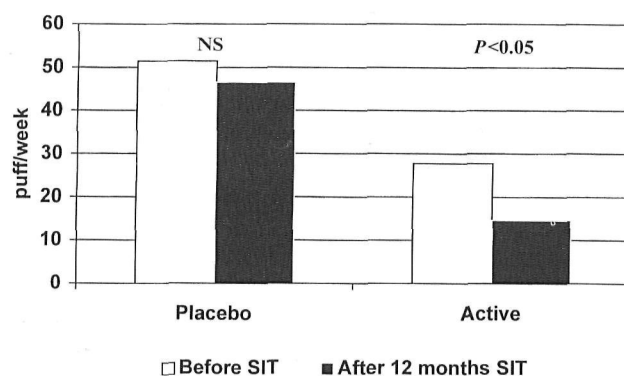


Fig. 2. Rescue medication (inhaled β_2 -agonists, mean number of puffs per week) in allergen-treated and placebo groups before and after treatment. NS=nonsignificant.

treatment were found in the placebo group, although a tendency to increased use of inhaled steroids was observed (Fig. 3). Only a few patients were using oral β_2 -agonists at inclusion, and no significant changes were observed.

Patients' overall effect evaluation

Among the actively treated patients, 82.4% classified the treatment effect as good (3) or very good (4). In the placebo group, all patients classified the treatment effect as no effect (0) or slight effect (1), and the difference between groups was statistically significant ($P=0.0022$) (Fig. 4).

Lung-function measurements and peak-flow registrations

No clinically relevant changes in spirometric values were observed before and after treatment (Table 3). However, for both groups, a small increase in FEV₁ or FVC was observed. In the active group, these changes were statistically significant ($P=0.0059$ and 0.019 , respectively), whereas they were nonsignificant for the placebo group ($P=0.465$ and 0.075 , respectively). The FEV₁/FVC

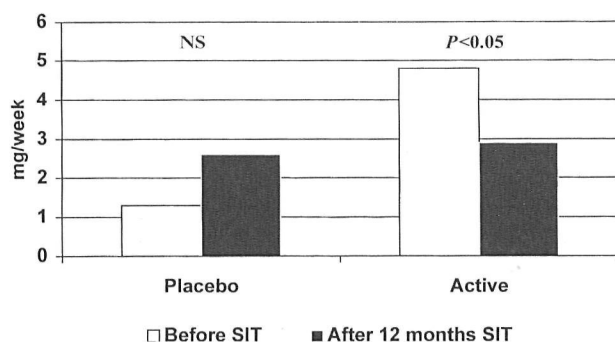


Fig. 3. Inhaled steroids, mean number mg per week, in allergen-treated and placebo groups before and after treatment. NS=nonsignificant.

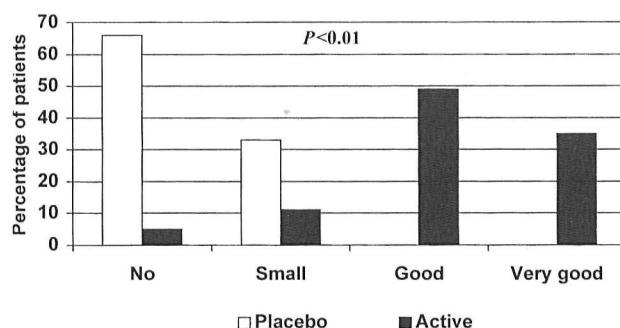


Fig. 4. Patients' overall effect evaluation after treatment in both allergen-treated and placebo group.

ratio increased for the active group, but decreased for the placebo group, thus rendering the difference between the groups statistically significant after treatment, but not before.

Peak flow measured morning and evening before and after treatment showed minor fluctuations for both groups (Table 3). However, none of the changes in peak flow were statistically significant.

Bronchial allergen challenge

Bronchial sensitivity decreased significantly to Dpt and Dfa ($P=0.022$ and 0.039 , respectively) in the

actively treated group (Figs. 5 and 6). However, no significant changes in bronchial sensitivity to Dpt ($P=0.60$) or Dfa ($P=0.75$) were found before and after treatment in the placebo group. Likewise, no significant differences between the active and the placebo groups ($P=0.20$ and 0.92 for Dpt and Dfa, respectively) were found before the initiation of SIT, but, after treatment, the actively treated patients had a significantly lower sensitivity ($P=0.037$ and 0.041 for Dpt and Dfa, respectively).

Skin prick tests

No clinically significant changes were found when testing before and after treatment with the standard panel of inhalant allergens for either of the groups.

Titration skin prick tests with extracts of Dpt and Dfa showed for all 10 dilutions for the actively treated patients a decrease in size (for Dpt: medians ranging from 2.5 to 7 mm before treatment and 0 to 5 mm after; for Dfa: medians ranging from 2.5 to 6.5 mm before treatment and 0 to 5.0 mm after). For Dpt, all differences were significant (P ranging from <0.0001 to 0.0220). For Dfa, the decrease was significant for seven dilutions (P ranging from 0.0029 to 0.0251), but not significant for three dilutions (P ranging from 0.1130 to 0.1252). For the placebo-treated group, both minor increases and decreases in size were observed (for Dpt: medians ranging from 1.5 to 7.5 mm before treatment and 0 to 6.5 mm after; for Dfa: medians ranging from 0 to 5.75 mm before treatment and 0.5 to 6.5 mm after), and none of these fluctuations were statistically significant.

IgE in sera

Total and specific IgE to Dpt and Dfa showed only minor fluctuations, and no significant differences within or between the groups were found.

Table 3. Spirometric lung function and peak-flow measurements before and after treatment in percentage of predicted values

	Before treatment % of predicted values				After treatment % of predicted values			
	Active group		Placebo group		Active group		Placebo group	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
FEV ₁	84.98	54.8–109.8	83.6	47.8–100.5	89.0	65.1–109.9	86.5	58.8–98.1
FVC	98.23	76.2–123.4	106.7	80.7–117.6	102.3	80.9–118.5	112.5	90.8–123.3
FEV ₁ /FVC	86.7	62.0–108.6	77.6	59.2–93.8	87.5	62.2–106.6	76.7	62.7–90.3
PF-M	86.6	62.8–126.4	85.4	69.5–113.0	87.4	61.5–127.9	86.6	59.6–115.2
PF-E	92.0	66.0–123.1	93.4	71.5–119.3	92.5	67.6–126.6	91.8	68.1–113.8

FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; PF-M: peak flow morning; PF-E: peak flow evening.

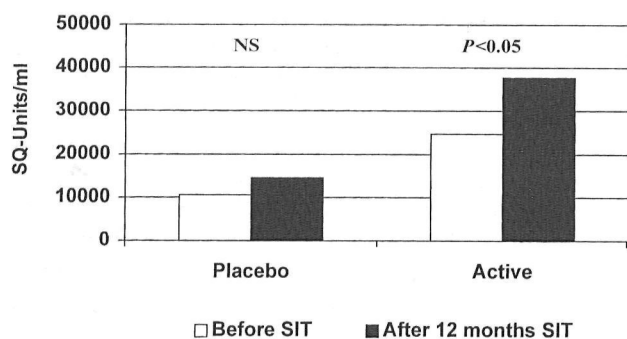


Fig. 5. Bronchial sensitivity (PC_{20}), mean number of SQ-U/ml to Dpt before and after treatment in allergen-treated and placebo groups. NS=nonsignificant.

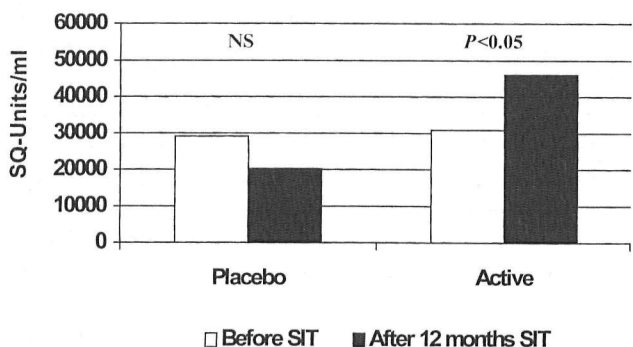


Fig. 6. Bronchial sensitivity (PC_{20}), mean number of SQ-U/ml to Dfa before and after treatment in allergen-treated and placebo groups. NS=nonsignificant.

Discussion

The present, placebo-controlled study shows a clinically relevant effect of SIT with extract of HDM in patients with bronchial asthma, with a reduction in symptom score as well as asthma medication. A reduction in the use of inhaled steroids was observed; in spite of this, lung-function measurements were stable or increased, and symptom score as well as use of rescue medication (inhaled β_2 -agonists) decreased. The clinical results were confirmed by paraclinical results: decreased immediate hypersensitivity to HDM in the target organ, the lungs. This was also observed in the skin. We did not find any significant changes in specific IgE. Others have reported an increase in specific IgE during the first months of immunotherapy followed by a later decline (13). However, we sampled only blood before treatment and after 1 year of treatment, a fact which probably explains why we were not able to detect this supposed transient increase.

The positive clinical results may be due to dosing and patient selection. We used the optimal maintenance dose, which was previously shown to be 100 000 SQ-U/ml ($\approx 7 \mu\text{g}$ Der p 1 or $\approx 10 \mu\text{g}$ Der f 1) (9). The patients were selected according

to international recommendations (14). The allergen used for SIT should be the one which causes most of the patients' symptoms, and patients with multiple allergies should not be included. The patients were stable due to adequate drug treatment, and none of the patients had severe asthma. Only patients with mild to moderate asthma were included. All patients had normal lung function when compared to age, sex, and height standardized values. Some of the previous double-blind, placebo-controlled SIT studies have shown the same results as ours (15, 16), whereas others found only a temporary effect (17). However, only a few double-blind, placebo-controlled SIT studies have been performed in patients with asthma due to HDM allergy, and most of these included relatively few patients (7, 14). This may reflect the difficulty in performing such SIT studies. A specific diagnosis can be hard to achieve, especially in HDM-allergic patients, because the symptoms often mimic the symptoms observed in patients with unspecific hypersensitivity in their bronchia. Therefore, patients suitable for this treatment are often difficult to identify by history alone – normally, many patients are tested, but only a few can be included.

Another problem seems to be patient compliance, which is often difficult to achieve in SIT studies. This problem could partly be due to the many visits to the clinic (including a 30-min observation after each injection), and the long study period. During this 1-year study, the patients came to approximately 22 injection visits, and, in addition to this, there were several visits for purposes such as provocation tests. The risk of receiving placebo treatment for a long time, and fear of needles on the part of some patients are also factors which contribute to the difficulty of recruiting patients for SIT studies. In this study, six patients dropped out for unexpected poor compliance, and two dropped out because they moved out of the area; four of these patients were in the placebo group (40%) and four of them were in the active group (19%). Thus, the dropout rate was highest in the placebo group, a fact which could present a problem for the study; nevertheless, the results indicate a treatment effect in favor of the allergen-treated group.

We conclude that SIT with a standardized HDM extract is effective in the treatment of adult asthmatics when a proper dose is used and patients are carefully selected.

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