

Effect of One-Year Subcutaneous and Sublingual Immunotherapy on Clinical and Laboratory Parameters in Children with Rhinitis and Asthma: A Randomized, Placebo-Controlled, Double-Blind, Double-Dummy Study

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Key Words

Children • Asthma • Rhinitis • Subcutaneous immunotherapy • Sublingual immunotherapy

Abstract

Background: It has been reported that both sublingual (SLIT) and subcutaneous (SCIT) allergen-specific immunotherapy have clinical efficacy, yet there are rather few comparative placebo studies of children. We aimed to investigate the clinical and immunological efficacy of mite-specific SLIT and SCIT versus a placebo in rhinitis and asthma in children.

Methods: The outcomes of this 1-year, randomized, placebo-controlled, double-blind, double-dummy study were symptom and medication scores, visual analog scores (VAS), titrated skin prick tests, nasal and bronchial allergen provocation doses, serum house dust mite-specific immunoglobulin E (HDM-sIgE), sIgG4, IL-10 and IFN- γ levels. **Results:** Clinical and laboratory parameters were evaluated in 30 patients. SCIT significantly diminished symptom and medication scores for rhinitis and asthma ($p = 0.03$ and $p = 0.05$ for rhinitis; $p = 0.01$ and $p = 0.05$ for asthma) and VAS. SLIT also reduced VAS, symptoms associated with rhinitis and asthma as well as medication usage for rhinitis, but this reduction was

not significant when compared with the placebo. Skin reactivity to HDM and HDM-sIgE levels was reduced significantly in both immunotherapy groups. Serum IL-10 levels and nasal provocative doses increased significantly with both SCIT and SLIT. Nasal eosinophil increments after nasal challenge decreased with two treatment modes, but bronchial provocative doses and sputum eosinophil increments after bronchial challenge were reduced only with SCIT. In both treatment arms, there was no change in IFN- γ levels. Serum sIgG4 levels increased significantly only in the SCIT group. **Conclusion:** Based on the limited number of patients at the end of the 1-year immunotherapy, the clinical efficacy of SCIT on rhinitis and asthma symptoms was more evident when compared with the placebo.

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Introduction

Allergen-specific immunotherapy is, along with allergen avoidance, the only specific treatment for allergic diseases [1, 2]. Subcutaneous immunotherapy (SCIT) has been in use since around 1910 and may prevent new sensitizations [3] and reduce the development of asthma in

patients with allergic rhinitis [4, 5]. Sublingual immunotherapy (SLIT) is a safe alternative to injective immunotherapy [6–12].

SCIT has been validated for the treatment of both asthma [13] and rhinitis [14]. Pollen allergy constitutes the best indication for this immunotherapy route, but efficacy has been also shown with standardized house dust mite (HDM) extracts in previous studies [14–16]. Multiple controlled SLIT studies have been performed [6, 8–11, 17–21] and two recent meta-analyses in children showed that the sublingual delivery of allergen vaccination constitutes an effective and safe alternative to the injectable route to reduce allergic respiratory symptoms and drug intake [22, 23]. SLIT has been reported to have promising evidence of efficacy in mite allergy [24] when validated with pollen extracts, and considering pediatric indications [25, 26].

The primary aim of this study was to investigate SLIT and SCIT in terms of their efficacy in children with rhinitis and asthma monosensitized to HDM. The secondary aim was to determine the immunological effects of both treatment modes. The design was a randomized, double-blind, placebo-controlled, double-dummy study over a 1-year immunotherapy period. In order to stabilize asthma and to assess the baseline level of symptom and medication scores, all patients were subjected to a follow-up over 1 year before the immunotherapy took place.

Methods

Patients

The patients were selected from among those referred to the Pediatric Allergy and Immunology Clinic on the basis of perennial nasal and bronchial symptoms. Recruitment criteria included a clinical history of at least 1 year of rhinitis with asthma related to HDM symptoms and no previous IT. Thirty-two children presenting with persistent rhinitis and asthma and monosensitized to HDM were included. Patients fulfilled the criteria for persistent mild asthma according to the Global Initiative for Asthma (GINA) guidelines [27], and the diagnosis of persistent allergic rhinitis was based on criteria in the WHO consensus statement on allergic rhinitis and its impact on asthma (ARIA) [25].

HDM allergy was verified by a positive skin prick test (a wheal >3 mm) (Allergopharma, Reinbeck, Germany) and the presence of specific immunoglobulin E (sIgE; radioallergosorbent test, RAST, class II or >0.70 kU/l) using the CAP system (Pharmacia, Uppsala, Sweden) for both *Dermatophagoides pteronyssinus* (*D.pt.*) and *D. farinea* (*D.f.*).

The study was approved by the ethical committee of the University Hospital and each patient and their caregivers gave written informed consent.

Study Design

The first year of the study (run-in period) consisted of the follow-up of patients to evaluate their baseline symptom scores and to optimize medication scores related to asthma and rhinitis. Physical examination, a visual analog score (VAS) for rhinitis and asthma, titrated skin prick tests, pulmonary function tests and allergen-specific nasal and bronchial challenges were performed both at the beginning of and 12 months after treatment. Nasal eosinophil counts were assessed both before and 24 h after nasal challenge; similarly, sputum eosinophil counts were obtained both before and 24 h after bronchial challenge. Additionally, the laboratory parameters evaluated before and after the 1-year treatment were HDM-sIgE, sIgG4, serum IL-10 and IFN- γ levels. Inhalation of budesonide (100–800 μ g/day) and salbutamol was recommended to the patients for the control of their asthma. Intranasal mometasone and antihistamines were given as needed to alleviate the symptoms of rhinitis. None of the patients were treated with oral corticosteroids or leukotriene antagonists. All the subjects were instructed to take antimite measures such as adequate cleaning of bedrooms and carpets and regular washing of teddy bears.

At the end of the first year, before the immunotherapy was started, 1 of the 32 patients withdrew his consent for the study, and the remaining 31 continued with immunotherapy.

The design of the second year or immunotherapy period was randomized, placebo-controlled, double-blind and double-dummy. Allocation to 3 study groups was performed by a computer-generated randomization method. All study personnel and participants were blinded to treatment assignment for the 1-year duration of the immunotherapy.

Based on the computer-generated randomization, the 31 patients were allocated to 3 groups: the SCIT group (10 patients) received active subcutaneous immunotherapy (injections) and placebo sublingual drops, the SLIT group (11 patients) received active sublingual immunotherapy (drops) and placebo subcutaneous injections, the placebo group (10 patients) received placebo sublingual drops and placebo subcutaneous injections.

Immunotherapy

For specific immunotherapy (SIT), active treatment involved standardized *D.pt.* and *D.f.* (50/50) mite extracts for sublingual use (NovoHelisen Oral, Allergopharma) and for subcutaneous administration (NovoHelisen Depot, Allergopharma). Identical placebo preparations included caramelized sugar for the sublingual therapy and histamine dihydrochloride 0.01, 0.1 and 0.5 mg/ml for injections to achieve a visual appearance and taste identical to SLIT preparations and the induction of local reactions to SCIT. The doctors carrying out the SIT had no knowledge of, nor access to the randomization code which was kept locked and sealed by a doctor not involved with the study.

SLIT was self-administered at home. The drops were taken at the same time each day, before meals, and were held under the tongue for 2 min before swallowing. The doses were increased for 12 weeks until the maintenance dose was reached. The initial dose was 1 drop of 10 TU/ml increasing to 28 drops on day 28, 1–28 drops of 100 TU/ml on days 29–56 and 1–28 drops of 1,000 TU/ml on days 57–84. After the patient reached this dose or the maximum tolerated dose, the maintenance dose was administered 3 times per week as 28 drops of 1,000 TU/ml. The cumulative 1-year dose for SLIT was approximately 173,733 TU (86,866.5 TU of *D.pt.* and 86,866.5 TU of *D.f.*).

SCIT was administered in the clinic and included a 12-week induction phase (weekly injections) starting with a dose of 0.2–0.8 ml of 50 TU/ml (weeks 1–3), 0.2–0.8 ml of 500 TU/ml (weeks 4–8) and 0.2–0.8 ml of 5,000 TU/ml (weeks 9–12). The maximum tolerated dose achieved during the induction phase was the maintenance dose, and it was repeated every fourth week. The cumulative 1-year dose for SCIT was approximately 43,770 TU (21,885 of TU *D.pt.* and 21885 TU of *D.f.*).

The ratio of dosages during maintenance therapy for SLIT and SCIT was 4.2.

Symptom and Medication Scores of Subjective Rhinitis and Asthma

Parents completed a self-assessment diary each day, scoring the symptoms of rhinitis (rhinorrhea, sneezing, nasal itching and blocked nose) and asthma (cough, wheezing, dyspnea and chest tightness) in the same way for the run-in and immunotherapy period. The symptoms were rated as: 0 = no symptoms, 1 = mild, 2 = moderate and 3 = severe. The combination of rhinitis and asthma symptoms was calculated as total symptom scores.

Prophylactic and rescue drug intake was recorded daily on the same diary card. One point was given if a β_2 -agonist rescue drug was taken on that day, and 0 if not. Similarly, if antihistamines such as cetirizine or loratadine were used for rhinitis on that day, it was scored as 1 point. The daily dose of inhaled budesonide and intranasal mometasone was scored as shown in table 1.

The combination of rhinitis and asthma medication scores was termed as total medication scores.

Nasal Provocation Test

Nasal provocation (NP) was performed according to the EAACI (European Academy of Allergy and Clinical Immunology) guidelines [28] using a Rhinospir 165 rhinomanometer (Sibelmel, Barcelona, Spain). The response was evaluated by measuring nasal resistance at 150 Pa with active rhinomanometry and by scoring the clinical symptoms. Total symptom scores represented the sum of the scores for: sneezing (0 points for 0–2 sneezes, 1 point for 3–5 sneezes, 2 points for >5 sneezes), rhinorrhea (1 point = moderate, 2 points = severe), tears in eyes or itching of eyes or throat (1 point) and conjunctivitis, cough, urticaria and/or dyspnea (2 points). Positive clinical nasal challenge was defined as >3 points [29].

After spraying 0.2 ml of the diluent, increasing concentrations of allergen extract (50, 500, 5,000 BU/ml; Allergopharma) were sprayed into the same nostril every 15 min until symptoms appeared and resistances doubled those induced by the diluent.

Nasal Eosinophils

Nasal cytologic specimens were obtained by using the same technique as described previously in another paper [30]. Nasal eosinophil counts were determined from cytospins (Cytospin 4; Shandon Corp., Pittsburgh, Pa., USA) and expressed as percentage of total cells.

Pulmonary Function and Allergen-Specific Bronchial Provocation Tests

Lung functions were assessed at each visit using ZAN 100 Spiromed (Germany).

Bronchial challenge tests were performed according to the general guidelines for standardization of bronchial challenge tests

Table 1. Doses of inhaled budesonide and intranasal mometasone for scoring

	Dose, μ g/day	Score
Inhaled budesonide	0	0
	0–200	1
	200–400	2
	400–800	3
	800–1,000	4
Intranasal mometasone	0	0
	50	1
	100	2
	200	3

with allergens [31] using standardized allergen solutions (Allergopharma) and following standardized procedures. The solution is inhaled with continuous nebulization (PARI, Proneb Compressor Nebulizer, Midlothian, Va., USA) over 60 s during tidal breathing. After 15 min, an FEV₁ value was obtained by spirometry and it was accepted as a reference value. Challenge continued with administering of the lowest allergen dose (5 BU/ml). After 15 min, a new FEV₁ value was obtained and then the increasing allergen concentrations were administered as 50 BU/ml. Spirometries were obtained after each increase in concentration. The test was stopped after a fall in FEV₁ of 20% or more had occurred, or the maximum concentration of 5,000 BU/ml had been administered.

Sputum Eosinophils

Sputum induction, processing and analysis were performed as described previously [30]. Cytospin preparations (Cytospin 4; Shandon Corp.) were made at 220 g for 6 min and stained with May-Grünwald Giemsa for an overall differential cell count of 400 nonsquamous cells. The eosinophil counts were expressed as percentages of total nonsquamous cells.

Serum HDM-sIgE, sIgG4, IL-10 and IFN- γ Levels

Serum HDM-sIgE and sIgG4 levels were determined using the CAP System (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

Serum IL-10 and IFN- γ concentrations were measured by means of ELISA (Assaypro, Mo., USA). The minimum detectable doses of IFN- γ and IL-10 were 30 and <100 pg/ml, respectively.

Visual Analog Questionnaire

Patients were instructed to assess their severity of complaints on a straight line which ranged from 0 to 10; '0' was no complaint and '10' was the most uncomfortable condition. VAS was evaluated for both rhinitis and asthma at the beginning and end of immunotherapy.

Assessment of Clinical Efficacy

Clinical efficacy was assessed separately for both symptom and medication scores and was calculated by comparing the immunotherapy period with the run-in year in order to take advantage of monitoring in the first year.

Table 2. Demographic data of patients evaluated at the end of immunotherapy

	SCIT	SLIT	Placebo	p
Number (male/female)	10 (6/4)	10 (5/5)	10 (6/4)	0.99
Age (mean $\pm$ SD), years	10.9 $\pm$ 3.2	9.2 $\pm$ 3.4	10.1 $\pm$ 2.7	0.51
Duration of rhinitis, months ^a	72 (36–144)	54 (12–144)	66 (36–96)	0.62
Duration of asthma, months ^a	60 (24–120)	48 (12–120)	36 (12–72)	0.25

^a Median (range). The Kruskal-Wallis test established p value differences between groups.

Adverse Events

Recording of adverse events included time of onset and resolution, severity, action taken and estimation of possible relationship with study drugs. Systemic side effects were classified and graded according to EAACI recommendations [1].

Exposure to HDM Allergens

Dust samples were taken from the mattresses with a vacuum cleaner equipped with a nozzle (Indoor Biotechnologies, UK) containing a collector with a filter paper [32]. A semiquantitative test (Acarex) was carried out with dust samples to assess the guanine dosage. Exposure was assessed in this way: 'none' (0), 'mild' (1), 'moderate' (2), and 'heavy' (3).

Statistics

All the analyses were performed using computer software (SPSS version 11.0; SPSS, Chicago, Ill., USA). Data was presented as medians and range or mean $\pm$ SD, as indicated. The Wilcoxon signed-rank test for intragroup analysis was performed; if there were two samples, the Mann-Whitney U test for between-group analysis was performed.

Results

Patients

Of the 32 randomized patients, only 30 were evaluable (1 patient withdrew consent at the end of the run-in period, and 1 patient randomized to active SLIT was excluded after refusing to take drops after 3 months of immunotherapy) for efficacy at the end of immunotherapy. Thus, 10 patients received active SCIT, 10 patients received active SLIT and 10 patients received the placebo.

The demographic characteristics of the patients are shown in table 2.

There was no significant difference between the 3 groups in terms of age ($p = 0.51$) and gender ($p = 0.99$).

Mite Exposure

The level of exposure to HDM was not found significantly different between the 3 groups of patients both at

the beginning of the run-in period ($p = 0.75$) and at the end ($p = 0.72$) of the 1-year immunotherapy.

Symptom and Medication Scores

The symptom and medication scores of both rhinitis and asthma were recorded daily during both the baseline year and the SIT period, and were calculated as monthly median values.

The changes in symptom and medication scores regarding rhinitis and asthma both with SCIT and SLIT compared with the placebo are shown in figure 1.

There was a statistically significant difference in symptoms associated with rhinitis and asthma with both SCIT ($p = 0.005$ for both) and SLIT ($p = 0.008$ and $p = 0.012$, respectively) in comparison to the baseline year.

Compared with the placebo group, the reduction in symptom scores concerning rhinitis was found significant in the SCIT group ($p = 0.03$). Although SLIT reduced rhinitis symptoms significantly in comparison to the baseline year ($p = 0.008$), this reduction was not found significant when compared with the placebo. Regarding asthma symptoms, SCIT significantly reduced complaints ($p = 0.01$), while SLIT yielded no statistically significant effect ($p = 0.48$) in comparison to the placebo group.

The total symptom scores were reduced significantly with SCIT ($p = 0.005$) and SLIT ($p = 0.005$) in comparison to the baseline year. As for SCIT, total symptom scores were significantly reduced in comparison to the placebo group ($p = 0.009$).

When compared with the baseline data, the median percentage improvement of rhinitis symptom scores and asthma symptom scores in SCIT was 31 and 100%, respectively, and in SLIT 6.6 and 3.3%, respectively.

The medication scores of both rhinitis and asthma significantly decreased in SCIT patients ($p = 0.05$ for both) when compared with the placebo group and with the baseline year ($p = 0.005$ and $p = 0.02$, respectively). There

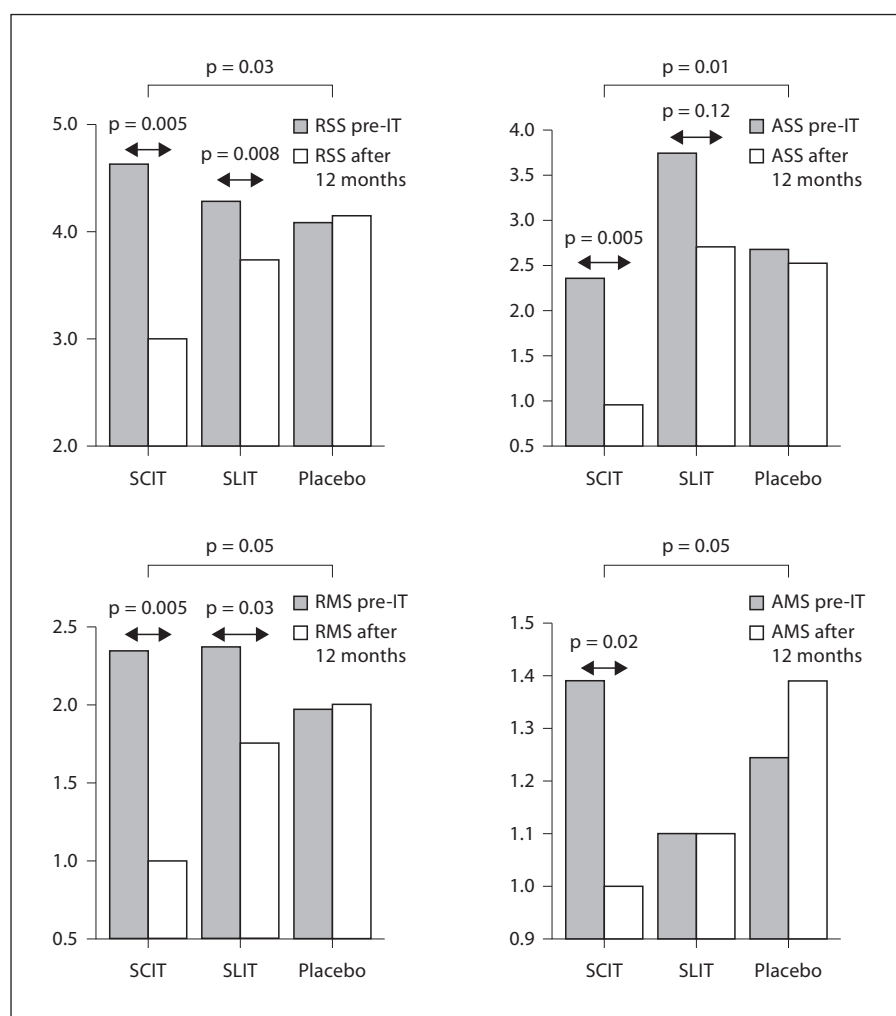


Fig. 1. Rhinitis and asthma symptom scores (RSS; ASS) and medication scores (RMS; AMS). IT = Immunotherapy.

was a significant decrease in medication scores regarding rhinitis ($p = 0.03$) and a tendency to a decrease ($p = 0.18$) in medication scores regarding asthma with SLIT in comparison to the baseline year.

Although a reduction in medication scores of rhinitis and asthma was observed with SLIT, this difference was not statistically significant ($p = 0.18$ for rhinitis and $p = 0.16$ for asthma). When total medication scores were analyzed, it was found that both SCIT and SLIT reduced ($p = 0.005$ and $p = 0.02$, respectively) the usage of medications in comparison to the baseline year.

No statistical difference between SCIT and SLIT was observed in terms of the reduction in symptoms of rhinitis ($p = 0.28$), or in medication scores associated with rhinitis ($p = 0.18$) and asthma ($p = 0.31$). Only asthma symptoms decreased significantly in the SCIT group ($p = 0.01$) when compared with the SLIT group.

Pulmonary Function Tests

The mean FEV_1 values of the SCIT, SLIT and placebo groups after the 12-month immunotherapy period were 99.2 ± 10.0 , 97.8 ± 7.6 and 96.2 ± 10.2 , respectively. FEV_1 values showed an average increase of 6.3% with SCIT, 5.5% with SLIT and 0.7% in the placebo group. When compared with the baseline year, FEV_1 values increased significantly with SCIT and SLIT ($p = 0.04$ for both), but did not change significantly in the placebo group ($p = 0.88$). The FEV_1 values of the SCIT and SLIT groups did not show a significant increase in comparison to the placebo group.

Visual Analog Scores

In comparison to the baseline year, VAS associated with both rhinitis and asthma improved with SCIT ($p = 0.005$ and $p = 0.007$, respectively) and SLIT ($p = 0.02$ for

Fig. 2. Wheal diameter values of the skin prick reaction (SPT) to *D.pt.* and *D.f.*

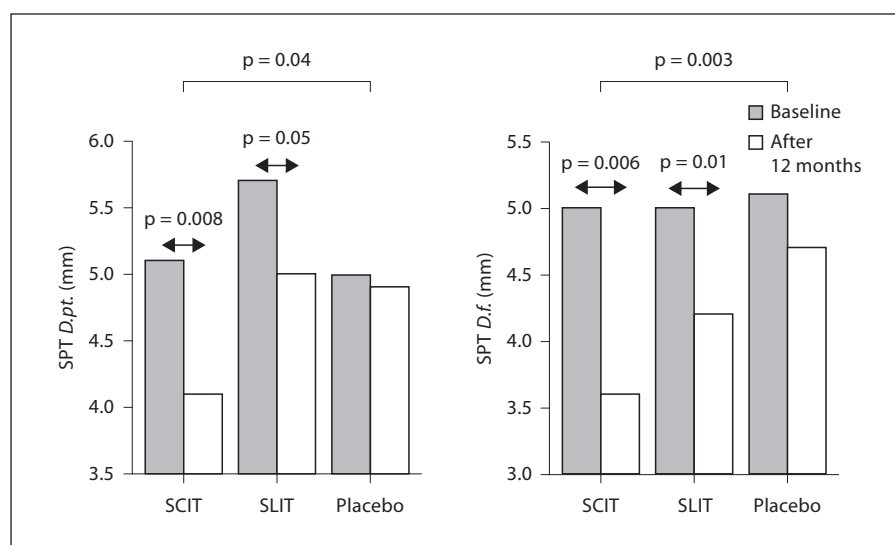
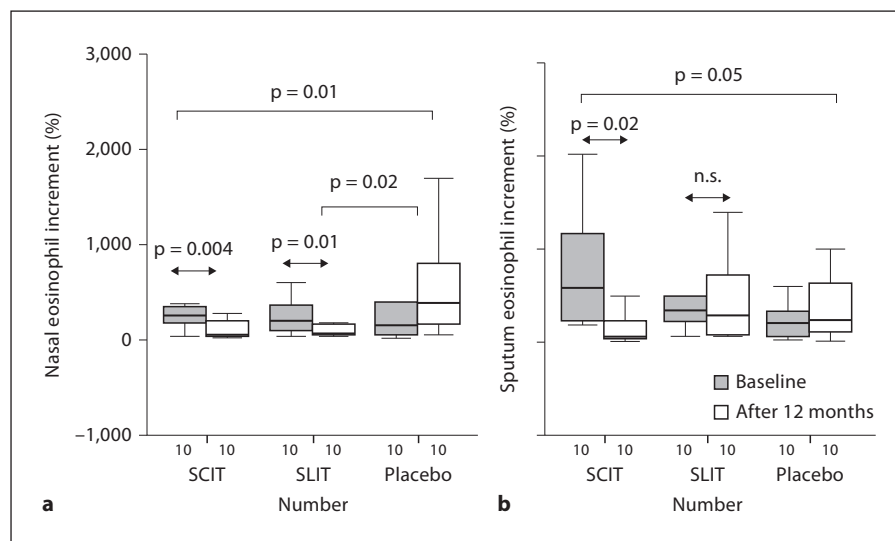


Fig. 3. Nasal eosinophil increment after NP therapy (a) and sputum eosinophil increment after BP therapy (b). n.s. = Not significant.



both). We found a significant difference regarding VAS of both rhinitis ($p = 0.05$) and asthma ($p = 0.02$) between patients receiving SCIT and the placebo. No statistically significant difference was detected between patients receiving SLIT in comparison to patients in the placebo group in terms of VAS of both rhinitis ($p = 0.14$) and asthma ($p = 0.39$).

Skin Prick Tests

When compared with the baseline, the wheal diameter of *D.pt.* and *D.f.* extracts decreased significantly in both the SCIT ($p = 0.008$ and $p = 0.006$, respectively) and SLIT ($p = 0.05$ and $p = 0.01$, respectively) groups. However, the patients receiving the placebo did not show a significant

decrease in wheal diameter of either *D.pt.* ($p = 0.70$) or *D.f.* ($p = 0.16$) (fig. 2).

Although statistically not significant ($p = 0.06$), the reduction in the wheal diameter of both *D.pt.* and *D.f.* was more prominent in the SCIT group when compared with the SLIT group.

HDM-Specific Nasal Provocation

Titration NP HDM doses increased significantly after 12 months of immunotherapy in both the SCIT ($p = 0.05$) and SLIT ($p = 0.01$) groups. No significant difference was observed between SCIT and SLIT regarding titrated NP HDM doses ($p = 0.31$). Nasal eosinophil increment after NP was significantly decreased in both the SCIT ($p =$

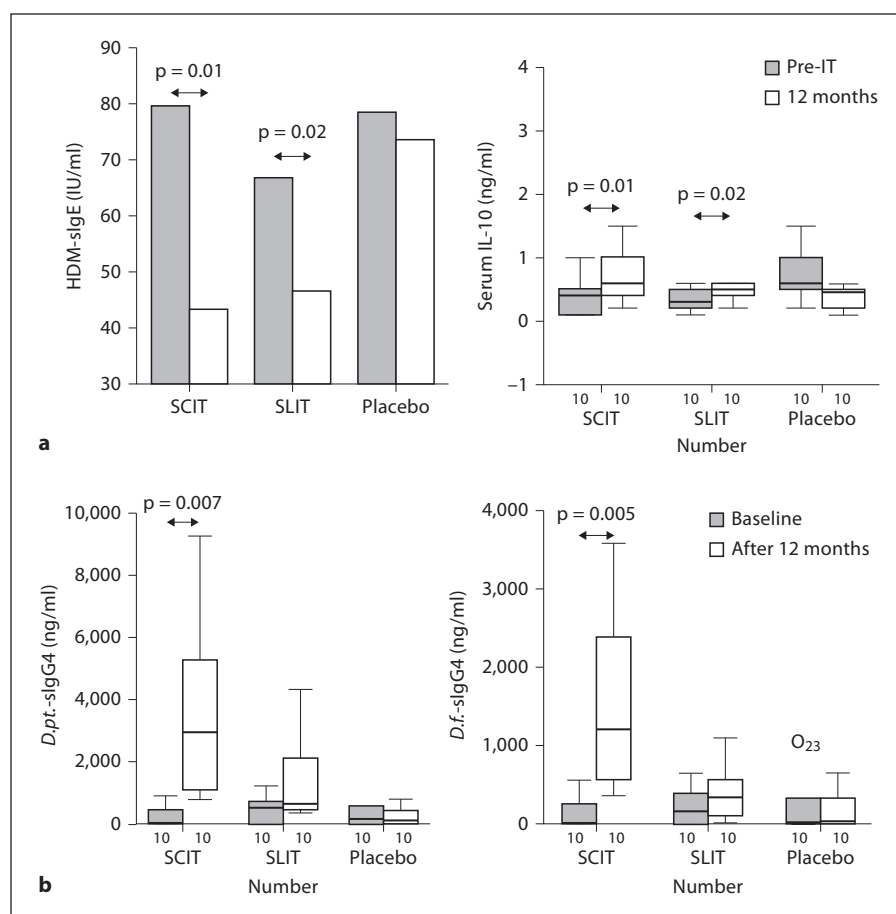


Fig. 4. a Serum HDM-sIgE and serum IL-10 levels. **b** Serum *D.pt.-* and *D.f.-*sIgG4 levels. IT = Immunotherapy.

0.004) and SLIT ($p = 0.01$) groups when compared with baseline ($p = 0.01$) and the placebo group ($p = 0.02$) (fig. 3). This increment did not change in placebo patients after 12 months of treatment ($p = 0.39$).

HDM-Specific Bronchial Provocation

The patients treated with SCIT ($p = 0.03$) showed a significant increase in HDM-specific bronchial provocation (BP) doses after 12 months, while the SLIT ($p = 0.56$) and placebo groups did not ($p = 0.78$). No significant difference was observed between SCIT and SLIT regarding titrated BP HDM doses ($p = 0.91$). Sputum eosinophil increment after BP showed a significant decrease in comparison to baseline in the SCIT group ($p = 0.02$), whereas this decrease was not observed in patients receiving SLIT ($p = 0.51$) and the placebo ($p = 0.20$) (fig. 3).

Serum HDM-sIgE, sIgG4, IL-10 and IFN- γ Levels

A significant decrease in HDM-sIgE levels was observed in SCIT ($p = 0.01$) and SLIT ($p = 0.02$) patients. We

did not detect a significant decrease in the HDM-sIgE levels of the placebo group ($p = 0.65$) (fig. 4a). Serum IL-10 levels increased significantly in patients treated with SCIT ($p = 0.01$) and SLIT ($p = 0.02$) (fig. 4a). Serum *D.pt.-* and *D.f.-*sIgG4 levels increased significantly only in the SCIT group ($p = 0.007$ and $p = 0.005$, respectively) (fig. 4b). We did not detect a significant change in IFN- γ levels in any group after 12 months of treatment. No significant difference was observed in terms of HDM-sIgE, IL-10 and IFN- γ levels between SCIT and SLIT groups. Serum *D.pt.-* and *D.f.-*sIgG4 levels increased significantly in the SCIT group in comparison to the SLIT group ($p = 0.003$ for both).

Adverse Events

No systemic side effects occurred in any of the 3 treatment groups. Local reactions related to injections appeared both in the SCIT group (2 patients) and in the placebo group (2 patients) during the induction phase. In the SLIT group there was an over-representation of itch-

ing or mild edema in the mouth and/or throat associated with drop intakes (3 patients; 2 in the placebo group). There was 1 withdrawal due to the local side effects of SLIT in the active group.

Discussion

In this study, we evaluated the efficacy of SCIT and SLIT in children with allergic rhinitis and asthma and monosensitized to HDM. This randomized, double-blind, double-dummy study compared treatment with an active allergen extract versus a placebo. This is the first immunotherapy study of children carried out in a double-dummy design with a placebo arm. Both the design and 1-year run-in period before the beginning of immunotherapy were significant contributing factors to the strength of the study. We found that 1 year of SCIT (compared to the placebo) was more effective in reducing symptoms and improving VAS related to both rhinitis and asthma.

The efficacy of SCIT has been shown in prospective studies [13, 33] and in the Cochrane analysis [33]. It has been demonstrated in some studies, including those of children, to be an effective treatment for patients with allergic rhinitis and asthma when the symptoms are caused by grass and HDM [34, 35]. Several systematic reviews [36, 37], meta-analyses [34, 38] and recently a Cochrane review [35], including both adults and children, conclude that SCIT significantly reduces asthma symptoms, allergen-specific bronchial hyperresponsiveness and the need for symptomatic asthma medication in asthma patients. In this study, 1 year of SCIT was observed to reduce asthma symptoms by 60.9% and rhinitis symptoms by 34.8%. The medication scores of asthma decreased by 28.6% and of rhinitis by 56.5% with 1 year of SCIT. In comparison to the patients receiving the placebo, a reduction in both symptoms and medication and an improvement in the VAS related to asthma and rhinitis were found to be statistically significant with SCIT.

A meta-analysis of pediatric studies reported that SLIT with standardized extracts is effective in children with allergic rhinitis compared with a placebo [25]. Two meta-analyses [23, 39], including children with asthma, demonstrated clinical benefits of SLIT with a significant reduction in symptoms and medication use related to asthma. Another trial with a 2-year SLIT, measuring lung function and symptom scores of 92 mite-sensitive asthmatic children, failed to demonstrate any effect [40]. In a recent meta-analysis on pediatric patients with asthma, it

was concluded that SLIT reduced both symptom scores and rescue-medication use compared to a placebo [41]. In all of these studies, treatment duration and regimens as well as doses of SLIT varied considerably. In this study, a 1-year SLIT was found to reduce rhinitis symptoms by 9.3% and asthma symptoms by 8.1%, which is a statistically significant decrease in comparison to the baseline year. Medication use related to rhinitis was reduced 29.2% following this 1-year SLIT. We did not find any significant effect on medication scores concerning asthma with the 1-year SLIT. Our results showed that SLIT had a significant effect on symptoms and VAS of both rhinitis and asthma in comparison to the baseline year, whereas this effect was not significantly different from the placebo at the end of the first year. Based on our observations, we suggest some possible explanations for this low efficacy of SLIT in our study. One shortcoming is that the dose of allergen administered was insufficient. In a double-blind placebo-controlled study performed in 30 mite-sensitive allergic children, the researchers did not observe any consistent, clinical benefit in actively treated patients receiving a mean cumulative dose of 0.57 mg of Der p 1 allergen [18]. In another double-blind placebo controlled study, Bousquet et al. [11] showed significant improvement of rhinitis symptoms, FEV₁ and PEFs with SLIT using a mean cumulative dose of 4.2 mg Der p 1. Guez et al. [19] showed no clinical benefit in comparison to the placebo in mite-allergic children with rhinitis with a mean cumulative dose of 2.2 mg Der p 1. In our study, the cumulative dose of allergen after the 1-year immunotherapy could not be expressed as micrograms, because it had not been provided by the manufacturer despite pressure from investigators.

It is also postulated that the appearance of clinical efficacy of SLIT needs much more time than SCIT (approximately 18–24 months of treatment). Thus, one should consider the fact that clinical efficacy may not yet show up after 12 months, the immunotherapy period in this study. The SCIT and SLIT treatment arms of this study continued for a second year and the results of these 2 years of therapy will be presented in another paper.

The successful effect of SIT was shown in rhinitis patients by a decrease in late-phase responses to local allergen challenge in the skin and nasal mucosa. In asthmatics, nonspecific airway hyperreactivity and bronchial responses to inhaled allergen challenge were decreased [42]. We also observed that the specific allergen reactivity in the skin decreased significantly in both the SCIT and SLIT groups when compared with the baseline year and the placebo.

In this study, both treatment modes increased the threshold dose of HDM to induce nasal hyperreactivity when compared with the placebo. Additionally, both SCIT and SLIT reduced nasal eosinophil increment after NP in comparison to baseline. The increase in the BP dose of HDM and the reduction in sputum eosinophil increment after BP were observed only with SCIT. These eosinophil increments after NP and BP did not decrease, but increased in patients receiving the placebo.

There are controversial immunological results with SCIT and SLIT. Many studies show a significant increase in IL-10 production and a decrease in allergen-specific IgE levels with both the treatment modes of immunotherapy in our study. We also detected a significant increase in allergen-specific IgG4 levels as reported with mite and pollen SCIT in the literature [43]. It has been shown that allergen-specific IgG4, IgG and IgA levels increased after two seasonal or preseasonal and prolonged (>3-year) grass pollen SLIT treatments [44]. However, SLIT with mites produces conflicting results about immunological parameters. In one study [45], 12 patients treated with *Dermatophagoides* monomeric allergoid SLIT for a year and a half showed significant downregulation in allergen-sIgE as well as an increase in IL-10 and IFN- γ production when sIgG1 and sIgG4 remained unchanged. Our observation was that a significant increase in sIgG4 levels occurred only in patients treated with SCIT, while IFN- γ levels did not change significantly in any group.

It should be considered that the significant placebo management effect could be observed in our study because of the close collaboration of investigators and families, regular follow-up, the education of both children and parents and the encouragement of self-management with diary cards. These factors also paved the way for a good adherence of patients and their families to the study, evidenced by no dropouts in the placebo group.

In our study, both SCIT and SLIT were found to have a good safety profile with no recorded systemic adverse effects and minimal local adverse events. Although we encountered some difficulties regarding compliance with treatment and the swallowing of drops in SLIT patients, thanks to regular, close follow-up and patient encouragement, there was fortunately only 1 dropout.

There are few studies in literature comparing the clinical efficacy of SCIT and SLIT. Two such studies compared injection immunotherapy with SLIT directly (1 with a further placebo arm) [8, 19]. These studies reported similar improvements in symptoms and medication requirements for the two administration routes. Another

study was randomized, double-blind, double-dummy and placebo-controlled, and was carried out in adult patients with birch pollen allergy. This particular study displayed that both methods of treatment were equally effective in the reduction of symptoms and drug intake [46]. In the study of Mungan et al. [18], it was shown that SCIT significantly improved symptoms and medication scores concerning rhinitis and asthma, while SLIT reduced only rhinitis symptoms in comparison to the placebo. In a recent study, it was reported that 1 year of SCIT and SLIT similarly improved symptom scores associated with rhinitis and asthma in comparison to untreated patients, but that the reduction of medication usage was more pronounced in SLIT [47].

The limitation of our study was the low number of patients. A potential reason for this is the fact that both the placebo arm and the double-dummy design of the study led to difficulties regarding the enrollment of children. Therefore, the risk of type II statistical error is notable for the limited number of patients.

In conclusion, this study, yet one more important contribution to the field, compared the clinical efficacy in children of both SCIT and SLIT with a placebo, with a double-blind and double-dummy design which was proposed for effective assessment and accurate results. We showed that both SCIT and SLIT had more clinical efficacy on the symptoms of both rhinitis and asthma compared to the baseline year. In comparison to the placebo arm, only SCIT was found to have a superior effect to the placebo on the reduction of rhinitis and asthma symptoms after the 1-year treatment. Further studies are needed to more precisely define doses and therapy duration, as well as the subgroups of patients who would be the ones to benefit more from the most appropriate type of immunotherapy.

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