Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial

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Summary

Background In children, the clinical efficacy and immunological mechanisms of sublingual immunotherapy (SLIT) compared with subcutaneous immunotherapy (SCIT) is still to be elucidated.

Objectives To compare SLIT, SCIT and pharmacotherapy in relation to clinical efficacy and immunological mechanisms that govern its effect in asthmatic/rhinitis children who were sensitized to house dust mite (HDM).

Methods In this single centre, prospective, randomized, controlled, open labelled, three parallel group trial, 48 patients mono-sensitized to HDM were randomized to receive either SLIT (n = 16), SCIT (n = 16) or pharmacotherapy alone (n = 16). Symptom, medication and visual analogue score (VAS) were collected and bronchial-nasal hyper-reactivity, skin prick tests, total-specific IgE were performed at baseline and 12 months after treatment. In addition, peripheral blood mononuclear cells were cultured with recombinant Der p 1 and Bet v 1 extracts and allergen-specific IL-4, IL-5, IL-13, IFN-γ, IL-10, and TGF-β secretions were measured.

Results SLIT and SCIT demonstrated a significant reduction of total rhinitis and asthma symptom score, total medication score, VAS and skin reactivity to HDM (P < 0.05) when compared with pharmacotherapy. A significant reduction of serum-specific HDM-IgE in SCIT and SLIT were observed. Moreover, titrated nasal provocative dose significantly increased in both immunotherapy groups when compared with the pharmacotherapy group. No adverse effects were reported in SLIT, while two patients demonstrated serious adverse events in SCIT. After 1 year of treatment, Der p 1–driven IL-10 significantly increased in SLIT compared with pharmacotherapy, whereas Bet v 1–driven TGF-β (negative control) increased significantly in SLIT only. No changes were observed for Th1–Th2 cytokines.

Conclusion Both SLIT and SCIT demonstrated clinical improvement compared with pharmacotherapy in asthma/rhinitis children sensitized to HDM.

Keywords asthma, cytokines, nasal provocation, rhinitis, sublingual–subcutaneous immunotherapy

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Introduction

The prevalence of allergic diseases including asthma, rhinitis and eczema are increasing for the last two decades in developed and developing countries, and its burden is substantial [1,2]. The mainstay treatments are patient education, allergen avoidance where feasible, pharmacotherapy for symptoms relief and, when appropriate, allergen-specific immunotherapy [3].

Specific allergen immunotherapy is the only treatment modality with the capacity of changing the natural course of the allergic disease, hence preventing its exacerbation [3], and might halt progression from rhinitis to asthma [4].
Subcutaneous immunotherapy (SCIT) in children has been shown to be effective [5] but due to the inconvenience of injection and the risk of serious side-effects, newer concepts of treatment have emerged. Sublingual immunotherapy (SLIT) appears to be associated with a lower incidence of systemic reactions and in a long-term treatment as an adjunct to pharmacotherapy results in the reduction of both the duration and the dose of inhaled corticosteroids and successful discontinuation along with an improvement of lung functions [6, 7].

SCIT induces changes that skew Th2- to Th1-type response (immune deviation) related to an increased IFN-γ and IL-2 production, with a reduction in Th2 activity, through a mechanism of anergy or tolerance, the latter being related to the generation of allergen-specific T regulatory (Treg) cells, which produce cytokines such as IL-10 and TGF-β [8]. The sublingual route of administration was suggested to have similar mechanisms as SCIT [9], with a particular involvement in mucosal dendritic cells [10].

The aim of the present study was to compare SLIT, SCIT and pharmacotherapy in relation to the clinical efficacy, frequency/severity of side-effects and immunological mechanisms that govern its effect in asthmatic/rhinitis children who were sensitized to house dust mite (HDM). The design was a single-center randomized, controlled, three parallel grouped in one set, prospectively followed for a period of 12 months.

Using a computer-generated randomization method, patients were randomized to one of the three treatment groups [SLIT and pharmacotherapy, SCIT and pharmacotherapy or pharmacotherapy only (positive control)] and followed-up prospectively for a period of 1 year. The primary outcome was the symptom/medication scores assessing upper and lower airways. Secondary parameters were the assessment of titrated SPT, new sensitizations, mtcBHR, ASNPT and the results of immunological parameters. The study protocol was approved by the Ethics Committee of the Medical Faculty of Marmara University and written consents were taken from all parents of children. The study design is summarized in Figure 1.

Patients
All patients had to have skin test positivity and serum sIgE only to D.pt and/or D.f. Patients with polysensitization to other aeroallergens, systemic immunological disorders, severe asthma with forced expiratory volume in 1 s (FEV₁) <70%, severe atopic dermatitis and previous use of allergen immunotherapy were excluded from the trial. All patients were instructed to take avoidance measures against HDM, including use of impermeable mattress and pillow covers, removal of carpets and curtains and hot water washing of bedding once weekly.

Methods

Study design
The study included 50 children (28 girls, 5–10 years of age) suffering from mild persistent asthma/rhinitis according to GINA guidelines [11], having HDM-related asthma/rhinitis symptoms, strictly mono-sensitized to Dermatophagoides pteronyssinus (D.pt) and Dermatophagoides farina (D.f) as confirmed by a positive skin prick test (SPT) and HDM-specific IgE (sIgE) level of ≥0.35 IU/mL. (Immulite method; Euro/DPC, Llberis, Wales, UK), who were being prospectively followed-up and received inhaled/intranasal steroids for at least 2 years with no reduction of symptoms, were enrolled. All eligible patients underwent an 8-week run-in period to evaluate their baseline clinical conditions by means of symptom and medication scores, visual analogue score (VAS), lung functions, methacholine bronchial hyper-reactivity (mtcBHR), allergen-specific nasal provocation (ASNPT) and SPTs. Also, the immunological parameters evaluated during the screening period were the total- and HDM-sIgE, from peripheral blood mononuclear cells (PBMC) allergen-induced IL-4, IL-5, IL-13, IFN-γ, IL-10 and TGF-β cytokines were evaluated before and 12 months after treatment. Patients were enrolled in May 2007, treatment was commenced on July 2007 and the evaluations of outcomes were assessed in July 2008.

The standardized extract used throughout the study was a 1 : 1 mixture of D.pt and D.f administered as a glycerinated solution (SLIT®; ALK-ABELLO, S.A., Madrid, Spain) or adsorbed on aluminium hydroxide (SCIT, ALUTARD® SQ, ALK-ABELLO, S.A). The SLIT was self-administered at home and included a 1-month induction phase followed by a maintenance phase of five drops three times a week. The drops were given early in the morning before breakfast and held under the tongue for 2 min before swallowing. SCIT was administered in the clinic and included a 16-week (weekly injections) induction phase followed by a monthly maintenance phase. The patients were observed at the clinic after each injection for at least 30 min for a possible side-effect of SCIT. The cumulative 1-year dose for SLIT was approximately 73 876.8 STU, which was equivalent to 295.5 µg of Der p 1 and 295.5 µg of Der f 1, and for SCIT was approximately 1131 540 SQ-U, which was equivalent to 111 µg of Der p 1 and 156 µg of Der f 1. All patients who were in SLIT and SCIT group received the same protocol as recommended by the manufacturers (Table 1). Also, all three groups were allowed to use rescue medications, inhaled/intranasal corticosteroids, antihistamines and oral steroids provided in a stepwise fashion depending on the persistence and severity of the symptoms as recommended [11].
Symptoms and medication scores

Patients or their parents were instructed to keep a diary during the treatment period, for a daily evaluation of symptoms according to a four-point scoring system: 0 (no symptoms) to 3 (severe symptoms) for each rhinitis symptoms (sneezing, nasal discharge, itching and nasal obstruction) and asthma symptoms (wheezing, breathlessness, dyspnoea and cough). The total score of all four rhinitis and asthma symptoms were termed as total rhinitis symptom scores (TRSS) and total asthma symptom score (TASS), respectively. The combination of TRSS and TASS was calculated as total symptom scores (TSS). When necessary, patients were allowed to use antihistamines, inhaled and/or intranasal corticosteroids. The patients had to record on the same diary card whenever they used

Fig. 1. Study design showing the flow of participants through each stage. SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SMS, symptoms-medication scores; VAS, visual analogue scores; PFT, pulmonary function test; SPT, skin prick test; NPT, nasal provocation test; Mtc, methacholine provocative test.

Table 1. Doses and duration of immunotherapy

<table>
<thead>
<tr>
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<th>SLIT</th>
<th>SCIT</th>
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<tbody>
<tr>
<td>Induction phase</td>
<td>Vial 0 (1.6 STU/mL): 1–10 drops</td>
<td>Vial 1 (100 SQ-U/mL): 0.2, 0.4, 0.8 cm³</td>
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<td></td>
<td>Vial 1 (8 STU/mL): 1–5 drops</td>
<td>Vial 2 (1000 SQ-U/mL): 0.2, 0.4, 0.8 cm³</td>
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<td></td>
<td>Vial 2 (40 STU/mL): 1–5 drops</td>
<td>Vial 3 (10 000 SQ-U/mL): 0.2, 0.4, 0.6, 0.8 cm³</td>
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<tr>
<td></td>
<td>Vial 3 (200 STU/mL): 1–5 drops</td>
<td>Vial 4 (100 000 SQ-U/mL): 0.1, 0.2, 0.4, 0.6, 0.8, 1 cm³</td>
</tr>
<tr>
<td></td>
<td>Vial 4 (1000 STU/mL): 1–5 drops</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>Given daily for 30 days</td>
<td>Given weekly for 16 weeks</td>
</tr>
<tr>
<td>Maintenance dose</td>
<td>Vial 4 (1000 STU/mL): 5 drops (3 × week)</td>
<td>Vial 4 (100 000 SQ-U/mL): 1 cm³ (monthly)</td>
</tr>
<tr>
<td>Daily maximum dose</td>
<td>Der p 1/Der f 1: 4/4 µg/1000 STU</td>
<td>Der p 1/Der f 1: 9.8/13.8 µg/100 000 SQ-U</td>
</tr>
<tr>
<td>Cumulative dose per year</td>
<td>Der p 1/Der f 1: 295.5 µg/295.5 µg (73876.8 STU)</td>
<td>Der p 1/Der f 1: 111 µg/156 µg (1131 540 SQ-U)</td>
</tr>
</tbody>
</table>

SLIT, sublingual immunotherapy; SCIT, subcutaneous immunotherapy.
medications (1 point: for β2 agonists and antihistamines, 2 points: inhaled/intranasal steroids, 3 points: one tablet of corticosteroid) and were calculated as the total medication score (TMS). The individual daily symptom and medication scores were recorded on a daily basis for the entire period of the study and the mean of 3 monthly scores were recorded during the 3-monthly study visit. Adverse reactions were classified according to the European Academy of Allergy and Clinical Immunology (EAACI) grading system [12].

**Visual analogue scale**

A 10 cm line to grade the severity of symptoms from ‘no symptoms’ (0 cm) to ‘the highest level of symptoms’ (10 cm) was given to the patients. Patients were asked to record a VAS in response to the question, ‘How has the severity of the asthma/rhinitis symptoms been during the last month?’ Patients were asked to grade their symptoms retrospectively for the last week on the every 3-month study visit and were assessed as baseline, and after 6th and 12th month of treatment.

**Skin prick testing**

The cutaneous response to allergens and new sensitizations were assessed before and 12 months after the treatment. SPTs were performed with 20 common aeroallergens; mites, latex, moulds, pollens, animal dander and insects (ALK-Abello, Lainate, Italy) as described previously [6].

**Nasal provocation test**

Patients were provoked in 10 min intervals with solutions in the following order: Test solution, 2, 4 and 8 BU/mL of **D.pt** extracts (negative control; ALK-diluent, allergen preparation; ALK-Abello) at the dosage of one puff (100 μL) into each nostril by a nasal spray applicator. These concentrations correspond, respectively, to about 0.016, 0.032 and 0.064 mg per puff of the major allergen. With each application, the response to provocation was recorded. A score was established (0: absent, 1: partial 2: moderate, 3: severe) for each of the rhinitis symptoms (sneezing, rhinorrhoea, blocked nose and itching). If a total score of at least 8 was not reached with the lowest concentration, the following ones were sequentially used. The test was performed at the beginning and end of the treatment.

**Pulmonary function test, methacholine challenge and immunoglobulin E levels**

Lung functions were assessed with a spirometer (Sensormedics, S3513, Yorba Linda, CA, USA). Starting from 0.031 mg/mL concentration, the subsequent methacholine dilutions were increased in a doubling manner until the provocative concentration of an inhaled agonist producing a 20% decrease in FEV1 (PC20) is achieved compared with the value recorded at baseline. A PC20 value <8 mg/mL was considered as positive for bronchial hyperresponsiveness. Serum total IgE (tIgE) and sIgE levels were determined using the Immulite 2000 (Immule; Euro/DPC, Linberis, UK), according to the manufacturer’s instructions. For the calculation of the ratio, ([sIgE/tIgE]×100 equation was used.

**Peripheral blood mononuclear cell isolation and detection of secreted cytokines**

PBMC were isolated by Ficoll–Hypaque density gradient centrifugation and then suspended in RPMI 1640 with 2 mmol/L L-glutamine, 100 U/mL penicillin/streptomycin, and supplemented with 10% foetal calf serum (FCS) (all from Sigma Chem Co., St Louis, MO, USA). To measure the release of cytokines, 6×10⁵ PBMC were incubated with 10 μg/mL recombinant Der p 1 (Indoor Biotechnologies Ltd., Cardiff, UK), and as a negative control 10 μg/mL recombinant Bet v 1 (Indoor Biotechnologies Ltd.) in 500 μL each in 48-well plates (Costar Corp., Cambridge, MA, USA) at 37°C with 5% CO2 for 5 days. The supernatants were collected and stored at −80°C until tested. IL-4, IL-5, IL-10, IL-13, IFN-γ (Endogen®), Rockford, IL, USA) and TGF-β (Assaypro®, St Peters, MO, USA) levels of PBMC culture supernatants were determined using commercial human ELISA kit, according to the manufacturer's instruction (sensitivity limits: IL-4: <2 pg/mL; IL-5: <2 pg/mL, IL-10: <7 pg/mL, IFN-γ: <2 pg/mL; IL-13: <3 pg/mL, TGF-β: <30 pg/mL).

**Statistical analysis**

Values are presented as mean±SD and median (range), unless otherwise specified. Comparisons for quantitative variables were performed by non-parametrical analysis, Mann–Whitney and Kruskall–Wallis tests for non-related samples. Comparisons at two different times were carried out using the Wilcoxon’s test for related samples. Pearson’s correlations were used to assess the relationships between the ratios of sIgE **D.pt** or **D.flt**-IgE and the symptoms medical scores. Significance was set at P<0.05. SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for analysis.

**Results**

**Patients**

Out of 50 included, 48 patients were randomized (median age 7 years). The number of patients who were evaluable...
for efficacy, safety and immunological comparison during the first 12 months of treatment were 16 for SCIT and SLIT each, and 16 pharmacotherapy-treated patients. At the end of the study, 43 patient’s data were evaluated (SCIT; 14, SLIT; 15 and 14 pharmacotherapy group). The number of patients completing the study and withdrawals are shown in Fig 1. The demographic characteristics are shown in Table 2. No significant differences were observed among the three groups with respect to gender, age, duration of asthma/rhinitis symptoms, total symptoms/medication score, VAS and bronchial or nasal hyper-responsiveness.

Clinical efficacy

Evaluation of clinical efficacy was based on daily symptoms/concomitant medication scores and VAS. After 12 months of treatment, the median TASS ($P = 0.02$), TRSS ($P = 0.03$), TSS ($P = 0.01$), TMS ($P = 0.03$) and VAS ($P = 0.02$) were significantly reduced in SLIT when compared with the pharmacotherapy group. The median percentage improvement of TRSS, TASS and TSS in SLIT was 47%, 100% and 77%, respectively, when compared with the pharmacotherapy group. Also, a significant reduction of VAS score within the SLIT group compared with the baseline was observed ($P = 0.02$) (Table 3, Fig. 2).

As for SCIT, the median TASS ($P = 0.04$), TRSS ($P = 0.01$), TSS ($P = 0.01$) and VAS ($P = 0.001$) were significantly reduced when compared with the pharmacotherapy group. The median percentage improvement of TRSS, TASS and TSS in SCIT was 67%, 93% and 81%, respectively, when compared with the pharmacotherapy group. There was no statistical difference in terms of TMS when compared with the pharmacotherapy group. When compared with the baseline data in SCIT group, VAS significantly reduced after 12 months of treatment ($P = 0.001$) (Table 3, Fig. 2). No statistical difference between the SLIT and the SCIT group was observed.

Adverse events

Two cases of systemic reaction were observed in the SCIT group, one being grade 3 and the other grade 4. The patient with grade 3 adverse reaction was a 5-year-old girl with both asthma and rhinitis. The patient had severe asthma symptoms occurring after every injection during the induction phase, which required rescue medication and was regarded as possible treatment related. The other patient with grade 4 adverse reaction was a 10-year-old girl having both asthma and rhinitis. Within 15 min after being given the 9th injection during the induction phase, flushing, wheezing, dyspnoea was observed and adrenalin was immediately administered. Both patients were discontinued from SCIT and withdrew from the study. One patient in the SCIT group had a local reaction at injection site which did not exceed 7 cm and did not require discontinuation of treatment. No adverse reactions were observed in the SLIT or the pharmacotherapy group during the study.

Lung functions, bronchial and nasal hyper-reactivity

No difference between/within the group of lung function and mtcBHR results were observed (data not shown), whereas titrated ASNPT revealed an increase in nasal provocative dose significantly in SLIT ($P = 0.01$) and SCIT ($P = 0.005$) when compared with the pharmacotherapy group at the end of 12 months of treatment (Fig. 3a). No difference between SLIT and SCIT group was observed.

Skin prick test response

When compared with the baseline, weal diameter of $D.f$ and $D.pt$ extract significantly decreased in SLIT group ($P = 0.01$, $P = 0.006$, respectively), whereas SCIT group demonstrated a significantly decreased weal diameter of $D.pt$ extract within 12 months of treatment ($P = 0.01$). There were no new sensitizations observed in all groups (Fig. 3b).

Serum-specific and total immunoglobulin E values and correlation between the ratio and symptom–medication scores

A significant decrease in sIgE $D.f$ in SCIT ($P = 0.03$) and SLIT ($P = 0.04$) group after 12 months of treatment was observed. sIgE $D.pt$ decreased significantly ($P = 0.03$) in SCIT when compared with pharmacotherapy. There was no significant difference in either between- or within-group comparisons in serum tIgE levels of the three groups (Fig. 4). Using the ratio of sIgE over tIgE of each allergen, no significant correlation was found in relation to the symptoms–medication scores or VAS (data not shown).

Analysis of allergen–induced cytokine responses

A significant decrease of Der p 1-induced-IL-10 in both pharmacotherapy and SCIT ($P = 0.03$, $P = 0.01$, respectively) was observed after 12 months of treatment, whereas there was no statistical difference in the SLIT group when compared with the baseline ($P = 0.43$) (Fig. 5a). At the end of 12 months of treatment, Der p 1-induced-IL-10 levels in the SLIT group was found to be significantly higher than the pharmacotherapy group ($P = 0.05$). Also, a significant increase was observed for Bet v 1 (negative control)-induced TGF-β in SLIT group after 12 months of treatment when compared with the baseline ($P = 0.03$) (Fig. 5d). Otherwise, no changes were observed for Der p 1− and Bet v 1-induced IL-4, IL-5, IL-13.
and IFN-γ, either in or between groups at 12 months of therapy (data not shown).

**Discussion**

This prospective, randomized, controlled study clearly revealed that SLIT and SCIT mode of treatment administered to allergic asthma/rhinitis children effectively reduce the daily symptoms and medication consumption, VAS, severity of skin sensitization, nasal sensitivity to specific allergen and sIgE production, with no significant effects on in vitro cytokine secretion.

Although there are elegant studies performed to compare the clinical efficacy and immunological outcome [13–18], to our knowledge, this is the first randomized, controlled, three parallel grouped in one set prospectively followed for the determination of efficacy and immunological mechanisms of subcutaneous and sublingual allergen immunotherapy in children. According to our results, using the estimated difference relative to the median values of the pharmacotherapy group, both SLIT and SCIT treated showed a significantly better outcome compared with pharmacotherapy alone. Results indicated that the SCIT and SLIT had reduced disease severity more than half the severity observed in pharmacotherapy-alone treated group. No difference between SCIT and the SLIT group was observed, which suggested that they are almost equally effective in...
controlling the severity of disease. Regarding safety, SCIT was the only treatment mode that resulted in two cases of severe reaction, which all occurred during induction period.

The main limitations of the current study are the low number of patients included in each arm, which increased the risk of a statistical error II and without using a placebo, as it could have raised ethical issues. Nevertheless, the strength was the application of the randomization method, selecting mono-sensitized patients and including the positive control group. Also, the period when the study was commenced needs to be accredited in that, during summer period (May–September) most patients are having their lowest level of symptoms, which can be attributed as a

Table 3. Primary outcomes of SCIT, SLIT and pharmacotherapy

<table>
<thead>
<tr>
<th></th>
<th>Pharmacotherapy</th>
<th>SLIT</th>
<th>SCIT</th>
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<td>T0</td>
<td>T1</td>
<td>T0</td>
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<tr>
<td>TRSS</td>
<td>1.56±1.05</td>
<td>2.9±0.7</td>
<td>1.3±0.9</td>
<td>1.5±1.0</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>1.78 (0–3)</td>
<td>2.8 (2–4)</td>
<td>2 (0–3)</td>
<td>1.5 (0–3)</td>
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<tr>
<td>TASS</td>
<td>0.95±0.62</td>
<td>2.5±1.6</td>
<td>1.4±1.5</td>
<td>0.2±0.4</td>
<td>0.02</td>
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<tr>
<td></td>
<td>1 (0–2)</td>
<td>2.7 (0–4)</td>
<td>1 (0–5)</td>
<td>0 (0–1)</td>
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<tr>
<td>TSS</td>
<td>2.5±1.3</td>
<td>5.4±1.7</td>
<td>2.8±2.2</td>
<td>1.4±1.5</td>
<td>0.01</td>
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<tr>
<td></td>
<td>3 (0–4)</td>
<td>5.5 (3–8)</td>
<td>3 (0–7)</td>
<td>1.3 (0–4)</td>
<td></td>
</tr>
<tr>
<td>TMS</td>
<td>2.5±1.5</td>
<td>2.8±1.1</td>
<td>2.8±1.2</td>
<td>1.2±0.9</td>
<td>0.03</td>
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<td></td>
<td>2 (0–4)</td>
<td>2 (2–4)</td>
<td>2 (0–4)</td>
<td>1.6 (0–2)</td>
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<tr>
<td>VAS</td>
<td>4.9±1.9</td>
<td>4.6±1.5</td>
<td>4.9±1.5</td>
<td>2.7±2.1*</td>
<td>0.02</td>
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<td></td>
<td>5 (1–8)</td>
<td>5 (2–7)</td>
<td>5 (2–7)</td>
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TRSS, total rhinitis symptoms score; TASS, total asthma symptoms score; TSS, total symptom score includes total rhinitis symptoms score (sneezing, runny nose, itchy nose, nasal congestion) and total asthma symptoms score (cough, wheezing, breathlessness, dyspnoea); TMS, total medical score; T0, at baseline; T1, at 12 months after treatment.

*P = 0.01 SLIT baseline vs. 12 months.

**P = 0.002 SCIT baseline vs. 12 months.

***SCIT or SLIT vs. pharmacotherapy at 12 months (Mann–Whitney U-test).

1Percentage of improvement was defined as the estimated difference of SCIT or SLIT group at 12 month in relative to the median of pharmacotherapy group at 12 month after treatment.

Fig. 2. Visual analogue score in pharmacotherapy, SCIT and SLIT group pre-treatment, 6 and 12 months after treatment. P < 0.05, statistical significant between the groups. Paired values for Wilcoxon’s signed-rank and Mann–Whitney U-test. ns, non-significant; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.
wash-out period for steroid effect and a period advised as the right time to start immunotherapy.

The clinical efficacy of SCIT is well established for both rhinitis and asthma. Meta-analyses relating to its efficacy on asthma [19] and rhinitis [20] are available. SLIT has also been validated in this respect [21]. Two recent meta-analyses in children showed that sublingual delivery of allergen vaccination constitutes a safe and effective alternative to the injectable route to reduce allergy respiratory symptoms and drug intake [22, 23].

Comparative clinical studies of sublingual and subcutaneous treatment yielded heterogeneous results. Khinchi et al. study [13] found that the SCIT group had a disease severity reduced to one-third and the SLIT group to half the severity observed in placebo treated. In the Quirino et al. [14] study, symptom and medication scores were reduced by approximately 50% in both SLIT and SCIT groups. The study by Mungan et al. [15], which includes a placebo group, showed in adults that in SCIT, both rhinitis and asthma symptom scores improved significantly, with reduction in medical scores while only rhinitis symptoms reduction was observed in SLIT when compared with the placebo. As for our study, total rhinitis and asthma symptoms were similarly reduced in both group but the reduction of medication usage was more pronounced in SLIT compared with SCIT.

Successful SIT reduces the symptoms of allergic disease and the need for medication. This was also shown in rhinitis patients with the decrease in late-phase responses to a local allergen challenge in the skin and nasal mucosa and in asthmatics, non-specific airway hyper-reactivity and bronchial response to inhaled allergen challenge are

\(\text{Fig. 3. (a)}\) Median values for the titrated allergen-specific nasal provocation dose (BU/mL). Comparing between the groups (SLIT, SCIT and pharmacotherapy) after 12 months of treatment (Mann–Whitney U-test). IT, immunotherapy; ns, non-significant. (b) Median (range) values for a weal diameter of skin prick reaction to *Dermatophagoides pteronyssinus* (*D.pt*) and *Dermatophagoides farinae* (*D.f*). Comparing baseline and 12 months of treatment within the groups (Wilcoxon’s signed-rank). ns, non-significant; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

\(\text{Fig. 4.}\) The median values for total IgE, specific IgE-*Dermatophagoides pteronyssinus* (*D.pt*) and *Dermatophagoides farinae* (*D.f*) before and 12 months after treatments. Paired values for Wilcoxon’s signed-rank and Mann–Whitney U-test*. P < 0.05 statistical significant, ns, non significance within the group.

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decreased [24, 25]. Our result confirms the effects of SIT, whereby both treatment modes decreased the specific allergen reactivity in the skin and increased the threshold dose to induce nasal hyper-reactivity when compared with untreated individuals.

Allergen-specific IgE antibodies are the hallmark of the immune response to allergens and an important diagnostic tool for allergic disease. Recently by using VAS, it was shown that a serum sIgE/tIgE ratio is an advantageous tool in predicting the clinical response to allergen-specific immunotherapy [26]. In our study, no correlation was observed between the symptoms–medication scores or VAS and sIgE/tIgE ratio, which might be due to the small sample size. On the other hand, other studies have shown that following immunotherapy, allergen-specific IgE antibody levels decrease with an increase in IgG, IgG4 and IgA [25, 27, 28]. In this comparative study, a decrease in sIgE was detected in both treatment modes compared with untreated, 12 months after treatment.

Clinical effects of SLIT and SCIT were not accompanied by alterations in peripheral blood T cell responsiveness to HDM allergen in vitro, in terms of cytokine productions. Studies of bee venom and pollen-sensitive patients suggest that SCIT can induce the suppression T cell responses and decrease the production of IL-4, IL-5 and IL-13 with an increase in IFN-γ production in the peripheral blood [29, 30]. Other studies showed that along with clinical improvement, SCIT significantly increased the ratio of IFN-γ: IL-5 mRNA-expressing cells in the nasal mucosa with no alterations in peripheral blood T lymphocyte responsiveness to grass allergen exposure in vitro, either in terms of proliferation or cytokine production [31]. As for SCIT studies with other allergens, still controversial immunological results have been more variable. There were no changes observed in IFN-γ or IL-4 production following HDM–SCIT but an increase in IL-10 production by CD4+ T cells was reported [32, 33]. As for SLIT, similar controversial results were obtained. Arikan et al. [34] demonstrated an increase in Th1 cytokines after 6 months HDM–SLIT when compared with the control. Cosmi et al. [28] used three doses (0.4, 2 vs. 10 μg/mL) of Der p 1 for in vitro stimulation of PBMC and for consequently analysing IFN-γ and IL-10. It was observed that IL-10 is suppressed on using a high dose of allergen extract in vitro with an increase in IFN-γ and vice versa with a low dose. Otherwise in studies including pollen allergen, lack of immune response in hand with our study was observed after 12 months of SLIT [35, 36]. These discrepancies might be due
to the small sample size or due to a non-standardization of extracts dose used of \textit{in vitro} protocols, which can suppress/over-express the cytokine production. An interesting result obtained in the present study is the increase in Bet v 1-induced TGF-\( \beta \) in birch pollen non-sensitized SLIT group. These increases may possibly be attributed as a mechanism for suppressing new sensitization, whereby this matter is worthy to be elucidated.

In conclusion, our study in HDM-sensitized asthmatic/rhinitis children treated with SIT and concomitant rescue medications demonstrated a vast clinical improvement when compared with untreated children. Because of to its non-adverse effect profile, SLIT seems to be favourable in children. More studies in children to address the long-term efficacy and cost-effectiveness of these two most-used modes of immunotherapy are needed in a larger scale.

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**References**
