

Long-Term Effect of Sublingual and Subcutaneous Immunotherapy in Dust Mite–Allergic Children With Asthma/Rhinitis: A 3-Year Prospective Randomized Controlled Trial

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■ Abstract

Background and Objective: Specific allergen immunotherapy is the only treatment modality that might change the natural course of allergic diseases in childhood. We sought to prospectively compare the long-term clinical and immunological effects of sublingual (SLIT) and subcutaneous (SCIT) immunotherapy compared with pharmacotherapy alone.

Methods: In this single-center, prospective randomized controlled trial, 48 children with mild persistent asthma with/without rhinitis, monosensitized to house dust mites (HDMs) were followed for 3 years. At baseline and years 1 and 3 of follow-up, patients were evaluated and compared for total rhinitis (TRSS) and asthma (TASS) symptom scores, total symptom scores (TSS), total medication scores (TMS), safety profiles, skin-nasal-bronchial reactivity, and immunological parameters.

Results: A significant reduction was observed in TASS for both HDM-SCIT and HDM-SLIT at year 3 of treatment compared with baseline and controls ($P<.05$ for both), with significant improvement in rhinitis symptoms for both groups compared with controls ($P=.01$ for both). TSS decreased significantly in both HDM-SCIT and HDM-SLIT at year 3 compared with baseline ($P=.007$ and $P=.04$, respectively) and controls ($P<.01$ for both). A significant reduction in TMS was observed in HDM-SCIT and HDM-SLIT compared with baseline and controls ($P=.01$ in all cases), with a reduction in skin reactivity to HDM ($P<.05$). Finally, a significant increase in allergen specific IgG4 was observed in the SCIT group at year 3 compared with baseline, the SLIT group, and controls ($P<.001$ in all cases).

Conclusions: HDM-sensitized asthmatic children treated for at least 3 years with either SCIT or SLIT showed sustained clinical improvement.

Key words: Asthma. Children. House dust mites. Immunotherapy. Subcutaneous immunotherapy. Sublingual immunotherapy.

■ Resumen

Antecedentes: La inmunoterapia con alérgenos es el único tratamiento que podría cambiar el curso natural evolutivo de las enfermedades alérgicas en la infancia. Nuestro objetivo era comparar, de manera prospectiva, la eficacia a largo plazo de la inmunoterapia sublingual (SLIT) y subcutánea (SCIT), con el tratamiento exclusivo con farmacoterapia convencional.

Métodos: En este ensayo clínico, prospectivo de tres años de duración, realizado en un solo centro y aleatorizado, se incluyeron 48 niños con asma leve persistente, con o sin rinitis asociada, monosensibilizados a los ácaros del polvo (HDM). Los pacientes fueron evaluados al inicio, al año y a los tres años de tratamiento, comparándose los cambios en la puntuación de síntomas nasales (TRSS), bronquiales (TASS), puntuación total de síntomas (TSS) y consumo de medicación (TMS), perfil de seguridad, reactividad frente al alérgeno cutánea, nasal y bronquial y diversos parámetros inmunológicos.

Resultados: Se observó una reducción significativa del TASS tanto para el grupo HDM-SCIT como HDM-SLIT al final del tercer año de tratamiento, tanto cuando se comparaba con la situación basal como con los cambios observados en el grupo control ($p<0.05$,

respectivamente). El TRSS también mejoró significativamente en ambos grupos HDM-SCIT y HDM-SLIT en el tercer año de tratamiento, cuando los cambios se compararon con los observados en el grupo control ($p=0,01$, en ambos). El TSS y el TMS disminuyeron también significativamente en ambos grupos HDM-SCIT y HDM-SLIT en el tercer año, comparado con la situación basal ($p=0,007$, $p=0,04$ / $p=0,01$, $p=0,01$ respectivamente) y con el grupo control ($p<0,01$, $p<0,01$ / $p=0,01$, $p=0,01$, respectivamente). Tras tres años de tratamiento la reactividad cutánea frente a los alérgenos de los ácaros disminuyó significativamente ($p<0,05$). Los niveles de IgG4 específica frente a ácaros se incrementaron en el grupo SCIT-HDM, comparados con la situación basal y con los cambios observados en el grupo SLIT-HDM y control ($p<0,001$, respectivamente).

Conclusiones: El tratamiento durante tres años con inmunoterapia específica tanto SCIT como SLIT se acompañó de una eficacia clínica sostenida, en este grupo de niños asmáticos sensibilizados a los ácaros del polvo. Ambas rutas de administración de la inmunoterapia parecen tener mecanismos de acción similares.

Palabras clave: Asma. Niños. Ácaros del polvo. Inmunoterapia. Inmunoterapia subcutánea-sublingual.

Introduction

The prevalence of atopic diseases such as allergic rhinitis and asthma has increased in children and adults over the past 3 decades [1]. House dust mites (HDMs) are common allergens worldwide and *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are major allergens that coexist in most geographical regions. Approximately 85% of asthmatics are typically HDM-allergic [2]. Acaricides and environmental control measures have been found to be of no effect in reducing asthma or rhinitis symptoms [3,4]. While treatment with rescue medications and corticosteroids can control allergic rhinitis and asthma symptoms, with improvement of lung function in asthmatics, it does not modify the natural course of disease.

Allergen-specific immunotherapy (SIT) can modify the course of allergic disease by preventing exacerbation [5], reducing the risk of new allergic sensitizations, and deterring the development of clinical asthma in children treated for allergic rhinitis [6]. Furthermore, one of the main goals of SIT is to achieve sustained clinical effects in post-treatment follow-up over a recommended period of 3 to 4 years [7,8].

The precise immunological mechanisms underlying the beneficial clinical effects of subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) remain a matter of research and debate. Changes to T-cell reactivity and induction of blocking antibodies such as IgG4 and IgA are regarded as immunological markers of clinical tolerance [9].

Despite the effectiveness of SCIT in children, new treatment modalities have emerged due to the discomfort (repeated injections) and risk of life-threatening adverse effects associated with subcutaneous administration. SLIT, for instance, which involves applying allergen extract to the mucosal surfaces of the immunologically rich sublingual area, has been accepted as a safe SIT method. Both SCIT and SLIT can reduce the duration and dose of inhaled corticosteroids and even lead to their successful discontinuation in the long-term treatment of HDM-induced asthma/rhinitis [10,11].

To our knowledge, no prospective studies have compared HDM-SCIT and SLIT in children for longer than 3 years, which is the currently recommended duration of treatment. The aim of this study was to evaluate and compare long-term clinical efficacy, safety, tolerability, and immunological mechanisms after 3 years of SCIT, SLIT, or pharmacotherapy alone in a

randomized prospectively followed-up 3-arm controlled trial of HDM-monosensitized children with asthma and/or rhinitis.

Methods

Patients

Patients with mild to moderate persistent asthma and/or rhinitis according to the Global Initiative for Asthma guidelines [12], monosensitized to HDM, who had been followed in the outpatient clinic for at least 2 years without improvement with inhaled corticosteroid treatment, were recruited and randomized to receive SCIT, SLIT, or pharmacotherapy only (control group). The inclusion and exclusion criteria applied during randomization have been previously reported [13]. The study protocol was approved by the ethics committee at our center (approval number B.30.2.MAR.0.01.02/AEK/707), and written consent was obtained from the parents of all the children included.

Study Design

At baseline and year 1 (data already published [13]), and year 3 of follow-up, all eligible patients were evaluated by means of symptom and medication scores, visual analog scale (VAS) scores, lung function tests, nonspecific bronchial hyperresponsiveness to methacholine (methacholine challenge), allergen-specific nasal provocation (ASNPT), and skin prick test (SPT) reactivities. Immunological parameters were evaluated at the same time points and included total and HDM-specific IgE, Der p 1- and Der p 2-specific IgG4, and allergen-stimulated production of IL-5, IFN- γ , and IL-10 from peripheral blood mononuclear cells (PBMCs). The primary outcome was the symptom and medication scores at year 3 of treatment. Secondary outcomes were SPT measurements, new sensitizations, methacholine challenge, ASNPT, and immunological readouts. The study design is summarized in Figure 1.

Treatment and Avoidance

The standardized allergen extract used throughout the study was a 1:1 mixture of *D pteronyssinus* and *D farinae* administered as a glycerinated solution (SLIT, SLIT; ALK-

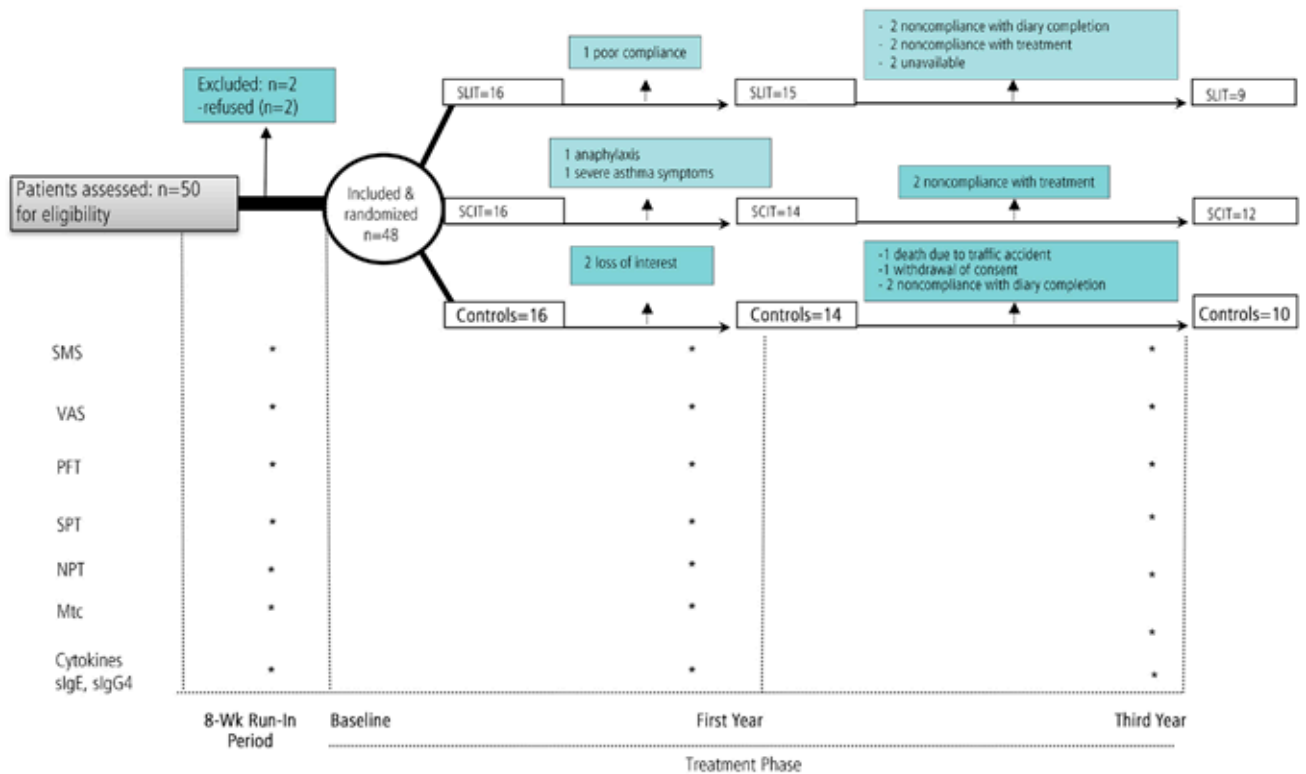


Figure 1. Study design showing the flow of participants through each stage of the study. SCIT indicates subcutaneous immunotherapy; SLIT, sublingual immunotherapy; Controls, pharmacotherapy only; SMS, symptom-medication scores; VAS, visual analog scores; PFT, pulmonary function test; SPT, skin prick test; NPT, nasal provocation test; Mtc, methacholine provocative test; slgE, specific IgE; slgG4, specific IgG4.

ABELLO, S.A., Madrid, Spain) or adsorbed on aluminium hydroxide (SCIT, ALUTARD SQ; ALK-ABELLO, S.A.). The dosing regimens and allergen equivalence for the SCIT and SLIT groups were as previously reported [13]. Briefly, SLIT was self-administered at home and included a 1-month induction phase followed by a maintenance phase of 5 drops administered 3 times a week. The drops were taken early in the morning before breakfast and held under the tongue for 2 minutes before swallowing. SCIT was administered in the clinic and included a 16-week induction phase (with weekly injections) followed by a monthly maintenance phase. The patients were observed at the clinic for at least 30 minutes after each injection for possible adverse effects. During the study, all 3 groups were allowed to use rescue medications, inhaled/intranasal corticosteroids, antihistamines, and oral corticosteroids provided in a stepwise fashion depending on the persistence and severity of the symptoms as recommended.

Symptoms and Medication Scores and VAS

Rhinitis symptoms (rhinorrhea, sneezing, itching, and nasal blockage) and asthma symptoms (cough, wheezing, breathlessness, and dyspnea) were recorded on a 4-point scale (0, no symptoms; 1, mild; 2, moderate; 3, severe). The total scores comprising all 4 rhinitis and asthma symptoms were termed total rhinitis symptom score (TRSS) and total asthma symptom score (TASS), respectively. These scores were then

combined to create the total symptom score (TSS). Patients scored their use of medications as follows: β -2 agonists, 1 point; inhaled/intranasal corticosteroids, 2 points; and 1 corticosteroid tablet, 3 points. The points were totalled to calculate the total medication score (TMS). Individual daily symptom and medication scores were recorded daily for the entire study period and mean monthly scores were recorded at every 3-monthly study visit. The severity of asthma and rhinitis symptoms was evaluated using a VAS consisting of a 10-cm line ranging from no symptoms (0 cm) to the highest level of symptoms (10 cm).

Skin Prick Testing

Skin prick tests were performed with 20 common aeroallergens, in addition to mites, latex, molds, pollens, animal dander, and insects (ALK-Abello, Lainate, Italy) as described previously [13].

ASNPT

The ASNPT was performed using a pressurized vehicle to spray allergen extract (100 μ L) into each nostril; the extract contained 2 to 8 BU/mL of purified HDM (*D pteronyssinus*) extract (negative control, ALK-diluent, allergen preparation; ALK-Abello). The response observed 10 minutes after each application was recorded. A score was recorded for each of

the rhinitis symptoms (sneezing, rhinorrhea, blocked nose, and itching) as follows: 0, no symptoms; 1, mild; 2, moderate; 3, severe. If a total score of 8 was not reached with the lowest concentration, a sequential doubling dose regimen was applied. The challenge was performed at baseline and at 1 and 3 years of treatment.

Pulmonary Function Tests and Methacholine Challenge

Pulmonary function tests were performed by means of maximal forced expiratory volume curves (Zan Flowhandy II, Zan Messgerate GmbH), as previously described [13]. Briefly, a nonspecific methacholine challenge was performed with a starting concentration of 0.031 mg/mL; the subsequent methacholine dilutions were increased in a doubling manner until the provocative concentration of the inhaled agonist produced a 20% decrease in FEV₁ (PC₂₀) compared with baseline. A PC₂₀ value of <8 mg/mL was considered positive for bronchial hyperresponsiveness [14].

Total and Specific IgE and Specific IgG4 Levels

Total serum and HDM-specific IgE levels were determined using the Immulite 2000 system (Euro/DPC), according to the manufacturer's instructions. Serum specific IgG4 levels for Der p 1 and Der p 2 were kindly quantified by ALK-Abello using ELISA. All the samples were coded to ensure the operators were blinded to the clinical information.

PBMC Isolation and Detection of Cytokines

PBMCs were isolated by Ficoll–Hypaque density gradient centrifugation and then suspended in RPMI 1640 with

2 mmol/L L-glutamine (Gibco, Invitrogen) and 100 U/mL penicillin/streptomycin, supplemented with 10% fetal calf serum. To measure the release of cytokines, 6x10⁵ PBMC were incubated with 10 µg/mL recombinant Der p 1 (Indoor Biotechnologies Ltd) in 500 µL each in a 48-well plate (Costar Corp) at 37°C with 5% CO₂ for 5 days. The supernatants were collected and stored at -80°C until tested. IL-5, IFN-γ, and IL-10 levels of PBMC culture supernatants were determined using the commercial human ELISA kit (Invitrogen) according to the manufacturer's instruction. All the specimens were coded so that the investigators were blinded to the patients' clinical characteristics.

Statistical Analyses

Values are presented as means (SD) and medians (interquartile range), unless otherwise specified. Comparisons for quantitative variables were performed by nonparametric analysis, with the Mann-Whitney and Kruskal-Wallis tests used for unrelated samples. Comparisons between 2 time points were carried out using the Wilcoxon test for related samples. Significance was set at *P*<.05 (GraphPad Prism 5).

Results

Patients

Forty-eight randomized patients who received at least 1 dose of immunotherapy were included in the intention to treat population for the first year of treatment [13]. Of these, 31 patients completed the 3-year treatment schedule and had full data on all variables of interest for the duration of follow-up. Twelve received SCIT, 9 received SLIT, and 10 received

Table 1. Demographic Characteristics of Patients Who Completed 3 Years of Follow-up

	SLIT	SCIT	Controls	<i>P</i> ^a
No. of patients	9	12	10	>.05
Sex F/M	5/4	6/6	4/6	>.05
Age, mean (SD), y				
Age, median (IQR), y	10.14 (1.16)	10.46 (1.95)	10.4 (2.66)	>.05
Symptom duration, ^b mean (SD), mo	9.9 (9.2-10.4)	10 (9.0-12.28)	10 (7.7-12)	
Symptom duration, ^b median (IQR), mo	22.3 (4.53)	31.0 (9.51)	28.5 (10.99)	>.05
	24 (24-24)	36 (24-36)	24 (24-36)	
Asthma only ^b	3	1	3	>.05
Rhinitis only ^b	1	3	1	>.05
Asthma + rhinitis ^b	5	8	6	>.05
FEV ₁ , ^b mean (SD)	96.6 (14.8)	98.7 (6.6)	98.3 (9)	>.05
FEV ₁ , ^b median (IQR)	93 (86-107)	100 (91-104)	98 (89-108)	
PC ₂₀ , ^b mean (SD)	2.5 (3.4)	1.72 (2.1)	1.4 (2.2)	>.05
PC ₂₀ , ^b median (IQR)	0.5 (0.06-6)	0.5 (0.06-4)	0.12 (0.06-4)	

Abbreviations: F, female; FEV₁, forced expiratory volume in the first second; IQR, 25%-75% interquartile range; M, male; PC₂₀, provocative dose causing a 20% reduction in FEV₁ compared with baseline; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

^aComparison between groups using Kruskal-Wallis H and χ^2 tests, *P*<.05.

^bBaseline data for patients who completed 3 years of follow-up.

pharmacotherapy only (control group). No treatment-related adverse reactions were observed or reported in either of the immunotherapy groups during the maintenance phase. The number of patients who completed the study and the withdrawals are shown in Figure 1. The reasons for withdrawal in the SLIT group were noncompliance with diary completion (n=2), noncompliance with treatment (n=2), and failure to attend more than 3 visits (n=2). In the SCIT group, the reasons were noncompliance with treatment (n=2) and in the control group they were death due to a traffic accident (n=1), withdrawal of consent (n=1), and noncompliance with diary completion (n=2). The overall demographics were similar between the 3 groups of patients who completed 3 years of treatment (Table 1).

Clinical Efficacy

The main clinical efficacy endpoints for patients completing year 3 of treatment with full data available are summarized in Table 2. Both SCIT and SLIT significantly reduced VAS scores at year 3 compared with baseline ($P<.01$ and $P=.03$ respectively, Table 2). The reduction in VAS scores was also significantly reduced in the SCIT group compared with controls ($P=.009$) (Figure 2A). Rhinitis symptoms significantly improved in both the SCIT and SLIT groups compared with controls ($P=.01$ in both cases) (Figure 2B). SCIT additionally improved TRSS significantly compared with baseline ($P=.007$), but no changes were observed in the either the SLIT or control groups.

Asthma symptoms significantly improved 3 years after treatment in both the SCIT and SLIT groups when compared with both baseline ($P=.03$ in both cases, Table 2) and the control group ($P=.03$ in both cases) (Figure 2C).

When compared with baseline, TSS significantly improved in the SCIT and SLIT groups after 3 years ($P=.007$ and $P=.04$,

respectively), but no significant changes were detected in the control group. When compared with the control group at year 3 of treatment, both the SCIT and SLIT groups had a significantly lower TSS ($P<.01$ in both cases) (Figure 2D). No statistical differences were observed between the SCIT and SLIT groups for TSS. A significant reduction in TMS was observed in both the SCIT and SLIT groups 3 years after immunotherapy when compared with baseline ($P=.01$ and $P=.01$, respectively, Table 2) and with the control group ($P=.01$ and $P=.01$, respectively) (Figure 2E). No significant differences were observed between the SLIT and the SCIT groups for TMS. Finally, no adverse events were noted in either immunotherapy group during the maintenance phase. Two cases of systemic reaction were observed in the SCIT group during the up-dosing phase and have been previously discussed [13].

Lung Function and Bronchial and Nasal Hyperreactivity

No significant differences were detected between the SCIT, SLIT, and control groups for nasal or bronchial hyperreactivity at year 3 of follow-up. Although a slight increase in peak expiratory flow, FEV₁, and provocation doses in the ASNPT and methacholine challenge were observed at year 3 compared with baseline in the SCIT and SLIT groups, these did not reach statistical significance (data not shown).

Skin Prick Test Reactivity

Skin reactivity against *D pteronyssinus* was significantly reduced in both the SCIT and SLIT groups after 3 years of treatment compared with baseline ($P=.01$ and $P=.02$, respectively). By contrast, reactivity against *D farinae* was significantly reduced in the SCIT group only at year 3 compared with baseline ($P<.01$). Additionally, when compared with the control group, the SCIT group had a significantly

Table 2. Clinical Efficacy Endpoints for Patients Who Completed 3 Years of Follow-up

	Controls (n=10)		SCIT (n=12)		%Imp ^b	SLIT (n=9)		%Imp ^b
	Baseline	Year 3	Baseline	Year 3		Baseline	Year 3	
VAS ^a	4.3 (1.8) 4.5 (3-5.7)	4.5 (2.7) 5 (1.5-6)	5 (1.5) 5.5 (3-6)	1.66 (1.87)** 1 (0-3.7)	63 %	4.8 (1.6) 5 (3-6.2)	2.5 (1.4)* 3 (2-4)	44 %
TRSS ^a	1.26 (1.0) 1.42 (0.1-2)	1.9 (1.4) 1.7 (0.5-3.5)	1.86 (1.0) 1.57 (1.3-2)	0.48 (0.51)** 0.3 (0.1-1.1)	74 %	0.98 (1.0) 0.9 (0-1.8)	0.38 (0.27) 0.3 (0.1-0.7)	80 %
TASS ^a	1.1 (0.6) 1 (0.7-1.7)	1.0 (1.0) 1.15(0-1.5)	1.1 (0.8) 1.1 (0.3-2)	0.14 (0.26)* 0 (0-0.3)	86 %	1.43 (1.9) 0.8 (0.2-2.3)	0.14 (0.25)* 0 (0-0.4)	86 %
TSS ^a	2.34 (1.4) 2.1 (1.4-3.5)	3 (1.9) 2.7 (1.4-4.7)	2.9 (1.4) 2.7 (2-3.4)	0.6 (0.7)** 0.3 (0.1-1.4)	80 %	2.42 (2.7) 1.7 (0.2-4.2)	0.5 (0.47)* 0.3 (0-0.8)	83 %
TMS ^a	2.3 (1.5) 2 (1.3-4)	1.7 (1) 1.8 (0.8-2.1)	2.5 (1.2) 2 (2-3.9)	0.3 (0.7)** 0 (0-0.4)	82 %	2.7 (0.9) 2.3 (2-3.6)	0.6 (0.5)** 0.6 (0-1)	64 %

Abbreviations: %Imp, Percent improvement; TASS, total asthma symptom score (cough, wheezing, breathlessness, dyspnea); TMS, total medication score; TRSS, total rhinitis symptom score (sneezing, runny nose, itchy nose, nasal congestion); TSS, total symptom score (TRSS+TASS); VAS, visual analog score.

^aData presented as mean (SD) and median (25%-75% interquartile range).

^bDefined as the estimated difference in the SCIT or SLIT group at year 3 of treatment relative to the mean of the control group at the same time.

* $P=.03$, ** $P<.01$, compared with baseline.

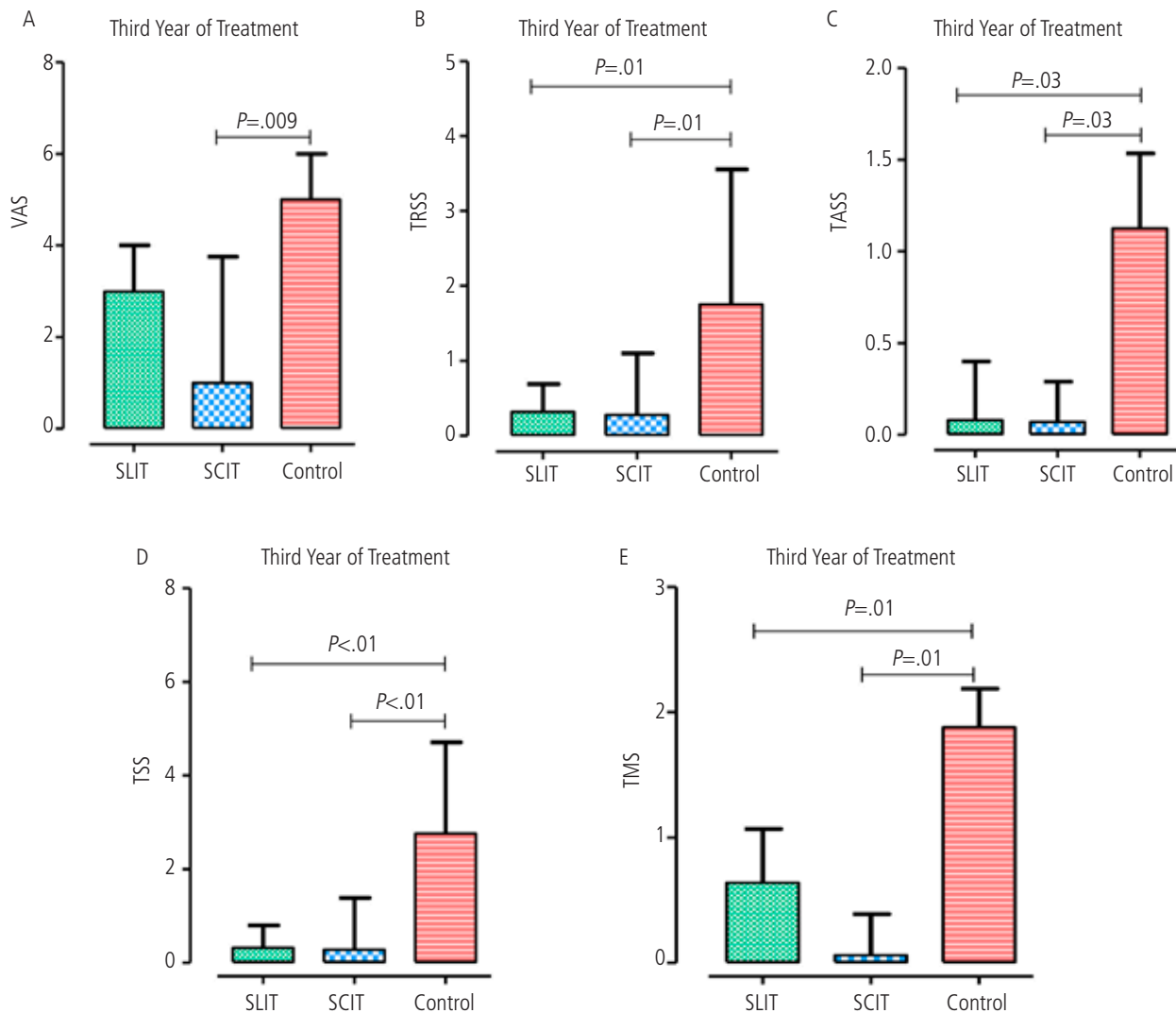


Figure 2. Comparison of primary endpoints between subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT), and control groups (pharmacotherapy only) after 3 years of treatment. A, Visual analog scores (VAS). B, Total rhinitis symptom scores (TRSS). C, Total asthma symptom scores (TASS). D, Total symptom scores (TSS). E, Total medication scores (TMS). Statistical significance between groups was set at $P<.05$.

smaller wheal diameter following exposure to *D pteronyssinus* ($P=.01$) and *D farinae* extracts ($P<.01$). When compared with the SLIT group, a smaller diameter was observed for the *D farinae* extract only ($P=.01$) (Figure 3A). No new sensitizations were observed in any of the groups at the end of 3 years of treatment.

Serum HDM-Specific Cytokines

No significant differences were observed for Der p 1-induced IL-5, IL-10, or IFN- γ either within or between any of the groups (data not shown).

Serum Specific IgG4 and IgE Levels

A significant and progressive increase in both Der p 1- and Der p 2-specific IgG4 levels were detected at years 1 and 3 of SCIT compared with baseline ($P=.001$ for both in year 1 and

$P=.002$ for both in year 3). Moreover, the SCIT group showed higher levels of Der p 1- and Der p 2-specific IgG4 at year 3 compared with the SLIT and control groups ($P<.0001$ for both) (Figure 3B). Neither the SLIT nor the control group showed any changes in specific IgG4. Finally, there were no significant changes in serum total IgE or HDM-specific IgE in either the between- or within-group comparisons (data not shown).

Discussion

This prospective, randomized, long-term follow-up controlled study demonstrates that SLIT and SCIT administered to children with HDM allergy leads to longitudinal clinical improvement, with a reduction in daily symptoms and the use of medication for both asthma and rhinitis. Significant suppression of skin reactivity to HDM, reflected by an increase

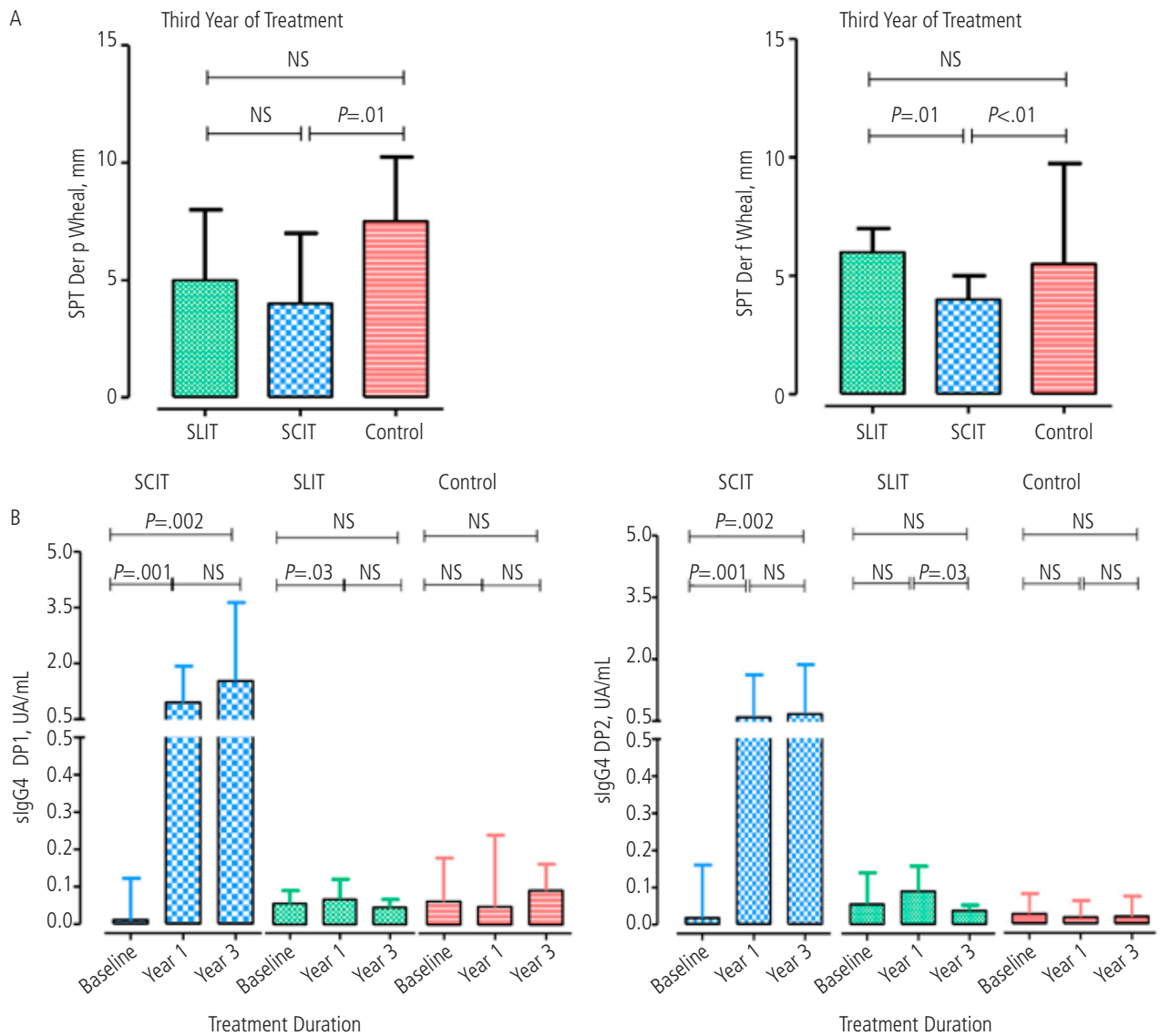


Figure 3. Skin reactivity against HDM (wheal diameter in mm) for *Dermatopharmagoides pteronyssinus* (Der p) and *Dermatopharmagoides farinae* (Der f) in subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT), and control groups (pharmacotherapy only) 3 years after therapy. B, Der p 1 and Der p 2 specific IgG4 levels (UA/mL) in SCIT, SLIT, and control groups at baseline and at 1 and 3 years after therapy. Statistical significance between groups was set at $P < .05$. NS indicates nonsignificant; SPT, skin prick test.

in specific IgG4 blocking antibodies, was observed in the SCIT group only.

The study demonstrates that SCIT and SLIT are effective in significantly reducing both asthmatic symptoms and medication usage compared with corticosteroid only treatment in children with HDM allergy. Our results are consistent with the findings of a recently published meta-analysis. The clinical efficacy of HDM-SCIT in asthma was recently confirmed in a systematic review by Abramson et al [15], who described a reduction in asthma symptoms and use of medications and an improvement in bronchial hyperreactivity following therapy. On the other hand, HDM-SLIT was reported to have favorable effects on asthmatic symptoms and medications with weak effect on rhinitis symptoms [16-18]. In our study, no adverse events were observed or reported during the 3-year

maintenance period for either HDM-SCIT or HDM-SLIT, although 2 patients in the HDM-SCIT group developed grade 3 and 4 reactions during the up-dosing phase at the beginning of this trial, as previously reported [13].

Comparative clinical studies of SLIT and SCIT are highly heterogeneous in terms of patient selection criteria, allergens used, and duration of follow-up. The few controlled studies that have compared HDM-SCIT and HDM-SLIT head to head have concluded that both routes of immunotherapy reduce clinical symptoms [13,19-22]. In the present study, the improvement in clinical symptoms and reduction in medication use was maintained for both SCIT and SLIT at 3 years of treatment and furthermore, both treatments were superior to pharmacotherapy alone. The long-term effect of HDM-SCIT has been demonstrated in both children [11,23]

and adults [24,25], with sustained clinical efficacy reported after at least 3 years of treatment and for as long as 3 to 6 years after treatment discontinuation. Recent studies have also shown similar long-term effects with HDM-SLIT [26-28]. Large-scale controlled trials of SLIT with other allergens have also recently confirmed the long-term clinical efficacy and disease-modifying effect of SLIT at least 2 years after completion of 3 years of treatment [29].

Skin reactivity to HDM decreased progressively in the immunotherapy groups compared with the control group, which is consistent with the findings of other studies investigating the long-term effects of SIT [5,30]. Our results showed no changes in allergen-specific IL-5, IL-10, or IFN- γ levels in HDM-sensitized asthmatic children treated with SCIT or SLIT for 3 years.

HDM-specific IgG4 levels, however, increased longitudinally with years of treatment in the SCIT group, while no differences were observed in the SLIT group. This is consistent with the results of previous prospective studies comparing seasonal or perennial allergen-specific immunotherapy comparing SCIT and SLIT prospectively [21,22,31]. The studies reported a significant increase in specific IgG4 levels in SCIT-treated but not SLIT-treated patients, and a gradual decrease was even observed in patients who received combined therapy with SCIT in the build-up phase and SLIT in the maintenance phase [21]. Our findings show that this difference is maintained after long-term treatment, but also that both modes of treatment result in a favorable response despite quantitative specific IgG4 differences. The exact function of specific IgG4 in SIT is still a subject of debate, and it is still not known whether it is a simple "bystander" or whether it has an immunomodulatory effect. A recent study showed that it was quality rather than quantity of pollen-specific IgG4 that correlated with clinical response after SIT [32], suggesting that serum inhibitory antibodies might be useful immunological markers for monitoring SIT response. Therefore, based on the results of the present study, it could be speculated that the quantitative increase in specific IgG4 in HDM-SCIT but not HDM-SLIT might reflect the mode of delivery and the allergen dose interval.

Very few comparative studies have explored the clinical efficacy and immunological outcomes of HDM-SCIT and HDM-SLIT. To our knowledge, this is the first randomized, controlled, 3-parallel grouped, prospective study of HDM-allergic children treated with SCIT or SLIT followed prospectively for 3 years. The main strengths of our study are the strict selection of HDM-monosensitized children, randomization, comparison of SCIT and SLIT with a pharmacotherapy only group, and the long-term prospective follow-up providing details about the clinical and immunological changes induced by immunotherapy. The main limitations, by contrast, are the absence of a placebo group and the small number of patients in each group. As the use of a subcutaneous placebo control in children is restricted by ethical issues, we used a pharmacotherapy only group for comparison.

In summary, HDM-sensitized children with asthma and/or rhinitis treated with either SCIT or SLIT showed improved clinical outcomes after 3 years of treatment compared with a pharmacotherapy only group. SLIT was found to have a better

safety profile than SCIT. Further large clinical prospective studies with different extracts are needed to determine the sustained long-term effects after cessation of treatment in asthmatic children.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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