

Original article

Clinical efficacy and safety of sublingual immunotherapy with tree pollen extract in children

Background: Subcutaneous immunotherapy has been the principal approach of immunotherapy in the treatment of allergic diseases. Several clinical studies with birch, alder or hazel pollen extract conducted as subcutaneous immunotherapy have been published suggesting a well-tolerated and clinically effective treatment. Only a few clinical studies of sublingual immunotherapy (SLIT) with these allergens have been published. This study investigated the clinical efficacy, safety and dose–response relationship of SLIT in children suffering from rhinoconjunctivitis with/without asthma.

Methods: Eighty-eight children (5–15 years) with a history of tree pollen-induced allergic rhinoconjunctivitis with/without seasonal asthma for ≥ 2 years were included. Allergy to tree pollen was confirmed by positive skin-prick test, positive specific IgE and positive conjunctival provocation test. The extract used was a glycerinated mixture of *Betula verrucosa*, *Corylus avellana* and *Alnus glutinosa* 100 000 SQ-U/ml. Children were randomized into three groups receiving SLIT 5 days a week for up to 18 months; dose group 1: accumulated weekly dose of 24 000 SQ-U; dose group 2: accumulated weekly dose of 200 000 SQ-U; and placebo.

Results: In the birch pollen season, dose group 2 showed a significant reduction of symptom ($P = 0.01$) and medication scores ($P = 0.04$) compared with placebo. Dose group 1 showed a significant reduction of symptom scores ($P = 0.03$). There were no statistical differences between dose groups 1 and 2. All children tolerated the treatment well.

Conclusion: SLIT with tree pollen extract provided dose-dependent benefits in tree pollen-allergic children in terms of significantly reduced symptoms and medication use. The treatment was well tolerated.

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Subcutaneous allergen injection immunotherapy has been the principal immunotherapy approach in the treatment of allergic respiratory airway diseases (1). Routes (local and non-injection) differing from subcutaneous injections have been investigated: oral, nasal, bronchial and sublingual immunotherapy (SLIT) (2). As a result of the potential high tolerability, SLIT is an attractive form of specific immunotherapy. The most common way to administer SLIT is to keep the allergen extract under the tongue for 1–2 min and then swallow it. At present, SLIT-swallow is the most used route of administration, and its use is supported by an increasing number of controlled studies investigating safety and efficacy. Studies have been conducted with various allergen extracts, e.g. house dust mites (3–6), cat (7), moulds (8) and different types of pollen: weeds (9–11), olive tree (12) and grasses (13–19). The dose of allergen used for SLIT is generally higher than that for subcutaneous allergen injection immunotherapy (20), but there are several

inconsistencies in the absolute dose used in the different studies. In order to establish a recommendation for the dose to be used for SLIT, information on the optimal dose must be achieved.

There are several published clinical studies with birch, alder or hazel pollen extract conducted as subcutaneous allergen injection immunotherapy. Results from these studies suggest that immunotherapy with tree pollen extracts is both well tolerated and clinically effective (21–27). It is expected that SLIT with birch, alder and hazel pollen extracts is also clinically effective and well tolerated. However, there are only few published clinical studies of SLIT with these allergens (28, 29).

In order to further elucidate the effect of SLIT, the present study was set up to investigate the efficacy, safety and dose–response relationship of tree pollen extract in children suffering from rhinoconjunctivitis with/without seasonal asthma caused by allergy to tree pollen. Our study is the first one using tree pollen extract in a

double-blind placebo-controlled setting evaluating clinical efficacy, safety and dose–response relationship of SLIT.

Materials and methods

Study subjects

This study was a single-centre, randomized, double-blind, placebo-controlled dose–response study including tree pollen-allergic children. The children were allowed to have a history of grass pollen allergy as the grass pollen season has no overlap with that of tree pollen, and the tree pollen season precedes the grass pollen season. The study was approved by the local ethics committee and written informed consent was obtained prior to the initiation of the study. The study subjects were enrolled based on screening investigations. The inclusion and exclusion criteria are presented in Table 1. Children were randomized to receive either of two doses of SLIT with a tree pollen extract or placebo 5 days-a-week. Ninety-eight subjects were randomized and the 88 subjects included in the efficacy analysis received SLIT for an average of 17 months (10–20 months). More than 90% of children received SLIT for more than 12 months.

Investigations

Specific IgE. Blood samples were taken at screening and analysed for specific IgE to *Betula verrucosa*, *Corylus avellana* and *Alnus glutinosa* using Magic Lite® (ALK-Abelló A/S, Hørsholm, Denmark).

Conjunctival provocation test. Conjunctival provocation test (CPT) was performed before and after treatment with SLIT. A drop of the test solution was applied in the lower conjunctival sac alternating between the two eyes, starting with sterile saline as a negative control. Aquagen SQ® (ALK-Abelló A/S) tree pollen extract was

administered in increasing concentrations from 100 SQ-U/ml to a maximum of 100 000 SQ-U/ml. A positive reaction was recorded when two of four of the following symptoms occurred: itching, redness of half the conjunctiva, tears or swelling.

Methacholine bronchial provocation test. Methacholine bronchial provocation test (MBPT) was performed during screening and after treatment with SLIT using a dosimeter technique (Spira Elektro 2, Hameenlinna, Finland) with controlled tidal breathing. After nebulization of isotonic saline, methacholine was delivered in five cumulative doses: 18, 72, 270, 810 and 2600 µg. MBPT was continued with 3-min interval between inhalations until a fall in forced expiratory volume in 1 s (FEV₁) of ≥20% was obtained. PD₂₀ was calculated by linear interpolation on a log-dose–response curve.

Skin-prick testing. Skin-prick tests (SPT) were performed using histamine-HCl (10 mg/ml) as a positive control and diluent as a negative control. The tested extracts were a mix of *C. avellana*, *A. glutinosa* and *Betula verrucosa* and a standard panel of *Phleum pratense*, *Artemisia vulgaris*, cat, dog, horse, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Soluprick® SQ 10 HEP, ALK-Abelló A/S), *Cladosporium herbarium* and *Alternaria alternata* (Soluprick 1:20 w/v, ALK-Abelló A/S). A wheal diameter ≥3 mm was regarded as a positive SPT result.

Late-phase skin reaction. Late-phase skin reaction (LPSR) was performed before and after treatment with SLIT. The allergen solution (Aquagen SQ® tree pollen extract diluted to 1000 SQ-U/ml, 0.05 ml corresponding to 50 SQ-U tree pollen extract) was injected intradermally. Early skin reaction was measured after 15 min, marking the reaction with a pencil and recording the marked size on the skin onto the overhead. LPSR was measured similarly after 24 h. A control intradermal injection using albumin diluent was performed for evaluation of unspecific reactions. The early- and late-phase skin reaction sizes were determined by using a computerized scanner. Only LPSRs were analysed.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age 5–14 years, both sexes	Perennial allergic symptoms
Clinical history of tree pollen-induced allergic rhinoconjunctivitis with/without seasonal asthma for at least 2 years	Clinically important symptoms of food allergy unless they are asymptomatic with elimination diet
Positive SPT ≥3 mm to tree mix and either one of <i>Betula verrucosa</i> , <i>Corylus avellana</i> and <i>Alnus glutinosa</i> (Soluprick® SQ 10 HEP, ALK-Abelló A/S)	Clinically important history of house dust mite and/or mould allergy
Positive specific IgE to tree mix and either one of <i>Betula verrucosa</i> , <i>Corylus avellana</i> and <i>Alnus glutinosa</i> (Magic Lite® SQ, ≥class 2)	Positive SPT ≥3 mm to <i>Dermatophagoides pteronyssinus</i> , <i>Dermatophagoides farinae</i> (Soluprick SQ 10 HEP) and <i>Cladosporium herbarium</i> , <i>Alternaria alternata</i> (Soluprick 1:20 w/v).
Positive CPT ≤ 100 000 SQ-U/ml to tree mix (Aquagen® SQ, ALK-Abelló A/S)	Daily contact with any pet to which the patient is sensitized
Bronchial provocation test with methacholine PD ₂₀ ≥ 150 µg	Any other clinically significant disease (as judged by the investigator) that might affect the outcome of the trial or the patient's health
Tree pollen allergy responsible for the majority of clinical symptoms	Treatment with beta-blockers or ACE inhibitors
Able to provide written informed consent (parents/legal guardian)	Non allergic chronic rhinitis or sinusitis
Ability to comply with treatment	Previous immunotherapy with any tree pollen extracts within the last 5 years
FEV ₁ ≥ 70% and PEF ≥ 80% of predicted normal value	Lack of cooperation or comprehension to participate in or complete the investigation
	Treatment with long-acting β ₂ agonist and/or antileukotrienes
	FEV ₁ < 70% and PEF < 80% of predicted normal value
	Severe atopic dermatitis
	Seasonal asthma requiring more than 400 µg budesonide/day, 400 µg beclomethasone/day, 200 µg fluticasone/day
	Nasal corticosteroid
	Cromones for asthma

Sublingual immunotherapy

The allergen extract used for SLIT was a tree pollen extract of SQ-standardized *B. verrucosa* (birch), *C. avellana* (hazel) and *A. glutinosa* (alder). An extract concentration of 1 000 000 SQ-U/ml (100 HEP) contains: 1.00 mg *B. verrucosa*, 1.40 mg *C. avellana* and 1.00 mg *A. glutinosa* corresponding to a major allergen content of 150 µg (Bet v 1/Aln g 1/Cor a 1). The study was conducted under double-blind conditions. There was no difference in colour and viscosity between the study drug and placebo. All blinding procedures were performed by the Quality Assurance Department at ALK-Abelló A/S.

Dosing. Sublingual immunotherapy was carried out at home. The study medication was a fluid in multiple-use vials. Each dose consisted of 10 drops (400 µl). Treatment was administered five times per week with a 5-week up dosing phase and a maintenance period of up to 18 months. The dose was administered sublingually and kept under the tongue for 3 min and then swallowed. The children were randomized equally to one of the three following groups: (i) *Dose group 1*: the active study medication for dose group 1 was, for the up dosing-phase, tree pollen extract in the concentrations 150, 400, 1500, 4000 and 12 000 SQ-U/ml and, for maintenance phase, a concentration of 12 000 SQ-U/ml. The accumulated weekly dose corresponded to 24 000 SQ-U or 3.6 µg major allergen Bet v 1/Aln g 1/Cor a 1. (ii) *Dose group 2*: the active study medication for this group was, for the up dosing phase, tree pollen extract in the concentrations 1500, 4000, 12 000, 35 000 and 100 000 SQ-U/ml and, for maintenance, a concentration of 100 000 SQ-U/ml. The accumulated weekly dose corresponded to 200 000 SQ-U or 30 µg major allergen Bet v 1/Aln g 1/Cor a 1. (iii) *Placebo*: the placebo medication was, for the up dosing and maintenance phase, a diluent containing 50% glycerol and 50% saline buffer.

Assessment of efficacy of SLIT

Patient diary. The children filled in their diary during the tree pollen season for 12 weeks. The following parameters were registered daily in the patient diary.

Symptom scores. Allergic, clinical symptoms were categorized as nasal symptoms (runny nose, sneezing, blocked nose), eye symptoms (streaming and swelling, redness and itching) and lung symptoms (breathlessness, cough, wheeze and chest tightness). Each symptom was scored by the children: 0, no symptoms; 1, slight symptoms; 2, moderate symptoms; 3, severe symptoms.

Medication. The children were supplied with medication for hay fever and asthma symptoms. The daily medication score for each child was calculated as the sum of medication administered at a particular day: cetirizine tablets (10 mg) – 2 points/tablet; cromoglicat eyedrops (40 mg/ml) – 1 point/drop; cromoglicat nasal spray (5.2 mg/dose), terbutaline inhalation (0.25 mg/dose) and salbutamol inhalation (0.2 mg/dose) – 1 point/puff; budesonide inhalation (200 µg/dose) and fluticasone propionate inhalation (100 µg/dose) – 4 points/puff and 80 points per course of prednisolone (5 mg/tablet).

Statistical methodology

Symptom scores were averaged over the 12-week tree pollen season for each child and similarly for medication

scores. The primary endpoint was the weighted sum of the average symptom and medication scores. Differences between groups were estimated by the Hodges-Lehmann estimation of the shift parameter and groups were compared with exact two-sided Wilcoxon two-sample rank-sum test. In order to adjust the overall type 1 error of 5% for multiple comparisons, a Bonferroni correction for testing three hypotheses was planned. *Post hoc*, the overall level of significance was set to 5%. Symptom and medication scores were also analysed separately as well as during the birch pollen season. Symptom scores were additionally analysed by nose, eye and lung symptoms. The secondary parameters were analysed in a similar way. The determination of the sample size of 3 × 33 patients was based on the standard deviation of symptom scores obtained in a previous two-armed subcutaneous immunotherapy study.

Results

Subject disposition is provided in Fig. 1. A total of 134 children were screened, of whom 36 children did not fulfil the inclusion criteria and a total of 98 children were randomized. Eighty-eight (32 in dose group 1, 27 in dose group 2 and 29 in the placebo group) were included in the efficacy analyses.

The demographics of study subjects are presented in Table 2. Overall, 40.9% and 30.7% of the children had tree pollen allergic history with symptoms of asthma and atopic dermatitis, respectively. The severity of symptoms was in most cases moderate for rhinitis and conjunctivitis, and mild for asthma and atopic dermatitis. The mean number of years with rhinitis symptoms were 4.6 years (range 2–10), with conjunctivitis symptoms 4.6 years (range 2–10), with asthma 4.2 years (range 2–10) and with atopic dermatitis 7.6 years (range 3–12). There was no difference between treatment groups regarding the distribution of allergic history to allergens other than tree pollen, and there was no difference between the three groups regarding general characteristics and demographics.

In this study, statistical analyses were planned to be performed on a Bonferroni-corrected 1.5% significance level. Retrospectively, we altered the significance level to 5% as the power of the study was considered insufficient to demonstrate any statistically significant differences at the conservative 1.5% significance level. At a 5% significance level, we demonstrated that SLIT with a tree pollen extract, when compared with placebo, reduced allergic symptoms during the tree pollen season in tree pollen-allergic children suffering from rhinoconjunctivitis with or without seasonal asthma.

Symptom and medication scores are illustrated in Fig. 2A and B. The symptom scores were significantly higher in the placebo group compared with the active treatment groups during the birch pollen season, whereas

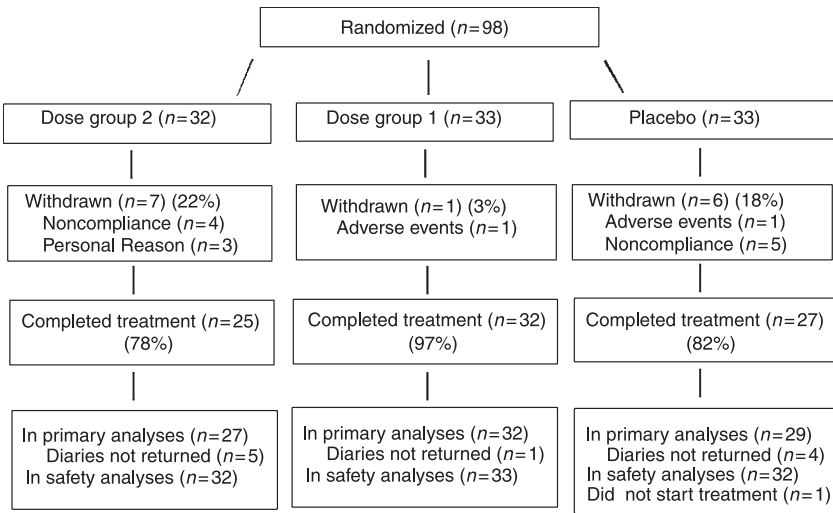


Figure 1. Subject disposition

Table 2. Subject demographics

	Dose group 2	Dose group 1	Placebo
Population	27	32	29
Male/female	13/14	19/13	18/11
Age in years, mean (SD)	9.0 (2.7)	9.6 (3.1)	9.9 (3.0)
Severity of rhinitis			
Mild, n (%)	3 (11.1)	7 (21.9)	3 (10.3)
Moderate, n (%)	17 (63.0)	16 (50.0)	18 (62.1)
Severe, n (%)	7 (25.9)	9 (28.1)	8 (27.6)
Duration of rhinitis in years, mean (SD)	4.1 (1.9)	5.0 (2.3)	4.6 (2.5)
Conjunctivitis, n (%)	26 (96.3)	31 (96.9)	29 (100)
Asthma, n (%)	9 (33.3)	17 (53.1)	10 (34.5)
Atopic dermatitis, n (%)	10 (37.0)	12 (37.5)	5 (17.2)
Allergic			
Grass pollen, n (%)	6 (22.2)	8 (25.0)	4 (13.8)
Animal dander, n (%)	5 (18.5)	5 (15.6)	7 (24.1)
Foods, n (%)	20 (74.0)	26 (81.3)	22 (75.8)
Duration of SLIT in months, mean (SD)	16.4 (3.5)	18.1 (2.5)	17.7 (3.1)

the medication scores remained lower in dose group 2 compared with dose group 1 and placebo group. The nose and lung symptom scores appeared higher during the birch pollen season, whereas difference was less clear in eye symptom scores.

Statistical comparison of the efficacy is presented in Table 3. Values were statistically significantly different in the reduction of symptom score ($P = 0.01$) during the whole tree pollen season for dose group 2 vs placebo. The same comparison revealed a marginal significance with the medication scores ($P = 0.06$). When the analysis was focused on the birch pollen season where the exposure and thereby the symptoms and need for medication was higher, a statistically significant difference in reduction of medication scores was found ($P = 0.04$). Significant

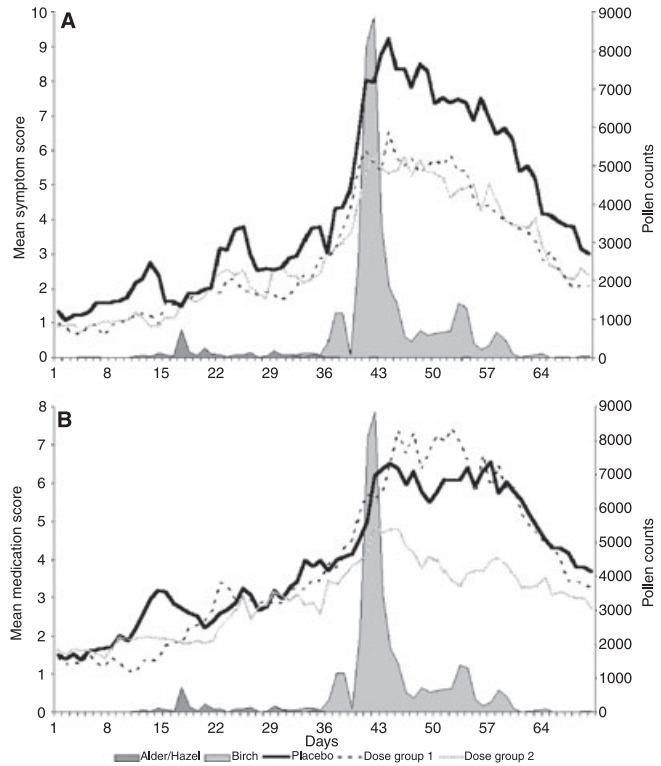


Figure 2. Average daily symptom score (A) and medication score (B) during the tree pollen season. Daily alder/hazel and birch pollen counts are included (grains per m³).

differences during the whole pollen season were found for nose, eye and lung symptoms, when analysed separately ($P = 0.04$, 0.04 and 0.02 , respectively).

No statistically significant differences in CPT, MBPT and LPSR between the treatment groups were seen. The analysis on MBPT was performed for asthma children only, but there was no statistical difference in this analysis.

Table 3. Comparison of Efficacy

	Dose group 2	Dose group 1	Placebo	Estimated reduction ^a (%) vs. placebo			
				Dose group 2	<i>P</i> -value ^b	Dose group 1	<i>P</i> -value ^b
Whole tree pollen season							
Symptom score, mean (SD)	2.9 (3.4)	2.9 (2.2)	4.3 (2.6)	1.77 (40.8)	0.01	1.36 (31.2)	0.03
Medication score, mean (SD)	2.9 (3.4)	3.8 (4.4)	3.9 (4.6)	0.94 (39.7)	0.06	0.16 (6.6)	0.72
Birch pollen season							
Symptom score, mean (SD)	4.0 (4.6)	4.2 (2.9)	6.0 (3.8)	2.36 (39.1)	0.01	1.69 (28.1)	0.04
Medication score, mean (SD)	3.6 (5.4)	5.3 (5.7)	5.1 (5.6)	1.47 (42.4)	0.04	0.14 (4.0)	0.83
Whole tree pollen season							
Nose symptoms, mean (SD)	1.5 (1.4)	1.6 (1.1)	2.2 (1.4)	0.74 (34.9)	0.04	0.66 (31.1)	0.04
Eye symptoms, mean (SD)	0.8 (1.2)	0.9 (1.1)	1.1 (0.9)	0.46 (47.7)	0.04	0.31 (32.0)	0.10
Lung symptoms, mean (SD)	0.6 (1.1)	0.5 (0.5)	0.9 (0.8)	0.40 (48.3)	0.02	0.28 (34.2)	0.07

^aHodges-Lehmann's estimate of the shift parameter.

^bTwo-sided *P*-value for an exact Wilcoxon two-sample rank-sum test.

Table 4. Adverse events – definitely or likely related to study medication

	Dose group 2 <i>n</i> ^a (%) <i>E</i> ^b	Dose group 1 <i>n</i> (%) <i>E</i>	Placebo <i>n</i> (%) <i>E</i>
Population	32 (100.0)	33 (100.0)	32 (100.0)
All events	17 (53.1) 143	13 (39.4) 41	8 (25.0) 25
Allergic reaction	2 (6.3) 2	–	–
Abdominal pain	1 (3.1) 1	2 (6.1) 2	–
Oral local reactions	16 (50.0) 137	12 (36.4) 38	8 (25.0) 24
Rhinitis	–	1 (3.0) 1	1 (3.1) 1
Flushing	1 (3.1) 3	–	–

^aNumber of study subjects suffering of adverse effects.

^bNumber of events in subjects together.

One child in the placebo group never had any study medication. All 97 children who received study medication were included in the safety analysis. The adverse events (AEs) definitely or likely related to study medication are shown in Table 4. SLIT was generally well tolerated and nearly all adverse events related to treatment were local allergic reactions in the mouth.

There was a dose–response relationship with highest frequency of AEs in dose group 2. However, no subjects from this dose group withdrew from the study because of AEs. It is therefore assumed that the occurrence and severity of local allergic reactions in the mouth is acceptable to the children. One child in dose group 1 withdrew from the study because of abdominal pain, related to treatment.

Discussion

We performed a double-blind, placebo-controlled study of clinical efficacy and safety of SLIT with tree pollen extract in children suffering from tree pollen-induced rhinoconjunctivitis in Finland where tree pollen is the most common cause of allergic rhinoconjunctivitis. Our

study is one of the largest SLIT studies ever performed among children and in addition to the double-blind placebo-controlled setting, the possible dose–response relationship of SLIT was also evaluated. In this study, we demonstrated that SLIT with a tree pollen extract, when compared with placebo, reduced allergic symptoms during the tree pollen season in tree pollen allergic children suffering from rhinoconjunctivitis with or without seasonal asthma. For dose group 2, this reduction was statistically significant ($P = 0.01$). Moreover, the comparison between dose group 2 and placebo during the birch pollen season showed a reduction in the asthma and hay fever medication ($P = 0.04$). No serious AEs with relationship to the study drug occurred during the study and no AEs represented a previously unsuspected important adverse effect of the study drug.

Therefore, this study demonstrated that SLIT is safe to use in children with tree pollen allergy. SLIT may cause local allergic reactions in the mouth. However, the severity and occurrence is acceptable to the children as no children stopped treatment because of the most frequently reported AE related to treatment.

The use of allergen-specific immunotherapy by the sublingual route, SLIT, has been increasing in clinical practice. This is due to the user-friendliness, its ease of administration and documented safety (30, 31). Clinical safety of SLIT has been analysed in two systematic reviews. The systematic review of the efficacy of SLIT in allergic rhinitis (32) shows that SLIT is a safe treatment which significantly reduces symptoms and medication requirements in allergic rhinitis in the adult population; but these results could not be confirmed in the paediatric group. A systematic review on the efficacy of SLIT in allergic asthma and rhinoconjunctivitis in children (33) showed that SLIT can be currently considered to have low to moderate clinical efficacy in children of at least 4 years of age, monosensitized to house dust mite, and suffering from mild to moderate persistent asthma.

No statistically significant differences in MBPT, CPT and LPSR were found. The systemic effect of SLIT is inducing the clinical efficacy, i.e. to decrease symptom and medication scores, even though we could not show any significant changes in the objective target organ, provocation test in the eye or in the non-specific bronchial hyperreactivity in these children. In LPSR, there was no change during the course of therapy in any of the groups. However, in a subpopulation of this study population, randomly chosen for an immunological study (34), it was observed that reduction in LPSR was associated with downregulation of IL-5 mRNA expression and absence of asthma symptoms 5 years later.

The WHO guidelines (1) recommend high-dose SLIT and that the dose level should be at least 50 times the dosage used for subcutaneous allergen injection immunotherapy. This is based on the assumption that allergen extracts from different sources and different manufactur-

ers are comparable; however, this is not the case. Because of the heterogeneity of allergens used, in the selection of patients, in the maintenance doses and in the time course of treatment, it is rather impossible to draw general conclusions on immunotherapy from studies performed with a single specific product and on therapies with different routes or methods of administration. In the future different immunotherapy products have to be proved to be effective and safe in individual clinical studies.

Overall, it is concluded from our study that SLIT with a SQ-standardised allergen extract of a mixture of the three most important tree pollen species, birch, alder and hazel, reduces allergic symptoms during the tree pollen season in tree pollen-allergic children. The treatment is well tolerated and useful for self-administration. The treatment outcome is dose-dependent with a more pronounced effect on reduction in symptoms and need for symptomatic medication in the highest dose tested.

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