

Original article

Five-year follow-up on the PAT study: specific immunotherapy and long-term prevention of asthma in children

Background: A 3-year course of specific immunotherapy (SIT) in children with hay fever to grass and/or birch pollen significantly reduced the risk of developing asthma. To investigate the long-term preventive effect, we performed a follow up – 2 years after termination of immunotherapy.

Methods: A total of 183 children, aged 6–14 years with grass and/or birch pollen allergy could be investigated 2 years after discontinuation of SIT or no treatment. Conjunctival provocation tests (CPTs) and methacholine bronchial provocation tests were carried out during the season and winter after 5 years. The development of asthma was assessed by clinical evaluation.

Results: The significant improvement in hay fever and CPT results observed after 3 years of SIT persisted at the 5-year follow-up. No difference in bronchial responsiveness to methacholine was found after 5 years because of spontaneous improvement during the follow-up period in the control patients. The immunotherapy-treated children had significantly less asthma after 5 years as evaluated by clinical symptoms [odds ratio 2.68 (1.3–5.7)] in favor of SIT for prevention of development of asthma and significantly less patients reported an increase in asthma scores ($P < 0.01$).

Conclusion: Immunotherapy for 3 years with standardized allergen extracts of grass and/or birch shows long-term clinical effect and preventive effect on development of asthma in children with seasonal rhinoconjunctivitis.

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Clinical efficacy of subcutaneous specific immunotherapy (SIT) for pollen allergy using SQ standardized allergen extracts (Alutard SQ; ALK-Abelló, Hørsholm, Denmark) has been confirmed in several studies (1–4). The connection between hay fever and asthma has been reviewed (5, 6), and the co-morbidity of upper and lower airway diseases carefully described by WHO (7). In 1968, Johnstone and Dutton were the first to describe the preventive potential of SIT in reducing the risk of asthma in children (8).

The preventive allergy treatment study (PAT) (9) has shown that SIT can prevent the development of asthma in children suffering from seasonal allergic rhinoconjunctivitis. The actively treated children had significantly less asthma after a 3-year course of SIT as evaluated by

clinical symptoms (odds ratio 2.52; $P < 0.001$), visual analog scale (VAS; $P < 0.001$ –0.05) and methacholine bronchial provocation test (MBPT; $P < 0.05$).

The aim of the present study was to investigate the potential long-term preventive effects on the development of asthma in children with seasonal allergic rhinoconjunctivitis 2 years after termination of SIT.

Methods

Patients

Initially, 205 children aged 6–14 years from six pediatric centers after a baseline season (0-season) were randomized to 3 years of subcutaneous SIT or to a control group (9). The children had a

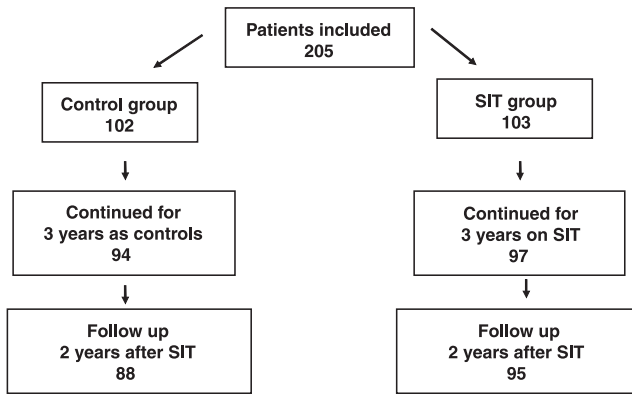


Figure 1. Flow chart of patient numbers.

clinical history of seasonal rhinoconjunctivitis caused by allergy toward birch and/or grass pollen. At 5-year follow-up, 183 children (121 males and 62 females) aged 11–20 years (mean 15.6) were investigated. One center did not participate in this follow-up study. The flow chart for the study is illustrated in Fig. 1. The children and their parents gave informed consent according to the Helsinki declaration. Ethical Committees approved the study in the respective countries.

Treatment

Patients were included and the treatment was initiated from 1992 to 1994. The children were stratified on the basis of bronchial responsiveness to methacholine during the 0-season, age, sex and years with pollinosis according to history and randomized into two groups. In order to reduce the influence of difference in pollen exposure, randomizations were performed center by center. In the controlled design, one group was treated with SIT for 3 years, while the other group served as an open control group. Both groups were followed by identical measures.

Both groups were allowed to take symptomatic medication limited to loratadine tablets 5–10 mg/day nasal levocabastine and/or ocular sodium cromoglycate. If necessary, nasal budesonide up to 100 µg/day in each nostril was allowed. In case of asthmatic symptoms, short-acting β₂-agonists were prescribed. When needed inhaled corticosteroids could be introduced. After discontinuation of SIT, all patients were offered the same drugs as before.

Specific immunotherapy was initiated after the 0-season with characterized and standardized allergen extracts of grass pollen (*Phleum pratense*) and/or birch pollen (*Betula verrucosa*). Up-dosing was performed with depot extracts (Alutard SQ), with weekly injections over 15–20 weeks or as rush immunotherapy with aqueous extracts (Aquagen SQ, ALK-Abelló). Maintenance injections with depot preparations were given every 6 weeks (±2 weeks) for a total period of 3 years. The contents of major allergen per maintenance injection (Alutard SQ; 100 000 SQ units/ml) corresponded to 20 µg Phl p 5 (grass) and 12 µg Bet v 1 (birch).

Conjunctival provocation test

Conjunctival provocation tests (CPTs) were performed outside the pollen seasons, always at the same time of the year, before the start of immunotherapy and after 1, 2, 3 and 5 years. Half log₁₀ increments at concentrations from 100 to 1 000 000 SQ units were used (Aquagen SQ) (10).

Methacholine bronchial provocation test

Methacholine bronchial provocation tests were performed during the 0-season(s) before randomization and in the relevant pollen season(s) and during winter. MBPT was performed using the reservoir method described by Matthys et al. (11). In brief, a high-quality nebulizer system (Pari Provocation Test 2; Pari, Starnberg, Germany) combined with a 10-l storage bag allowing standardized pulmonary aerosol deposition at saturated ambient temperature and pressure conditions was used. First 0.9 M NaCl solution and then test solutions 0.5, 1, 2, 4, 8, 16 mg/ml methacholine were nebulized and inhaled. Forced expiratory volume in 1 s (FEV₁) was measured three times before exposure after each inhalation; the highest value was recorded. The tests were stopped either after inhalation of the highest concentration of methacholine (16 mg/ml) or at the concentration giving a ≥20% decrease in FEV₁ in relation to baseline. The provocation dose (PC₂₀) was estimated by linear interpolation of the two last (log-transformed) concentrations tested. In each center, the same devices for measuring FEV₁ were used on all test occasions. Identical dilution instructions for methacholine were used at each center.

Visual analog scale

Symptoms of conjunctivitis, rhinitis and asthma compared with pre-treatment symptoms were evaluated on a 100-mm VAS after every season(s).

Asthma diagnosis

Asthma was defined as recurrence of at least two out of the three following symptoms within the last 12 months:

- Cough,
- Wheeze, and
- Shortness of breath.

Further demands for the conclusive diagnosis of asthma were that the symptoms were not only triggered by infections and that the patients responded to treatment with β₂-agonists. The clinical diagnosis was based only on the appearance of repeated symptoms and independent of the level of hyperresponsiveness.

Statistical methods

Changes from baseline of logarithmic transformed values of CPT were analyzed within groups by Wilcoxon sign rank test and between groups by Wilcoxon rank sum test. Changes from baseline of VAS scores of conjunctivitis and rhinitis were analyzed by the *t*-test. The difference in the number of patients that reported increased asthma symptoms on VAS was analyzed within groups by the Wilcoxon sign rank test and between groups by Wilcoxon rank sum test.

Changes from baseline of log-transformed values of MBPTs were analyzed within groups by Wilcoxon sign rank test and between groups by Wilcoxon rank sum test. Clinically diagnosed asthma was analyzed per center by Fischer's exact test, and homogeneity of odds ratios between centers was tested by Zelen's exact test. The mean odds ratio of clinically diagnosed asthma was calculated in accordance with the ICH E9 guideline (12) weighing centers equally, weighing by the precision of the odds ratios, and using a mixed logistic model with random center and treatment-by-center effects.

The statistical analysis was performed using SAS version 6.12, SAS version 7.0 and StatXact version 3.0. Two-sided tests and a test significance level of 5% were used.

Results

Of the 183 patients investigated in this follow-up study after 5 years, 142 had no asthma at inclusion. Eight children were drop-outs as they were lost for follow-up; they derived from four different centers.

The clinical effect on rhinitis and conjunctivitis achieved during SIT was persistent 2 years after termination of treatment (5-year follow-up) (Fig. 2). According to the VAS scores of conjunctivitis, the active group improved significantly more from baseline to 5 years compared with controls (-29.4 and -11.8 mm, respectively, $P < 0.001$). Similar results were found for rhinitis VAS scores (-21.5 and -7.4 mm, respectively, $P < 0.01$).

The conjunctival sensitivity measured by provocation tests was significantly reduced in the active group compared with the control group ($P < 0.001$). This result was similar to that observed after 3 years (termination of SIT). The mean threshold values for CPT are illustrated in Fig. 3.

Patients without asthma before the start of SIT ($n = 142$) were analyzed for the development of asthma after the 5-year period. A statistical homogeneity between individual centers was found. The final outcome of

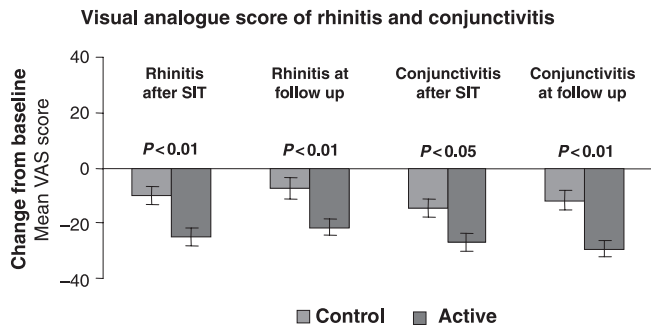


Figure 2. Rhinitis and conjunctivitis visual analog scores at the end of specific immunotherapy (SIT) and 2 years after termination (change from baseline and standard error of the mean).

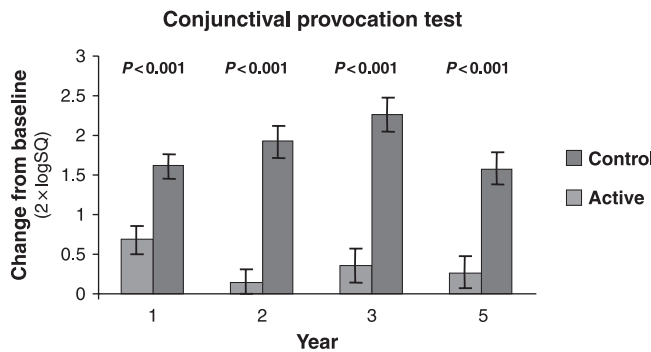


Figure 3. Conjunctival provocation test. For children allergic to both grass and birch, the challenge with both allergens is included (change from baseline and standard error of the mean).

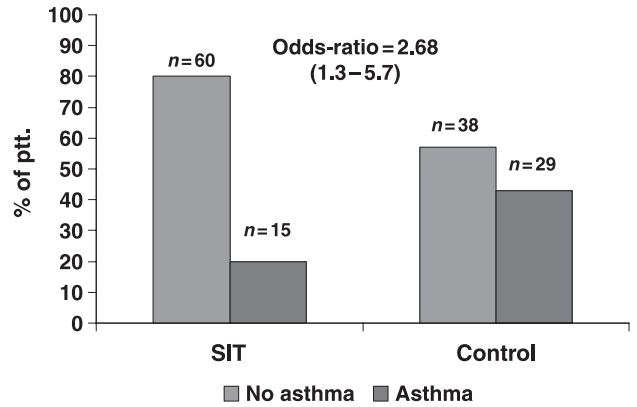


Figure 4. The percentage of children with and without asthma 2 years after termination of immunotherapy. Based on the patients without asthma before treatment ($n = 142$). The absolute number of children is shown above the bars.

asthma development according to our definition showed an odds ratio of 2.68 (1.3-5.7, $P < 0.05$) in favor of the hypothesis that SIT can prevent the long-term development of asthma (Fig. 4). In the control group, the number of patients that reported increased asthma symptoms after 5 years were 39% ($P < 0.01$). In the SIT group, the number of patients reporting more symptoms was not significant.

Concerning bronchial responsiveness to methacholine no significant differences in change from baseline of PC_{20} FEV_1 were observed between the two groups after 5 years (Fig. 5) during seasonal exposure as well as during wintertime.

Of those 36 patients with asthma at inclusion that were followed up with regard to final asthma diagnosis, seven were without asthma after 5 years. Two of these (controls) were those already free of asthma after 3 years. From 3 to 5 years, five additional patients lost their asthma (four controls and one active).

Discussion

This study has shown that the good clinical outcome achieved by SIT was persistent 2 years after termination of treatment in children suffering from hay fever caused by allergy to grass and/or birch. Despite the increasing age of the children included in this study with several children growing into adolescence, we found a very high compliance and a minimal drop-out rate during the follow-up period.

A crucial question is the definition of asthma. Although the entity of bronchial asthma includes mucosal inflammation, bronchial hyperresponsiveness and clinical symptoms, the diagnosis of allergy is primarily clinical – especially in clinical studies as in the present study. Indirect measurements could be used to add information on bronchial inflammation, but have drawbacks. ECP or

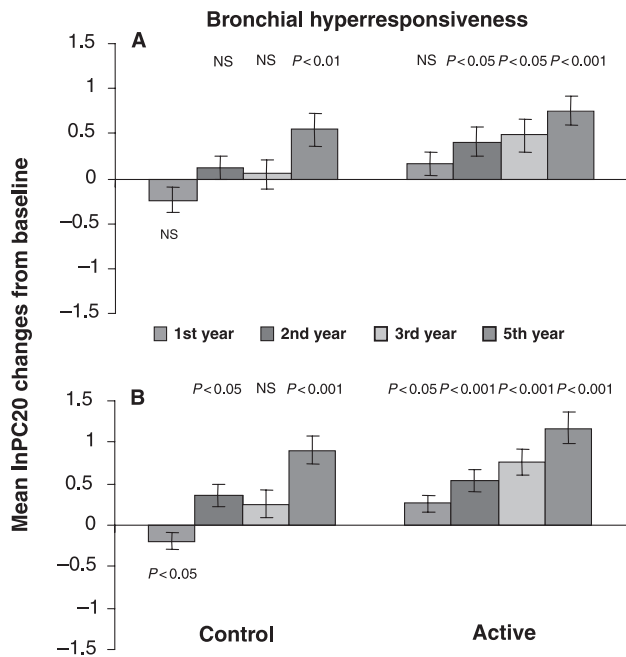


Figure 5. Development of bronchial responsiveness (methacholine challenge test) within each group measured as $\ln PC_{20}$ -change from baseline in (A) the seasons of pollen exposure and (B) during winter (change from baseline and standard error of the mean).

similar from blood samples is neither sensitive nor precise enough. Exhaled nitric oxide might be preferable, but was not in use when the study was started. We used the identical definition of asthma for the 5-year follow-up as chosen for the original PAT-Paper (9).

The persistent long-term reduction in allergic symptoms and increased tolerance to allergen exposure is in accordance with other studies. Up to 8 years clinical effect after termination of 2–3 years of SIT has been shown for grass pollen, tree pollen as well as animal hair and dander, and house dust mite (13–17).

In this study, the long-term clinical effect was strengthened by a long-term reduction of specific conjunctival responsiveness. This is the first time that long-term reduction in symptoms has been confirmed by *in vivo* challenge of an affected organ. The CPT could therefore be considered as a useful monitoring parameter of the improvement of clinical symptoms during SIT. Further

studies should be initiated to confirm a potential correlation between CPT and clinical symptoms.

The most important finding of the PAT study was that SIT with characterized and SQ-standardized allergen extracts could prevent the development of asthma in children (9). This preventive capacity is now shown to be persistent for at least 2 years after termination of treatment. Although not specifically designed for this purpose, other studies have indicated the preventive potential (14, 18, 19). A recent study on a 3-year course of co-seasonal sublingual immunotherapy has also shown the potential of prevention of seasonal allergic asthma in grass pollen allergic children suffering only from rhinitis (20). The fact that the treatment of grass pollen allergy by SIT reduced the risk of newly developed asthma, as well as the bronchial reactivity during season and during winter may indicate that there are nonspecific immunomodulating effects.

In contrast to the results after 3 years, bronchial responsiveness to methacholine showed no statistical significant differences between active and control patients at the 5-year follow-up. Previously, one study in cat allergic patients with mild to moderate asthma showed a persistent reduction of reactivity to histamine during the 5-year follow-up period (15). In our study in children, there was no difference between the SIT group and control group 2 years after termination of treatment. This might be explained by a spontaneous improvement of bronchial responsiveness over time as a natural improvement of bronchial responsiveness from infancy to adulthood has been reported (21, 22). This study showed that children in the included age group did not spontaneously improve bronchial hyperresponsiveness until 5 years after inclusion. In the active group, bronchial hyperresponsiveness improved from the first year after inclusion indicating that SIT may have accelerated the natural improvement. From our data, we cannot assess the long-term effect of having accelerated the improvement of bronchial hyperresponsiveness.

In conclusion, this follow-up study indicate that SIT for 3 years with standardized allergen of grass and/or birch (maintenance dose 20 μg Phl p5, 12 μg Bet v1, respectively) shows long-term effect on symptoms and a reduction of the risk of developing asthma in children with seasonal rhinoconjunctivitis. Therefore, subcutaneous SIT may not only be indicated as a first line therapeutic treatment for allergic rhinoconjunctivitis but should also be considered as a secondary preventive measure.

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