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Original article

Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study

Background: 3-year subcutaneous specific immunotherapy (SIT) in children with seasonal allergic rhinoconjunctivitis reduced the risk of developing asthma during treatment and 2 years after discontinuation of SIT (5-year follow-up) indicating long-term preventive effect of SIT.
Objective: We evaluated the long-term clinical effect and the preventive effect of developing asthma 7-years after termination of SIT.
Methods: One hundred and forty-seven subjects, aged 16–25 years with grass and/or birch pollen allergy was investigated 10 years after initiation of a 3-year course of SIT with standardized allergen extracts of grass and/or birch or no SIT respectively. Conjunctival provocations were performed outside the season and methacholine bronchial provocations were performed during the season and winter. Asthma was assessed by clinical evaluation.
Results: The significant improvements in rhinoconjunctivitis and conjunctival sensitivity persisted at the 10-year follow-up. Significantly less actively treated subjects had developed asthma at 10-year follow-up as evaluated by clinical symptoms [odds ratio 2.5 (1.1–5.9)]. Patients who developed asthma among controls were 24/53 and in the SIT group 16/64. The longitudinal treatment effect when adjusted for bronchial hyper-responsiveness and asthma status at baseline including all observations at 3, 5 and 10 years follow-up (children with or without asthma at baseline, $n = 189$; 511 observations) was statistically significant ($P = 0.0075$). The odds ratio for no-asthma was 4.6 95% CI (1.5–13.7) in favor of SIT.
Conclusion: A 3-year course of SIT with standardized allergen extracts has shown long-term clinical effects and the potential of preventing development of asthma in children with allergic rhinoconjunctivitis up to 7 years after treatment.
Clinical implication: Specific immunotherapy has long-term clinical effects and the potential of preventing development of asthma in children with allergic rhino conjunctivitis up to 7 years after treatment termination.

L. Jacobsen[1], B. Niggemann[2], S. Dreborg[3], H. A. Ferdousi[4], S. Halken[5], A. Høst[6], A. Koivikko[7], L. A. Norberg[6], E. Valovirta[7], U. Wahn[2], C. Möller[8] (The PAT investigator group)

[1]ALK-Abelló, Hørsholm, Denmark; [2]Department of Pediatric Pneumology and Immunology, Charité, Berlin, Germany; [3]Department of Pediatrics, University Hospital of Oslo, Oslo, Norway; [4]Department of Pediatrics, University of Linköping, Linköping, Sweden; [5]Department of Pediatrics, Hospital of Sønderborg, Sønderborg, Denmark; [6]Department of Pediatrics, University Hospital of Odense, Odense, Denmark; [7]Turku Allergy Centre, Turku, Finland; [8]Department of Pediatrics, Umeå University, Umeå, Sweden

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L. Jacobsen
Krokushaven 18
DK-2765 Smørum
Denmark

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Allergic rhinoconjunctivitis is a major risk factor for later development of asthma (1, 2). More than 20% of all children with rhinoconjunctivitis develop asthma later in life and rhinitis frequently precedes the onset of asthma. Although not specifically designed for this purpose, other studies have indicated the preventive potential of specific immunotherapy (SIT) in reducing the risk of asthma in patients with allergic rhinoconjunctivitis (3–6). 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 (7).

The Preventive Allergy Treatment study (PAT) is the first prospective long-term follow-up study that tested whether SIT can prevent the development of asthma and whether the clinical effects persist in children suffering from seasonal allergic rhinoconjunctivitis caused by allergy to birch and/or grass pollen as these children grow up. The total SIT period was 3 years, after which the children were evaluated for the development of asthma. The patients were re-evaluated after a total of 5 years. The evaluation showed that immunotherapy impedes progression from allergic rhinoconjunctivitis to asthma after 3 years of SIT (8) and at the 5 year follow-up 2 years after treatment termination (9). The actively

treated children had significantly less asthma after 3 years of SIT (odds ratio 2.5; $P < 0.001$) and at the 5 year follow-up (odds ratio 3.1; $P < 0.01$) as evaluated by clinical symptoms in favor of SIT for prevention of development of asthma and significantly less patients reported an increase in asthma scores. Furthermore, the significant improvement in allergic rhinoconjunctivitis and conjunctival provocation test (CPT) results observed after 3 years of SIT persisted at the 5 year follow-up.

The present study investigated whether these clinical effects along with the preventive effect of developing asthma persisted 7 years after termination of SIT (at 10 year follow-up).

Methods

Patients

A total of 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. The children had a clinical history of birch and/or grass pollen induced seasonal allergic rhinoconjunctivitis. Further inclusion criteria were positive skin prick test and CPT results. For a further description of inclusion criteria see Moller et al. (2002)(8). The study design is illustrated in Fig. 1. The patients and/or their parents gave informed consent according to the Helsinki declaration and Ethical Committees approved the study in the respective countries.

Treatments

Patients were included and the treatment was initiated from 1992 to 1994. The patients were stratified on the basis of bronchial responsiveness to methacholine during the 0-season, age, sex and years with allergic rhinoconjunctivitis according to history and then randomized into two groups. To reduce the influence of difference in pollen exposure, randomizations were performed centre for centre. 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 the identical measures, except the administration of the allergen injections.

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 inhaled β_2-agonists were prescribed. When needed inhaled corticosteroids could be introduced. After discontinuation of SIT, all patients were offered the same drugs as before.

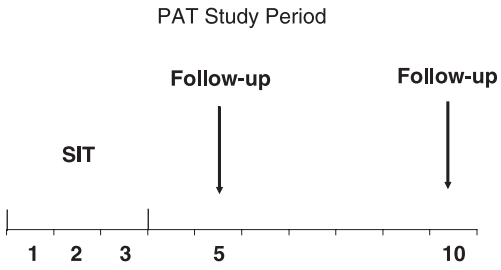

Figure 1. Study design.

After a 0-season, SIT was initiated 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; ALK-Abelló, Horsholm, Denmark), with weekly injections over 15–20 weeks or as rush immunotherapy with aqueous extracts (Aquagen SQ; ALK-Abelló). Maintenance injections with the depot preparation 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).

Skin prick test

Skin tests were performed on the flexure aspect of the forearm in duplicate before the start of SIT. The following allergen extracts were used: timothy, birch, mugwort, dog, cat, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cladosporium herbarum* and *Alternaria alternata* (Soluprick SQ, 10 HEP/molds 1/20 w/v, ALK-Abelló).

Conjunctival provocation test

Conjunctival provocation tests were performed outside the pollen seasons, always at the same time of the year, before the start of immunotherapy and after 1, 2, 3, 5 (5-year follow-up) and 10 years (10-year follow-up). Half ^{10}log increments at concentrations from 100 to 1 000 000 SQ units were used (Aquagen SQ; ALK-Abelló) (10).

Methacholine bronchial provocation test

Methacholine bronchial provocation tests (MBPT) 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 (11). The method involves 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. First 0.9 M NaCl solution and then test solutions 0.5, 1, 2, 4, 8, 16 mg/ml methacholine were nebulized and inhaled. $FEV_1$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 FEV_1 in relation to baseline. The provocative dose (PC_{20}) was estimated by linear interpolation of the two last (log-transformed) concentrations tested. In each center the same devices for measuring FEV_1 were used on all test occasions. Identical dilution instructions for methacholine were used at each center.

Visual analogue scale

Symptoms of conjunctivitis, rhinitis and asthma compared with pretreatment symptoms were evaluated on a 100 mm visual analog scale (VAS) after every season(s).

Asthma diagnosis

Asthma was defined as recurrence of at least two 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 β_2-agonists. Thus, the clinical diagnosis was independent of the level of hyper-responsiveness to methacholine.

Statistical methods

The effect of SIT at 3, 5 and 10 years on prevention of asthma was addressed specifically by analyzing the children without asthma at baseline. Clinically diagnosed asthma was analyzed per centre by Fischer's exact test, and homogeneity of odds-ratios between centers was tested by Zelens Exact test.

Additionally, a longitudinal data analysis of the probability for 'no asthma' was performed with a mixed logistic regression model. By using a mixed model we obtain information not only from complete observations, but also from incomplete ones, through the conditional expectation of the missing measurements given the observed ones. In the analysis all children randomized and all available data for all follow-up time points have been included. The mixed model included treatment effect (SIT *vs* control), baseline bronchial hyper-responsiveness and asthma status at baseline as explanatory variables.

Changes from baseline of logarithmic transformed values of CPT were analyzed by ANOVA. Changes from baseline of VAS scores of conjunctivitis and rhinitis and bronchial hyper-responsiveness were analyzed by ANOVA adjusted for baseline values. Two-sided tests and a test significance level of 5% were used.

Results

Initially, 205 children aged 6–14 years were randomized after a baseline season to 3 years subcutaneous SIT or to a control group. One hundred and eighty-three subjects (121 males, 62 females) aged 11–20 years (mean 15.6) participated at the 5-years follow-up. At 10-year follow-up 147 subjects (117 of those had no asthma at inclusion) aged 16–25 (mean 21.0) years were included in the analysis. One centre did not participate in the 10-year follow-up study and 36 subjects were lost for follow-up at 10-year. The flow chart of patient numbers for the study is illustrated in Fig. 2.

Patients without asthma before the start of SIT (n = 117) were analyzed for the development of asthma after the 10 year period. The number of patients who developed asthma among controls was 24/53 and in the actively treated group 16/64.

A statistical homogeneity between individual centers was found. According to the definition, asthma development showed an odds ratio of 2.5 (1.1–5.9) in favor of the hypothesis that SIT could prevent the long-term development of asthma (Fig. 3). The odds ratio after 3 years of immunotherapy was 2.5 (1.3–5.1) and 3.1 (1.4–6.9) at 5-year follow-up. Out of those 40 patients reporting asthma at 10 year follow-up, 73% reported asthma during the summer and 55% during the winter.

The final statistical model included treatment, baseline bronchial hyper-responsiveness and asthma status at baseline. The longitudinal treatment effect when adjusted

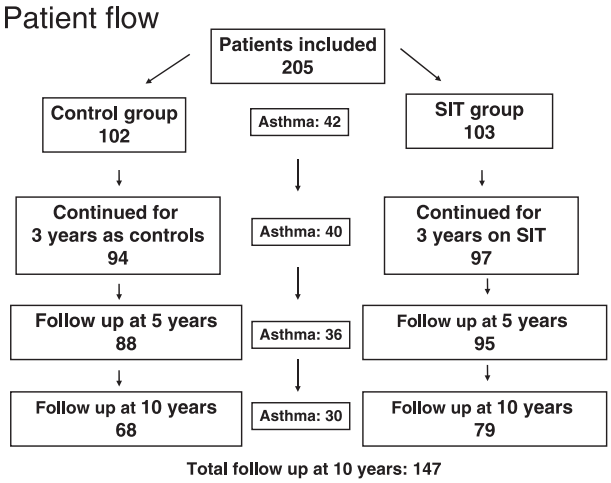

Figure 2. Flow chart of patient numbers. The children with mild to moderate seasonal asthma at baseline are illustrated separately.

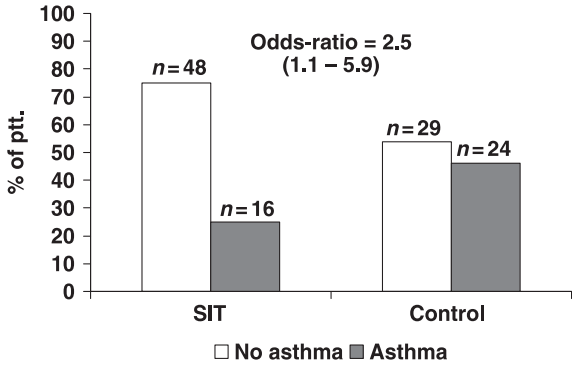

Figure 3. The percentage of children with and without asthma 7 years after termination (10-year follow-up) of specific immunotherapy. Based on the patients without asthma before treatment (n = 117). The absolute number of children is shown above the bars.

for bronchial hyper-responsiveness and asthma status at baseline including all observations at 3, 5 and 10 years follow-up (n = 511) and including all children with or without asthma at baseline (n = 189) was statistically significant (P = 0.0075). The odds ratio for no asthma was 4.6 [95% CI (1.5–13.7)] in favor of SIT. Bronchial hyper-responsiveness at baseline was associated with increased risk of later development of asthma (P = 0.002). Also an increased probability for having no asthma after 3, 5 and 10 years was demonstrated if the child had no asthma at baseline ($P < 0.0001$).

The clinical effect on conjunctivitis and rhinitis following SIT was persistent 7 years (10-year follow-up) after the termination of treatment. According to the VAS of conjunctivitis and rhinitis, the active group improved significantly more from baseline to 10-year follow-up compared with the control group (−20.9 and −12.4 mm, $P < 0.05$ for conjunctivitis; and −19.9 and −11.5 mm, $P < 0.05$ for rhinitis) (Fig. 4).

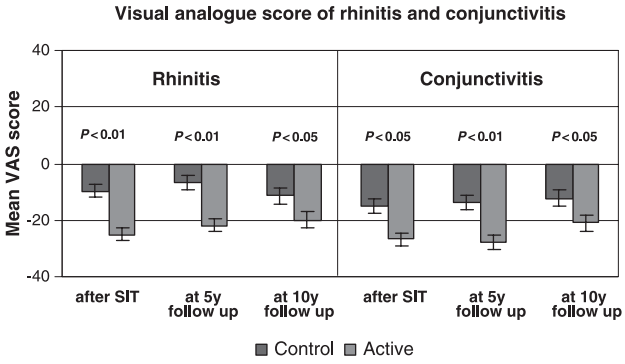

Figure 4. Change from baseline and standard error of the mean for rhinitis and conjunctivitis visual analogue scores at the end of specific immunotherapy, 2 and 7 years after termination.

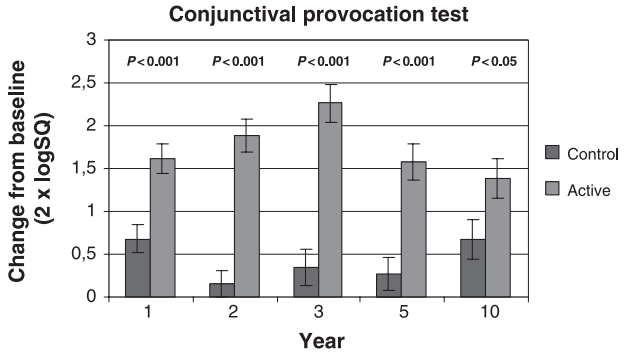

Figure 5. Change from baseline and standard error of the mean for the conjunctival provocation test. For children allergic to both grass and birch, the challenge with both allergens is included.

The conjunctival sensitivity measured by provocation test was significantly reduced in the active group compared with the control group ($P < 0.05$) (Fig. 5).

The groups did not show significant bronchial hyperresponsiveness after 5 and 10 years. The mean (range) seasonal PC_{20} values for the SIT and control groups at 5 year were 16.7 (0.37–32) and 15.6 (0.03–32) mg/ml. The corresponding winter PC_{20} values were 22.0 (0.51–32) and 18.9 (0.55–32) mg/ml. At 10-year follow-up the seasonal PC_{20} values for SIT and control groups were 20.9 (0.34–32) and 21.4 (0.08–32) mg/ml. The winter PC_{20} values were 22.6 (0.93–32) and 23.9 (0.39–32) mg/ml.

There were no significant differences between the SIT and control group in bronchial responsiveness to methacholine in change from baseline of PC_{20} after 10 years.

A potential association between the development of new perennial allergies and development of asthma was analyzed. Of those children who developed asthma during the 10-year follow-up, 30% (18 of 61) also developed a positive skin prick test to house dust mites compared with 17% (15 of 86) of the children who did not develop asthma. The difference was not significant. The same picture was demonstrated for the development of sensitivity to cat or dog allergens. Of those children who developed asthma during 10-year follow-up, 57% (35/61) also developed a positive skin prick test to one or more of the following allergens: house dust mite, cat or dog allergen compared with 50% (43 of 86) of the children who did not develop asthma.

Discussion

This study has demonstrated that the significant clinical outcome achieved during SIT persisted not only at termination of treatment (8) and 2 years after (9) but also 7 years after termination of treatment and that SIT reduced the risk of developing asthma in children suffering from allergic rhinoconjunctivitis at 10-year follow-up indicating a long-lasting benefit of SIT as these children grow up.

In the longitudinal statistical analysis including all subjects at all occasions, we found that bronchial hyperresponsiveness in childhood increased the risk for later development of asthma and that allergen SIT with standardized allergens can prevent the development of asthma.

Various strategies for the prevention of the development of allergic rhinoconjunctivitis and asthma have been proposed including allergen avoidance, pharmacological treatment (antihistamines and steroids) and SIT. Allergen avoidance is hardly applicable to grass and birch pollen allergy and only a limited reduction in exposure can be achieved by the modification of life habits. In contrast, as an inverse relationship between levels of allergen exposure in early life and allergy symptoms has been indicated in some studies, suggesting that exposure to high levels of allergen may provide protection against sensitization (12–14), a new approach in primary prevention has recently been initiated by the Immune Tolerance Network (collaborative research under the National Institute of Allergy and Infectious Diseases) (15). This study is the first in which high-risk children who are sensitized to food but not sensitized to inhalants is given an inhalant allergen mixture (grass, house dust mites and cat) sublingually to prevent them from developing further allergic sensitization and asthma.

Secondary prevention addressing diseased children to prevent symptom and further disease progression involves traditional pharmacotherapy with antihistamines. While this treatment provides symptomatic relief and disease control, this does not modify long-term outcomes in children as the natural course of the disease is not altered. A recent large multi-centre trial in which children with atopic dermatitis were given cetirizine failed to reduce the development of asthma (16).

As a potential tertiary preventive measure for worsening of asthma by early treatment with inhaled steroids in

children with episodic wheezing has been suggested, but recent studies on the capacity of inhaled steroid therapy during early symptomatic episodes of wheezing to delay progression to persistent disease has failed to show any preventive potential (17, 18).

While the persisting long-term clinical effect after termination of SIT has been demonstrated previously (5, 19–23), the PAT study is the first prospective long-term follow-up study to demonstrate that SIT can prevent the development of asthma 7 years after the termination of treatment in children suffering from seasonal allergic rhinoconjunctivitis, and that it is possible to interfere with the natural course of allergic disease.

In contrast to the results at the termination of SIT in the present study (8) and other studies e.g. (4) but in concordance to 5-year follow-up (9), bronchial responsiveness to methacholine showed no statistical significant differences between active and control groups at the 10-year follow-up. As at 5-year follow-up, this may be explained by a spontaneous improvement of bronchial responsiveness over time as a natural improvement in bronchial responsiveness from infancy to adulthood has been reported (24, 25). In the literature it is suggested that patients with rhinitis who also have bronchial hyper-responsiveness are more likely to develop asthma (26). Our study demonstrated that children with rhinitis and bronchial hyper-responsiveness at baseline were those most likely to develop asthma demonstrating that bronchial hyper-responsiveness may predict the risk for later asthma development.

We also investigated if development of new perennial allergen sensitivities were associated with the development of asthma. Although our data indicated that more children with asthma had developed sensitivity to house dust mites, our study can not confirm this hypothesis. Testing of this hypothesis will require more investigations.

In conclusion, this 10-year follow-up study demonstrates that SIT for 3 years with high-dose standardized allergen extracts shows persistent long-term effect on clinical symptoms after termination of treatment and long term, preventive effect on later development of asthma in children with seasonal rhinoconjunctivitis. In this light, SIT should be recognized not only as first line therapeutic treatment for allergic rhinoconjunctivitis but also as secondary preventive treatment for respiratory allergic diseases.

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