

Original article

The safety and efficacy of subcutaneous birch pollen immunotherapy – a one-year, randomised, double-blind, placebo-controlled study

Background: There is only very limited documentation of the efficacy and safety of high-dose subcutaneous birch pollen immunotherapy (IT) in double-blind, placebo-controlled (DBPC) studies. Birch pollen is a major cause of allergic morbidity in northern Europe and in eastern parts of North America.

Methods: Thirty-five patients with severe rhinoconjunctivitis (hay fever) to birch pollen were allocated to double-blinded clustered IT with a depot birch pollen extract (*Betula verrucosa*) or placebo injections. Seven patients in each group had concomitant self-reported seasonal asthma. Treatment was conducted as a clustered regimen and was performed in a specialist unit. Symptom scores from nose, eyes, and lungs, and use of oral and topical antihistamines, beta-2-agonists, and oral corticosteroids were recorded daily during the season of 2000. Sensitivity to allergen provocation in skin, conjunctiva, and nasal mucosa was measured before and after 10 months of treatment. Post-seasonal assessment of symptom severity was performed using a simple questionnaire.

Results: IT reduced the symptom score for both rhinoconjunctivitis and asthma (P -values < 0.05), total medication score ($P < 0.02$) and use of oral antihistamines ($P < 0.01$). IT reduced specific conjunctival sensitivity ($P < 0.05$), skin prick test, and especially cutaneous late-phase response diameters ($P < 0.00001$), and increased general well-being on post-seasonal evaluation ($P < 0.01$). IT was safe, with side-effects at the same level as placebo.

Conclusions: High-dose, subcutaneous IT is efficacious and safe in patients with severe birch pollen rhinoconjunctivitis and asthma.

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Birch pollen is second to grasses as the most important outdoor allergen causing IgE-mediated allergic disease (1) in Northern Europe and constitutes a major cause of allergic disease in certain areas of North America (2, 3). Subcutaneous, high-dose immunotherapy (IT) is recommended as the treatment of choice in birch pollen allergy that is uncontrollable by conventional rescue medication (4). To our knowledge, no papers on studies investigating the clinical efficacy and safety of high-dose subcutaneous birch pollen (IT) in adults in a

double-blind and placebo-controlled (DBPC) design have been published (5). Previous studies on efficacy have been conducted with low-dose immunotherapy (6), grass pollen immunotherapy as control treatment (7), no placebo (8, 9), sublingual or oral treatment (10, 11) or in children (12). Safety data are even more sparse (6, 9, 11–13). The potential for a fatal anaphylactic response is the major concern of subcutaneous IT, and it is important to estimate the risk of hazardous side-effects in treatments for nonlife-threatening disease (14, 15). The incidence and severity of side-effects differ between allergens, perhaps as a consequence of variations in content of major allergen in the extract, which makes it necessary to investigate safety for each allergen (13).

The clinical efficacy of IT can be monitored by measuring symptoms and recording use of medication

Abbreviations: CPT: conjunctival provocation test; DBPC: double-blind, randomised, placebo-controlled study; IT: allergen-specific immunotherapy; LPR: late phase skin reaction; NPT: nasal provocation test; QoL: quality of life; SPT: skin prick test; SQ-U: standard quality units; VAS: visual analog scale.

during the season, or clinical sensitivity to specific allergen-challenge in a target organ outside the season (5). No single challenge test has been found to yield predictive information on the outcome of IT, but a change in sensitivity between pre- and post-treatment challenges has been found to be associated with changes in clinical sensitivity (5). However, a preferential challenge test has not been defined, and major differences exist between the sensitivity, specificity, simplicity, and safety of challenge tests (16, 17). Paraclinically, IT is associated with decreased allergen-induced basophil histamine release, whereas total and specific IgE levels tend to rise initially (4, 18, 19). We wanted to estimate the relationship of several types of challenge and laboratory tests to the clinical effect of immunotherapy.

Inhalation allergy is accompanied by a significant decrease of in-season quality of life (QoL), which is perhaps not reflected in the scoring of individual symptoms from the involved organs (eyes, nose, and lungs) (20). QoL-studies have been performed during season. We wanted to investigate if post-seasonal assessment could identify efficacy of IT.

Material and methods

Patients

Out of 40 screened patients, 35 patients (14 men, 21 women) of median age 27 years (range 19–46) were enrolled in the study. The median duration of birch pollen allergic rhinoconjunctivitis was 10 years (range 2–31). They all had positive histories of at least two seasons of severe allergic symptoms in April and May (corresponding to the local birch pollen season) and poor symptom control in the previous seasons on regular antiallergic treatment. All had positive skin prick tests (SPT) with a wheal diameter greater than 3 mm to *Betula verrucosa* (Soluprick SQ, ALK-Abelló, Hørsholm, Denmark) and a positive serum test for birch pollen-specific IgE of class 2 or greater (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden). Fourteen patients reported seasonal asthmatic symptoms, but all had normal lung function spirometry outside season (Vitalograph, Buckingham, England). Twenty patients had self-reported allergy to grass pollen exposure. One screened patient was skin test-negative and was not included. Exclusion criteria were in addition to general recommendations (4) (number of screened, excluded patients): previous IT towards birch; lactation or pregnancy at start of injection therapy ($n=1$); perennial rhinitis ($n=1$) or asthma ($n=1$); or continuous use of systemic beta-blockers ($n=1$).

The study was approved by the local ethics committee of Copenhagen, Denmark, and written informed consent was obtained from all patients prior to admission to the study.

Treatment allocation and design

At inclusion, patients graded their overall symptom severity in the season of 1999 on a visual analog scale (VAS). Based on gender, the overall symptom severity, and the presence of self-reported seasonal asthma, the patients were randomised to immunotherapy or placebo treatment by the method of minimization (21). The three parameters were equally weighted. Clinical data at inclusion are shown in Table 1. The randomization was performed by and the result was known only by a non-clinical investigator (L.K.P.). Injection therapy began in January 2000. Throughout the birch pollen season 2000 (April–May) patients completed daily diaries on symptom severity and medication use. After one year of follow-up, in autumn 2000, tests that had been conducted at inclusion were repeated, and patients completed a questionnaire on efficacy of the treatment. The clinical co-ordinator of the study (U.B.) and a study nurse conducted all inclusion, treatment, and control visits. Both were blinded to the randomization throughout the study.

Clinical and paraclinical tests

Conjunctival challenge test (CPT) was performed and interpreted as described by Möller et al. (22). The nasal challenge test (NPT) was performed as described by Jacobi et al. (23), and scored as follows: the severity of nasal itching, blockage, and secretion were each scored from 0 to 3 points (corresponding to none, mild, moderate, and severe symptoms), and the number of sneezes was counted: >5 sneezes scored 3 points, <5 sneezes scored 0 points. The NPT was considered positive if the sum of scores after 10 min exceeded 5 points. The immediate skin reactions after SPT (see above 'Patients') was recorded after 10 min. The late-phase reaction (LPR) was recorded within 18–24 h after intradermal injection of 50 SQ-U Aquagen SQ (ALK-Abelló, Hørsholm, Denmark). Skin reactions were recorded as described by Varney et al. (24). Specific IgE was measured using the CAP System (Pharmacia Diagnostics, Uppsala, Sweden). Total IgE was measured by a microparticle enzyme immunoassay (IMX System, Abbott Diagnostics, Abbott Park, IL). Birch pollen-induced basophil histamine release was measured as described by Skov (25). The titre giving 50% of the maximal response was registered. All tests were performed with the same allergen batch throughout the study.

Immunotherapy

From January 2000 to November 2000, the patients received placebo-controlled, double-blind, clustered IT with *Betula verrucosa* pollen extract (Alutard SQ, ALK-Abelló, Hørsholm, Denmark). The administration of IT followed the guidelines of the EAACI (4). At each visit, the patient's clinical condition and suitability for injection was assessed by the clinical investigator. The clustered-injection

Table 1. Clinical data of patients at inclusion, $n=35$

	IT	Placebo	P-value
Male/female	7/10	7/11	$>0.7^*$
Age (years) median (range)	28 (21–46)	26 (19–25)	$>0.6^{**}$
Duration of birch pollinosis (years) median (range)	11 (2–31)	7 (2–23)	$>0.2^{**}$
Seasonal asthma (yes/no)	7/10	7/11	$>0.7^*$
FEV ₁ (% of predicted) median (range)	105 (77–139)	109 (85–140)	$>0.7^{**}$
Allergic to hazel/alder (yes/no†)	0/17	4/13	$<0.05^*$
Allergic to grass (yes/no†)	11/6	9/9	$>0.2^*$

† Self-reported symptoms. Groups compared with * Chi-squared test (categorical data), and ** Mann–Whitney U-test (continuous variables).

Table 2. Schedule for increment and maintenance dosing in clustered dosage immunotherapy

Week	Dose (SQ-U)
1	10, 100, 1000
2	2000, 2000
3	5000, 5000
4	20 000
5	40 000
6	60 000
7	100 000
9	100 000
13	100 000
21	100 000

schedule is shown in Table 2. From treatment week 13 all maintenance doses (100 000 SQ-U) were given with eight-week intervals. On days with several injections, at least 30 min separated the injections. A 30-minute observation period followed each injection. Dose modifications were made if systemic or local side-effects occurred, with either no increment, or reduction in the dose in up to four steps, depending on the severity of the side-effects. Side-effects were graded in four categories (I–IV) according to the EAACI position paper (4).

Pollen counts

Pollen grains were collected by a standard Burkard Volumetric 7-Days Spore Trap in Copenhagen, Denmark (55°43' N, 12°34' E) 15 m above ground level. Pollen grains were counted daily (expressed as number/m³ air) by the Danish Meteorological Institute, Copenhagen (26).

The birch pollen season was defined as the period including the mid 90% of total birch pollen.

Assessments

The severity of symptoms from each organ (nose, eyes and lungs) were assessed daily on diary cards using a four-step scale (0 = none, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms). Diaries were completed from 13 March to 21 May. Symptoms were attributed to birch pollen from the first day of the birch pollen season to the first day of the grass pollen season pollen count > 10 grains/m³, since several patients had self-reported grass pollen hay fever. This period extending the season was called the potential symptom period. For each patient, the daily symptom scores were summed to a total symptom score with a maximum of nine points. The symptom scores for the eyes and nose were added to a rhinoconjunctivitis score, and the symptom scores for the lungs were regarded as the asthma score. For further analysis, the two week-period with the highest total symptom score during the birch pollen exposure were selected for each patient and each symptom as described by Zenner (27), and this score was compared to the symptom scores for the season and the potential symptom period.

The basic rescue medications were: oral antihistamines (acrivastine 8 mg), topical antihistamines (levocabastine eye drops, 0.5 mg/ml and nasal spray 50 µg/dose), and topical beta-agonists (salbutamol inhalations, 200 µg/dose). Daily consumption was recorded on diary cards. To avoid excessive medication use, patients were instructed to use medication only when symptoms were present. If symptoms were not controllable with the maximal dosage of the basic drug treatment, a one-week course of oral prednisolone (12.5 mg/day) was prescribed. Topical corticosteroids were not allowed in the study (24). Drugs were scored as follows: each eye drop or nasal spray of levocabastine, and each inhalation of salbutamol scored 1, and each dose of acrivastine (8 mg) and prednisolone (12.5 mg) scored 2 (24, 27). The total medication score for each patient was calculated as the

sum of medication scores; the hay fever medication score was calculated as the sum of scores for oral, conjunctival, and nasal antihistamines, and oral prednisolone. The use of medication in the two weeks where the patient suffered the most symptoms was used for further analysis (27). The period from 1 April to 14 April served as the preseason baseline for comparison of symptoms and medication between the groups. Post-seasonal assessment of the severity of the season was performed at the one-year follow-up, using the VAS and a simple nonvalidated questionnaire: 'In the season, did you experience: a) any effect of the given treatment, b) a decrease in symptoms, c) a reduction in medication usage, or d) an increased general well-being compared to previous seasons?'.

Extracts

The extracts for SPT (Soluprick SQ, 100 000 SQ-U/ml), for challenge tests (Birch Aquagen SQ), and for IT (Birch Alutard SQ) were commercially available and produced by ALK-Abelló, Hørsholm, Denmark. The content of the major birch pollen allergen Bet v 1 was 12 µg/100 000 SQ-U. The placebo was produced and packed by ALK-Abelló, Hørsholm, Denmark, and consisted of the same suspension buffer as in the Alutard SQ product, but with ascending concentrations of histamine dihydrochloride (1 : 100 000 w/v to 1 : 100 w/v). The active (Alutard SQ) and placebo treatment packages were identical. All extracts were kept at + 4°C.

Statistics

A power calculation revealed that an expected decrease in total symptom score of at least 25%, a pooled standard deviation of 30%, a power (1-beta) of 0.8, and an error rate (alpha) of 0.05 in the one-tailed *t*-test, demanded at least 19 patients in each treatment group (21).

All data were collected before termination of the study, and were analyzed using a statistical software program available from the Internet (R, version 1.1.1; <http://www.r-project.org>) (28). The baseline characteristics of the IT and placebo group were compared by the *chi*-squared test for categorical variables, and by the *t*-test and the Mann-Whitney *U*-test for continuous variables. The daily mean scores for symptoms and medication (area under the curve, AUC) in a given period were compared between the groups using the Mann-Whitney *U*-test. An arbitrary level of 10% missing data in the diaries was accepted. Missing data were omitted from data analysis, which means that no fitted values were constructed. If more than 10% of data were missing in the diaries, data analysis was considered inconclusive. Patients who did not complete the treatment were excluded from the final calculations.

Data were statistically analyzed by minimum, maximum, and median values, arithmetic means, standard deviation (SD), standard error of the mean (SEM), and frequency distributions.

Results

Patients

Figure 1 describes the patient flow through the study. A total of 34 (97%) patients completed the study. One patient (placebo-treated) was excluded due to noncompliance resulting in uncompleted diary cards and unacceptably long intervals between maintenance doses. One patient (IT-treated) failed to complete useful diary cards because of noncompliance to registration but completed the treatment and was not excluded from the follow-up visit. The missing data in diaries constituted less than 4%.

Table 3. Injection therapy and side-effects

	IT	Placebo	P-value
Injection/pt (season)	13 (12–16)	14 (11–18)	>0.4*
Dose (SQ-U†) (season)	435 110 (110 210–515 110)	435 110 (235 110–645 110)	1*
Injection/pt (follow-up)	17 (15–23)	17 (15–21)	>0.4*
Dose (SQ-U†) (follow-up)	735 110 (615 100–815 100)	735 110 (616 100–945 100)	1*
Total number of injections	290	301	–
Patients with side-effects	4	10	<0.01**
Severity grade 3–4	0	0	1**
Severity grade 1–2	7	16	<0.03**
Immediate side-effects	7	14	0.06**
Late side-effects	0	2	0.13**

† SQ-U as stated on vials. * Mann–Whitney U-test. ** Chi-squared test.

Immunotherapy

All but one patient had completed the dosage increment phase before the start of the birch pollen season (18 April). One patient in the IT group suffered from a viral upper airway infection during the winter, and the onset of treatment was postponed until the patient was clinically healthy (early March). However, there was no statistical significant difference between the groups concerning the number of injections or doses given at the start of the season or at the one-year follow-up (Table 3).

Side-effects

A total of 591 injections were administered in the period from January to October 2000, and 23 episodes of possible side-effects were observed in 14 patients (Table 3). Significantly more reactions occurred in the placebo group. All reactions were either unspecific or local (grade I) or mild systemic (grade II) and were relieved by oral antihistamines. Two episodes were delayed (> 30 min after injection). Large local reactions *per se* were not counted as side-effects.

Pollen count

The birch pollen season in 2000 in the Copenhagen area commenced 18 April and terminated 11 May. This 24-day season had an average of 185 pollen grains/m³/day, which was representative for a season with an

average pollen load (7, 29). Eighty percent of the birch pollen count was collected during a two-week period (22 April–6 May). The grass pollen count exceeded 10 grains/m³ on 16 May, defining the potential symptom period to 18 April to 15 May. Of the total number of days in the potential symptom period with symptoms, 85% (range: 77% to 100%) in the IT group and 86% (range: 79% to 100%) in the placebo group were during the season (Mann–Whitney U-test, $P > 0.1$). The season in 1999 had an average pollen count of 108 grains/m³/day, and a length of 19 days, comparable to a season with a low pollen load (29).

Symptom score and medication score

Fig. 2 depicts the total symptom and medication score during the diary registration period. The occurrence of symptoms and use of medication followed the increase in pollen count with a delay of approximately four days in both groups. The total symptom score in the IT group comprised 71% (range: 57% to 100%) of the total symptom score observed in the potential symptom period, compared to 73% (range: 48% to 100%) in the placebo group (Mann–Whitney U-test, $P > 0.7$). There was no pre-seasonal difference between the groups in symptom scores, but the placebo group had a significantly higher use of topical nasal antihistamines (Mann–Whitney U-test, $P < 0.05$, data not shown). Self-reported sensitivity to hazel and alder occurred only in the placebo group, but these patients did not have significantly more severe symptoms (median total symptom score 4.0 vs 3.6; $P > 0.6$). Table 4 shows the effect of IT on symptoms and medication use. Three patients (IT: $n = 1$, placebo: $n = 2$) needed a one-week course of oral prednisolone 12.5 mg late in the season. Statistical comparison of difference in corticosteroid use in the two groups was omitted because of the small sample size.

Post-seasonal assessment

At one-year follow-up, there was a significant reduction in the visual analog scale grading (VAS) of the severity of the season in the IT group as compared to the

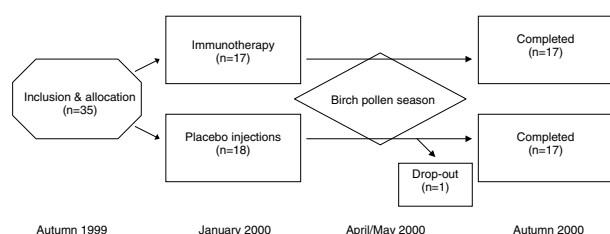


Figure 1. Study flow chart 1999–2000. Timing of allocation, treatment, season, and termination of study. Number of patients in parentheses. Pre- and post-seasonal assessment were performed in autumn 1999 *res.* 2000. In-season assessment of symptom severity was in April/May 2000.

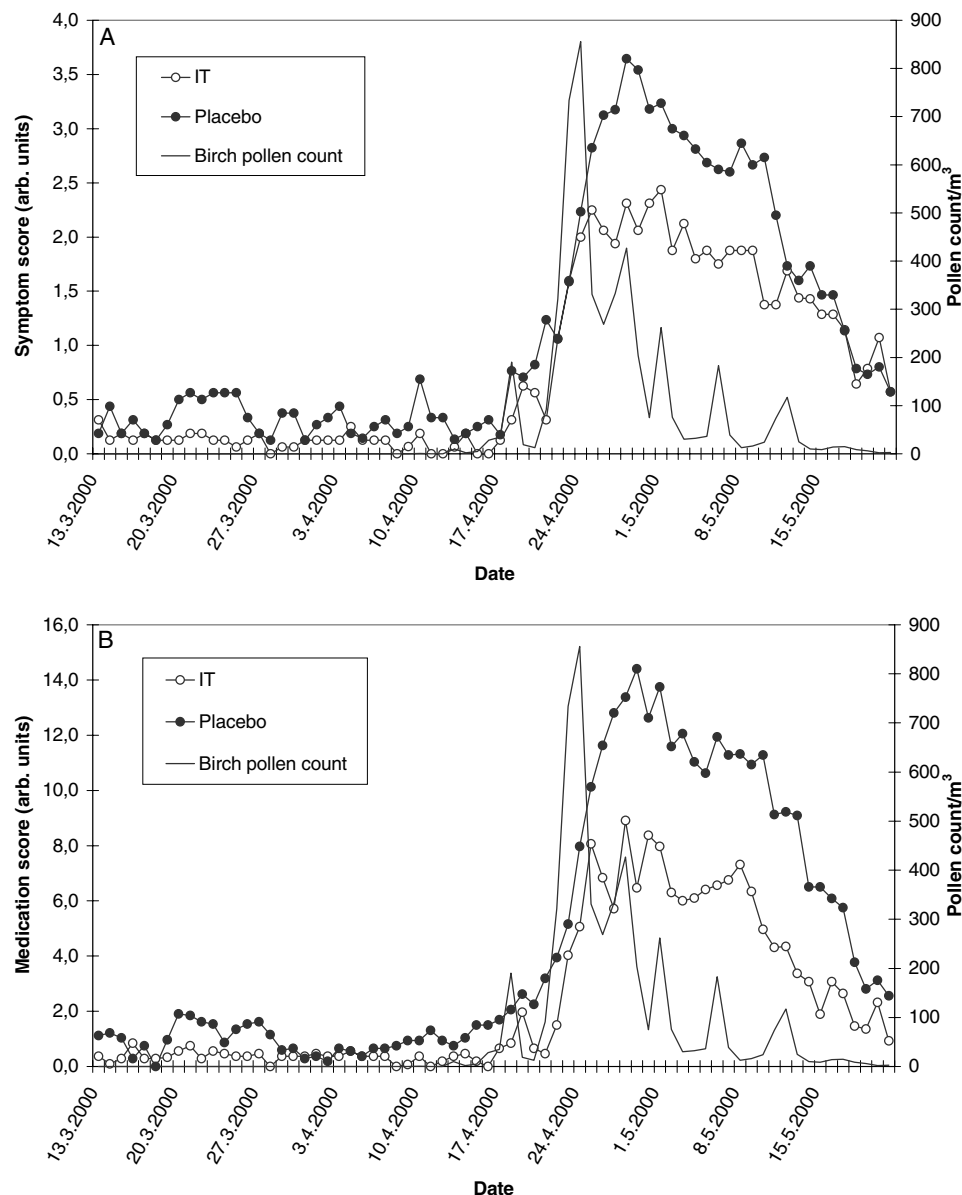


Figure 2. The daily pollen count in the observation period, and the daily group mean in each treatment group: (A) total symptom score, and (B) total medication score. Open circles: IT group. Black circles: placebo group. Solid line: birch pollen count/m³.

placebo group (Table 5A). The distribution of answers to the four-item questionnaire is listed in Table 5B. Two IT patients denied an effect: one patient (A) experienced reduced rhinoconjunctivitis but aggravated night-time coughing and an increased need for inhaled beta-2-agonists (decrease in VAS), whereas the other patient (B) had severe rhinoconjunctivitis and was treated with oral corticosteroids (increase in VAS). Both patients received maintenance doses at the beginning of the season.

Clinical and paraclinical tests

Table 6 shows the effect of IT on clinical and paraclinical parameters. The change in sensitivity to

CPT in the IT group was only significant as a paired analysis and not between the treatment groups. Observe that the median value did not change whereas the range did. A positive NPT was not present without a positive CPT, and a change in NPT was not present without a concomitant change in CPT, while the opposite was found (data not shown). The major differences between the groups were in the sizes of the SPT and especially the LPR. Patient A (see above: 'Post-seasonal assessment') experienced a decrease in CPT but not in SPT, LPR, NPT or S-IgE. Patient B experienced a decrease in LPR but not in any other parameter. The titre of allergen-induced basophil histamine-release was not influenced by the treatment. Neither the birch pollen-specific IgE nor the total-IgE differed significantly

Table 4. Median symptom and medication score (range), $n=33$

	IT	Placebo	<i>P</i> -value*
Symptom score			
Total	32.5 (6.0–71.0)	51.0 (14.0–76.0)	< 0.04
Hay fever	31.5 (6.0–50.0)	44.0 (14.0–75.0)	< 0.05
Asthma	0.0 (0.0–13.0)	1.0 (0.0–15.0)	< 0.03
Medication score			
Total	52.0 (2.0–114.0)	102 (2.0–186.0)	< 0.02
Acrivastine	22.0 (0.0–66.0)	62.0 (0.0–92.0)	< 0.01
Levocabastine eye drops	3.5 (0.0–56.0)	20.5 (0.0–44.0)	> 0.2
Levocabastine nasal spray	3.0 (0.0–44.0)	10.0 (0.0–41.0)	> 0.1
Prednisolone†	–	–	–
Hay fever medication‡	46.0 (2.0–114.0)	102.0 (2.0–177.0)	< 0.02
Salbutamol	0.0 (0.0–12.0)	0.0 (0.0–34.0)	> 0.4

Scores are the area under curve for the two individual weeks with maximal symptoms.

* Groups compared with Mann–Whitney . two-sided U-test.

† Only three patients received corticosteroids. Comparison between groups was omitted.

‡ The sum of scores of local and systemic antihistamine, and prednisolone intake.

between the groups at any measurement. Both parameters increased in both groups but only the increase in total-IgE levels in the placebo group reached significance.

Discussion

To our knowledge, no DBPC study on high-dose birch pollen IT has been published. We have demonstrated that subcutaneous specific immunotherapy with a commercially available extract of *Betula verrucosa* is a safe and efficacious treatment of IgE-mediated birch pollen allergic rhinoconjunctivitis and asthma. Our results support earlier findings in grass pollen IT (30), and in one high-dose birch pollen IT study using grass pollen IT as control treatment (7). The actively treated group experienced a significant decrease in symptoms paralleled by a concomitant decrease in antihistamine use, and in hay fever medication. The use of asthma

medication did not differ between the groups. The described differences between the groups were only apparent when considering the two weeks with the highest symptom scores. Our treatment duration was short, resulting in administration of relatively low total doses of allergen as compared to other IT studies (7, 30), and the size of the study was small, making the risk of type II errors substantial (21). However, we consider the two-week data representative for the birch pollen season, since the investigated two-week period comprised almost 75% of symptoms registered in the period from start of the birch to the start of the grass pollen season. Furthermore, we have presented all *P*-values as two-sided test values, even though the power calculation demonstrated that significant differences were only to be expected in one-sided tests.

We observed a delay in peak symptoms compared to the peak pollen count followed by a slow decline extending beyond termination of the pollen season. This pattern seems to be typical for birch (7, 8, 29) in contrast to other pollen allergens (27, 31, 32). The exposure to birch pollen is sudden and intense: approximately 80% of the total birch pollen count was collected in a two-week period, and the delay and prolongation of symptoms could be a result of the allergic priming of involved mucosal surfaces (33). The phenomenon makes it important to extend symptom registration beyond pollen exposure when evaluating severity of birch pollen induced symptoms.

Post-season VAS-scoring was used to assess the overall severity of the season. In the IT group, there was a significant reduction in VAS score (4, 24). In contrast, the VAS score in the placebo group tended to increase, possibly reflecting the higher pollen load in the 2000 season compared to 1999.

Based on the simple questionnaire, significantly more patients in the IT group confirmed having an increased

Table 5A. Subjective, out-of-season assessment: visual analog scale 1999 and 2000

	1999 VAS (mm)	2000 VAS (mm)	<i>P</i> -value
IT	46 (5–83)	30 (8–70)	< 0.06
Placebo	41 (8–92)	56 (8–92)	> 0.7
<i>P</i> -value†	> 0.7	< 0.05	

† Mann–Whitney U-test.

Table 5B. Subjective, out-of-season assessment: answers to questionnaire on impact of treatment at one-year follow-up

	Effect of treatment?		Symptom decrease?		Medication reduction?		Increased well-being?	
	Yes	No	Yes	No	Yes	No	Yes	No
IT	15	2	14	3	10	7	14	3
Placebo	8	9	8	9	5	12	5	12
<i>P</i> -value‡	< 0.03		> 0.07		0.17		< 0.006	

‡ Chi-squared test.

Table 6. Results of clinical and paraclinical tests at baseline (1999) and at one-year follow-up (2000)

	Immunotherapy [median(range)]			Placebo [median(range)]			Immunotherapy vs placebo	
	1999	2000	<i>P</i> value* 1999 vs 2000	1999	2000	<i>P</i> value* 1999 vs 2000	<i>P</i> value† 1999	<i>P</i> value† 2000
CPT (SQ-U)	10 ⁴ (10 ² -10 ⁵)	10 ⁴ (10 ⁴ -10 ⁵)	<0.05	10 ⁴ (10 ³ -10 ⁵)	10 ⁴ (10 ³ -10 ⁵)	>0.8	>0.6	>0.1
NPT (SQ-U)	10 ⁵ (0-10 ⁵)	10 ⁵ (0-10 ⁵)	0.40	10 ⁴ (0-10 ⁵)	10 ⁵ (0-10 ⁵)	>0.7	>0.4	>0.7
SPT (mm)	7.5 (5.5-11)	6 (3-9)	<0.002	9 (4.5-13.5)	7 (3.5-14)	>0.5	>0.1	<0.02
LPR (mm)	68.5 (0-109.5)	33.5 (0-57.5)	<0.05	71.5 (0-139)	70.5 (45.5-107)	>0.2	>0.3	<0.00001
HR (titre)	3 (0-4)	3 (0-4)	0.30	3 (0-5)	3 (0-5)	>0.6	>0.6	0.60
Spec-IgE (kU/ml)	38.8 (2.7-268)	45.5 (6.9-210)	>0.2	22.0 (4.8-123)	34.8 (17.7-124)	>0.1	>0.5	>0.4
Total-IgE (kU/ml)	140 (25-453)	222 (28-506)	>0.05	86 (20-433)	120 (34-1043)	0.001	>0.1	>0.3

* Mann Whitney U -test for paired comparison within groups. † Mann Whitney U -test for comparison between groups.

CPT, conjunctival provocation test; NPT, nasal provocation test; LPR, late-phase reaction; SPT, skin prick test for birch; HR, histamine release; Spec-IgE, birch pollen-specific IgE.

general well-being in the season, than having subjective symptom reduction or decreased use of medication. This indicates that IT is associated with improvement not described in symptom scoring, and we interpret this as increased quality of life (QoL) during the season (30). QoL has in other studies been measured during the season with validated questionnaires (34, 35). In-season assessment of symptoms and QoL is uncommon in routine, non-investigative IT (4), making the implementation of monitoring of QoL in routine IT difficult. In this respect, our findings are promising since consecutive post-seasonal VAS-scoring and the simple questionnaire were sufficient to monitor improvement. The power of our questionnaire and the possible presence of a season-of-response phenomenon remains to be elucidated.

The blinding of the study was evaluated using the questionnaire: eight out of 17 patients in the placebo group experienced a positive, unspecified effect of the treatment, compared to significantly more patients in the IT group (15 out of 17). In conclusion, the blinding in the IT group was influenced by clinical efficacy, whereas the blinding was maintained in the placebo group.

IT-treated patients experienced a decrease in conjunctival sensitivity to allergen exposure, as shown in previous studies (10). We were unable to demonstrate a decrease in nasal sensitivity in the IT group, but direct comparison between studies is difficult since different methods of allergen application have been used (24, 24, 36–39). In our study, the NPT produced no information that was not contained in the CPT. Furthermore, CPT is associated with an easier application of standardized amounts of allergen and has higher sensitivity and specificity in allergic disease than the NPT (16, 38). Taken together, CPT seems to offer the most convenience and reliability as a routine test for target organ sensitivity.

A highly significant reduction in the size of the intradermal late phase skin reaction (LPR) in the IT

group was found. The change in LPR size has been demonstrated to correlate with clinical efficacy of IT (5, 18, 30). The LPR was investigated on the anterior side of the antebrahium in an area not used for injection therapy, which excludes that the LPR change was due to local skin hyposensitization. There was a less significant reduction in the diameter of the SPT (30, 40, 41).

We report no significant change in total IgE or birch pollen-specific IgE during IT. The response to IT is characterized by an initial increase followed by a slow decline in specific and total IgE (6, 18, 41, 42). The lack of change in specific IgE could explain the insignificant changes in allergen-induced basophil histamine release since a strong correlation exists between decreases in these parameters (19, 43).

Our study fails to demonstrate useful predictors of the individual efficacy of IT. Only two patients described a lack of effect of IT, and they did not differ from the responders in any of the above mentioned parameters including the LPR decrease. It is not known if the patients were truly nonresponders to IT, since nonresponse requires at least 12 months of maintenance treatment (more than one season) – a period exceeding our study period (4).

We conducted IT according to international guidelines (4, 5), and the number of side-effects was at the placebo level. To our knowledge, no DBPC papers about side-effects during subcutaneous high-dose birch pollen IT in adults have been published. In a controlled study using grass pollen IT as control treatment in double-sensitized patients, only grade I and II side-effects were reported in the birch pollen group (13). Unexpectedly, significantly more side-effects were seen in the placebo group, suggesting that the few side-effects in the IT group were not due to type II errors. Placebo extract did not contain any additives not present in the Alutard extract except histamine dihydrochloride, and the observed difference cannot be explained as anything other than

random. Our observations support the emphasis on the need for placebo-controlled safety studies on each allergen, since side-effect frequency and severity of birch pollen IT have been found to differ from that of grass IT, for example (5, 13).

By using only three criteria obtained out-of-season (sex, presence of asthma, VAS score for previous season), the treatment groups became comparable in all baseline parameters except for the pre-seasonal use of nasal antihistamines. Thus our study confirms that it is possible to randomise patients to a phase III study using simple information on previous seasons obtained out-of-season – even though the risk of a type II error is present in our study (21, 24). The advantage of a run-in season is the possibility of using baseline symptom scores in allocation and subsequent statistical pairing of data. However, long-term studies might imply low patient compliance, and they do not abrogate the impact of inter-seasonal differences in pollen counts (7).

The higher pre-seasonal use of nasal antihistamines in the placebo group possibly reflects the uneven distribution of patients with self-reported allergy to earlier pollinating members of the *Fagales* family: alder (*Alnus glutinosa*) and hazel (*Corylus avellana*). Self-

reported allergy to hazel/alder was not associated with increased symptom severity in the birch pollen season. Birch pollen IT has been found effective in reducing hazel and alder symptoms in patients clinically sensitive to all three *Fagales* pollens, and it cannot be excluded that the observed difference was influenced by a clinical effect of IT (10).

In conclusion, we found that subcutaneous high-dose birch pollen IT is an efficacious and safe treatment for birch pollen IgE-mediated allergic rhinoconjunctivitis and asthma. Our DBPC study supports results in previous, non-DBPC studies. Importantly, it supports the single study moving the burden of evidence from category IIa to Ib: from uncontrolled to controlled evidence (7, 44).

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