

Original article

Allergen-specific immunotherapy in birch- and grass-pollen-allergic rhinitis. I. Efficacy estimated by a model reducing the bias of annual differences in pollen counts

Background: Evaluation of the efficacy of allergen-specific immunotherapy (IT) with pollen extracts is complicated by annual variation in pollen intensity. Our study aimed to evaluate the efficacy of birch and grass IT, taking into consideration these variations.

Methods: After 1 year of observation, 52 patients with rhinoconjunctivitis and allergy to birch as well as grass pollen were allocated to double-blinded clustered IT with aluminum-adsorbed extract produced from either birch (*Betula verrucosa*) pollen or grass (*Phleum pratense*) pollen. After 1 year of treatment, the patients continued IT with their original extract and also received the other extract. During the three consecutive pollen seasons, the rhinoconjunctivitis symptom score and the use of antihistamines, eye-drops, and oral prednisolone were recorded. Longitudinal data analysis was used to investigate the relation between different pollen counts and the magnitude of clinical efficacy.

Results: An effect of IT was found on symptom score, antihistamine intake, and eye-drop use for both birch and grass (P values <0.05). The mean reduction in symptom score/medication by IT ranged from 24% to 95%, depending on mean seasonal pollen counts. A minimum mean seasonal grass-pollen count of 20–30 pollen grains m^{-3} was required for the efficacy of grass IT to emerge.

Conclusions: A model was developed for evaluation of efficacy in longitudinal IT studies, taking the differences in annual pollen counts into consideration. The model showed a significant beneficial role of pollen IT in rhinoconjunctivitis patients allergic to birch and grass pollen.

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A relevant method to evaluate the efficacy of allergen-specific immunotherapy (IT) with pollen is to monitor the clinical manifestations of the allergic disease (rhinitis, conjunctivitis, and asthma) by diaries during the pollen seasons (1, 2). One-year, placebo-controlled pollen IT studies with comparison of seasonal symptom score and medication of two matched patient groups (actively and placebo-treated) are a relevant and commonly used design (3–6). However, the matching of groups according to parameters such as sex, age, duration of allergy, and results of specific IgE, skin prick test (SPT), and conjunctival provocation test

(CPT) does not imply identical clinical severity of the allergic disease. A difference in clinical severity before treatment could mask a significant difference between groups in symptom score/medication after treatment.

Another approach is to investigate the reduction in symptom scores/medication in relation to a pretreatment period, enabling the investigator to stratify according to the clinical parameters in question, and to follow the patient groups before and during treatment (7). This gives a stronger evidence of efficacy. However, pollen studies carried out over several years are complicated by annual variations in seasonal pollen counts, a complication which could interfere with an observed reduction in symptom score/medication (8–11).

In this IT study, we monitored symptom score and use of medication in patients allergic to birch and grass

Abbreviations. AUC: area under the curve; CPT: conjunctival provocation test; EAACI: European Academy of Allergology and Clinical Immunology; IT: allergen-specific immunotherapy; PEF: peak expiratory flow; SPT: skin prick test; SQ-U: standardized quality units.

pollen for three consecutive pollen seasons, one before and two during IT. A statistical model with longitudinal data analysis was developed to investigate the clinical effect of IT, and to reduce the bias of the differences in pollen counts. Included in this model was the means to test for differences in clinical severity of the allergic disease between the groups.

Material and methods

Patients

Fifty-two patients (28 men, 24 women) median age 26 years (range 18–52), with both birch- and grass-pollen-allergic rhinoconjunctivitis for a median of 16 years (range 5–33), were included in a 3-year controlled IT study. They all had a history of severe allergy to birch and grass pollen with symptoms in April and May, and May through July, corresponding to the local birch- and grass-pollen seasons, respectively. Seventeen of the patients also suffered from mild to moderate asthma with occasional symptoms; six of these patients had symptoms only during the pollen season. Ten patients were using inhaled corticosteroids. All the patients had a positive skin prick test (SPT) response (wheal area greater than 7 mm²) to *Betula verrucosa* and *Phleum pratense* (Soluprick SQ, ALK-Abelló, Hørsholm, Denmark) and a positive serum test for specific IgE of class 2 or greater (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden) for both

allergens (Table 1). All patients fulfilled the criteria for IT according to the position paper of the European Academy of Allergology and Clinical Immunology (EAACI) (2). Exclusion criteria were, in addition to those generally recommended (2): perennial rhinitis, clinical allergy to animal dander with more than weekly contact to the animal in question, pregnancy, and IT within the previous 5 years.

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

Symptom score and medication

In 1992–4, from April to August, the patients once a day recorded symptom score and medicine intake. The severity of each of five symptoms (sneezing, rhinorrhea, nasal congestion, itchy nose and/or throat, and itchy eyes) was scored on an arbitrary scale of 0–3 (0: no symptoms, 1: mild, 2: moderate, and 3: severe symptoms). The daily total symptom score was calculated. The patients were instructed to use medication only when needed, and to observe changes in medication requirement. The basic drug treatment was the antihistamine acrivastine (8 mg), with a maximum intake of three capsules daily, and antazoline-naphazoline eye-drops, with a maximum dosage of six drops in each eye daily. If symptoms were not sufficiently alleviated at the maximum dosage of antihistamine tablets and

Table 1. Patient characteristics at inclusion

Patient parameters	Group 1	Group 2	P value
Sex (M/F) number	12/14	13/13	0.781
Age (years) median (range)	27 (18–52)	26 (19–41)	0.313
Duration of allergy (years) median (range)	16 (5–30)	16 (5–33)	0.621
Asthma number			0.108
No	20	15	
Allergen contact only	3	3	
With or without allergen contact	3	8	
Birch SPT (mm ²) median (range)	36.7 (20.3–81.1)	44.2 (7.12–95.9)	0.77
Grass SPT (mm ²) median (range)	47.0 (18.5–76.3)	41.0 (15.8–182.8)	0.942
Birch-specific IgE number			0.929
Class 2	5	4	
Class 3	13	14	
Class 4	8	8	
Grass-specific IgE number			0.581
Class 2	3	1	
Class 3	10	11	
Class 4	13	14	
Birch CPT number			0.337
Not done	2	1	
10 ³ SQ-U/ml	2	0	
10 ⁴ SQ-U/ml	14	16	
10 ⁵ SQ-U/ml	8	9	
Grass CPT number			0.152
Not done	1	2	
10 ³ SQ-U/ml	1	3	
10 ⁴ SQ-U/ml	19	21	
10 ⁵ SQ-U/ml	5	1	

eye-drops, oral prednisolone in 10-mg tablets (Nycomed DAK, Copenhagen, Denmark) was prescribed. If more than 20 mg was needed the same day, the patient were asked to contact the investigator for a 1-week prednisolone treatment.

Pollen counts

The Burkard recording volumetric spore trap (Burkard Manufacturing Ltd, Rickmansworth, Hertfordshire, UK) was used for collecting pollen grains. The method is described elsewhere (12). The trap was placed on a roof, 15 m above ground, in Copenhagen city. Pollen grains were counted daily during the pollen seasons, and the amount of pollen was expressed as number of pollen grains/m³ air. The birch- and grass-pollen seasons were defined as the period including the mid 90% of total pollen of birch and grass, respectively.

Patient assignment for IT

After the birch- and grass-pollen season in 1992 (base line), the patients were assigned to active IT treatment with birch (group 1) or grass (group 2) by the method of minimization, to secure two balanced treatment groups (13, 14). This method took into account numerous variates (potential risk factors), and in this study seven variates were considered to be relevant to secure two equally balanced treatment groups: total symptom score for 1 week in the birch-pollen and grass-pollen peak seasons, specific IgE and SPT for birch and grass, and age. The variates were weighted, with 3 for symptoms and 1 for the remaining factors.

Twenty-six patients were assigned to group 1 (birch IT) and 26 patients to group 2 (grass IT). Patient groups were comparable with respect to sex, age, duration of allergy symptoms, specific IgE, and SPT and CPT sensitivity (Table 1).

Extracts

The *B. verrucosa* and *P. pratense* extracts for SPT (Soluprick SQ, 100 000 SQ-U/ml), for CPT (Aquagen SQ), and for IT (Alutard SQ) were supplied by ALK-Abelló, Hørsholm, Denmark. The activity of the extracts was given in standardized quality units (SQ-U). The content of the major birch-pollen allergen Bet v 1 was 12 µg per 100 000 SQ-U, and the content of the major grass-pollen allergen Phl p 5 was 20 mg per 100 000 SQ-U.

IT

From November 1992 to autumn 1993, the patients received double-blinded clustered IT with either *B. verrucosa* (group 1) or *P. pratense* (group 2) pollen extracts (Fig. 1). From November 1993 to the end of 1994, the patients continued IT with their original extract and in addition received the other extract, and

were subsequently treated with both *B. verrucosa* and *P. pratense* pollen extracts. The administration of IT followed the EAACI guidelines (2). Before each injection, the physician evaluated the patients' clinical condition, including peak expiratory flow (PEF), and assessed their suitability for IT. The clustered induction schedule is shown in Table 2. The interval between maintenance doses was gradually increased to 2 months. The patients received the injections at 30-min intervals and stayed in the clinic for at least 30 min after the last injection. Dose modifications were made if systemic reactions occurred, with either no increment, or reduction of the dose by up to four steps. Patients experiencing severe systemic reactions, repeated allergic rhinitis, or inconvenient, large local reactions during cluster IT were transferred to a schedule of one injection a week.

Statistics

Group characteristics were analyzed by chi-square test (categorical variables) and two-tailed Mann-Whitney U test (continuous variables). Daily mean symptom score and medication (area under the curve [AUC]) were compared groupwise for each year by the Mann-Whitney rank sum test. Patients who did not complete the treatment were excluded from final calculations.

Longitudinal data analysis was used to investigate the relation between different pollen counts and the magnitude of clinical efficacy. For each patient, seasonal means of symptom scores and medication were calculated. This was done to simplify the model. The seasonal means of each variable were treated as dependent variables as a function of the treatment, and the natural logarithm of the mean seasonal pollen count. A square root transformation was applied to normalize the residuals. For the square root response, a statistical fixed-effect model was formulated, basically following the structure of the usual multiple regression

Table 2. Schedule for dose increment

Day	Dose (SQ-U)
1	10
	100
	1000
7	2000
	4000
14	6000
	10 000
21	10 000
	20 000
28	30 000
	30 000
35	50 000
	50 000
42	100 000

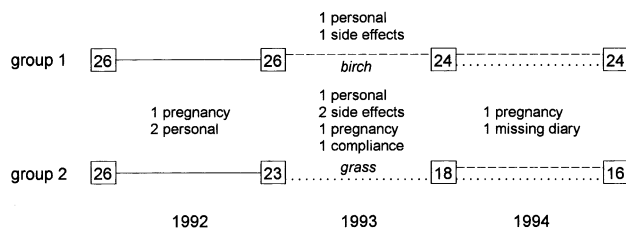


Figure 1. Study flowchart 1992–4. Number of remaining patients at beginning and end of each year for groups 1 and 2. Reason for dropouts described for each year. Straight line: baseline year; broken line: birch IT; dotted line: grass IT.

analysis:

$$Y_{ijk} = \mu + \alpha_i + \delta_1 p_j + \delta_2 t_{ij} + \delta_3 tp_{ij} + \gamma_{ik} + \xi_{ijk}(1)$$

Here i denotes the treatment group, i.e., group 1 or 2; j denotes the year, i.e., 1, 2, or 3; and k identifies patients. μ is a constant, and α_i is the group influence. α_i sums to zero over all i . p_j is the influence of pollen level; t_{ij} is the treatment effect, with the value 0 for no treatment, and 1 for treatment; tp_{ij} is the treatment-pollen interaction; and δ_1 , δ_2 , and δ_3 are their coefficients. γ_{ik} is random patient influence assumed to be independent and normally distributed with mean zero and variance σ_s^2 [$N(0, \sigma_s^2)$]. The errors ξ_{ijk} are independent and normally distributed with mean zero and variance σ^2 [$N(0, \sigma^2)$]. γ_{ik} and ξ_{ijk} are independent. The expected symptom score/use of medication, with and without IT, can be calculated according to the estimated δ s in the above model. A significant δ_1 indicated an influence of pollen level on symptom score/use of medication. A significant δ_2 indicated a treatment effect of IT. A significant δ_3 indicated an IT treatment effect dependent on pollen levels. The probability that each parameter of equation 1 could be omitted from the model was tested by the chi-square-type statistics Wald test (15).

The nonlinear optimization routines Newton-Raphson and REML were used, with similar response. The residuals for symptom score were normally distributed. The residuals for antihistamine use were only approximately normally distributed in the birch-pollen seasons 1992 and 1994, and, similarly, for eye-drop use, the residuals were only approximately normally distributed for all seasons. Hence these results should be interpreted with some caution.

As part of the analyses, the compound symmetry structure for the 3×3 within-subject covariance matrix was tested. This residual structure allowed an estimate of the year-to-year correlation between symptom score and use of medication for individual patients.

The analyses were performed with BMDP-5V, release 7 (BMDP 1992). Missing values were estimated by BMDP from covariate information. It was assumed that the reason an observation was missing was not related to the variable that was missed.

Results

Patients

The patient flow during the study is described in Fig. 1. Three patients from group 2 were excluded from the study after assignment, but before the start of IT: one patient due to pregnancy, and two patients due to personal reasons. During the first year, two patients from group 1 dropped out (one because of travel abroad and one due to subcutaneous nodules), and five patients from group 2 dropped out (one because of personal reasons not related to the clinical protocol, one because of pregnancy, one because of compliance problems, one after mild asthma symptoms after the first injection, and one because of repeated urticaria). During the second year, one patient from group 2 left the study because of pregnancy. Forty-one patients completed the 2-year IT treatment (24 in group 1 with 2 years of birch and 1 year of grass IT, and 17 in group 2 with 2 years of grass and 1 year of birch IT). One patient from group 2 was excluded from calculations due to missing diaries, leaving 24 patients from group 1 and 16 patients from group 2 for final calculations. The difference in number of dropouts between the birch and grass groups was significant ($P=0.02$). The dropouts did not, however, differ with respect to symptom score and medication during the baseline year, and with respect to patient characteristics (data not shown).

IT

During the first year of IT, the cumulated doses before the corresponding pollen season were medians of 613 110 SQ (range 266 210–645 110 SQ-U) and 724 110 SQ-U (range 68 110–948 110 SQ-U) for groups 1 and 2, respectively. One patient in group 1 did not reach the maintenance dose before the birch-pollen season (dose 80 000 SQ-U, cumulated dose 266 210 SQ-U), and two patients in group 2 did not reach the maintenance dose before the grass-pollen season (patient 1: dose 40 000 SQ-U, cumulated dose 436 110 SQ-U; patient 2: dose 10 000 SQ-U, cumulated dose 68 110 SQ-U). During the second year, the cumulated doses before the corresponding pollen season for the second allergen were medians of 715 110 SQ-U (range 358 710–869 110 SQ-U) and 548 665 SQ-U (range 141 210–773 110 SQ-U) for groups 1 and 2, respectively. All patients but one (group 2, dose 40 000 SQ-U, cumulated dose 141 210 SQ-U) reached the maintenance dose of the second allergen before the pollen season. The detailed course of IT and side-effects is described separately (16).

Symptom score, medication, and pollen counts

The mean daily symptom score and use of medication for the birch- and grass-pollen seasons 1992–4 for group 1 (birch IT in 1993) and group 2 (grass IT in 1993) are

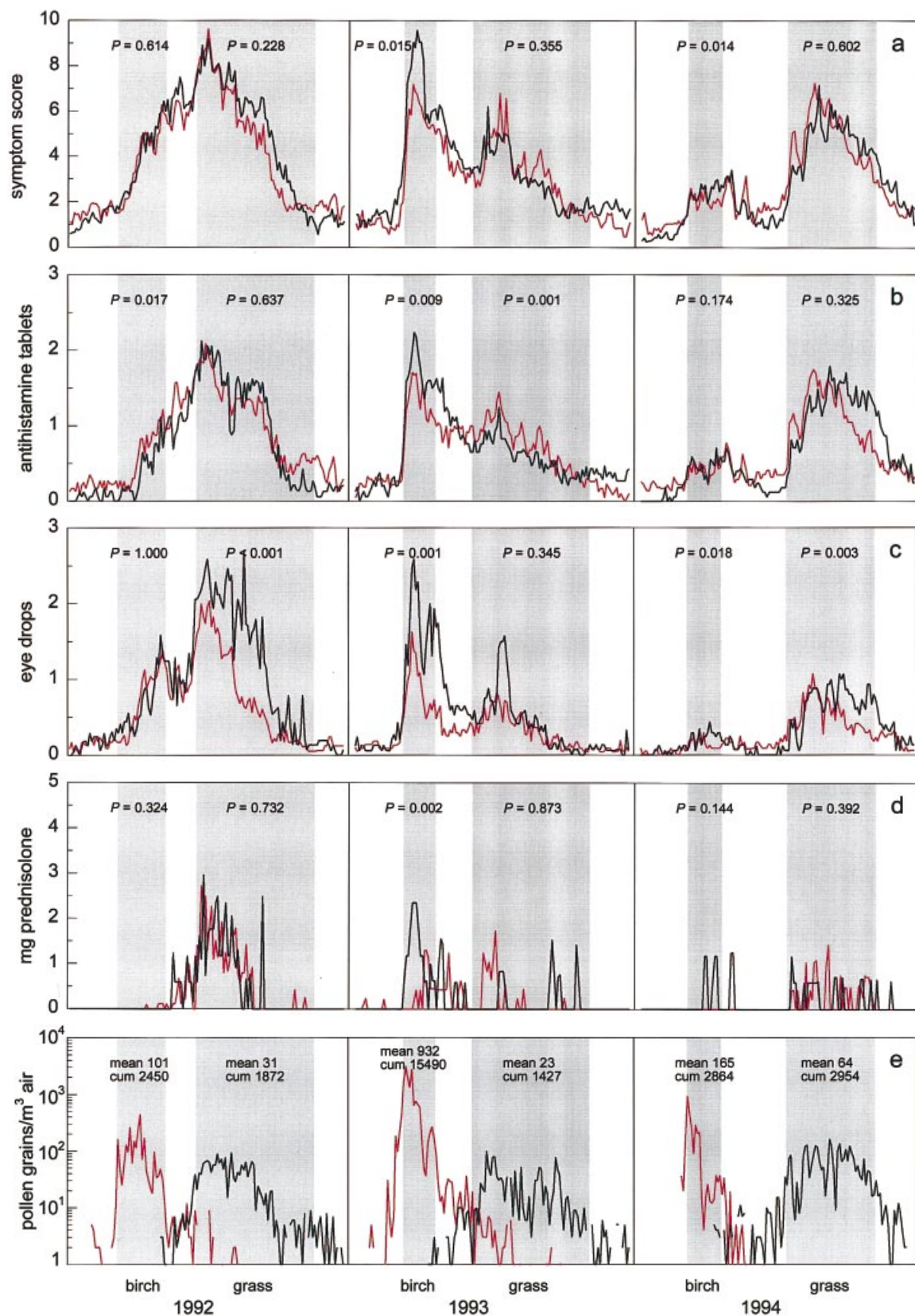


Figure 2. Daily group mean of **a**) symptom scores; **b**) number of antihistamines; **c**) number of eye-drops; **d**) mg prednisolone, during birch- and grass-pollen seasons 1992–4. Red line: group 1 (birch IT in 1993 and 1994; grass IT in 1994); black line: group 2 (grass IT in 1993 and 1994; birch IT in 1994). Daily birch- and grass-pollen counts (**e**). Red line: birch-pollen counts; black line: grass-pollen counts. Gray background: defined birch- and grass-pollen seasons. *P* values determined by Mann–Whitney rank sum test.

Table 3. Results from longitudinal variance analysis (equation 1). Values shown are estimated values, standard error in parenthesis, and *P* values in italics

Dependent variable	μ Constant	σ_s^2 Between patient variance	σ^2 Residual variance	α Group effect	δ_1 Pollen effect	δ_2 Treatment effect	δ_3 Pollen-treatment interaction
Birch							
Symptom score	284	0.274 (0.079)	0.224 (0.036)	−0.024 (0.101) <i>0.81</i>	0.334 (0.071) <i><0.0001</i>	−2.703 (0.687) <i>0.0001</i>	0.348 (0.122) <i>0.0043</i>
Antihistamine tablets	−771	0.106 (0.030)	0.083 (0.013)	0.032 (0.062) <i>0.61</i>	0.292 (0.043) <i><0.0001</i>	−0.912 (0.419) <i>0.029</i>	0.100 (0.074) <i>0.18</i>
Eye-drops	−210	0.122 (0.038)	0.130 (0.021)	−0.008 (0.070) <i>0.91</i>	0.170 (0.054) <i>0.0017</i>	−1.441 (0.523) <i>0.0059</i>	0.175 (0.093) <i>0.060</i>
Grass							
Symptom score	−1893	0.234 (0.066)	0.173 (0.027)	0.006 (0.091) <i>0.94</i>	1.111 (0.242) <i><0.0001</i>	2.386 (1.050) <i>0.023</i>	−0.738 (0.285) <i>0.0096</i>
Antihistamine tablets	−1411	0.116 (0.031)	0.057 (0.009)	−0.019 (0.062) <i>0.76</i>	0.658 (0.140) <i><0.0001</i>	1.398 (0.606) <i>0.021</i>	−0.411 (0.164) <i>0.012</i>
Eye-drops	−2423	0.199 (0.052)	0.094 (0.015)	−0.044 (0.080) <i>0.59</i>	0.878 (0.178) <i><0.0001</i>	2.726 (0.774) <i>0.0004</i>	−0.826 (0.210) <i>0.0001</i>

described in Fig. 2 (a–d). Before treatment, groups 1 and 2 were, as expected, similar in symptom score ($P=0.614$ and $P=0.228$ for the birch- and grass-pollen seasons, respectively). But minor differences were seen in medication, with group 1 higher in antihistamine tablets during the birch-pollen season ($P=0.017$) and group 2 higher in eye-drops during the grass-pollen season ($P<0.001$). The defined pollen seasons for 1992–4 are shown in Fig. 2 (e). As one would expect, the seasonal pollen counts and the duration of the pollen season differed from year to year (birch 1992: mean 101 pollen grains, 24-day season; 1993: mean 932 pollen grains, 16-day season; 1994: mean 165 pollen grains, 17-day season) (grass 1992: mean 31 pollen grains, 58-day season; 1993: mean 23 pollen grains, 58-day season; 1994: mean 64 pollen grains, 44-day season).

The birch-pollen season in 1993 had the highest birch-pollen counts ever measured in Denmark, with a

maximum daily pollen count of 3155 pollen grains per m^3 air. After 1 year of treatment (1993), the birch IT group 1 had statistically significantly fewer symptoms and used less medication during the birch-pollen season than the untreated group 2 (symptom score, $P=0.015$; antihistamine tablets, $P=0.015$; eye-drops, $P=0.001$; mg prednisolone, $P=0.002$) (Fig. 2, a–d). This strongly indicates a beneficial role of birch IT, even though the symptom score and medication for both groups seemed to follow the changes in pollen counts with higher values in 1993 (Fig. 2, a–e).

The grass-pollen count of 1993 seemed smaller than that of 1992, although the difference was not statistically significant ($P=0.272$, Mann–Whitney). The symptom score and medication seemed to follow the grass-pollen counts irrespective of treatment, with a decrease from 1992 to 1993 (Fig. 1, a–e). After 1 year of treatment (1993), the grass IT group 2 and the untreated group 1 were almost similar in symptom score and use

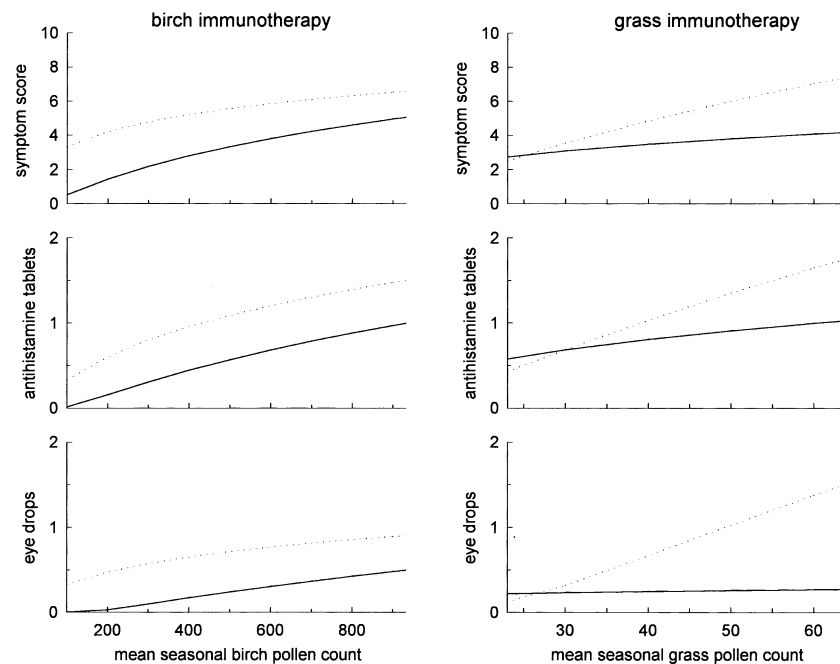


Figure 3. Calculated seasonal mean of symptom score, and seasonal mean of medication of average birch- and grass-pollen-allergic patient at different pollen counts, based on model. Dotted line represents untreated group, and solid line treated group.

of medication during the grass-pollen season (symptom score, $P=0.355$; antihistamine tablets, $P=0.001$; eye-drops, $P=0.345$; mg prednisolone, $P=0.873$). As such, one could question the benefit of the grass IT, even though the treated group and untreated group seemed to “change places”, with higher values during the grass-pollen season in 1992 and lower values during the grass-pollen season in 1993 for the grass IT group 2 (Fig. 2, a–d). No significant difference in changes (pretreatment year – treatment year 1) of seasonal mean symptom score and medication was found between the grass IT group 2 and the nontreated group 1 (symptom score, $P=0.194$; antihistamine tablets, $P=0.361$; eye-drops, $P=0.253$; mg prednisolone, $P=0.318$). The pollen seasons of birch and grass in 1994 seemed more intense than in 1992, with higher daily mean and cumulated seasonal pollen counts in 1994. An effect of IT was therefore suggested, as the symptom score/use of medication seemed to be reduced from 1992 to 1994 for both groups.

A longitudinal model was made to reduce the bias of the differences in pollen counts, and further to strengthen the reliability of the results by including the single patient as his own reference (see equation 1 in statistics). Table 3 shows the estimated parameters of the model. Fig. 3 shows the effect of treatment, as a function of mean seasonal pollen counts calculated from equation 1, and the estimated parameters in Table 3. No significant effect of treatment was observed for the dependent variable prednisolone intake because of too few patients using prednisolone, and the detailed analysis results are not included in Table 3 and Fig. 3.

No significant group effect (α) was observed; i.e., the two groups of patients could be regarded as taken from the same population of patients (Table 3). The variance between patients (σ_s^2) was larger than the residual variance (σ^2). Although we had a small number of patients in our study, the model was able to make conclusions on treatment effect.

There was a significant pollen effect (δ_1) (Table 3), and the symptom score and medication were positively dependent on pollen counts; i.e., the higher the pollen count, the higher the symptom score/medication (Fig. 3). A significant effect of IT (δ_2) was found for both birch and grass; i.e., if the patients were treated, they had lower symptoms and used less medication (Table 3 and Fig. 3). However, a seasonal grass-pollen load above a count of 20–30 pollen grains/m³ was necessary for an effect on symptom score/medication to emerge (Fig. 3). In most cases, the IT effect was dependent on pollen counts (δ_3). For grass, the difference in symptom score and medication between the treated and untreated groups increased with increasing pollen intensity. This phenomenon was not observed for birch (Fig. 3). The magnitude of improvement induced by active treatment is visualized in Fig. 3. Depending on mean seasonal pollen count, a reduction in symptom

response from 24% to 84% was observed for birch IT treatment, and up to 39% reduction for grass IT treatment. The reductions in antihistamine response were 34–95%, and up to 20% for birch and grass IT treatment, respectively. The reductions in eye-drop response were 48–88%, and up to 82% for birch and grass IT, respectively.

The compound symmetry structure within patients from year to year was fully justified for the dependent variables symptom score, and antihistamine and eye-drop use, with correlation values between 0.5 and 0.7, indicating that 25–50% of one year's value could be explained by the last year's value for the same patient; that is, a given patient's mean seemed to be constant above or below the group mean.

Discussion

Like several other IT studies (7–10, 17, 18), this study also showed an important annual variation in pollen counts. The duration of the defined pollen season, as well as the pollen counts, differed during the 3-year period. As a consequence, difficulties in the evaluation of the efficacy of treatment emerged in this study. For birch IT, symptom score and medication rose after the first treatment year, questioning the efficacy of treatment. However, the birch-pollen season during the first treatment year was the most intensive of the seasons registered in Denmark. A statistical difference between the treated and untreated group was found this year, indicating efficacy after all. For grass, symptom score and medication decreased after the first treatment year, but for both groups. No statistical difference was seen, after the first treatment year, between the treated and untreated groups, except for antihistamine intake. This could be caused by a low grass-pollen season in 1993, as we could not expect an effect of grass IT to emerge below a certain mean seasonal pollen count. A tendency toward higher grass-seasonal values of symptom score/medication before IT was seen for group 2 (not significant); this could contribute to the missing difference between the grass IT group 2 and the untreated group 1 in the first treatment year. Another explanation could be that group 2 had a higher symptom score and medication use during the preceding birch-pollen season, and thereby was not comparable at the beginning of the grass-pollen season (priming). One could argue that dropouts could contribute to the missing difference after grass IT, as a higher dropout rate was seen in the grass group. However, these patients did not differ in symptom score and medication during the baseline year, and with respect to patient characteristics. Furthermore, the number of presumably more sensitive patients leaving the study due to side-effects did not differ between the birch and grass groups.

To overcome the indicated problems in the efficacy evaluation of pollen IT, which arose when pollen counts differed from year to year, we used a combined model, including the pollen counts and the patient results of the different years. A significant efficacy of both birch and grass IT was shown, with a reduction in symptom score and antihistamine and eye-drop use in the treated patients. The difference between the two groups might have been higher if the interaction between birch and grass had been investigated. However, in the statistical model, the birch- and grass-pollen seasons were investigated independently to make the model as simple as possible.

The study indicated that the efficacy of grass IT did not show below a certain low seasonal mean grass-pollen count, whereas the efficacy of birch IT could be demonstrated even at low birch-pollen counts. Similarly, other studies were able to show a significant difference in symptom and medication scores between actively treated and placebo-treated grass-pollen-allergic patients only during the period with high pollen exposure (4, 19). In a multicenter study, a significant difference between actively treated and placebo-treated grass-pollen-allergic patients was found only in centers with an average weekly pollen count of more than 40 pollen grains per cubic meter (6). It is not clear whether this was due to the natural history of the pollen seasons, birch being an abrupt and short season with great variance in pollen counts, and grass a long season with less variation in pollen counts.

An increasing difference in symptom score and medication between the treated and untreated groups was observed with increasing grass-pollen intensity. In our study, the highest mean seasonal grass-pollen count was observed during the second year of treatment (1994). The observed change in treatment effect, which was dependent on low and high pollen levels, may have been influenced by a placebo effect during 1994, as only the first year of treatment was truly double-blinded in this study design (during the second year of treatment, the patients knew they were treated). The statistical analysis, however, clearly identified a significant treatment effect (and pollen effect), despite any placebo effect during 1994.

Our model specifically estimated the variation that came from the single patient, i.e., it used the patient as his/her own control, but still the statistical testing of efficacy was done by testing differences between the two groups. To the clinician and the patient, a statistically significant reduction in number of patients with a reduction in severity of the disease, and not a statistical difference between groups, may be a more relevant parameter. However, this requires a clinically relevant definition of the size of reduction in disease severity. An arbitrary grading of the magnitude of clinical efficacy has been proposed (1), with a reduction in symptom/medication score of 30–44% as low efficacy, 45–59% as

moderate efficacy, and more than 60% as high efficacy. With respect to the question of whether differences in results depend on the point of origin, i.e., whether there are higher percentage reductions when the pretreatment values are small, our results of 20–95% reduction in responses, depending on mean seasonal pollen count, indicate a clinically important role of IT. The question of whether these arbitrary gradings are useful in daily life and correspond to the patients' own interpretation of the treatment remains to be investigated.

In this study, the patients were randomized according to age, specific IgE, and SPT and symptom score. Even though the two groups were comparable before treatment according to symptom score, they still differed in antihistamine intake during the birch-pollen season, and eye-drop use during the grass-pollen season before treatment. These possible group differences in disease severity before treatment might bias the results of 1-year placebo-controlled studies, and could include false-positive or false-negative treatment effects. In the present study, the statistical model allowed us to test whether the patient groups were actually alike, and no differences between the groups were observed after adjustments for patients leaving the study.

Symptom scores depend upon medication. Several investigators have combined symptom scores and use of medication in a symptom-medication score, but the relative weight of scores has varied from one study to another (4, 5, 7, 9, 19). This study showed a large between-patient variation in symptom score and medication, and showed that the response of a given patient was related to the response of previous years, indicating that if a patient, for example, tended to use much medication before treatment, this attitude toward medication remained, irrespective of the efficacy of IT. In the study population, a tendency was observed toward higher use of antihistamine at the end of a long pollen season, with the same degree of symptoms (20). This indicated that the patients' tolerance of symptoms changed during the season, and that they were more willing to use drugs at the end of the season. For these reasons, we avoided a somewhat arbitrary weight, and looked at symptom score and medication separately, even though problems could have arisen if symptoms and medication showed discordant changes. The model showed the efficacy of the treatment in both symptom scores, and antihistamine and eye-drop use. The model could not determine the prednisolone parameter because only a few patients were using prednisolone.

In conclusion, birch IT proved effective in a longitudinal, blinded, controlled study of patients with dual sensitivity to birch and grass pollen. Pollen IT appeared to be specific for the pollen employed, with a reduction of symptoms and medication during the birch-pollen season in the birch IT patients compared to the grass IT patients. The results of the study revealed that the

evaluation of clinical efficacy in pollen IT studies lasting several years is complicated by differences in annual pollen counts. The ability to demonstrate clinical efficacy of grass-pollen IT appeared to be dependent upon the magnitude of the pollen counts. A statistical longitudinal model for the evaluation of the efficacy of pollen IT, which takes into consideration the differences of pollen counts, has been presented and proven useful. The model demonstrated a beneficial role of both birch- and grass-pollen IT in patients with dual sensitivity. However, the model should be used in

further longitudinal IT studies lasting several years, with other seasonal pollen courses, to confirm its effectiveness.

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