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# Effects of sublingual immunotherapy for multiple or single allergens in polysensitized patients

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**Background:** Sublingual immunotherapy (SLIT) has proven efficacy in treating respiratory allergy.

**Objective:** To compare the clinical and functional effects and the effect on nasal eosinophils of SLIT with either single or combination allergens.

**Methods:** We performed an open-labeled, controlled, 4 parallel-group randomized study with 58 patients sensitized to birch and grasses only who had rhinitis and bronchial hyperreactivity in both pollen seasons. Patients were recruited for the study from January 1, 1999, to June 30, 2001. The patients received SLIT for birch, SLIT for grass, SLIT for birch and grass, or drugs only. Symptom and medication scores, forced expiratory volume in 1 second, bronchial hyperreactivity, and nasal eosinophil counts were evaluated in both pollen seasons at baseline and after 2 and 4 years.

**Results:** Ten patients dropped out and 48 completed the study. No change in all the considered parameters vs baseline was seen in patients treated with drugs only. Those patients receiving SLIT for grass or birch had a significant clinical improvement and nasal eosinophil reduction vs baseline and vs patients who did not receive SLIT in the target season ( $P < .01$ ) but also in the unrelated pollen season ( $P < .05$ ). The patients receiving SLIT for grass and birch improved as well, and their improvement in clinical symptoms and inflammation was significantly greater than in patients treated with SLIT for the single allergens. Minor changes were seen in the forced expiratory volume in 1 second, since it remained within the reference range in the whole population.

**Conclusion:** In patients sensitized to grass and birch, SLIT with the 2 allergens provided the best clinical results. Nevertheless, SLIT with birch only or grass only also provided a measurable improvement in the grass season and birch season, respectively.

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## INTRODUCTION

Allergen immunotherapy is capable of modifying the immunological response to the offending allergen at the earliest stages, probably acting via the T regulatory cells.<sup>1,2</sup> This modification results in decreased inflammatory phenomena and, in parallel, in symptomatic improvement during the exposure to allergens themselves.<sup>3</sup> Although the efficacy of the subcutaneous route is well established, safety concerns<sup>4,5</sup> have favored the development of sublingual immunotherapy (SLIT). Currently, convincing evidence exists of the efficacy of SLIT in respiratory allergy<sup>6,7</sup> and of its excellent safety profile in young children.<sup>8,9</sup> Based on these results, the clinical use of SLIT has been accepted in the World Health Organization and Allergic Rhinitis and its Impact on Asthma guidelines.<sup>3,10</sup>

As opposed to infancy, in adulthood respiratory allergy is often characterized by the presence of multiple sensitizations, with only approximately 20% of patients being monosensitized.<sup>11</sup> Since immunotherapy is specific for the allergen, rather than for the disease, its use in polysensitized patients is still a matter of debate, and no clinical trial has been designed to dissect the response to SLIT with single vs multiple allergens. Indeed, in clinical practice it is usually possible to identify the clinically relevant allergen, for instance, based on the distribution of symptoms over the year or with allergen-specific challenges. Nevertheless, when more than one allergen is causative, it is still not clear if it is more efficacious to use multiple allergen extracts or if immunotherapy for a single allergen can extend its effects to other allergens. This study was designed to compare, in patients sensitized to birch and grasses, the clinical and functional effects and the effect on nasal eosinophil counts of SLIT with either single or combination allergen therapy. This study could be performed in our region because the pollen seasons of grass and birch do not overlap.

## METHODS

### Study design

This study was an open-labeled, randomized, controlled, 4 parallel-group clinical trial. The inner ethical committee did not allow *any* double-blind, placebo-controlled design mainly

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because of the duration of the study and the availability of other effective treatments for the patients. The study was not intended to demonstrate the clinical efficacy of SLIT but rather to compare single and associated allergens. To achieve the maximum effect, the first evaluation was made after 2 years of treatment. Patients sensitized to both birch and grasses, after a baseline evaluation (year 2001), were randomly allocated to receive either drug therapy alone (controls), drugs and SLIT for birch (SLITB), drugs and SLIT for grass (SLITG), or drugs and mixed SLIT (SLITBG). The treatment was continued for 4 years. Diary cards, pulmonary function test (PFT) with methacholine challenge, and nasal scraping for eosinophil count were evaluated at baseline, after 2 years, and after 4 years during both pollen seasons (birch and grasses). The study design is shown in Figure 1.

#### Patients and diagnosis

Adults (>18 years) were recruited among outpatients from January 1, 1999, through June 30, 2001. Mandatory inclusion criteria were as follows: (1) clinical history of rhinoconjunctivitis from at least 2 years, with symptoms limited to the pollination period of birch and grasses (February to July); (2) skin test (>5 mm) positivity to birch and grasses only; (3) normal spirometry results (forced expiratory volume in 1 second [FEV<sub>1</sub>] >79% of predicted with bronchial hyperreactivity [methacholine provocation dose that caused a decrease in FEV<sub>1</sub> of 20% (PD<sub>20</sub>) <1,200 µg]) during pollen seasons; and (4) mild intermittent asthma associated with rhinitis. Patients sensitized to allergens other than birch and grass, with persistent asthma, or who had impaired pulmonary function were not admitted and neither were patients with malignancies, chronic obstructive pulmonary disease, or systemic immunological disorders. The diagnosis of rhinitis was made according to clinical criteria,<sup>8</sup> including sneezing, itching,

rhinorrhea, and nasal congestion with or without eye symptoms. Asthma was diagnosed based on the presence of one or more episodes of wheezing, cough, or chest tightness (diurnal or nocturnal) during the pollen season.<sup>12</sup> Skin prick tests were performed, according to guidelines,<sup>13</sup> with a panel of allergens (ALK-Abellò, Milan, Italy), including mite, grasses, olive, birch, cat and dog dander, ragweed, mugwort, Parietaria, *Alternaria*, and *Cladosporium*. These allergens are responsible for most sensitizations in Italy.

#### SLIT and concomitant treatments

The SLIT (Anallergo, Florence, Italy) was prepared as a glycerinated solution to be administered as sublingual drops in the morning (because patients had empty stomachs). The vaccine had to be kept under the tongue for approximately 2 minutes and then swallowed. The SLITBG patients took the 2 extracts, from separate vials, at the same time. The extracts were standardized through combined radioallergosorbent test inhibition and bioequivalence method: the potency was expressed as radioallergosorbent test units per milliliter (RU/mL). The build-up phase (approximately 50 days) involved the administration of increasing concentrations, from 100 through 3,000 RU/mL. In the maintenance phase, SLIT was administered on alternate days for approximately 4 years until 2005. The dose per month was approximately 100 µg of Bet v 1 and 70 µg of Phl p 1 (on average 5 to 10 times greater than the recommended subcutaneous immunotherapy dose). All patients were prescribed drug treatment according to the Allergic Rhinitis and its Impact on Asthma guidelines during pollen seasons: cetirizine or loratadine (10 mg once daily), nasal cromolyn (10 mg/d), and inhaled salbutamol on demand. Intranasal beclomethasone, 2 puffs per nostril twice daily (400 µg/d), was prescribed by the physician only in the case of poor response to antihistamines and cromolyn.

#### PFT and methacholine challenge

Spirometry and methacholine challenge were performed at baseline and at the second and fourth years both in the birch (March) and grass (June) pollen seasons. PFTs were performed by a plethysmographic cabin and computerized spirometer (Masterlab Yaeger, Wurtzburg, Germany). FEV<sub>1</sub> and forced vital capacity were measured. The methacholine challenge was performed when the patient was asymptomatic and had been free of salbutamol for at least 12 hours. A dosimeter (Masterlab Yaeger) activated by the inhalation effort was used to give increasing doses of methacholine from 30 to 1,200 µg. Hyperreactivity was considered severe for a PD<sub>20</sub> less than 400 µg, moderate for a PD<sub>20</sub> of 400 to 800 µg, and mild for a PD<sub>20</sub> less than 1,290 µg.<sup>14</sup>

#### Clinical scores

All patients were followed up with regular clinical visits during the whole study and completed a 1-month diary card in March (birch season) and May (grass season). The following symptoms were considered: nasal itching, sneezing, rhinorrhea, nasal obstruction, cough, wheezing, and eye itching or redness. Each symptom was scored as follows: 0, absent;

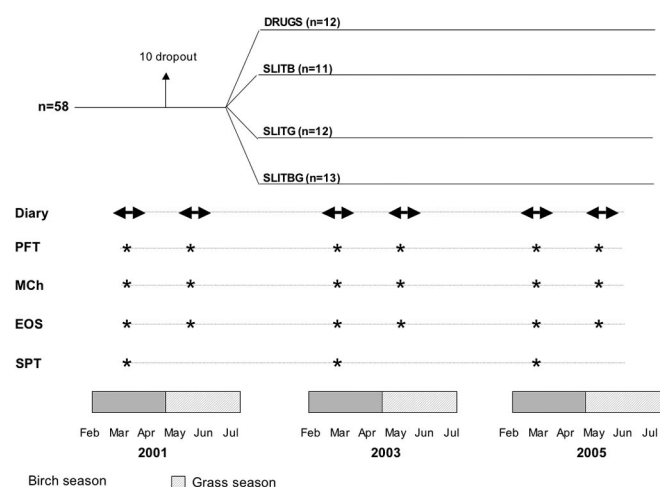


Figure 1. Study design. PFT indicates pulmonary function test; MCh, methacholine challenge; EOS, nasal eosinophils count; SLITB, sublingual immunotherapy for birch; SLITBG, sublingual immunotherapy for birch and grass; SLITG, sublingual immunotherapy for grass; and SPT, skin prick test.

1, mild; 2, moderate; and 3, severe. The use of medications was scored with 1 point for each dose of salbutamol, nasal steroid, cromolyn, or antihistamine. The total monthly symptoms plus medication score was then calculated for each patient and used for analysis. The maximum possible monthly score for symptoms was 720.

#### Nasal eosinophils

Nasal smears were collected from the anterior third of the inferior turbinate. The smears were transferred on a glass slide, air-dried, stained by May Grünwald-Giemsa, and read in optical microscopy. Eosinophils were expressed as the percentage of the total white cells per 10 fields. Smears were performed during birch and grass pollen seasons. Patients were advised to discontinue use of intranasal nasal steroids (if any were being taken) at least 7 days before the nasal scraping.

#### Pollen count

Pollen counts were performed daily by the Associazione Italiana di Aerobiologia at the Busto Arsizio Hospital, located in the center of the geographic region. The mean weekly count of the grains per cubic meter was calculated.

#### Statistical analysis

Comparison of sex ratio at baseline was tested by Pearson  $\chi^2$ , whereas the Kruskal-Wallis test for independent groups was used to verify differences among the treatment groups,<sup>15</sup> regarding the patient age and clinical parameters ( $FEV_1$ , symptoms plus medication score, methacholine reactivity). Median values at different times (2001, 2003, and 2005) were previously tested using the Kruskal-Wallis test, and then pair-wise comparisons were performed by the Mann-Whitney  $U$  test. Changes in symptoms plus medication score,  $FEV_1$ , nasal eosinophils, and methacholine after 2 and 4 years were tested by the Friedman test for paired samples, and then pair-wise comparisons were computed by the Wilcoxon test.<sup>16</sup> The Dunn-Sidak significance level correction method was used to determine the  $\alpha$  level:  $\alpha' = 1 - (1 - \alpha)^{1/k}$ , where  $k$  is the number of comparisons.<sup>17</sup> Because our purpose was to compare the 4 groups of SLIT treatment,  $k = 6$  and subsequently  $\alpha'_{0.05, 6} = 0.0085$ ,  $\alpha'_{0.01, 6} = 0.00167$ , and  $\alpha'_{0.001, 6} = 0.000167$ . When variations were tested inside each treatment group, 3 comparisons were made and then the corrected  $\alpha$  values were  $\alpha'_{0.05, 3} = 0.0169$ ,  $\alpha'_{0.01, 3} = 0.0033$ , and  $\alpha'_{0.001, 3} = 0.00033$ . The probability level was computed using a complete randomization method (permutation or exact test;  $P_{\text{Exact}}$ ) or by a Monte Carlo simulation based on a 1,000,000 sampled tables ( $P_{\text{MC}}$ ; 26, 27) when computation was not possible. All the statistical analyses have been computed using SPSS statistical software, version 12.01 (SPSS Inc, Chicago, IL).

## RESULTS

The pollen seasons are illustrated in Figure 2. Peak pollination occurred in March for birch and in May for grasses, and no overlapping occurred between the 2 pollen seasons. In

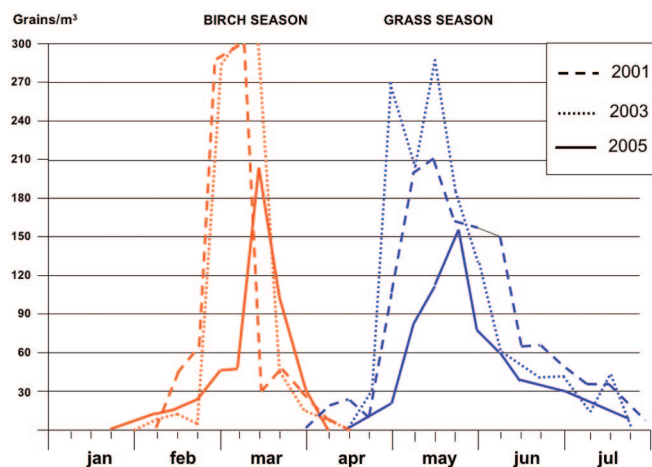


Figure 2. Pollen counts for birch and grasses in the years 2001, 2003, and 2005.

2005 the pollen counts were approximately 30% lower than in 2001 and 2003.

Fifty-eight patients (mean age, 27 years; age range, 19–43 years; 35 men) were enrolled and allocated to the 4 groups; 10 patients dropped out. Five of the dropouts were from the drug group (1 retired consent, 1 worsening of symptoms, 3 noncompliance with clinical visits) and 5 from the SLIT groups (2 retired consent, 2 noncompliance with visits, 1 worsening of symptoms). A mild oral itching was reported by 3 patients (1 in each SLIT group). No pharmacologic intervention or dose adjusting was required for these events. Age and clinical parameters (nasal eosinophils,  $FEV_1$ , and clinical scores) at baseline did not significantly differ in the 4 groups as indicated in Table 1. Sex ratio also did not differ ( $\chi^2 = 0.033$ ,  $P_{\text{Exact}} > .05$ ).

As shown in Figure 3A the symptoms plus medication score of the 4 groups of patients were homogeneous at baseline. No significant change was found in the symptoms plus medication score over time in the patients treated with drugs only (Friedman tests;  $P_{\text{MC}} > .05$ ). In all 3 groups receiving SLIT, a significant clinical improvement was found vs baseline at 2 and 4 years, irrespective of the allergen. This finding means that the patients treated with grass SLIT improved also in the birch pollen seasons and the patients receiving birch SLIT improved also in the grass season. The same results were obtained if the symptoms scores (Fig 3B) and medication scores (Fig 3C) were analyzed separately.

A similar behavior was detected for methacholine challenge, as shown in Figure 4. The bronchial hyperreactivity measured with methacholine improved significantly in all the SLIT groups in both pollen seasons and vs baseline, whereas no change was seen in patients treated with drugs only. The  $FEV_1$  remained within the reference range ( $>79\%$  of the predicted) during the whole study period in all groups. It displayed a significant overall increase (Friedman tests;  $P_{\text{MC}} < .001$ ) in the mixed SLIT group in both pollen seasons,

Table 1. Baseline Clinical Characteristics\*

| Characteristics                 | Controls     | SLIT for birch | SLIT for grass | SLIT for birch and grass |
|---------------------------------|--------------|----------------|----------------|--------------------------|
| Age                             | 27.1 (1.9)   | 27.7 (1.8)     | 27.3 (1.6)     | 26.2 (1.4)               |
| No. with asthma                 | 4            | 5              | 5              | 6                        |
| Eosinophils, birch season       | 28.2 (2.2)   | 33.9 (5.3)     | 34.7 (2.4)     | 35.6 (2.3)               |
| Eosinophils, grass season       | 27.8 (2.6)   | 29.0 (2.5)     | 32.8 (2.7)     | 34.0 (1.4)               |
| FEV <sub>1</sub> , birch season | 98.1 (5.7)   | 100.6 (3.23)   | 97.9 (6.4)     | 97.8 (0.8)               |
| FEV <sub>1</sub> , grass season | 102.6 (2.2)  | 105.7 (3.0)    | 98.5 (1.7)     | 96.8 (0.9)               |
| SMS, birch season               | 355.8 (14.8) | 391.2 (32.2)   | 387.4 (14.5)   | 382.8 (14.3)             |
| SMS, grass season               | 360.8 (18.4) | 389.2 (19.5)   | 416.7 (42.2)   | 374.6 (19.9)             |
| Asthma score birch season       | 36 (10)      | 52 (16)        | 39 (12)        | 49 (12)                  |
| Asthma score grass season       | 39 (12)      | 38 (12)        | 28 (9)         | 45 (11)                  |

Abbreviation: FEV<sub>1</sub>, forced expiratory volume in 1 second; SLIT, sublingual immunotherapy; SMS, symptoms plus medication score.

\* Data are presented as mean (SEM).

although the mean values at 2 and 4 years did not differ vs baseline. Furthermore, a significant decrease in FEV<sub>1</sub> vs baseline was noticed in the patients receiving drugs only and a significant increase after 4 years of SLIT vs baseline was apparent in the grass pollen season in patients receiving mixed SLIT (data not shown). Nasal eosinophils (Fig 5) remained unchanged in the patients treated with drugs only. In the SLIT-treated patients, nasal eosinophils were significantly decreased in both pollen seasons at 2 and 4 years, but for the patients who received SLIT for birch a significant decrease of eosinophils during the grass pollen season was found only at the fourth year.

Considering the 4 parameters (symptoms plus medication score, FEV<sub>1</sub>, methacholine, and eosinophil count), in the birch season no difference was found between the patients treated with birch only and those receiving both extracts. The same happened in the grass season, during which grass SLIT and combined SLIT were equivalent. Nevertheless, the improvement of symptoms plus medication score, eosinophil count, and methacholine reactivity (not FEV<sub>1</sub>) was significantly greater in the birch season than in the grass season in patients receiving SLIT for birch and the same happened in the SLIT for grass group during the grass season (Table 2). This particular effect is exemplified for symptom plus medication scores in Figure 6.

## DISCUSSION

Immunotherapy with multiple allergens in polysensitized patients is a common practice in the United States, whereas in Europe the treatment with one or a few relevant allergens is preferred. On the other hand, most clinical trials assessing the efficacy of immunotherapy have been performed with a single allergen. Few studies have specifically addressed the effect of mixed allergens,<sup>19–22</sup> and 2 of these studies<sup>19,22</sup> failed to demonstrate a significant clinical efficacy, as exemplified by the study by Adkinson et al<sup>22</sup> that was performed using conventional immunotherapy with the maximum tolerated doses. In addition, no study has compared the efficacy of immunotherapy with one vs multiple allergens. In current clinical practice, the causal role of an allergen should be

clearly ascertained before prescribing immunotherapy for the allergen itself.<sup>1</sup> Of note, because of the cross-reactivity within related species (eg, grasses, weeds), it is often sufficient to vaccinate with the relevant allergen(s).<sup>18</sup> The only study similar to the present one was performed in children to evaluate the value of adding omalizumab to immunotherapy<sup>21</sup> for birch and/or grasses. That study showed some allergen selectivity of the clinical response, although this was not the test hypothesis of the trial, and in the study the pollen seasons partially overlapped. Another study assessed the efficacy of cat and dog extracts used alone or together,<sup>23</sup> but because of the small population size and the low potency of dog extract, the results were inconclusive.

Taking advantage of the clear separation of the pollen seasons of birch and grasses in our geographical region, we attempted to dissect the effects of immunotherapy for the single and mixed allergens with the sublingual route that is safe and well accepted by patients. In previous studies, SLIT has been shown to be able to down-regulate the inflammatory infiltration at the target organ<sup>24,25</sup> and to reduce the degree of bronchial hyperresponsiveness during pollen seasons.<sup>25,26</sup> Thus, we evaluated, in addition to clinical symptoms, nasal eosinophil counts and methacholine reactivity. Indeed, this study is not double-blind and placebo controlled because of practical and ethical reasons and because our aim was not to demonstrate the efficacy of SLIT but to obtain information about the performance of single vs multiple allergens. In this regard, the use of objective parameters (nasal eosinophils and pulmonary function) allowed minimizing the possibility of investigator-induced biases.

The results of our comparative study suggest that, in the case of birch-grass sensitization, the SLIT with both allergens performs globally better than SLIT with the single allergens. In fact, the birch and grass SLIT provided a significantly greater improvement of symptoms plus medication score in both seasons compared with the single allergens, which were anyway effective in their target seasons. One challenging and unexpected result (Figs 2 to 5) was that patients treated with SLIT for birch or grass improved also in the grass and birch season, respectively. This fact was consistently reproducible

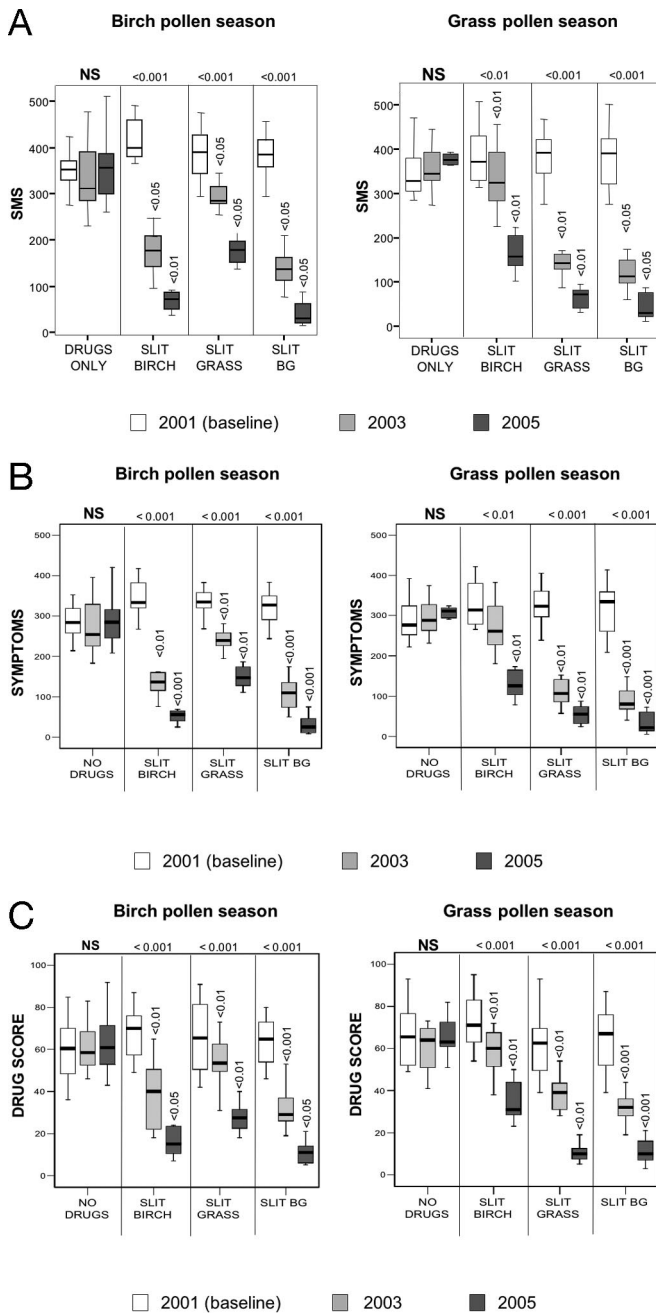


Figure 3. Symptoms and medication scores (SMSs) (A), symptoms scores (B), and medication scores (C). Scores are plotted separately for the birch pollen season (left) and grass pollen season (right) at baseline and after 2 years and 4 years of treatment. Boxes represent the 1 to 3 interquartile range and the median. Whiskers are the extreme values. Significance levels of Friedman tests within each group are reported outside the graphs; those of the Wilcoxon test for pairwise comparisons are reported near the boxes. NS indicates nonsignificant; SLIT, sublingual immunotherapy.

also for bronchial hyperreactivity and nasal eosinophils, which are reliable markers of allergic inflammation.<sup>27</sup> Thus,

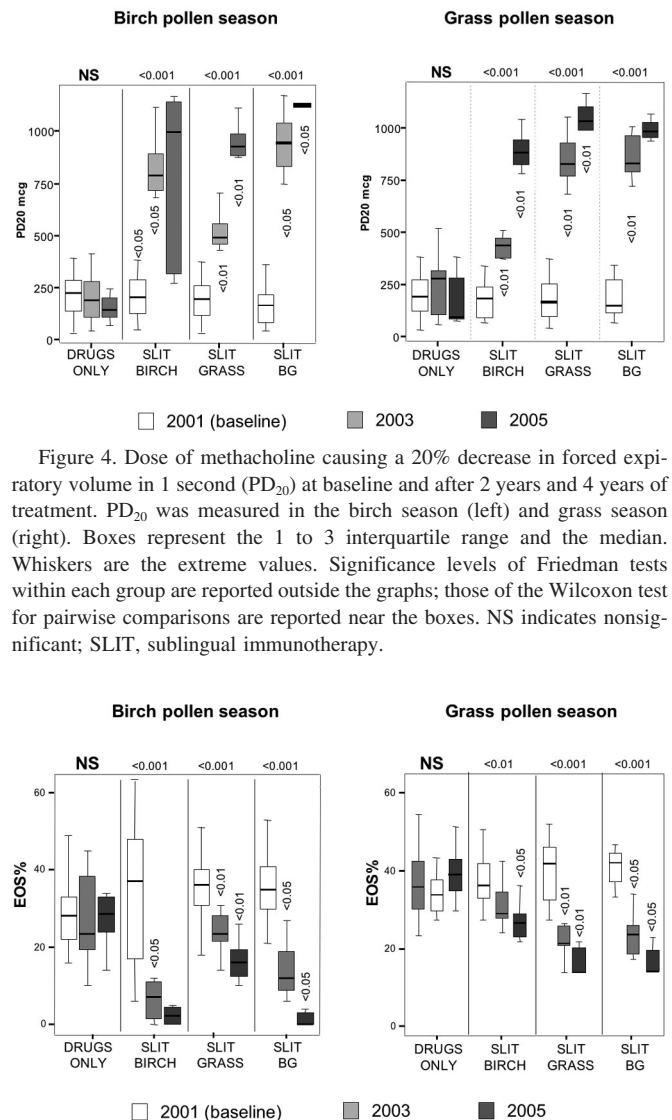


Figure 4. Dose of methacholine causing a 20% decrease in forced expiratory volume in 1 second (PD<sub>20</sub>) at baseline and after 2 years and 4 years of treatment. PD<sub>20</sub> was measured in the birch season (left) and grass season (right). Boxes represent the 1 to 3 interquartile range and the median. Whiskers are the extreme values. Significance levels of Friedman tests within each group are reported outside the graphs; those of the Wilcoxon test for pairwise comparisons are reported near the boxes. NS indicates nonsignificant; SLIT, sublingual immunotherapy.

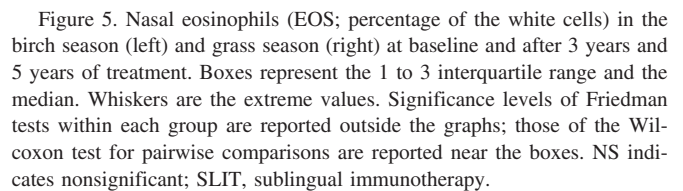


Figure 5. Nasal eosinophils (EOS; percentage of the white cells) in the birch season (left) and grass season (right) at baseline and after 3 years and 5 years of treatment. Boxes represent the 1 to 3 interquartile range and the median. Whiskers are the extreme values. Significance levels of Friedman tests within each group are reported outside the graphs; those of the Wilcoxon test for pairwise comparisons are reported near the boxes. NS indicates nonsignificant; SLIT, sublingual immunotherapy.

an extended or carryover effect of the administration of a single allergen should be hypothesized. In this regard, we have no reasonable explanation for the observed effect, because our study did not evaluate specific immunological parameters. We can hypothesize that the SLIT-induced increase in interleukin 10 as previously reported<sup>28,29</sup> plays a critical role, since interleukin 10 exerts its regulatory effect in a partially aspecific way.<sup>30</sup> In addition, a role of cross-reactive proteins could be invoked to explain the nonallergen specific

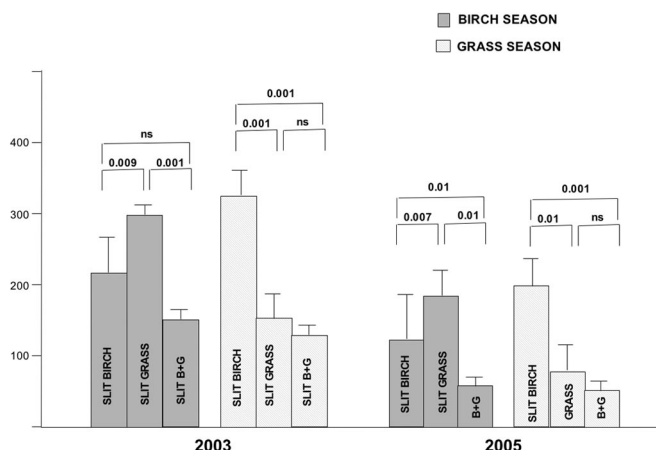


Figure 6. Comparison among the 3 sublingual immunotherapy treatments concerning the effect on symptom and medication scores (mean and SD) in years 2003 and 2005. ns indicates nonsignificant; SLIT, sublingual immunotherapy.

effect. It is well known that profilins are partly shared by birch and grasses,<sup>31–33</sup> although their clinical role seems not to be of great relevance with the considered allergens.

In conclusion, this open study suggests that the effect of SLIT with one allergen is extended also to the other allergens (birch or grass). Since this result could have relevant clinical and economic implications, it needs more rigorous confirmations. First, the observation should be repeated in double-blind trials, with larger samples of patients. Second, it is important to know if the effect is present also with other unrelated allergens (eg, pollens and mites). Finally, an immunological approach, including the evaluation of the behavior of T regulatory cells and cytokines, could help elucidate the clinical observation.

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Table 2. Comparison Among Treatments for Each Considered Outcome

|                 | Clinical score           |                          | FEV <sub>1</sub>        |              | Nasal eosinophils        |                          | Methacholine reactivity  |                          |
|-----------------|--------------------------|--------------------------|-------------------------|--------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                 | Birch season             | Grass season             | Birch season            | Grass season | Birch season             | Grass season             | Birch season             | Grass season             |
| Year 2003       |                          |                          |                         |              |                          |                          |                          |                          |
| SLITB vs SLITG  | 0.09<br>(Better SLITB)   | 0.01<br>(Better SLITG)   | NS                      | NS           | 0.008<br>(Better SLITB)  | 0.001<br>(Better SLITG)  | 0.009<br>(Better SLITB)  | 0.001<br>(Better SLITG)  |
| SLITB vs SLITBG | NS                       | 0.001<br>(Better SLITBG) | NS                      | NS           | NS                       | 0.001<br>(Better SLITBG) | NS                       | 0.002<br>(Better SLITBG) |
| SLITG vs SLITBG | 0.001<br>(Better SLITBG) | NS                       | NS                      | NS           | 0.001<br>(Better SLITBG) | NS                       | 0.001<br>(Better SLITBG) | NS                       |
| Year 2005       |                          |                          |                         |              |                          |                          |                          |                          |
| SLITB vs SLITG  | 0.007<br>(Better SLITB)  | 0.01<br>(Better SLITG)   | NS                      | NS           | 0.008<br>(Better SLITB)  | 0.001<br>(Better SLITG)  | NS                       | NS                       |
| SLITB vs SLITBG | 0.01<br>(Better SLITBG)  | 0.001<br>(Better SLITBG) | NS                      | NS           | NS                       | 0.001<br>(Better SLITBG) | NS                       | NS                       |
| SLITG vs SLITBG | 0.001<br>(Better SLITBG) | NS                       | 0.01<br>(Better SLITBG) | NS           | 0.001<br>(Better SLITBG) | NS                       | NS                       | NS                       |

Abbreviations: FEV<sub>1</sub>, forced expiratory volume in 1 second; NS, nonsignificant; SLITB, sublingual immunotherapy for birch; SLITG, sublingual immunotherapy for grass; and SLITBG, sublingual immunotherapy for birch and grass.

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