

ORIGINAL PAPER

Cluster immunotherapy with a glutaraldehyde-modified mixture of grasses results in an improvement in specific nasal provocation tests in less than 2.5 months of treatment

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Summary

Background Cluster immunotherapy is becoming increasingly used. It allows for a rapid build up phase and the administration of higher doses of allergen in a shorter period of time.

Objectives To evaluate the effect of short-term pre-seasonal immunotherapy using a glutaraldehyde-modified allergen vaccine in reducing specific nasal hyperreactivity in nasal challenge tests.

Materials and Methods Thirty-three patients were selected. All patients had a positive history of allergic rhinitis and skin tests to grass pollen, although most of them (72.7%) were sensitized to other allergens as well. The study was conducted outside of the pollen season and the patients did not receive any pharmacological treatment during this period of time. Two randomized groups of patients were established; Group A: 22 patients (13 females and nine males) and Group B, 11 control patients (seven females and four males). Patients in Group A received immunotherapy with a vaccine containing 50% of the wild grasses *Trisetum paniceum* and *Dactylis glomerata*. All patients underwent titrated nasal provocation tests (NPT) before and after completion of the study (2.3 and 2.8 months for Groups A and B, respectively). The administration schedule consisted of 0.1 and 0.2 mL at day 1, followed by 0.3 and 0.5 mL at day 7, 0.5 mL after 2 weeks followed by 0.5 mL monthly. A single vial was used containing an allergen concentration of 10 000 TU/mL (105 µg of total protein and 24.6 µg of Group 1 plus 5 allergens/mL). A mean of 6.5 injections were administered to Group A patients between NPTs.

Results There were no significant differences between both groups at the beginning of the study ($P=0.48$). At the end, only Group A patients needed significant greater threshold concentrations for a positive NPT than at the beginning ($P=0.002$).

Conclusions A short-term cluster pre-seasonal immunotherapy with a modified vaccine containing a mixture of grass pollen is effective as determined by an objective measure after only a mean 2.3 months of treatment.

Keywords allergens, allergic rhinitis, cluster immunotherapy, Dactylis, nasal challenges, Trisetum

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Introduction

Immunotherapy is safe and effective to treat allergic respiratory diseases produced by the inhalation of grass pollen allergens [1, 2]. Cluster immunotherapy is increasingly being used as a safe and effective method of immunotherapy. It allows for a rapid and safe build up phase, reaching maintenance in just a few injections. It was first described by Norman et al. [3] using the recently

developed allergoids. Although it has also been successfully explored with native allergens [4–6], the commercial availability of allergoids in Europe is considered the most important reason for the increased use of cluster immunotherapy. It has been proposed, that immunotherapy with allergoids allows the administration of higher doses of allergen [7, 8] and, therefore, clinical effects of immunotherapy with allergoids may start earlier than with conventional immunotherapy.

Several studies have been published using grass allergoids for sub-cutaneous immunotherapy and showing various degrees of efficacy. The comparison of these studies is difficult since the use of formaldehyde [9–16] or glutaraldehyde [17–20] produces allergoids with different characteristics. Other important differences are the lengths of the studies, the doses given, the administration schedules and the ways in which efficacy was assessed. While some studies have used rush, or cluster protocols and evaluated safety, others have used more traditional build-up schemes and evaluated efficacy using different parameters. To the best of our knowledge, studies addressing a pre-seasonal clinical effect on grass specific nasal hyperreactivity of a short-term cluster grass immunotherapy schedule using glutaraldehyde-modified allergoids have not been published. Pre-seasonal immunotherapy schedules usually use a cluster immunotherapy protocol, which consists of the grouped administration of two or more doses of immunotherapy in 1 day. Maintenance can be reached within 1–2 weeks.

In the present study, we have assessed the pre-seasonal clinical effect of a glutaraldehyde-polymerized grass allergen vaccine administered following a cluster protocol. Titrated nasal provocation tests (NPTs) performed immediately before and after immunotherapy were used as objective outcome measure. The vaccine formulation contained a 50% mixture of *Trisetum paniceum* and *Dactylis glomerata*. These wild grasses were selected based on their wide distribution in Madrid and surrounding areas [21]. *T. paniceum* is a grass species belonging to the Poaceae family, which is characteristic of the Mediterranean continental climate. It has also been described as one of the major causes of type I allergy in the Madrid region [22]. This species, and mainly *Trisetum flavescens*, is also present in the middle and north of Europe [23]. *D. glomerata* also belongs to the Poaceae family and is widely distributed in Spain and most European countries. While the allergenicity of *D. glomerata* is well established [24], less is known about the allergenicity of *T. paniceum*, but it also seems to contain Groups 1 and 5 allergens [25].

For the present study, patients from an outpatient clinic were selected for their clinical history of allergic rhinitis, and positive skin tests, to grasses without any restriction to other sensitizations. The results indicate that this treatment is effective, as detected by NPTs, in just 2.3 months of immunotherapy even in polysensitized patients.

Materials and methods

Study design, inclusion and exclusion criteria

The study was performed as a prospective, controlled, open trial. Patients were randomized and allocated to the treatment, or control group. The study was conducted outside of the pollen season between November and

February and the patients did not receive any pharmacological treatment, or rescue medication since patients were free of symptoms. Before the inclusion in the study, all patients were skin tested with a battery of allergens and selected based on a clinical history of allergic rhinoconjunctivitis and positive skin prick tests to grass pollen allergens. The inclusion criteria were the following: (1) age range between 12 and 60 years; (2) positive history of allergic rhinitis with or without asthma at least during the last 2 grass pollen season, (3) positive skin prick tests to grasses, (4) and no prior immunotherapy and (5) a positive nasal challenges test at ≤ 1852 BU/mL and negative to saline. The exclusion criteria for receiving immunotherapy were those outlined in the WHO position paper on allergen immunotherapy. Furthermore, patients needed to complete baseline and final nasal challenges with grass allergen extracts in order to be properly evaluated and the data analyzed. The research protocol was approved by the Review Board of the Clinical Center, and written informed consent was obtained from each patient.

Patient population

Thirty-three patients (20 females and 13 males, mean age 30.2 years) met the above mentioned criteria and were randomized using the random utility from the Corell Word Perfect software (Corel Corporation, Ottawa, Canada) and included in the study. Random numbers comprised from 1 to 9. Those patients assigned 1, 3 or 5 were included in the control group, and those assigned 2, 4, 6, 7, 8 or 9 were included in the immunotherapy group. Overall, 24 individuals had rhinoconjunctivitis alone and nine rhinoconjunctivitis and asthma. After randomization, 22 individuals (13 females and nine males, mean age 29.7 years) were allocated to Group A and 11 individuals (seven females and four males; mean age 30.8 years) to Group B. The clinical histories and previous therapies were recorded at the first visit. None of the patients was receiving pharmacological treatment. The mean duration of allergic rhinitis was 6 years (range, 2–9 years).

Skin tests

All 33 patients were skin tested with a standardized commercially-available battery of aeroallergens at 50 HEP (Immunotek, Madrid, Spain) including *T. paniceum*, *D. glomerata*, *Olea europaea*, *Platanus hispanica*, *Cupressus arizonica*, *Chenopodium album*, *Dermatophagoides pteronyssinus*, *Blatta orientalis*, cat and dog dander, and non-standardized extracts of *Alternaria alternata* and *Aspergillus fumigatus*. Major allergen content (Group 1 plus 5) in *T. paniceum* and *D. glomerata* was approximately 80 µg/mL. Histamine chlorhydrate at 10 mg/mL was used as a positive control, and 50% glycerol-saline as a negative control. All skin-test sites were evaluated

after 20 min. The area of each weal was measured by planimetry using the Prick-Film system (Immunotek, Madrid, Spain). Briefly, the area of the weals was drawn on the skin device and scanned using the Prick-Film software. The skin prick test results were expressed in mm². A positive reaction was defined as a weal with at least half the size of the histamine lacking a reaction to the negative control [26].

Vaccine and treatment schedule

The vaccine contained a 50% mixture of two standardized grass pollen (*D. glomerata* and *T. paniceum*) extracts in form of polymerized allergoids (Clustoid[®], Roxall Medizin/Dr Beckmann Pharma GmbH) manufactured by Immunotek, Madrid, Spain. Clustoid is an aluminum hydroxide-adsorbed depot allergen preparation produced by modification of the allergen by glutaraldehyde, which is presented in a single vial at a concentration of 10 000 TU/mL. Major allergen content was estimated by scanning densitometry [27] in both grass species in the step prior to modification, as suggested in the European Pharmacopoeia [28]. Groups 5 plus 1 grass allergen content of the vaccine was 24.6 µg/mL.

The administration schedule consisted in the injection on the first day of 0.1+0.2 mL in different arms at a 30 min interval, followed by 0.3+0.5 mL on the seventh day and another 0.5 mL on day 15th. Afterwards, 0.5 mL was administered at monthly intervals during the length of the study. After the last injection, patients were scheduled for the final challenge test.

Safety evaluation

Safety was evaluated following the position paper on Allergen Immunotherapy of the European Academy of Allergy and Clinical Immunology (EAACI) [29]. Local reactions were quantified by measuring the diameter of erythema of the reaction. The reactions were categorized as immediate (onset during the first 30 min after the administration) and delayed (onset afterwards). Immediate reactions with a diameter smaller than 5 cm and delayed reactions smaller than 10 cm were considered to be clinically irrelevant. Systemic reactions were graded as follows: grade 0: no symptoms; grade 1: unspecific symptoms – reactions probably not IgE mediated, i.e. discomfort, headache or arthralgia; grade 2: mild systemic reactions – mild rhinitis or asthma responding adequately to antihistamines or β_2 -agonist therapy; grade 3: non-life-threatening systemic reactions – urticaria, angioedema or severe asthma, responding well to treatment; and grade 4: anaphylactic shock – rapidly evoked reaction of itching, flushing, erythema or bronchial obstruction, requiring intensive treatment. Immediate reactions were evaluated by the responsible allergist. Patients were in-

structed to immediately contact the clinic, or the responsible allergist, in case of delayed reactions.

Titrated nasal provocation test

NPT were performed following methods previously described [30]. Peak nasal inspiratory flow (PNIF) was measured in litres per minute using the In-Check Nasal inspiratory flow meter (Clement Clarke International Ltd, London, UK). Three consecutive measurements were performed, and only the maximal value was used for calculation. Each volunteer was trained to use the technique before the nasal challenge. Challenge tests always started with the determination of baseline for PNIF and by using Immunotek diluent as a control. A challenge test with serial allergen concentrations was performed to determine the allergen concentration that elicited a reaction based on the presence of at least two of the three following criteria: (1) a decrease in PNIF of 40% of baseline; (2) five or more sneezes over a period of 10 min; and (3) at least 0.5 g of nasal secretions (evaluated by weighing the tissue). Allergen (0.2 mL/nostril) was nebulized at increasing concentrations into each nostril every 15 min with a DeVilbiss atomizer. PNIF (best of three measurements), sneezes, and nasal secretions were recorded 10 min after challenge with each allergen concentration until the reaction threshold was reached. Patients with a positive reaction, and who met the entry criteria (positive reaction with an allergen concentration of ≤ 1852 BU/mL), were randomly assigned to receive, or not, immunotherapy.

The evaluation of the patient was conducted in a blinded fashion. The same batch of standardized allergens (mixture of two grass pollens; Immunotek, Madrid, Spain) was used throughout the study. Serial dilutions of the allergen extract (206, 617, 1852 and 5556 BU/mL) were prepared immediately before the challenge test in sterile diluents (pyrogen-free saline solution with 0.05% human albumin). Major allergen content (Groups 1 plus 5) in 5556 BU/mL was 9 µg/mL. Allergen nasal challenges were not conducted if any of the following were applicable: (1) positive reaction with saline solution, (2) presence of unstable allergic rhinitis and or asthma, peak flow rate below 20% of normal values, (3) sign or symptoms of allergic, viral, or infectious rhinitis 2 weeks before the nasal challenge tests; (4) symptoms or signs of allergic rhinitis during the course of the study; (5) nasal pathology (major nasal septum deviation or major poliposis), and (6) any medication that may affect the test parameters (oral, or topical antihistamines, steroids, or antidepressants with antiallergic properties).

Pollen counts

Pollen grains were sampled with volumetric spore trap according to previously described techniques [21]. A

Burkard trap (Burkard Manufacturing Co., Rickmansworth, Herst, UK) was installed on the roof of the clinic (20 m high), which is located in the east of the urban area of Madrid with no parks or gardens nearby. Pollen identification was conducted by expert personnel and was based on comparison with reference slides and photographs. This information is available at www.pole-n.es.com.

Statistical analysis

Descriptive statistics were expressed as the mean with the 95% confidence intervals. The Wilcoxon matched-pairs signed-ranks test (two-tailed), the Mann-Whitney (two-tailed) and ANOVA tests were used for comparative statistics. Contingency table analysis (Fisher's exact test) was used to analyze the percentage of patients that had improved, or not, at the end of the study. The sample size was calculated by estimating that at the end of the study 60% of the patients would need more than three times the initial amount of allergen extract to experience a positive nasal test in the active group and 10% in the control group for an $\alpha = 0.05$ (two-tailed) and a power of $\beta = 0.2$ (two-tailed). The number of patients was increased in 20% order to cover the possible drop-outs. The final estimated sample size was 20 patients in the active and 10 in the control group. A 2 : 1 ratio was selected for the study due to ethical reasons.

Results

Patients

Thirty-three patients were recruited for the study and randomized. Thirty-one patients (93.9%) completed the trial. Two patients (one in each group) were excluded from the final analysis due to a lack of sensitivity in the first nasal challenge test. Four additional (three in the active and one in the control group) had a positive nasal reaction with the saline solution at the second nasal challenge test and, therefore, were excluded from the statistical analysis. Overall, the severity of the allergic rhinitis was considered as moderate to severe according to the Aria classification [31]. A flow chart of the study design and the patients included in each group is shown in Fig. 1.

Pollen Counts

Pollen counts were conducted for grasses, *C. arizonica*, *P. hispanica* and *O. europaea*, because allergic patients in Madrid are frequently sensitized to these pollens. Results are shown in Fig. 2. Small amounts of grass pollen were detected in the air during the length of the study. Higher peaks of *C. arizonica* pollen were detected. *O. europaea*

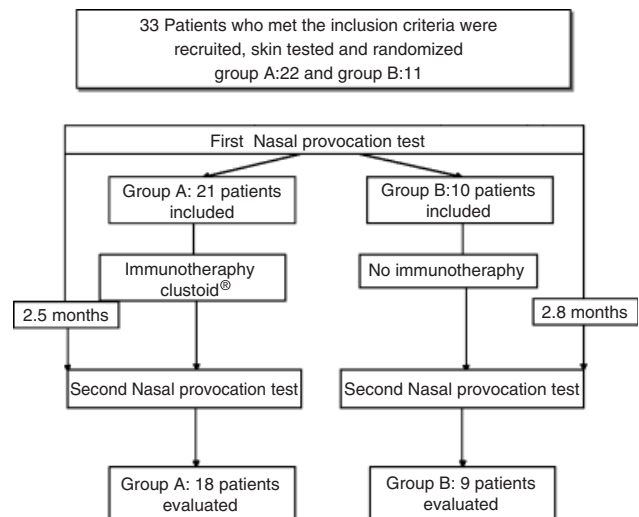


Fig. 1. Schematic diagram showing the design of the study. At the end of the study, a total of 27 patients could be analysed; 18 in Group A and nine in Group B.

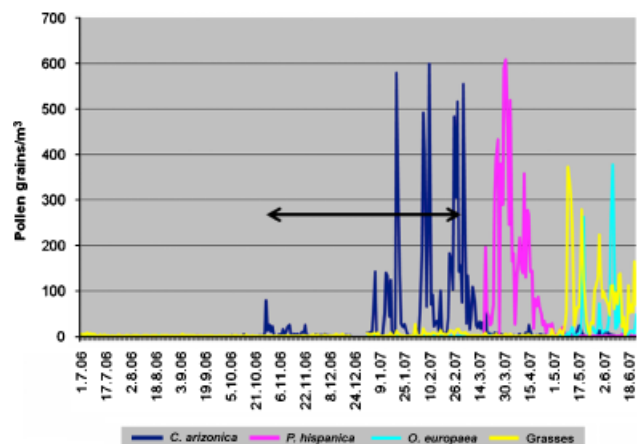


Fig. 2. Pollen counts in the area of Madrid, including *Cupressus arizonica*, *Platanus hispanica*, Grasses, and *Olea europaea* pollens from July 1st 2006 to June 30th 2007. The arrow indicates the period in which the study was conducted.

and *P. hispanica* clearly pollinated outside of the period in which the study was conducted.

Immunotherapy group and safety

Twenty-one patients (13 females and eight males) were treated with the vaccine. Patients in Group A received a mean of 6.5 (five to seven) injections during a mean period of 2.3 (1.2–3.5) months. Overall, the treatment was well tolerated. No systemic reactions were recorded. One patient experienced one immediate large local reaction, with a diameter of 8 cm after 10 min of the injection. It was successfully treated with a cold pad application and 5 mg of desloratadine. Six delayed local reactions with a

mean diameter of 11 cm were recorded and treated also with a cold pad application and 5 mg of desloratadine.

Control group

Ten patients from Group B (six females and three males) completed the study. The mean time between the initial and final provocation test in Group B was 2.8 (1.7–3.5) months.

Skin tests

Grass pollen skin prick test responses to grasses were positive in all 33 patients. No significant differences were seen between both groups regarding the mean size of skin test reactions for *T. paniceum*, *D. glomerata* or histamine. Detailed information about the skin test results at the time of patients' recruitment is shown in Table 1. As can be seen most of them were also sensitized to other aeroallergens.

Nasal challenges

Thirty-one (93.9%) patients had a positive response at baseline. There were no significant differences in the threshold concentrations needed to produce a positive NPT between both groups at the beginning and at the end of the study.

In the first challenge, the mean drop in PNIF was –42% (–72% to 0%); 66% of the patients scored positively for a drop of more than 40% in PNIF. The mean weight of nasal secretions was 2316 mg (410–9208); 96%

of the patients scored positively for a weight greater than 500 mg. The mean number of sneezes was 6.7 (1–27); 63% of the patients had five or more sneezes after the challenge.

In the second challenge, the mean drop in PNIF was –40% (–67% to 0%); 59% of the patients scored positively for a drop of more than 40% in PNIF. The mean weight of nasal secretions was 2381 mg (567–7966); 100% of the patients scored positively for a weight greater than 500 mg. The mean number of sneezes was 6.8 (1–21); 59% of the patients had five or more sneezes after the challenge. The ANOVA test demonstrated that the differences were not statistically significant.

The mean $\pm$ standard deviation (SD) allergen concentration needed to produce a positive reaction in Group A was 597.7 ± 559.3 vs. 823.1 ± 732.3 BU/mL in Group B ($P = 0.52$; Mann–Whitney test). However, the differences between both groups became significant at the end of the study. The mean $\pm$ SD allergen extract needed in Group A was 1097.3 ± 619.51 BU/mL and 617.2 ± 503.98 BU/mL in Group B ($P = 0.022$). Individual results are shown in Fig 3a and b.

The Mann–Whitney test was also used to compare the differences of nasal threshold levels for a positive NPT at the beginning and at the end of the study. At the end of the study, patients in Group A needed a significantly

Table 1. Mean skin test results to different allergens and histamine, expressed in mm², and rates of sensitization to other aeroallergens

	Group A (n = 22)	Group B (n = 11)
Mean skin test size <i>Trisetum paniceum</i> in mm ²	40.4	50.4
Mean skin test size <i>Dactylis glomerata</i> in mm ²	47.0	43.1
Mean size reaction to histamine in mm ²	17.2	16.4
Other sensitizations (%)	77.26	63.6
<i>Olea europaea</i>	59.1 (29 mm ²)	54.6 (29 mm ²)
<i>Platanus hispanica</i>	45.4 (21 mm ²)	32.2 (21 mm ²)
<i>Cupressus arizonica</i>	18.2 (19 mm ²)	36.4 (13 mm ²)
<i>Chenopodium album</i>	27.3 (12 mm ²)	18.2 (5 mm ²)
<i>Dermatophagoides pteronyssinus</i> (%)	9.1 (26 mm ²)	18.2 (34 mm ²)
<i>Blatta orientalis</i>	9.1 (18 mm ²)	0
Cat	27.3 (20 mm ²)	9.1 (17 mm ²)
Dog	22.7 (11 mm ²)	18.2 (10 mm ²)
<i>Alternaria alternata</i>	9.1 (15 mm ²)	9.1 (7 mm ²)
<i>Aspergillus fumigatus</i>	9.1 (8 mm ²)	0

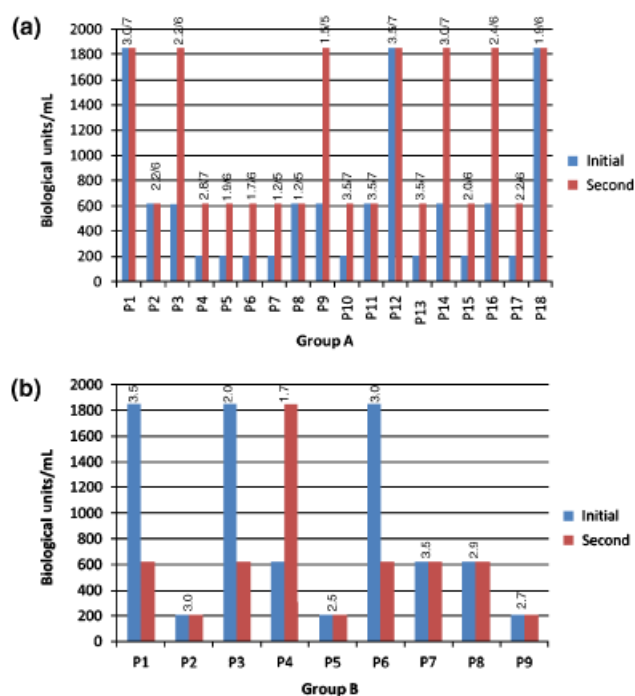


Fig. 3. (a, b) Individual results of all the participants in the study. Blue bars (first bar) denote the allergen concentration at which they reacted at the beginning and the red bar (second bar) the concentration needed at the end of the study to experience a positive reaction. Numbers on the columns denote number of months in the study before initial and second nasal provocation test/number of injections. The Y-axis represents Biological U/mL.

larger threshold concentration than at the beginning (525.89 ± 519.63 vs. 960.1 ± 677.1 BU/mL at the end; $P=0.013$) (Fig. 2a). The difference was not significant for Group B (891.67 ± 741.9 vs. 617.2 ± 503.9 BU/mL; $P=0.31$) (Fig. 3b).

The Wilcoxon Matched-Pairs Signed Rank test was used to search for differences within the groups at the first and second nasal challenges. These differences were highly significant when analysing Group A patients ($P=0.0005$). By contrast, no significant differences were detected for Group B patients ($P=0.375$).

The Fisher's exact test was used to compare the numbers of patients who improved, or not, in both groups after the second nasal challenge. Twelve patients (66.6%) experienced an improvement in Group A vs. 1 (11.1%) in Group B ($P=0.008$). All eight patients who reacted at the lowest dose of 206 BU/mL experienced a significant improvement, while none of the three, who reacted at the highest screening dose of 1852 BU/mL, did (see Fig. 3a).

Discussion

We have demonstrated that a short course of preseasonal immunotherapy (mean of 6.5 injections given in an average time of 2.3 months) with a modified grass pollen vaccine induces objective improvement on nasal challenges in a significant number of patients. In the present study we used a cluster pre-seasonal schedule, which is being increasingly used to treat allergic patients against seasonal allergic rhinitis. This schedule has advantages over the classical immunotherapy build up schemes. Maintenance is reached with a fewer number of injections, increasing compliance and producing a clinical benefit after a short period of time. Cluster immunotherapy is commonly used with allergoids, which allows for the administration of higher allergen doses. In spite of these advantages, a limited number of cluster pre-seasonal immunotherapy studies using grass pollen allergoids have been published.

A continuing problem in the evaluation of immunotherapy is that most allergic patients have multiple sensitivities, whereas the treatment tends to be allergen specific. It has been shown that immunotherapy is less effective in polysensitized than in monosensitized patients. Studies using polysensitized paediatric patients with asthma have failed to demonstrate efficacy [32]. It has been suggested that monosensitization may differ both immunologically and clinically from polysensitization, and specific immunotherapy is more effective in patients sensitized only to a single pollen than in patients with multiple pollen sensitization [33]. Thus, polysensitization may have a negative influence on the results of immunotherapy, especially if clinically relevant allergens are excluded. On the other hand, the effect of immunotherapy in polysensitized patients may also be compro-

mised, if the allergen vaccine contains a large number of allergens, thus producing a dilution effect which may have an influence on the optimal dose. In this regard, however, Marogna et al. [34] have demonstrated that patients sensitized to grasses and birch have the best clinical results when both allergens are included.

The study presented herein used a pragmatic approach, and polysensitized patients were included. Thus, 78.3% were polysensitized in Group A and 63.6% in Group B (see Table 1). This approach is closer to the real situation in the daily practice of allergology, when polysensitized patients are evaluated.

Several parameters have been used to demonstrate clinical efficacy, including subjective (symptom and medication scores, visual analog scales and quality of life parameters), as well as objective parameters. The most commonly used objective parameters include nasal and bronchial provocation tests, which are a reflection of the sensitivity of the target organ. When the patients are asymptomatic NPT reproduce the disease due to single allergen exposure. Therefore, it has been proposed that the better way to assess the extent to which patients are protected by immunotherapy against specific allergen exposure is the use of specific nasal challenges [35]. Nasal challenges out of the pollen season, may simulate natural exposure, and provide a method for comparing responses without interference from other allergens.

The present study was conducted outside of the grass pollen season and, therefore, patients were asymptomatic and not receiving pharmacological treatment. NPT were conducted at the beginning and at the end of the study. At baseline, all patients had a positive response and there were no significant differences between groups. The difference became significant at the end of the study, when patients in Group A needed a significant larger threshold concentration than at the beginning of the study ($P=0.013$). Furthermore, 12 patients (66%) experienced an improvement in Group A, vs. one patient (11%) in Group B. By contrast, six patients (33%) did not improve in Group A, whereas in Group B five patients (55%) did not improve and three of them (33%) worsened (χ^2 analysis $P=0.005$). It is noteworthy that all eight patients who reacted at the lowest dose of 206 BU/mL experienced a significant improvement after immunotherapy, while none of those who reacted at the highest dose initially tested of 1852 BU/mL did. This data may suggest a higher protective role of immunotherapy for more severely allergic patients.

We acknowledge that clinical symptoms were not evaluated in the study, because it was conducted outside of the pollen season to obtain objective measures of efficacy through NPT. Nevertheless, it has been suggested that nasal challenges are a good indicator of clinical efficacy and a good predictor of symptoms during the pollen season [14]. Successful short-term pre-seasonal

immunotherapy to birch pollen, showing a significant decrease in symptoms and use of anti-allergic drugs under natural pollen exposure, was also accompanied by an increase in the threshold dose necessary to induce a positive NPT [36].

At the end of the study, the data from four patients (three in Group A and one in Group B) could not be analysed, because they experienced a positive nasal reaction with the saline solution. It is possible that these four patients, who were also sensitized to other aeroallergens, including *C. arizonica* flowering at the time of the second nasal challenge, had a nasal hyperreactivity due to this fact. This also might be the case for the three patients in Group B that worsened as detected in the second challenge. Moreover, it should be noted that some small peaks of grass pollen counts were already detected in Madrid during February and March (Fig. 2), and therefore a nasal hyperreactivity due to true grass pollen cannot be excluded. However, we believe that mite sensitivity may not have played a role in the overall results, because mites are not important environmental allergens in Madrid, as has been demonstrated in previous publications [37].

The above results thus indicate that the effectiveness of cluster immunotherapy with grass allergoids modified with glutaraldehyde in reducing specific nasal reactivity can be scored in a short period of time using objective measures like specific NPT even in polysensitized patients. The efficacy was achieved after a mean of 6.5 injections and in less than in 2.5 months (in average) in patients suffering from rhinoconjunctivitis with or without asthma. In these patients, the clinical significance of other sensitivities should also be evaluated and considered when choosing the appropriate allergens for immunotherapy.

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