

## ORIGINAL PAPER

# Safety of immunotherapy with therapeutic vaccines containing depigmented and polymerized allergen extracts

M. Casanovas, R. Martín, C. Jiménez, R. Caballero and E. Fernández-Caldas

Laboratorios LETI, S.L., Tres Cantos, Madrid, Spain

## Clinical and Experimental Allergy

### Summary

**Background** The major complication of allergen immunotherapy is a severe reaction.

**Objective** To evaluate the safety of depigmented and glutaraldehyde-modified allergen extracts in a large group of patients undergoing immunotherapy treatment.

**Material and methods** Seven hundred sixty-six patients, having rhinoconjunctivitis and/or asthma, were entered in a prospective, multi-centre, observational cohort study, to evaluate the safety of immunotherapy with modified allergen vaccines. Patients were sensitized to mites and/or pollen and received a therapeutic vaccine containing depigmented and polymerized allergen extracts of mites and/or pollens adsorbed onto aluminium hydroxide. The schedule of administration consisted of a build-up phase of 4- to 6-weekly injections, followed by 12-monthly injections of the maintenance dose. Tolerance was assessed by recording all side reactions related to immunotherapy.

**Results** All patients completed the study. Fifty-four clinically relevant local reactions (43 immediate and 11 delayed) were observed (0.4% of injections). The systemic reactions were 34 in 12 patients. Six reactions were immediate (all of grade 2) and 28 delayed (18 of grade 1 in two patients, nine of grade 2 and one of grade 3). The systemic reactions of grade 2 or 3 occurred in 0.12% of the injections. All systemic reactions were mild and resolved spontaneously without the need for medication.

**Conclusion** Specific immunotherapy using modified allergen vaccines is safe to treat allergic patients. The percentage of adverse reactions detected is lower than those reported in the literature with native-unmodified allergen extracts.

**Keywords** allergen immunotherapy, asthma, mites, modified allergen extracts, pollens, rhinoconjunctivitis, safety

*Submitted 16 June 2006; revised 22 November 2006; accepted 29 December 2006*

### Correspondence:

Miguel Casanovas, Laboratorios LETI, S.L.  
Sol, 5, 28760 Tres Cantos, (Madrid),  
Spain.

E-mail: mcasanovas@leti.com

### Introduction

The efficacy of therapeutic vaccines containing allergen extracts administered through the subcutaneous route has been demonstrated in several double-blind placebo-controlled (DBPC) studies and open trials [1, 2]. It is the only treatment that ameliorates symptoms and medication scores, modifies the natural course of the illness [3–5] and prevents the worsening of specific bronchial hyper-reactivity [6]. The main concern related to the subcutaneous administration of specific immunotherapy (SIT) is safety, which has been extensively evaluated and reviewed from published studies [7–9], or specifically evaluated in prospective studies [10–15]. These studies have assessed the safety of allergen vaccines from one or

various manufacturers in large groups of patients. The risk of side-effects related to SIT with allergen extracts has been recognized since early studies [16, 17]. The intrinsic properties of the allergens (IgE-binding epitopes) and the susceptibility of the allergic patient are responsible for the side-effects seen in allergic patients after the administration of immunotherapy. The risk of these adverse side-effects is intrinsic to SIT, but they are not necessarily acceptable. Severe side-effects may be acceptable if the degree of benefit is large and the risk of side-effects is minimized and treated promptly and effectively [16]. Overall, the incidence of type 4 reactions using immunotherapy is rare. The reasons why patients do not experience type 4 reactions using carefully standardized IT are kept open for debate.

Methods to reduce the risk of adverse reactions associated with allergen immunotherapy based on genetic engineering are being envisaged [18–21]. Other possibilities, currently in use, include the modification of allergens with glutaraldehyde [22, 23], which produces extracts with decreased IgE-binding capacity. Depigmented and polymerized allergen extracts have also been proven to be safe and clinically efficacious [6, 24–30], allowing for the administration of high doses in a short period of time. The safety of these vaccines has also been confirmed using a rush build-up phase in a large group of patients [31]. Other routes of administration, such as sublingual immunotherapy, are also being used.

The objective of the study was to evaluate the safety of therapeutic vaccines containing modified allergen extracts in a statistically representative cohort of patients. Safety was evaluated following the criteria of the European Academy of Allergy and Clinical Immunology (EAACI) [32].

## Material and methods

### Design

The study was phase IV prospective and open label. It was conducted in 35 allergy centres in Spain, under the supervision of a trained allergist. The sample size was calculated based on the results obtained in the study of Gastaminza et al. [10]. This study described an incidence of 79 systemic reactions in 1212 patients (6.52%). These reactions were of grades 2, 3 and 4. Expecting to reduce the incidence of systemic reactions at least by half, for an  $\alpha = 0.05$  and a power of 80% (error protection of 7.84) [33], the cohort of patients needed was 682 patients. Assuming 20% of drop-outs, the sample size was adjusted to 819 patients. The final number of patients included in the study was 766.

### Patients

A cohort of 766 patients (365 males, 401 females) with a mean age of 23.2 years [95% confidence interval (CI) 22.4–24.1] was studied. The age distribution of the patients was as follows: 17% between 4 and 10 years of age; 24.4% between 11 and 18; and 38.6% between 19 and 35 and 18.3% older than 35 years: age data were not available for 1.7% of the patients. Patients were sensitized to mites (*Dermatophagoides pteronyssinus*, *D. farinae*, *Euroglyphus maynei*) or pollens (grasses, *Olea europaea*, *Parietaria judaica*, *Cupressus arizonica*, *Salsola kali*, *Artemisia vulgaris*, *Plantago lanceolata* and/or *Chenopodium album*). The inclusion and exclusion criteria to enter the study were those outlined in the EAACI and WHO Position Papers on allergen immunotherapy [34, 35]. Three hundred and sixteen patients (41.3%) were

diagnosed as having rhinoconjunctivitis (RC), 377 (49.2%) rhinoconjunctivitis and asthma (RC+A) and 73 (9.5%) asthma alone.

### Immunotherapy schedule

Each patient received an individualized commercial therapeutic vaccine (Depigoid<sup>®</sup>, Laboratorios LETI, SL, Tres Cantos, Spain) of the modified allergen extract. Extracts were prepared according to the methods described previously [28]. Two consecutively numbered vials containing a mixture of the polymers of the allergen extracts were prepared for each patient. In vial 1, the polymer concentration was the result of depigmenting and polymerizing the vial of maximum concentration of the corresponding non-modified vaccine. Vial 2 contained a 10-fold higher polymer concentration. The build-up phase was initiated with vial 1 (2- or 3-weekly injections), followed by 2 or 3 weekly injections of vial number 2 until the maintenance dose was reached. The maintenance doses were 12 injections of 0.5 mL of vial number 2. None of the patients received pre-treatment with drugs (antihistamines or corticosteroids) in order to prevent adverse reactions. The injections were administered subcutaneously on the extensor surfaces of the upper arms using an insulin syringe with a 26-gauge, 1/2 in. non-removable needle. All patients had a 30-min waiting period after each injection, following the recommendations of the European Academy of Allergology and Clinical Immunology (EAACI) [34]. The composition of the vaccines administered to the patients is shown in Table 1. Immunotherapy was administered without interruption or reduction of the planned dose during the pollen season.

The contents of the major allergens in the final preparation could not be detected because of the modification process. However, following the recommendations of the European Pharmacopoeia [36], major allergen content was determined in the corresponding native preparation before modification and expressed in mcg/mL. The contents in mite extracts were determined by ELISA using monoclonal antibodies (Indoor Biotechnologies Ltd., Charlottesville, VA, USA) and in pollen extracts by means of scanning densitometry [37]. The contents in the freeze-dried native extract needed to produce the maximum concentration of the modified preparation were 17.3, 10.5 mcg and 12.3 of Der p1, Der p2 and Der f1, respectively. In pollen, the figures were 131 mcg of Ole e 1 (*O. europaea*), 17 mcg of Par j 1 (*P. judaica*), 66 mcg of Sal k 1, 15.5 mcg of Phl p1 and 32 of Phl p5 (*Phleum pratense*), 60.0 mcg of Dact g 2 and 20.0 of Dact g 1+5 (*D. glomerata*).

### Safety assessment

Patients were instructed before the initiation of the study to warn the attending physician of new prescriptions

Table 1. Composition of the vaccines administered to the patients

| Composition                                                                        | <i>n</i> | Percentage |
|------------------------------------------------------------------------------------|----------|------------|
| Mites                                                                              |          |            |
| <i>Dermatophagoides pteronyssinus</i>                                              | 99       | 12.92      |
| <i>D. farinae</i>                                                                  | 3        | 0.39       |
| <i>D. pteronyssinus</i> + <i>D. farinae</i>                                        | 317      | 41.38      |
| <i>D. pteronyssinus</i> + <i>Euroglyphus maynei</i>                                | 1        | 0.13       |
| Total patients receiving immunotherapy with mite extracts                          | 420      | 54.83      |
| Pollen                                                                             |          |            |
| Grasses (mix or single grass pollen extracts)                                      | 48       | 6.27       |
| Individual or mixture of grasses+ <i>Olea europaea</i>                             | 94       | 12.27      |
| Weeds (individual pollen extracts)                                                 | 55       | 7.18       |
| Weeds (mixtures of two or more pollen extracts)                                    | 1        | 0.13       |
| Trees (individual pollen extracts)                                                 | 88       | 11.49      |
| Trees (mixtures of two or more pollen extracts)                                    | 4        | 0.52       |
| Mixtures of various pollen extracts                                                | 30       | 3.92       |
| Total patients receiving immunotherapy with pollen extracts                        | 320      | 41.78      |
| Total patients receiving immunotherapy with a mixture of mites and pollen extracts | 26       | 3.39       |

Weeds: *Parietaria judaica* was the most important, followed by *Artemisia vulgaris*, *Salsola kali*, *Chenopodium album* and *Plantago lanceolata*.

Trees: *Olea europaea* was the most important, followed by *Cupressus arizonica*.

(including vaccines) received out of the allergy unit, and on the existence of new status, such as pregnancy and intercurrent disease(s). At any point in time, the general exclusion criteria for immunotherapy were followed.

The reactions were categorized as immediate when the onset was during the first 30 min after the administration, and delayed when the onset was afterwards. Local reactions were quantified by measuring the diameter of erythema of the reaction. Immediate reactions with a diameter smaller than 5 cm and delayed reactions smaller than 10 cm were considered to be clinically irrelevant. The systemic reactions were graded following the classification of the EAACI Position Paper on Immunotherapy [34]. The grades were: *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  $\beta_2$ -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. In case of a late reaction, the patient was instructed to contact the personnel of the immunotherapy unit (the responsible allergist).

### Statistics

Descriptive statistics included the frequency of the incidence of the reactions per patient and per injection,

including the 95% lower and upper CI. Fisher's exact *P*-value was used to evaluate the significance of side reactions between mites and pollen, or between the build-up and maintenance phases.

## Results

### Patients and number of injections

All 766 patients received the planned doses. A total of 14 022 injections were administered; 3714 corresponded to the build-up phase and 10 308 to the maintenance dose. It was necessary to repeat a build-up phase dose due to mild side reactions in three patients, who continued with the planned schedule without further adverse events. Table 2 shows the description of the reasons for these decisions.

### Adverse reactions

A total of 54 local reactions (one per 14 patients, and one per 241 injections) were recorded. Twenty-two occurred in the build-up phase and 32 in the maintenance phase (Fisher's exact *P*-value = 0.02). No statistically significant differences were observed between mites and pollen (Fisher's exact *P*-value = 0.13). No local reactions were recorded with the vaccines containing a mixture of mites and pollen. Table 3 shows the number and the intensity (mm of diameter) of clinically relevant reactions per group of allergen extracts (mite or pollen) and per dose injected.

Eighteen reactions were of grade 1 (non-IgE mediated) and occurred in two patients; all were delayed and mild.

Table 2. Patients who needed the repetition of one dose and description of the cause

| Dose modification |           |                                                           |      |      |                                                                     |
|-------------------|-----------|-----------------------------------------------------------|------|------|---------------------------------------------------------------------|
| Patient           | Diagnosis | Composition of the vaccine                                | Dose | Vial | Comments                                                            |
| 37                | R, A      | <i>Parietaria judaica</i>                                 | 0.1  | 2    | First dose was repeated due to local reaction and rhinitis symptoms |
| 617               | R         | <i>Dermatophagoides pteronyssinus</i> + <i>D. farinae</i> | 0.1  | 1    | The dose was repeated due to rhinitis                               |
| 773               | R         | <i>P. judaica</i>                                         | 0.3  | 2    | The dose was repeated due to rhinitis                               |

R, rhinoconjunctivitis; A, asthma.

There were 16 systemic reactions in 10 patients. The frequency was 1.31% per patient (95% CI, 0.50, 2.12) and 0.12% per injection (95% CI, -0.13, 0.37). These systemic reactions of grades 2 and 3 were 15 and 1, respectively (one reaction per 48 patients, and one per 814 injections), and eight occurred with the administration of pollen extracts and eight with mites (Fisher's exact  $P$ -value = 0.62). Six of the systemic reactions were immediate (all of grade 2) and 28 delayed (18 of grade 1, nine of grade 2 and one of grade 3). All reactions were mild and resolved spontaneously without the need for rescue medication. Two reactions in one patient were recorded with a vaccine containing a mixture of *D. pteronyssinus* and *O. europaea*. Table 4 shows a description of these reactions.

## Discussion

A cohort of 766 patients was treated subcutaneously with a therapeutic vaccine containing depigmented and polymerized allergen extracts to evaluate safety in this prospective open study. We included a large number of centres to avoid the bias of a single, or few physicians administering the treatment and to be more representative of real-life conditions.

In the present study, we detected that 0.4% of the total injection number caused local reactions and that 0.1% caused a systemic reaction. None of these reactions was of grade 4, and all were mild and resolved spontaneously without the need for medication. None of the patients withdrew from the study due to adverse events. In a similar study [38] involving less patients, the same type of modified allergen extracts was administered to 175 patients for a total of 2261 injections. The incidence of local reactions with a diameter between 5 and 10 cm was 1.4%, whereas for those with a diameter larger than 10 cm, it was 0.1%. Thirteen reactions were systemic, which represents 0.6% of the total number of injections administered. All these reactions were judged as mild and no type 4 reactions were observed.

Gastaminza et al. [10] provided data from 1212 patients in which SIT with unmodified standardized allergen

extracts adsorbed to alum was administered in a single immunotherapy unit. These patients were diagnosed with allergic asthma and/or RC, or hymenoptera venom anaphylaxis, and received a total of 29 754 injections. Seventy-nine systemic reactions (0.3% per injection) occurred in 2.9% of the patients receiving mite extracts, 7.5% of patients with pollen extracts and 6.7% in the case of hymenoptera venoms. No withdrawals were reported. A multi-centric study by Moreno et al. [11] evaluated 488 patients treated with biologically standardized allergen extracts for respiratory allergies; 433 patients completed the study and four were asked to withdraw by the physicians. In three cases, the withdrawal was suggested due to recurrent reactions and in one due to a single reaction. Four more patients left the trial by their own decision due to local reactions during the build-up phase. The total injections administered were 17 526 and the number of systemic reactions was 50 (0.3% of the injections): 30 of grade 2 and 21 of grade 3. In this study, the investigators detected more systemic reactions in patients receiving mite extracts. Winther et al. [12] evaluated the side-effects of SIT of a single manufacturer in a multi-centre study involving 1038 patients, treated with extracts of mite, pollen, dog or cat or with wasp and bee venoms. The total number of injections was 23 047. Forty-one patients (4%) stopped the treatment due to complications (local or systemic reactions, or worsening of allergic symptoms). Twenty-three per cent of the patients treated with one allergen extract did not reach the maximum dose, and 29% when the treatment was with more than one allergen extract. A total of 582 side-effects were reported in 341 patients (2.5% of the injections). Seventy-eight per cent of these reactions were of grade 2%, 20% of grade 3% and 1% (eight reactions) of grade 4. Tabar et al. [14] analysed 419 patients, observing that 4.8% of the patients experienced a systemic reaction (0.4% of the injections), these reactions being mainly in patients with asthma and sensitized to *D. pteronyssinus*. In another study [13], 239 patients were evaluated in a DBPC study using an allergen extract of *D. pteronyssinus* from a single manufacturer administered using two types of build-up phases (conventional and cluster). During the

Table 3. Number and percentage (with 95% CI) of local reactions

| Size      | Build-up         | % per patient | 95% CI |       | % per injection | 95% CI |       | % per patient | 95% CI |       | % per injection | 95% CI |       |       |      |
|-----------|------------------|---------------|--------|-------|-----------------|--------|-------|---------------|--------|-------|-----------------|--------|-------|-------|------|
|           |                  |               | Lower  | Upper |                 | Lower  | Upper |               | Lower  | Upper |                 | Lower  | Upper |       |      |
| Immediate |                  |               |        |       |                 |        |       |               |        |       |                 |        |       |       |      |
| Mites     |                  |               |        |       |                 |        |       |               |        |       |                 |        |       |       |      |
|           | 5 cm < Ø < 10 cm | 5             | 0.65   | 0.08  | 1.22            | 0.04   | -0.10 | 0.17          | 11     | 1.44  | 0.59            | 2.28   | 0.08  | -0.12 | 0.28 |
|           | Ø > 10 cm        | 0             | 0.00   | 0.00  | 0.00            | 0.00   | 0.00  | 0.00          | 4      | 0.52  | 0.01            | 1.03   | 0.03  | -0.09 | 0.15 |
|           | Total            | 5             | 0.65   | 0.08  | 1.22            | 0.04   | -0.10 | 0.17          | 15     | 1.96  | 0.98            | 2.94   | 0.11  | -0.12 | 0.34 |
| Pollen    |                  |               |        |       |                 |        |       |               |        |       |                 |        |       |       |      |
|           | 5 cm < Ø < 10 cm | 10            | 1.31   | 0.50  | 2.11            | 0.07   | -0.12 | 0.26          | 11     | 1.44  | 0.59            | 2.28   | 0.08  | -0.12 | 0.28 |
|           | Ø > 10 cm        | 2             | 0.26   | -0.10 | 0.62            | 0.01   | -0.07 | 0.10          | 0      | 0.00  | 0.00            | 0.00   | 0.00  | 0.00  | 0.00 |
|           | Total            | 12            | 1.57   | 0.69  | 2.45            | 0.09   | -0.12 | 0.29          | 11     | 1.44  | 0.59            | 2.28   | 0.08  | -0.12 | 0.28 |
| Delayed   |                  |               |        |       |                 |        |       |               |        |       |                 |        |       |       |      |
| Mites     |                  |               |        |       |                 |        |       |               |        |       |                 |        |       |       |      |
|           | Ø > 10 cm        | 3             | 0.39   | -0.05 | 0.83            | 0.02   | -0.08 | 0.12          | 2      | 0.26  | -0.10           | 0.62   | 0.01  | -0.07 | 0.10 |
| Pollen    |                  |               |        |       |                 |        |       |               |        |       |                 |        |       |       |      |
|           | Ø > 10 cm        | 2             | 0.26   | -0.10 | 0.62            | 0.01   | -0.07 | 0.10          | 4      | 0.52  | 0.01            | 1.03   | 0.03  | -0.09 | 0.15 |

incremental phase, there were 19 adverse reactions in 15 patients receiving the allergen extract; eight reactions (0.2% per injection) were systemic (three immediate and five delayed). All reactions were of grade 2. During the maintenance phase, nine delayed adverse reactions (0.5% per injection) were reported in eight patients. Two reactions were local and seven systemic (one with non-specific symptoms and six with asthma). As a consequence, the dose schedule for these patients was modified. Machin et al. [15] evaluated the safety in 339 patients carefully treated in an immunotherapy unit, observing 21 systemic reactions in 14 patients (4.1% of the patients, 0.4% of the injections).

The tolerability of these modified extracts was compared in double-blind studies with the corresponding unmodified preparations [29, 30]. Doses 10 times higher than those used in the present study were achieved, and no adverse events were reported with the doses used in normal clinical conditions, confirming the wide therapeutic margin for this chemically modified allergen vaccine and the safer profile than regular vaccines based on native-unmodified extracts.

Although this study was not designed to compare with the sublingual route of administration, the rate of side reactions described in this paper is lower than the one described in some studies using sublingual immunotherapy. The safety of this immunotherapy, using drops or tablets, has been reviewed in a study including eight DBPC trials [39]. A total of 690 patients (347 active and 343 placebo) were studied; 145 adverse events were reported in the active and 79 in the placebo group ( $P < 0.01$ ), with a higher incidence of adverse events in the mouth (18% and 4% of the patients in the active and placebo groups, respectively) and in the gastro-intestinal tract (14% and 4% of the patients in the active and placebo groups, respectively). Two laryngeal oedemas were reported in patients receiving the active treatment (0.6% of the patients).

In a DBPC study using sublingual tablets [40] of *P. pratense*, there were three non-explained withdrawals due to adverse events in the group of 74 patients who received the active tablet. Finally, in a large study using tablets [41], 855 patients were included and 451 (53%) had adverse events, judged by the investigators as probably, or possibly related to the treatment; 26 participants (3%) withdrew due to adverse events.

Considering the results presented in this study and in the context of the data available in the literature, we can conclude that the SC administration of these modified allergen extracts is safer than the use of unmodified allergen vaccines. Furthermore, the available published data suggest that the safety profile of these modified allergen vaccines administered subcutaneously could be better than the one observed with the sublingual administration of allergen extracts.

Table 4. Description of the systemic reactions

| Patient | Vial | Dose | Allergen extract                             | Date       | Description           | Type      | Grade |
|---------|------|------|----------------------------------------------|------------|-----------------------|-----------|-------|
| 37      | 2    | 0.1  | <i>Parietaria judaica</i>                    | 03/05/2000 | Rhinitis symptoms     | Immediate | 2     |
| 60      | 2    | 0.5  | <i>Dermatophagoides pteronyssinus</i>        | 14/03/2000 | Bronchial symptoms    | Delayed   | 2     |
| 60      | 2    | 0.5  |                                              | 13/04/2000 | Bronchial symptoms    | Delayed   | 2     |
| 101     | 1    | 0.5  | <i>Olea europaea</i>                         | 16/10/2000 | Nervousness           | Delayed   | 1     |
| 101     | 2    | 0.3  |                                              | 06/11/2000 | Nervousness           | Delayed   | 1     |
| 101     | 2    | 0.5  |                                              | 21/12/2000 | Nervousness           | Delayed   | 1     |
| 101     | 2    | 0.5  |                                              | 22/01/2001 | Nervousness           | Delayed   | 1     |
| 195     | 2    | 0.3  | Grass mix + <i>O. europaea</i>               | 19/01/2001 | Rhinitis symptoms     | Delayed   | 2     |
| 200     | 2    | 0.5  | Grasses                                      | 04/05/2001 | Rhinitis symptoms     | Immediate | 2     |
| 208     | 1    | 0.1  | <i>D. pteronyssinus</i> + <i>D. farinae</i>  | 02/11/2000 | Urticaria             | Delayed   | 3     |
| 227     | 2    | 0.1  | <i>D. pteronyssinus</i> + <i>D. farinae</i>  | 09/05/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.3  |                                              | 16/05/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 23/05/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 23/06/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 23/07/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 22/08/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 21/09/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 21/10/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 20/11/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 20/12/2000 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 19/01/2001 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 18/02/2001 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 20/03/2001 | Nervousness, headache | Delayed   | 1     |
| 227     | 2    | 0.5  |                                              | 19/04/2001 | Nervousness, headache | Delayed   | 1     |
| 319     | 2    | 0.1  | <i>D. pteronyssinus</i> + <i>O. europaea</i> | 15/11/2000 | Rhinitis symptoms     | Delayed   | 2     |
| 319     | 2    | 0.3  |                                              | 22/11/2000 | Rhinitis symptoms     | Delayed   | 2     |
| 617     | 1    | 0.3  | <i>D. pteronyssinus</i> + <i>D. farinae</i>  | 28/12/2000 | Rhinitis symptoms     | Delayed   | 2     |
| 617     | 1    | 0.5  |                                              | 04/01/2000 | Rhinitis symptoms     | Delayed   | 2     |
| 617     | 1    | 0.5  |                                              | 11/10/2000 | Rhinitis symptoms     | Delayed   | 2     |
| 644     | 2    | 0.1  | Grasses                                      | 05/10/2001 | Rhinitis symptoms     | Immediate | 2     |
| 651     | 2    | 0.1  | <i>D. pteronyssinus</i> + <i>D. farinae</i>  | 08/09/2000 | Rhinitis symptoms     | Delayed   | 2     |
| 773     | 1    | 0.1  | <i>P. judaica</i>                            | 21/07/2000 | Rhinitis symptoms     | Immediate | 2     |
| 773     | 2    | 0.3  |                                              | 18/08/2000 | Rhinitis symptoms     | Immediate | 2     |
| 773     | 2    | 0.4  |                                              | 01/09/2000 | Rhinitis symptoms     | Immediate | 2     |

## Acknowledgements

The authors wish to thank the collaboration of all the participants in the study, including the prescribing doctors and nurses. This study was sponsored by Laboratorios LETI, S.L.

## References

- Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med* 1995; 151:969–74.
- Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2000: CD001186.
- Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. WHO position paper. *J Allergy Clin Immunol* 1998; 102:558–62.
- Moller C, Dreborg S, Ferdousi HA *et al.* Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002; 109:251–6.
- Williams AP, Krishna MT, Frew AJ. The safety of immunotherapy. *Clin Exp Allergy* 2004; 34:513–4.
- Ameal A, Vega-Chicote JM, Fernández S *et al.* Double-blind and placebo-controlled study to assess efficacy and safety of a modified allergen extract of *Dermatophagoides pteronyssinus* in allergic asthma. *Allergy* 2005; 60:1178–83.
- Bousquet J, Michel FB. Safety considerations in assessing the role of immunotherapy in allergic disorders. *Drug Saf* 1994; 10:5–17.
- Nettis E, Giordano D, Ferrannini A, Tursi A. Systemic reactions to allergen immunotherapy: a review of the literature. *Immunopharmacol Immunotoxicol* 2003; 25:1–11.
- Malling HJ. Immunotherapy as an effective tool in allergy treatment. *Allergy* 1998; 53:461–72.
- Gastaminza G, Algorta J, Audicana M, Etxenagusia M, Fernández E, Muñoz D. Systemic reactions to immunotherapy: influence of composition and manufacturer. *Clin Exp Allergy* 2003; 33:470–4.

- 11 Moreno C, Cuesta-Herranz J, Fernandez-Tavora L, Alvarez-Cuesta E. Immunotherapy safety: a prospective multi-centric monitoring study of biologically standardized therapeutic vaccines for allergic diseases. *Clin Exp Allergy* 2004; 34:527–31.
- 12 Winther L, Arnved J, Malling HJ, Nolte H, Mosbech H. Side-effects of allergen-specific immunotherapy. A prospective multi-centre study. *Clin Exp Allergy* 2006; 36:254–60.
- 13 Tabar AI, Echechipia S, García BE et al. Double-blind comparative study of cluster and conventional immunotherapy schedules with *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 2005; 116:109–18.
- 14 Tabar AI, García BE, Rodríguez A, Olaguibel JM, Muro MD, Quirce S. A prospective safety-monitoring study of immunotherapy with biologically standardized extracts. *Allergy* 1993; 48:450–3.
- 15 Machín IS, Robaina JCG, Bonnet C et al. Immunotherapy units: a follow-up study. *J Invest Allergol Clin Immunol* 2001; 11:167–71.
- 16 Frew AJ. Immunotherapy: how safe is safe? *Clin Exp Allergy* 2006; 36:251–3.
- 17 Freeman J. Vaccination against hayfever: report of results during the first three years. *Lancet* 1914; 1:1178.
- 18 Vrtala S, Hirtenlehner K, Susani M et al. Genetic engineering of recombinant hypoallergenic oligomers of the major birch pollen allergen, Bet v 1: candidates for specific immunotherapy. *Int Arch Allergy Immunol* 1999; 118:218–9.
- 19 Pauli G, Purohit A, Oster JP et al. Clinical evaluation of genetically engineered hypoallergenic rBet v 1 derivatives. *Int Arch Allergy Immunol* 1999; 118:216–7.
- 20 Niederberger V, Valenta R. Molecular approaches for new vaccines against allergy. *Expert Rev Vaccines* 2006; 5:103–10.
- 21 Kraft D, Ferreira F, Vrtala S et al. The importance of recombinant allergens for diagnosis and therapy of IgE-mediated allergies. *Int Arch Allergy Immunol* 1999; 118:171–6.
- 22 Patterson R, Suszko IM, Zeiss CR, Pruzansky JJ. Development and evaluation of polymerized allergens for immunotherapy. *Int Arch Allergy Appl Immunol* 1981; 66 (Suppl. 1):293–6.
- 23 Grammer LC, Shaughnessy MA, Patterson R. Modified forms of allergen immunotherapy. *J Allergy Clin Immunol* 1985; 76:397–401.
- 24 Guerra F, Daza JC, Almeda E. Immunotherapy with a depigmented, polymerized vaccine of *Olea europaea* pollen allergens significantly reduces specific bronchial and skin test reactivity in sensitized patients after one year of treatment. *J Invest Allergol Clin Immunol* 2003; 13:108–17.
- 25 García-Sellés J, Pascual A, Funes E et al. Clinical efficacy and safety of a depigmented and glutaraldehyde polymerized therapeutic vaccine of *Parietaria judaica*. *Allergol Immunopathol (Madrid)* 2003; 31:63–9.
- 26 Álvarez-Cuesta E, Aragonese-Gilsanz E, Martín-García C, Berges-Gimeno P, González-Mancebo E, Cuesta-Herranz J. Immunotherapy with depigmented glutaraldehyde-polymerized extracts: changes in quality of life. *Clin Exp Allergy* 2005; 35:572–8.
- 27 Ibero M, Castillo MJ. Significant improvement of specific bronchial hyperreactivity in asthmatic children after 4 months of treatment with a modified extract of *Dermatophagoides pteronyssinus*. *J Invest Allergol Clin Immunol* 2006; 16:194–202.
- 28 Ferrer A, García-Sellés J. Significant improvement in symptoms, skin test, and specific bronchial reactivity after 6 months of treatment with a depigmented, polymerized extract of *Dermatophagoides pteronyssinus* and *D. Farinae*. *J Invest Allergol Clin Immunol* 2003; 13:244–51.
- 29 Casanovas M, Sastre J, Fernández-Nieto M, Lluch M, Carnés J, Fernández-Caldas E. Double-blind study of tolerability and antibody production of unmodified and chemically modified allergen vaccines of *Phleum pratense*. *Clin Exp Allergy* 2005; 35:1377–83.
- 30 Casanovas M, Fernández-Caldas E, Alamar R, Basomba A. Comparative study of tolerance between unmodified and high doses of chemically modified allergen vaccines of *D. Pteronyssinus*. *Int Arch Allergy Appl Immunol* 2005; 137:211–18.
- 31 Casanovas M, Martín R, Jiménez C, Caballero R, Fernández-Caldas E. Safety of an ultra-rush immunotherapy build-up schedule with therapeutic vaccines containing depigmented and polymerized allergen extracts. *Int Arch Allergy Immunol* 2005; 139:153–8.
- 32 Malling HJ, Weeke B. EAACI position paper: immunotherapy. *Allergy* 1993; 48:7–35.
- 33 Carné X, Moreno V, Porta Serra M, Velilla E. Calculating the number of patients necessary for designing a clinical study. *Med Clin (Barcelona)* 1989; 92:72–7.
- 34 EAACI. Position paper: Immunotherapy. (EAACI) The European Academy of Allergology and Clinical Immunology. *Allergy* 1993; 48:7–35.
- 35 World Health Organization. Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 1998; 53 (Suppl.):1–42.
- 36 Council\_of\_Europe. *European pharmacopeia. Allergen products*, 3rd Edn. Council\_of\_Europe, 1997.
- 37 Boluda L, Sastre J, Casanovas M, Fernández-Caldas E. Determination of Ole e 1 by enzyme immunoassay and scanning densitometry. Validation by skin-prick testing. *J Immunol Methods* 1999; 223:17–26.
- 38 Sager A, Renner BG. Safety aspects and course of allergen-specific immunotherapy with a depigmented, polymerized allergen extract. *Allergo J* 2004; 13:386–91.
- 39 Andre C, Vatrinet C, Galvain S, Carat F, Sicard H. Safety of sublingual-swallow immunotherapy in children and adults. *Int Arch Allergy Immunol* 2000; 121:229–34.
- 40 Dahl R, Stender A, Rak S. Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. *Allergy* 2006; 61:185–90.
- 41 Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006; 117:802–9.