

Original article

Double-blind and placebo-controlled study to assess efficacy and safety of a modified allergen extract of *Dermatophagoides pteronyssinus* in allergic asthma

Background: A randomized double-blind, placebo-controlled study was conducted in patients allergic asthma sensitized to *Dermatophagoides pteronyssinus*.

Objective: To evaluate the efficacy and safety after 1-year of immunotherapy with a modified *D. pteronyssinus* extract compared with placebo.

Material and methods: Fifty-five patients were randomly allocated to receive the active treatment ($n = 29$), or placebo ($n = 26$). The main outcome was the specific bronchial provocation test (BPT). Other parameters analysed were dose-response skin prick test (SPT), symptom and medication scores and asthma quality of life (AQLQ).

Results: At the end of the study, the active group showed a significant increase in the $PD_{20}FEV_1$ compared with placebo ($P = 0.0029$). Nineteen patients of the active vs 10 of the placebo group needed more than twice the initial amount of allergen extract to have a positive BPT ($P = 0.0293$); seven patients in the placebo vs one in the active group needed less than half ($P = 0.0137$). In SPT, a significant improvement ($P = 0.0049$) was found in the active group. This group also had a median reduction of 91.5% in symptom scores, whereas the placebo group increased by 86%. Medication scores decreased in both groups (56% in the active and 11.4% in the placebo). In AQLQ, the differences between both groups were significant ($P = 0.0234$) at the end of the study.

Conclusion: After 1 year of treatment, the modified extract of *D. pteronyssinus* demonstrated to be safe and efficacious to treat patients with asthma and allergic rhinoconjunctivitis sensitized to this mite.

A. Ameal, J. M. Vega-Chicote, S. Fernández, A. Miranda, M. J. Carmona, M. C. Rondón, E. Reina, J. J. García-González

Allergy Department, Hospital 'Carlos Haya', Málaga, Spain

Key words: allergen vaccine; bronchial provocation; clinical efficacy; *Dermatophagoides pteronyssinus*; immunotherapy.

J. J. García-González
Allergy Department
Hospital 'Carlos Haya'
Plaza del Hospital Civil
s/n, Primera Planta
29009 Málaga
Spain

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The treatment of mite allergic asthmatic patients focuses primarily on environmental control, pharmacological treatment, immunotherapy and education (1). Allergen-specific immunotherapy has shown clinical benefit in double-blind, placebo-controlled trials (2). In successful studies, immunotherapy has increased the threshold dose eliciting bronchial obstruction and has shown a significant reduction in symptom and/or medication scores (2). In an attempt to overcome a long series of injections over a lengthy period of time, glutaraldehyde-modified allergenic extracts were developed (3). However, there are few studies showing efficacy with glutaraldehyde-modified mite vaccines in the treatment of allergic bronchial asthma (4, 5). A new allergoid, which includes a depigmentation step in which the enzymatic activity is inactivated, pigments removed and the solubility of the allergoid enhanced has recently been developed (6–8). One open and controlled study (8) using a mite mixture extract of *Dermatophagoides pteronyssinus* and *D. farinae* demonstrated a significant reduction ($P < 0.05$) of the

mite-specific bronchial hyperreactivity after 6 months of treatment.

There are few studies on mite allergy and exposure in Malaga, Spain. However, in one study, 28.2% of the student population had a positive skin test to *D. pteronyssinus* (9), which is the most prevalent mite in this area (10).

The objective of this study was to evaluate the clinical efficacy and safety of a depigmented, polymerized vaccine-containing *D. pteronyssinus* in a prospective, randomized, double-blind and placebo-controlled clinical trial.

Material and methods

Patient population

Sixty-six patients with mild/moderate asthma, diagnosed according to GINA guidelines (11) and rhinoconjunctivitis because of sensitization to *D. pteronyssinus* were initially planned to be included in

the study; 63 were recruited and 55 concluded the study. This study was approved by the Ethics Committee of the University Hospital ‘Carlos Haya’, Málaga and the Spanish Health Authorities. The inclusion criteria were: clinical history suggestive of house dust mite allergy, positive skin tests using standardized extract and negative to other common aeroallergens, positive specific bronchial provocation test (BPT) and detectable specific immunoglobulin E (IgE) to this mite. The exclusion criteria were those outlined in the World Health Organization (WHO) position paper on allergen immunotherapy (2), pregnancy, lactation and aspirin intolerance.

Study design and patient assignment

The study was randomized, double blind and placebo controlled. The active group received a modified allergen extract of *D. pteronyssinus* and the control received placebo. The duration of the study was 12 months per patient. Figure 1 gives an outline of the study design.

The sample size was calculated based on the number of patients that, after 1 year, could achieve an improvement of 100% in the values needed to produce the PD₂₀FEV₁ in the BPT. Assuming that 30% of the patients receiving placebo and 70% in the active group will achieve this improvement, for an $\alpha = 0.05$ (two-tailed) and $\beta = 0.2$ (one-tailed), the sample size must be of 50 patients, divided into two groups of 25 (12). Assuming a 25% of withdrawals, the sample size was adjusted to 66 patients.

After the initial diagnostic tests, the patients were blindly randomized and treated with active or placebo. Both groups received pharmacological treatment, if needed, consisting of oral ebastine, salbutamol and topical steroids (budesonide).

Modified allergen extract of *D. pteronyssinus*

The modified vaccines and placebo were supplied by the allergen manufacturer (Laboratorios LETI, S.L., Tres Cantos, Spain) The vaccines were prepared and standardized as previously described (8). The 50% IgE inhibition points were measured by enzyme-linked immunosorbent assay (ELISA; 13) using the in-house reference preparation and a serum pool of mite allergic individuals, in

accordance with the recommendations of the European Pharmacopoeia (14). The figures were 295 ng for native extract and of 18.52 µg (63 times more) for the modified. The 50% IgG inhibition points were also measured by ELISA (15) using the same serum pool; 13 ng were needed of the native and 15 ng of the modified extract.

Der p 1 and Der p 2 allergens were measured in the native and polymerized extracts by ELISA (Indoor Biotechnologies Ltd, Charlottesville, VA, USA). The native extract contained 20.35 µg of Der p 1 and 12.30 µg of Der p 2 per mg of freeze-dried extract. Because of the polymerization process, Der p 1 and Der p 2 were not detectable in the modified extract.

Treatment schedule

The modified extract was adsorbed onto aluminium hydroxide. Two consecutively numbered vials containing the polymer were prepared for each patient. In vial number 1, the polymer concentration was 7 µg/ml. Vial number 2 contained 70 µg/ml, which is the result of depigmenting and polymerizing 10 times the top concentration of the nonmodified extract (14.25 µg of Der p 1/ml and 8.61 of Der p 2). The trial medication was administered subcutaneously at regular intervals based on the following dosage schedule: three administrations of 0.1, 0.25 and 0.5 ml of vial 1 (1 injection/week); followed by three administrations of 0.1, 0.25 and 0.5 ml of vial 2 (1 injection/week). Finally, 12 administrations of 0.5 ml of vial 2 were administered in monthly intervals. The placebo contained all the components of the vaccine with the exception of the modified extract.

Safety

Side reactions were recorded and classified according the localization (local or systemic), and the time of appearance (immediate or delayed). Local reactions were expressed as the length of the diameter. Systemic reactions were graded in accordance with the The European Academy of Allergology and Clinical Immunology (EAACI) guidelines (16).

Bronchial provocation test

This test was considered as the main outcome to document the clinical efficacy of the treatment (17). It was performed at baseline and after 1 year according to the method of Cockcroft et al. (18) with modifications to adapt a specific allergens (19). The same batch of native, standardized unmodified allergen extract of at 0.1, 1, 10 and 100 HEP/ml (40.7 µg of Der p 1/ml and 17.22 of Der p 2; Laboratorios LETI, S.L.) was used throughout the trial. It was supplied freeze-dried and vacuum-closed to be reconstituted just before use. All patients were tested between 8 AM and noon and none was pretreated with drugs or challenged under circumstances that could affect the result of the test (20–22). Only immediate reactions were recorded and evaluated.

The results were expressed as PD₂₀FEV₁. Differences between baseline and after 12 months of immunotherapy were calculated for each patient. The results were also expressed as the number of patients that needed at the end of the study more than double or less than the half of the initial amount of allergen extract to experience a positive BPT.

Skin prick tests

Patients were skin tested at baseline and at the end. These tests were conducted in duplicate, on the volar surface of the forearm at

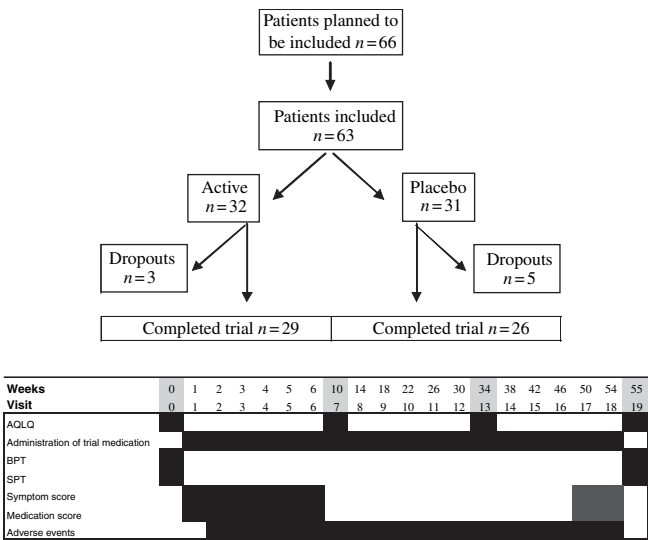


Figure 1. Flow chart and schedule of the clinical trial.

the concentrations of 0.002, 0.02, 0.2 and 2 mg/ml. The vial of maximum concentration had a potency of 100 HEP/ml and contained 40.70 µg of Der p 1/ml and 17.22 of Der p 2 (Laboratorios LETI, S.L.). Histamine HCl 10 mg/ml and a glycerinated saline solution were used as positive and negative controls, respectively. All the reactions were recorded after 15 min of application (17). The area of each wheal was measured by planimetry using a Wacom palette (Wacom Tehnology Co., Vancouver, WA, USA) and the computer program MacDraft (Microspot, Inc., Boca Raton, FL, USA). For each patient, the dose of allergen extracts to produce the same wheal size as the positive control (10 HEP) was calculated (17).

Symptom and medication scores

All patients recorded daily symptom scores (nose, eyes and lungs) during the build-up phase of the treatment (visits 2–6) and 1 month before the end of the study. Nose (sneeze, blockage and running), eye (itching, redness and swelling) and chest (breathlessness, wheeze, chest tightness), were scored on a scale from 0 to 3 (0 = none; 1 = slight, the symptom is present but not troublesome; 2 = moderate, the symptom is troublesome, but not disabling or insufferable and 3 = severe, the symptom disabling and/or insufferable). The daily total symptom score was calculated as the sum of all individual symptom scores. The intake of medication was recorded and quantified according to Dreborg et al. (23).

Asthma quality of life assessment

The questionnaire developed by Marks et al. was used (24). It was formulated four times during the trial: baseline (visit 0); when the maintenance dose was repeated for the first time (visit 7); after 6 months (visit 13) and at the end of the trial (visit 19).

Statistical methods

The Excel spreadsheet (Microsoft, Inc., Seattle, WA, USA) and the statistical software spss v.11.0 (SPSS, Inc., Chicago, IL, USA) were used. The results were analysed for normality (Shapiro-Wilk), demonstrating that all the outcomes did not follow a normal distribution. Descriptive statistics were expressed as the median with the first and third quartiles (interquartile range, IQR), and non-parametric tests were used for comparative statistics (Wilcoxon, Friedman and Mann-Whitney). Contingency table analysis (chi-square *P*-value) was used to evaluate the statistical significance of the number of patients who needed at the end more than twice, or less than half the baseline dose of native extract to reach a positive BPT. Hodges-Lehmann estimator [with the 95% lower and upper confidence limits (CI)] was used to measure the effect size of the differences between the two groups.

Results

Patient population

Sixty-three patients were entered and 55 completed the study (26 males and 29 females; mean age 23 years, range: 14–48). Twenty-nine patients were treated with the active preparation and 26 received placebo. At baseline, there were no statistically significant differences between the two groups in age, sex and duration of asthma. Eight

patients withdrew the study, three in the active and five in the placebo group. Five abandons were due to the patient's personal decision, one for pregnancy, one to bad compliance and one to relocation.

Bronchial provocation test

At baseline, the active group needed a median of 2.56 HEP (IQR: 0.54–5.61), and placebo 2.77 (IQR: 1.69–4.02) ($P = 0.9173$). At the end of the study, these values were 7.14 (IQR: 4.29–14.38) and 2.76 HEP (IQR: 1.50–10.81), respectively ($P = 0.0029$).

The differences of the PD₂₀FEV₁ values between those obtained after 12 months with those at baseline in the active group had a median value of 4.27 HEP (IQR: 1.74–8.45), and of –0.05 HEP (95% IQR: –1.17 to 5.19) in the placebo ($P = 0.0004$). The results of the Hodges-Lehmann estimator were –0.21 (95% CL: –2, 0.95) at baseline and 3.11 (95% CL: 0.4–5.88) at the end; and 4.41 (95% CL: 1.78–8.44) for the differences between baseline and at the end.

The active group showed a significant difference from baseline ($P < 0.0001$), whereas the placebo group did not ($P = 0.9292$). After 12 months, the active group needed a median of 2.8 times more allergen to achieve the PD₂₀FEV₁ value. In contrast, in the placebo group this value was 1, indicating no changes. Figure 2 shows the evolution of the PD₂₀FEV₁. At the end of the study, 19 patients in the active group needed more than twice the amount of the native allergen to obtain a positive BPT vs 10 in the placebo group ($P = 0.0293$). One patient in the active group and seven in the placebo group needed less than half the amount of the native allergen to achieve a positive BPT at the end ($P = 0.0137$).

Skin prick test

At baseline, the median value to produce a wheal size of 10 HEP in the active group was 0.05 mg (IQR: 0.02–0.08)

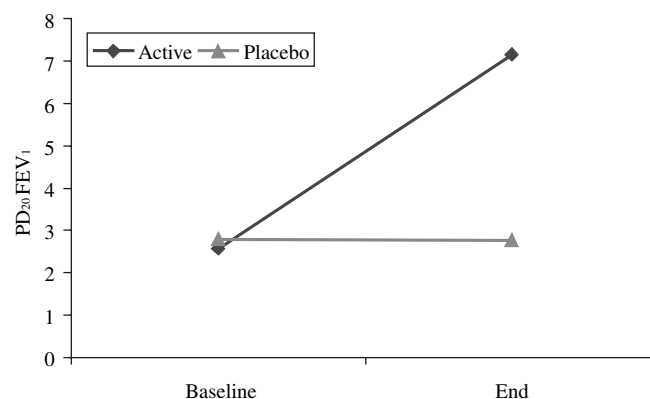


Figure 2. Evolution of the PD₂₀FEV₁ values of the bronchial provocation test (BPT) from baseline to the end of the study.

vs 0.07 mg (IQR: 0.02–0.18) in the placebo group ($P = 0.3366$). At the end of the study, these values were 0.22 (IQR: 0.11–0.32) and 0.10 mg (IQR: 0.03–0.17), respectively ($P = 0.0049$). The results of the Hodges–Lehmann estimator were -0.02 (95% CI: $-0.1, 0.02$) at baseline, 0.09 (95% CI: $0.03, 0.16$) at the end, and 0.13 (95% CI: $0.04, 0.21$) for the difference between baseline and at the end. The active group showed a significant difference from baseline ($P < 0.0001$), whereas the placebo group did not ($P = 0.4237$). After 12 months, the active group needed a median of 4.5 times more extract to achieve the value of 10 HEP. Figure 3 shows the evolution of the skin prick test (SPT).

Symptom score

At baseline, the median value of the active was 1.57 (IQR: 0.50 – 2.35) vs 1.43 (IQR: 0.33 – 2.40) for the placebo group ($P = 0.7341$). At the end, the values were 0.14 (IQR: 0.00 – 1.93) and 2.93 (IQR: 1.11 – 4.19), respectively ($P = 0.0001$). The results of the Hodges–Lehmann estimator were -0.14 (95% CI: $-1.1, 0.86$) at baseline, -1.83 (95% CI: $-3.10, -0.60$) at the end, and -1.71 (95% CI: $-0.1, -3.4$) for the difference between baseline and the end. Only the group treated with the active vaccine showed a significant difference from baseline ($P = 0.0001$), whereas the placebo group remained without significant changes ($P = 0.7341$). After 12 months the active group had a 78% decrease of symptoms over placebo.

Medication score

At baseline, the median of the active group was 3.57 (IQR: 2.58 – 5.29) vs 4.0 (IQR: 2.54 – 5.36) for placebo ($P = 0.8306$). At the end of the study (12 months) the values were 1.00 (IQR: 0.29 – 1.50) and 3.13 (IQR: 2.47 – 3.63), respectively ($P < 0.0001$). The results of the

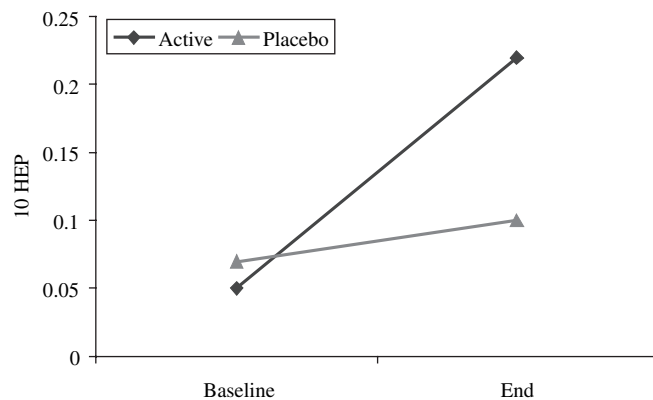


Figure 3. Evolution of the quantity of nonmodified allergen extract needed to obtain the 10 HEP value from baseline to the end of the study.

Hodges–Lehmann estimator were -0.50 (95% CI: $-1.70, 0.75$) at baseline, and -2.00 (95% CI: $-2.60, -1.30$) at the end and -1.56 (95% CI: $-0.2, -2.8$) for the difference between baseline and the end. The active group showed a statistically significant difference from baseline ($P = 0.0005$), whereas the placebo group did not ($P = 0.4650$). The group treated with the active treatment had, after 12 months, a 68% decrease of medication over placebo.

Asthma quality of life questionnaire

The results of the median values of the questionnaire, the Hodges–Lehmann estimator and the Mann–Whitney’s test results are shown in Table 1. Both groups showed improvement when the results of the four visits were analysed ($P < 0.0001$). When the values obtained from visits 7, 13 and 19 were used, the active group showed a significant improvement ($P = 0.0233$), whereas the placebo group did not ($P = 0.8517$). In the active group, the analysis of paired values of visits 13 and 7, and of visits 19 and 7) were not significant ($P > 0.05$), whereas the difference between visits 13 and 19 was significant ($P = 0.0234$). In the placebo group, all these differences were not significant ($P > 0.05$). Figure 4 shows the evolution of asthma quality of life (AQLQ).

Table 1. Descriptive [median and interquartile (IQ) range] and comparative statistics (Hodges–Lehmann and Mann–Whitney) of the values obtained with the asthma quality of life (AQLQ)

	Active	Placebo	Hodges–Lehmann	Mann–Whitney (P-value)
Visit 0	17.00 (13.00–30.00)	27.00 (15.25–36.25)	–5 (–13; 2)	0.1771
Visit 7	7 (3.25–11.00)	9.00 (5.00–16.00)	–2 (–6; 2)	0.2369
Visit 13	7 (3.00–9.00)	9.00 (5.00–14.00)	–2 (–5; 1)	0.1891
Visit 19	4 (1.00–8.00)	10.50 (5.00–17.00)	–5 (–9; –2)	0.0025

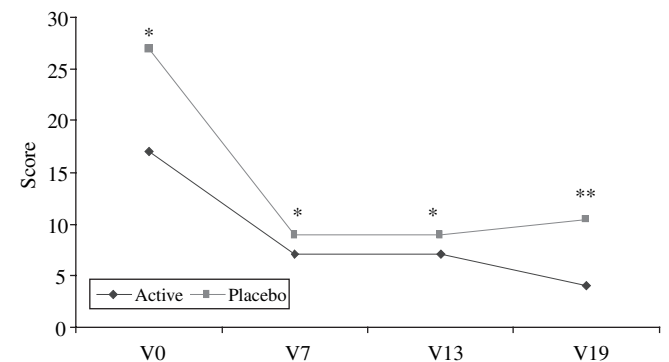


Figure 4. Evolution of the asthma quality of life (AQLQ) throughout the entire study * $P > 0.05$ (Mann–Whitney’s test); ** $P < 0.05$ (Mann–Whitney’s test).

Table 2. Systemic reactions classified according the grade and time of appearance

	Grade 1		Grade 2		Grade 3	
	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed
Active	1	3	0	1	0	1
Placebo	0	2	2	0	0	0

Safety assessment

The treatment was well-tolerated and no patient withdrew because of adverse events. Two local reactions (one immediate and one delayed) with a diameter between 5 and 10 cm were recorded in the active group, and three immediate in the placebo group. Systemic reactions were recorded in five patients in the active and three in the placebo group (Table 2). All reactions of grade 2 were mild pruritus; the grade 3 reaction was urticaria, which appeared 2 h after the administration of the vaccine in a patient who had a clinical history of other episodes of urticaria unrelated to the administration of the vaccine.

Discussion

This clinical trial was designed to evaluate safety and efficacy of a depigmented, glutaraldehyde-modified *D. pteronyssinus* allergen extract. The results obtained demonstrate that the treated group had a significant decrease in allergen-specific bronchial hyperreactivity. This group needed at baseline a median of 2.56 to achieve the PD₂₀FEV₁, whereas after 12 months of treatment the median value was 7.14 HEP. The placebo group remained without modification. Between groups, the differences from baseline were highly significant ($P < 0.05$); in the active group, the median difference from baseline was 4.27, whereas in the placebo group this value was -0.06. The result of the Hodges-Lehmann estimator demonstrates the effect size of these findings, in which the zero value is not included between the 95% CI. These findings reflect the effect of the treatment on the target organ, because environmental measures to control mite allergens were not in place and the symptomatic medication was carefully adjusted before the performance of BPT in order to avoid any influence in the results.

The reproducibility of the PD₂₀FEV₁ is between the limits of a twofold concentration of the antigen (22). Therefore, we also studied the number of patients who needed twice the amount of native allergenic extract to obtain a positive BPT. After 12 months, 19 patients in the active and 10 in the placebo group met this criterion ($P = 0.0293$). This results agree with the findings of Ferrer and García-Sellés (8), who observed a significant improvement after 6 months of treatment with a therapeutic vaccine containing a mixture of modified *D. petronysinus* and *D. farinae*. Guerra et al. (6) obtained

similar results after 1 year of immunotherapy with a modified vaccine of *Olea europaea*.

An important clinical finding was that seven patients of the placebo group needed less than half the amount of the native unmodified extract to achieve a positive specific BPT at the end of the study, and only one patient of the active group ($P < 0.05$). This result is also in agreement with the findings of Guerra et al. (6) who reported similar results in untreated asthma patients allergic to the pollen of *O. europaea*. This report suggests that specific immunotherapy also acts as a preventive treatment for the worsening of allergen-induced bronchial hyperreactivity.

It has been previously reported (2, 25) that the effects of allergen immunotherapy on the natural course of allergic disorders include: (i) prevention of a reaction following re-sting in insect venom allergy; (ii) prevention, or decrease in the natural progress of allergic rhinitis to asthma and (iii) inhibition of new sensitization in monosensitized children. Herein, we report the effect of preventing the worsening of allergen-specific bronchial hyperreactivity. We acknowledge that more studies are needed to fully understand this finding.

The SPT results showed the same evolution as BPTs, demonstrating a significant decrease in skin reactivity in the active group. The quantity of native allergen extract to achieve the value of 10 HEP increased in a factor of 4.4 in the active group, whereas in the placebo group the factor was 1.4. The result of the Hodges-Lehmann estimator demonstrated the effect size of these findings, in which the zero value is not included between the 95% CI.

Symptom and medication scores were improved only in the group treated with the modified allergen extract, whereas the group treated with placebo remained without modification. The symptom score improved by 78% in the active group and the medication score by 68%. These improvements should be considered in the category of high efficacy (26).

The analysis of the AQLQ shows that between the two groups there was no significant difference at baseline ($P > 0.05$). After the inclusion in the study, both groups experienced an improvement that could not be explained as a consequence of the treatment. However, it could be explained as the Hawthorne effect (27, 28). This effect was first noticed in the Hawthorne plant of Western Electric and described as an initial improvement in a production process caused by the obtrusive observation of this process. The special attention received, increased the production rate of the workers, not as a consequence of changes in the working conditions introduced by the plant's management, but because management demonstrated interest in such improvements. In our study, the differences between both groups were significant only at the end ($P = 0.0025$), being the improvement exclusively in the active group.

In conclusion, in this study we demonstrate that the treatment with a depigmented and polymerized allergen

extract of *D. pteronyssinus* is safe and shows significant efficacy in allergic respiratory disease as measured by objective and subjective clinical parameters. It also demonstrates the role of immunotherapy in preventing the worsening of specific bronchial hyperreactivity and respiratory symptoms.

Acknowledgments

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