

Double-blind comparative study of cluster and conventional immunotherapy schedules with *Dermatophagoides pteronyssinus*

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Background: The conventional schedule for subcutaneous immunotherapy with allergen extracts, although efficacious and safe, is slow during the dose-increase phase.

Objective: We sought to compare the efficacy and safety of subcutaneous immunotherapy with *Dermatophagoides pteronyssinus* standardized extract given in a 6-week cluster period and a conventional 12-week schedule during the incremental-dose phase.

Methods: Of 239 patients with rhinitis, allergic bronchial asthma, or both caused by *D pteronyssinus*, 120 were randomly assigned to the cluster schedule, and 119 were randomly assigned to the conventional schedule. A biologically standardized *D pteronyssinus* depot extract (ALK-Abelló S.A., Madrid, Spain) was administered in a placebo-controlled, double-blind fashion during the initial phase of cluster or conventional treatment. We recorded adverse reactions, clinical efficacy, cutaneous reactivity, and serum specific immunoglobulins to *D pteronyssinus* before immunotherapy, when the maximum dose was reached in the cluster and conventional schedules, and after 1 year of treatment.

Results: The cluster schedule reduced the time to maintenance dose by 46% and caused systemic adverse reactions (all mild) after only 0.15% of injections, with no differences in comparison with the conventional schedule. Cluster immunotherapy led to decreases in asthma and rhinitis symptoms, reduced the cutaneous reactivity, and produced the increase in specific IgE and IgG₄ levels on reaching the maintenance dose in the sixth week, 6 weeks earlier than with the conventional schedule.

Conclusion: The cluster schedule for the initial phase of immunotherapy with incremental doses of *D pteronyssinus* is a safe alternative to conventional immunotherapy and offers

the further advantage of achieving clinical and immunologic improvements sooner. (J Allergy Clin Immunol 2005;116:109-18.)

Key words: Cluster immunotherapy, conventional immunotherapy, double-blind study, clinical efficacy, safety, children, adults, skin prick test, specific immunoglobulins, kinetics

The clinical efficacy of subcutaneous immunotherapy with standardized allergen extracts for the treatment of allergic rhinitis and bronchial asthma is now well documented.¹⁻⁴ However, conventional schedules for the initial phase of incremental doses have the disadvantage of the time needed to reach the maintenance dose, with its attendant costs in terms of time and resources. Moreover, it remains to be shown whether conventional schedules are the best approach available. In recent years, studies have been performed to identify the ideal schedule that allows the dose considered effective to be reached in the shortest possible time with the fewest adverse effects. Cluster schedules, thanks to their lower rate of adverse effects in comparison with other faster schedules for the initial phase, are an alternative to conventional immunotherapy. Many different cluster schedules have been used, with a number of visits that ranged from 3 to 8, once or twice a week, and 1 to 5 shots per visit. Most studies of cluster schedules with different allergen extracts have set out to determine tolerance,⁵⁻⁷ whereas few studies have evaluated efficacy parameters.⁸⁻¹² Fewer still have compared the efficacy of cluster schedules versus conventional approaches.¹³ No study to date has investigated whether reaching the maximum dose in less time achieves clinical improvements in less time. Finally, little is known about how the schedule type used in immunotherapy administration affects the changes in immunologic parameters, although the most aggressive schedules (cluster or rush) seem to produce these changes more quickly than conventional ones.^{14,15}

In this study we used a double-blind design to compare a conventional and a cluster schedule designed at our center. Our aims were to determine whether the cluster treatment was as safe as the conventional schedule¹³ and

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Abbreviations used

BU: Biologic units
PEF: Peak expiratory flow

whether the former approach, in which the maintenance dose is reached in half the time, yielded clinical benefits and immunologic changes sooner, a feature that would constitute an additional advantage.

METHODS**Study design**

This was a prospective, randomized, placebo-controlled, double-blind phase IV clinical trial. Physicians and patients were unaware of which of the 2 immunization schedules was used during the initial dose-increase phase of immunotherapy.

Patients

The sample size needed to compare the safety of the cluster and conventional schedules was determined on the basis of data for adverse reactions obtained in a pilot study.¹³ All patients given a diagnosis of slight-to-moderate rhinitis or bronchial asthma caused by *Dermatophagoides pteronyssinus* at our allergology department during a 1-year period for whom immunotherapy was indicated were invited to take part in the study.

The diagnosis of and pharmacologic treatment for rhinitis and asthma were based on the criteria of the International Consensus for the Diagnosis and Treatment of Rhinitis¹⁶ and the National Heart, Lung, and Blood Institute of the National Institutes of Health,¹⁷ respectively. Inclusion required a positive skin prick test response (wheal ≥ 3 mm in diameter) and *D pteronyssinus*-specific IgE in serum of 0.7 kU/L or greater (CAP Pharmacia, Uppsala, Sweden).

The indication for immunotherapy was established on the basis of the guidelines of the Immunotherapy Subcommittee of the European Academy of Allergology and Clinical Immunology² and World Health Organization recommendations.³

Ethical issues

The study schedule complied with the International Declaration of the World Medical Association and was approved by the hospital's clinical trials committee and by the Spanish Drug Agency (Dirección General de Farmacia). All patients provided written informed consent to take part in the study.

Allergen extract

Treatment was given with a commercial extract, Pangramin Depot UM *D pteronyssinus* (ALK-Abelló, Madrid, Spain). This depot extract is adsorbed on aluminum hydroxide and is standardized in biologic units (BU). The highest-concentration vial had an allergenic activity of 10 BU/mL and contained 4 μ g/mL Der p 1 and 2 μ g/mL Der p 2.¹⁸

A *D pteronyssinus* extract, glycerinated to 50% (vol/vol) and containing 0.4% phenol (vol/vol) and 0.9% NaCl (wt/vol), with a 500 BU/mL biologic activity was prepared for cutaneous tests. This extract was kept at -20°C to maintain biologic activity during the whole study period.

Preparation of immunotherapy and placebo doses

All patients were given the same number of doses, some containing allergen extract and some containing the placebo, according to the cluster or conventional schedule used to blind physicians and patients to the treatment. The dose schedules for the initial phase in both treatments are shown in Table I. All doses were prepared weekly at the pharmacy service of the hospital as single doses and were labeled in a way that ensured that immunotherapy was administered in a double-blind fashion.

In both groups the maximum dose of 8 BU was administered at week 15, week 18, and every 4 weeks thereafter until the end of the first year. All patients came to the immunotherapy unit of the hospital for treatment throughout the study period.

Patients were excluded from the study if treatment was interrupted for longer than 5 weeks during the initial phase or longer than 16 weeks in the maintenance phase or if severe systemic adverse reactions occurred.

Evaluation of efficacy

Clinical efficacy was evaluated by grading the severity of disease on the basis of the physician's evaluation, diary cards, use of medication, and peak expiratory flow (PEF) and with a visual analog scale.

Scoring of clinical symptoms and use of medication by the physician. The severity of asthma and rhinitis was graded as the sum of the scores for symptoms,^{19,20} use of medication, and respiratory function (for asthma), as described in earlier studies.^{13,21-23}

Diary cards, use of medication, and PEF. Symptoms and use of medication were recorded by patients on a diary card, and in patients with asthma, PEF results were recorded during 2-week periods at different times during the study. Each patient was given a standardized card to note symptoms and medications used for symptoms.²⁴ Patients with asthma were given a PEF meter (Mini-Wright peak-flow meter; Clement Clark, London, United Kingdom). Patients measured PEF in the morning on rising and before going to bed.

Medication use was recorded daily. Each puff with a short-acting bronchodilator (rescue medication) was scored arbitrarily as 1 point, and the scoring for other drugs was based on the reduction in β_2 -agonist use for rescue therapy as reported in earlier studies²⁵⁻²⁹: 1 point for 25 μ g of salmeterol administered through inhalation or the equivalent amount administered through other routes; 1 point for 250 μ g of beclomethasone dipropionate administered through inhalation or its equivalent; 1 point for 5 mg of oral montelukast; and 0.25 points for 2 mg of nedocromil administered through inhalation. A score of 1 point was assigned arbitrarily to 1 tablet of antihistamines for rhinitis, and 1.4 points was assigned to 400 μ g/d nasal beclomethasone or its equivalent.³⁰ When the dose of drug was higher than those mentioned before, we calculated the score of points in proper relation (ie, 500 μ g of beclomethasone dipropionate administered through oral inhalation should be 2 points). Scores were calculated for symptoms and use of medication separately, as was mean PEF and variability according to the method of Quackenboss et al³¹ by using the following formula:

$$(\text{Maximal PEF} - \text{Minimal PEF}) \times 100 / \text{Mean PEF}.$$

Visual analog scale. Patients evaluated their clinical status subjectively on a 10-point visual analog scale² from very poor (0) to very good (10).

Cutaneous tests

Cutaneous tests were carried out by using the skin prick test with the *D pteronyssinus* extract at 500, 100, 20, and 4 BU/mL and with histamine chlorohydrate at 10 mg/mL and saline solution.

TABLE I. Cluster and conventional schedules

Week	Cluster schedule			Conventional schedule		
	Dose (BU)	Total dose (BU)	Total dose (μg of Der p 1)	Dose (BU)	Total dose (BU)	Total dose (μg of Der p 1)
0	0.02 0.04 0.06 0.08	0.2	0.08	0.01	0.01	0.004
1	0.1 0.2 0.4	0.9	0.36	0.02	0.03	0.012
2	0.6 0.8	2.3	0.92	0.04	0.07	0.028
3	1 2	5.3	2.12	0.08	0.15	0.06
4	2 3	10.3	4.12	0.1	0.25	0.1
5	3 4	17.3	6.92	0.2	0.45	0.18
6	8	25.3	10.12	0.4	0.85	0.34
7	0	25.3	10.12	0.8	1.65	0.66
8	0	25.3	10.12	1	2.65	1.06
9	0	25.3	10.12	2	4.65	1.86
10	0	25.3	10.12	4	8.65	3.46
11	0	25.3	10.12	6	14.65	5.86
12	0	25.3	10.12	8	22.65	9.06

Interval of injections = 30 minutes. Only placebo doses were administered to the cluster group from weeks 7 to 12. The maintenance immunotherapy doses were administered at weeks 15 and 18 and were continued every 4 weeks up to the end of 1 year.

Tests were duplicated with a standardized lancet (Prick-Lancett BN; Dome/Hollister-Stier, Miles Ltd, United Kingdom) according to the recommendations of the EAACI Subcommittee on Skin Tests^{32,33} and were all performed by the same person throughout the study. The variation coefficient in cutaneous tests was 15%. The wheal areas were measured with a scanner, and data were transformed to logarithms so as to apply parametric tests.

In vitro parameters

Serum specific IgE, IgG, and IgG₄ concentrations in serum against *D pteronyssinus* were determined through fluoro-enzyme-immuno assay, according the Pharmacia CAP System RAST FEIA method (Pharmacia Diagnostics, Uppsala, Sweden). The quantification of specific IgE, IgG, and IgG₄ was assessed in a unique assay at the end of the study.

Safety evaluation

Each dose administered and all local and systemic adverse reactions were recorded and classified in accordance with the guidelines of the Immunotherapy Subcommittee of the European Academy of Allergy and Clinical Immunology.²

Time course of the study

Clinical efficacy was evaluated at T0 (before immunotherapy began), T1 (sixth week of treatment, when the maximum dose was reached with the cluster schedule), T1' (12th week of treatment, when the maximum dose was reached with the conventional schedule), and TF (after 1 year of treatment).

For each parameter, clinical efficacy was recorded at the beginning (T0) and end (TF) of the study in all patients. The T1 and T1' measures were recorded for a subgroup of patients chosen randomly before treatment began.

Skin prick tests were performed in 5 phases: P0 (before immunotherapy initiation), P6 (in the sixth week of treatment, when the maximum dose was reached in the cluster schedule), P12 (in the 12th week of treatment, when the maximum dose was reached in the conventional schedule), P18 (in the 18th week, after the third maintenance dose), and P52 (after 1 year of treatment). The first and final skin prick tests (before immunotherapy initiation and after a year, respectively) were carried out on all patients. Time controls during the remaining periods were on patients selected at random before beginning treatment.

Finally, specific IgE, IgG, and IgG₄ levels were determined before immunotherapy initiation (or T0), every 2 weeks up to the 16th week, and after a year of treatment. Intermediate control times were carried out on a randomly selected subgroup of patients in such a way that each patient had 3 serum extractions at the following times: before immunotherapy initiation, after 1 year, and at a randomly selected time between the two time points.

Statistical analysis

Statistical analyses were done with SPSS version 10.0 for Windows (SPSS Inc, Chicago, Ill), the BMDP Statistical Software release 7 (Statistical Solutions, Saugus, Mass), and the Stat Xact program (Cytel Software Corp, Cambridge, Mass). We used Wilcoxon and Mann-Whitney tests to assess the differences in the same group and between both treatment groups, respectively.

A parallel-line assay for skin tests (AIASA-CRS-PLA)³⁴ was used for individual data, calculating differences in the cutaneous tolerance index at each control time, to study the evolution of cutaneous sensitivity. The cutaneous tolerance index is the factor for which allergenic extract concentration must be multiplied when tested in a group so as to obtain the same wheal area as in another group. The evolution in the same treatment group and between different treatment groups (cluster and conventional) was compared through

TABLE II. Demographic and clinical data

	Cluster schedule (n = 120)	Conventional schedule (n = 119)
Sex (male/female)	75/45	71/48
Age, y (mean $\pm$ SD)	19.34 $\pm$ 9.8	18.47 $\pm$ 9.49
Disease duration, mo (median [interquartile range])	48 (24-120)	48 (24-60)
Bronchial asthma (Mi/Mo/Se)	94 (55/39/0)	82 (54/28/0)
Rhinitis (Mi/Mo/Se)	107 (16/88/3)	102 (10/90/2)
Smoker	13	22

Mi/Mo/Se, Mild/moderate/severe.

ANOVA. Each patient's increase or decrease in specific IgE, IgG, and IgG₄ variables was evaluated in each control time period compared with values before treatment initiation. Correlation between cutaneous test modification and specific immunoglobulins was analyzed by using the Spearman correlation coefficient. Significance required for all tests was .05.

RESULTS

Patients

Informed consent to participate in the study was obtained from 239 patients. They were randomly assigned to receive cluster (120 patients) or conventional (119 patients) therapy in the initial phase of treatment. There were no differences between the 2 groups in clinical or demographic characteristics at the start of the study. (Table II).

Compliance with treatment

Nine patients (5 in the cluster group and 4 in the conventional group) did not complete the initial phase and were excluded from further study. One patient in the cluster group was withdrawn because of worsening asthma that contraindicated the administration of the remaining scheduled doses of immunotherapy. Another 8 patients dropped out because of problems traveling to the immunotherapy unit for scheduled treatment.

During the maintenance phase, 13 patients were lost to the study before the end of the first year (8 in the cluster group and 5 in the conventional group). One patient was withdrawn because she became pregnant during the maintenance phase, and the other 12 dropped out for reasons unrelated to adverse reactions or worsening disease. The data for these patients were included in the statistical analysis for the period up to the day they dropped out or were withdrawn but were excluded from the 1-year analysis of efficacy of immunotherapy.

Doses used and duration of different stages of immunization

The time (mean $\pm$ SD) needed to reach the pre-established dose of 8 BU was 50.4 $\pm$ 0.97 days in the cluster group and 93.45 $\pm$ 1.41 days in the conventional group (46% shorter in the cluster group). A total of 5198 injections were given during the initial phase, 3544 of

which were for the allergen (1590 in the conventional group and 1954 in the cluster group); the rest of the injections contained the placebo. Cumulative dose was 25.3 $\pm$ 0.40 BU in the cluster group and 22.45 $\pm$ 0.33 BU in the conventional group. During the maintenance phase, a total of 2037 doses of allergen were given until the end of the first year of treatment.

Evaluation of clinical efficacy

Scoring of clinical symptoms and use of medication by the physician. Patients in the cluster group had a higher mean baseline score for asthma symptoms. The reduction in this score during T1 was greater in the cluster group (52% vs 32%). During T1', the reduction was greater with conventional therapy, such that the improvement in symptoms was similar in both groups at the end of this period (Fig 1). During T1, use of medication for asthma symptoms decreased significantly only in the cluster group (30%). In the conventional group no significant decrease was seen until TF (data not shown). For rhinitis, the global reduction in symptoms and medication during T1 use was 43% in the cluster group and 35% in the conventional group. During T1', these figures were 59% in the cluster group and 42% in the conventional group; this difference was statistically significant. In TF the effects of both schedules were similar (Fig 2).

Symptom cards, use of medication, and PEF. During T1, respiratory symptoms decreased by 45% in the cluster group, and no appreciable reduction was seen in the conventional group. The difference between groups disappeared at T1', with a 40% reduction in symptoms in the conventional group (Fig 3). There were no decreases of note in use of medication for asthma. Mean PEF increased starting at T1 in the cluster group. In contrast, mean PEF in the conventional group showed no increase until T1'. However, mean PEF values did not differ between groups in any phase of the study (data not shown). PEF variability decreased during T1' to the end of the first year of immunotherapy in the cluster group and remained unchanged in the conventional group (Fig 4).

Rhinitis global symptom and medication use scores decreased by 43% in the cluster group but showed no decrease in the conventional group during T1. There was an additional reduction in T1' in the cluster group, reaching 54%, and there was a 37% decrease in the conventional group. In T1' there was no significant difference between the groups (Fig 5).

Visual analog scale. The only significant change in patients' subjective evaluations of the symptoms was seen in the cluster group after 1 year of treatment, with an improvement of 21% with respect to T0 (data not shown).

Cutaneous tests

Before immunotherapy initiation, cutaneous sensitivity to the allergen was 1.36 (95% CI, 1.06-1.73) times greater in the cluster group than in the conventional group. There was then a significant decrease in cutaneous sensitivity to the allergen with the cluster schedule: 1.83 (95% CI,

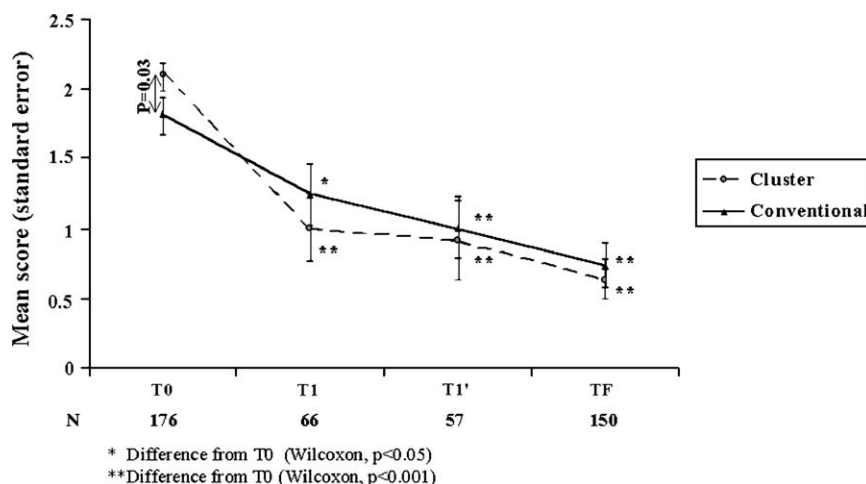


FIG 1. Asthma symptom scores from T0 to TF in the cluster and conventional groups of treatment.

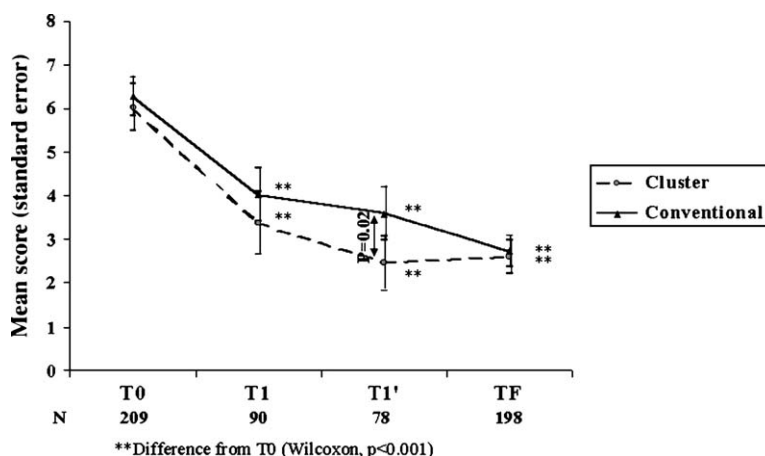


FIG 2. Rhinitis symptom and medication scores from T0 to TF in the cluster and conventional groups of treatment.

1.30-2.56) and 2.94 (95% CI, 2.07-4.19) times less at P6 and P12, respectively. This reduction was maintained at P18 and P52. The decrease in cutaneous sensitivity with the conventional schedule was not significant until P12, when it was 1.84 (95% CI, 1.40-2.41) times less, continuing to decrease at P18 and reaching 3.18 (95% CI, 2.54-4.00) times less after a year of treatment.

The differences observed in the evolution of both treatment groups was almost significant at P6 ($P = .055$) and significant at P12 ($P = .041$). Subsequently, cutaneous responses were similar (Fig 6).

In vitro tests

Table III shows specific IgE, IgG, and IgG₄ results at each control time period. There was a significant increase in specific IgE levels with the cluster schedule from the sixth week, coinciding with the end of the build-up phase. With the conventional schedule, this did not occur until the 12th week of treatment.

Specific IgG₄ showed the most homogenous behavior, registering an increase from the sixth week with the cluster

schedule. This increase was also evident from the 10th week with the conventional schedule but was only statistically significant from the 14th week. This continuous increase during treatment reached a 7-fold increase in both groups by the end of the year.

Cutaneous immunoglobulin correlation tests

There was a good correlation between specific IgG and IgG₄ ($r = 0.94$, $P < .0001$), which was not the case with specific IgE (data not shown).

There was no correlation between change in skin prick test results and change in specific IgE, IgG, or IgG₄ levels at any of the control time periods.

Adverse effects

During the incremental-dose phase, there were 19 adverse reactions (triggered by 0.54% of the allergen doses) in 15 patients (6.28% of the group). There were no adverse reactions with the placebo injections. Eight reactions were systemic (0.22% of all allergen injections), and of these, 3 were immediate (0.08% of all allergen

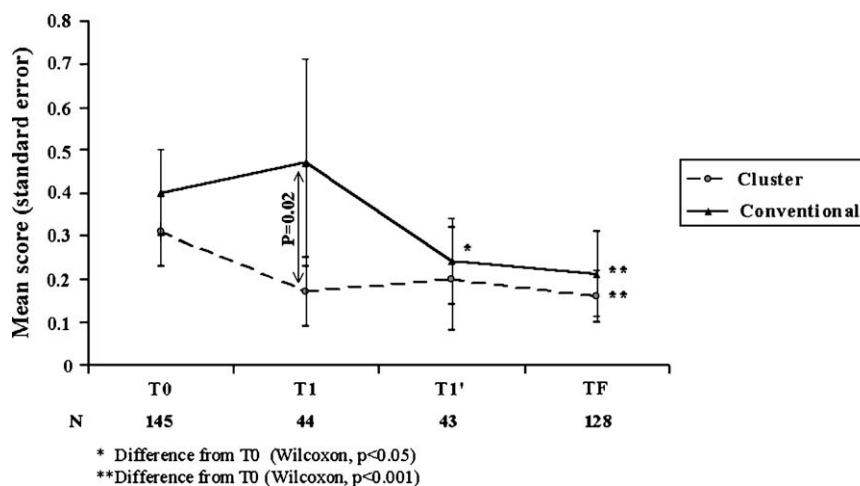


FIG 3. Asthma symptom scores recorded in diary cards from T0 to TF in the cluster and conventional groups of treatment.

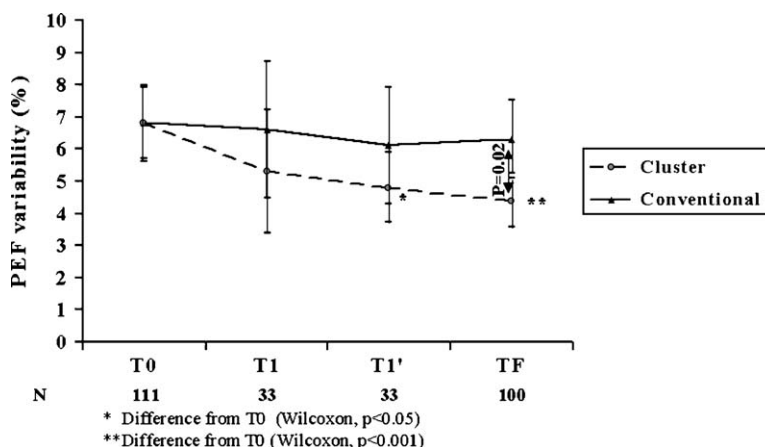


FIG 4. Variability of PEF from T0 to TF in the cluster and conventional groups of treatment.

injections). All adverse reactions were mild (grade 2). There were no differences between the 2 schedules in frequency or type of adverse reaction.

During the maintenance phase, there were 9 adverse reactions (0.45% of all doses) in 8 patients (3.54% of the group). Two were local delayed reactions, appearing later than 30 minutes after the injection. The other 7 were systemic (0.35% of the doses), none were immediate (1 with nonspecific symptoms and 6 with asthma), and all occurred with the highest dose given (8 BU). As a result of these reactions, the dose schedule for these patients was modified. However, all patients eventually tolerated the maximum dose of 8 BU.

DISCUSSION

The main objective of this study was to compare 2 schedules, cluster and conventional, for the incremental-dose phase of immunotherapy. We did not set out to

determine the efficacy of immunotherapy for mite allergy because this has been well documented.^{3,4} Therefore the study design did not include a separate placebo or control group. Placebo doses were used for all patients during the initial phase of immunotherapy to blind physicians and patients alike to the schedule that was being tested. This way, we ensured that any placebo effect would be similar in the 2 treatment groups.

The only parameters that actually measure treatment effectiveness are clinical parameters.¹ There is no universally accepted method for measuring clinical efficacy, and experts generally recommend a combination of different methods.² The methods used in this study were a severity of disease score, diary cards, PEF, and a visual analog scale. Ideally, entries should be made regularly throughout the period of study, but given the difficulty in getting patients to make entries reliably and consistently during an 8-week period,³⁵ we opted to select 2-week periods at different times during the study. Diary cards used in studies of the efficacy of cluster or rapid schedules for

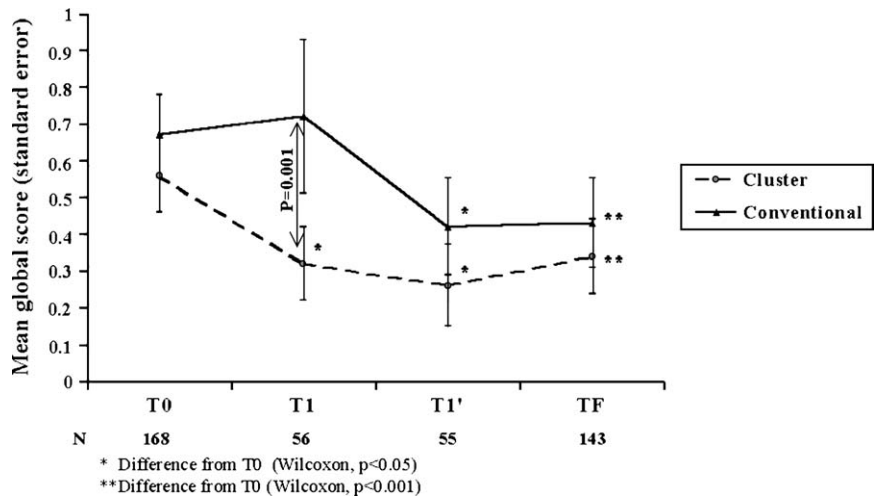


FIG 5. Rhinitis global symptom and medication scores recorded in diary cards from T0 to TF in the cluster and conventional groups of treatment.

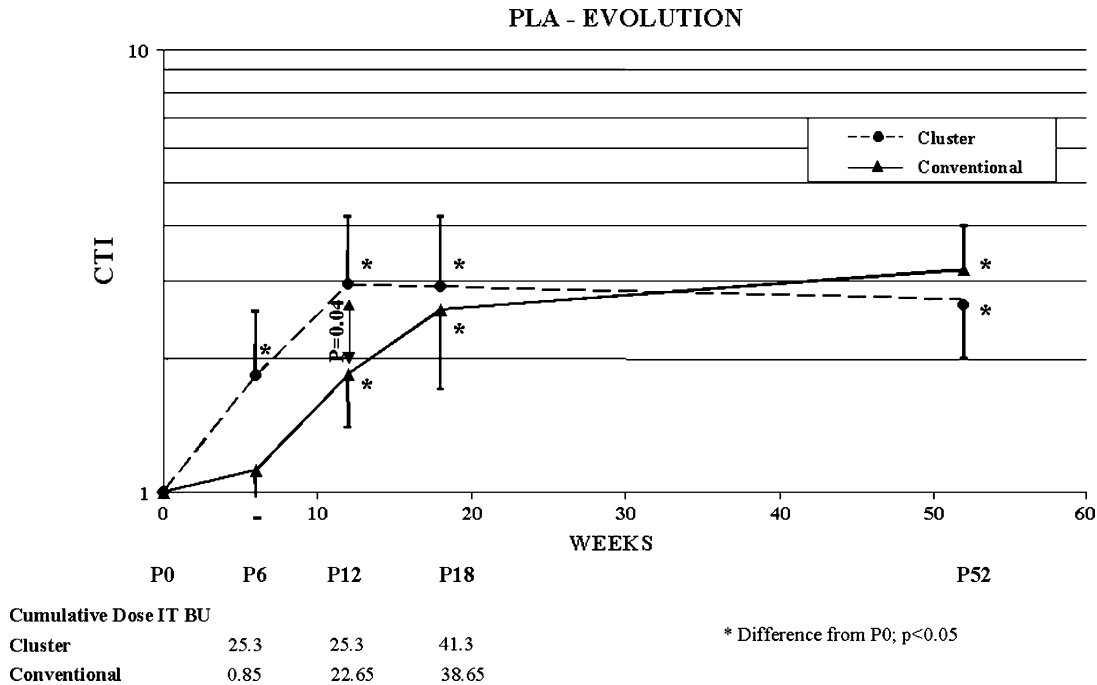


FIG 6. Evolution of cutaneous tolerance index in skin prick tests from P0 to P52 in the cluster and conventional groups. *IT*, Immunotherapy.

pollen allergy have shown that symptoms and use of medications were both reduced during the pollination period in the immunotherapy group in comparison with the placebo or control groups.⁹⁻¹¹

In the present study the symptoms of asthma and the global score for rhinitis were reduced in both immunotherapy groups at T1. This prompt reduction might be due in part to an external (placebo or Hawthorne) effect or to better pharmacologic control of asthma. The reduction in symptoms in the conventional immunotherapy group cannot be attributed to the lower doses used for immuno-

therapy at that point during the treatment schedule (cumulative dose, 0.85 BU; ie, one-thirtieth the dose given to patients in the cluster group). However, in patients treated with the cluster schedule, the decrease in asthma symptoms at T1 was greater than in the conventional schedule group (52% vs 32%). Malling¹ has suggested, on the basis of double-blind, placebo-controlled studies of the efficacy of immunotherapy, an arbitrary grading system to evaluate efficacy. On the basis of this system, the efficacy of the cluster schedule at T1 in reducing asthma symptoms was moderate, and the

TABLE III. *D pteronyssinus*-specific IgE, IgG, and IgG₄ at the different control times for the cluster and conventional groups

Week	Group (N)	Dp IgE	Dp total IgG	Dp IgG ₄
0	Cluster (106)	26.9 (2.2-314.8)	14.2 (3.1-55.9)	405.7 (65.2-1681.1)
	Conventional (106)	24.5 (1.5-372.2)	14.2 (4.1-56.0)	317.2 (68.4-2112.5)
2	Cluster (16)	26.1 (1.6-421.9)	16.4 (4.9-51.2)	477.6 (84.0-1977.0)
	Conventional (13)	18.8 (0.7-284.5)	13.2 (2.2-82.0)	333.0 (45.6-3473.8)
4	Cluster (12)	22.1 (1.4-343.2)	15.4 (2.7-82.2)	436.1 (25.8-3376.0)
	Conventional (11)	26.3 (1.4-494.4)	17.6 (10.1-35.8)	240.9 (71.7-2212.1)
6	Cluster (14)	38.8 (9.0-161.7)	21.9 (4.9-46.8)	620.6 (71.8-2654.6)
	Conventional (11)	32.6 (4.6-347.4)	12.5 (7.9-48.1)	307.0 (58.7-3729.1)
8	Cluster (12)	34.4 (3.6-323.6)	24.8 (3.7-65.1)	939.0 (61.4-3564.5)
	Conventional (15)	27.7 (3.3-229.7)	11.9 (6.9-50.3)	307.6 (51.4-5613.1)
10	Cluster (17)	40.8 (8.4-197.7)	19.9 (3.0-132.8)	777.8 (229.9-3150.7)
	Conventional (14)	31.6 (9.4-107.0)	22.1 (8.7-52.7)	564.3 (53.8-5365.4)
12	Cluster (6)	21.6 (2.0-233.8)	16.4 (6.7-34.1)	1081.4 (122.2-5926.5)
	Conventional (8)	46.2 (8.6-242.8)	23.2 (7.8-81.2)	761.1 (109.2-6943.8)
14	Cluster (11)	47.8 (10.6-207.4)	20.6 (6.9-41.9)	1846.0 (71.1-10185.9)
	Conventional (17)	49.8 (7.5-333.6)	21.1 (7.8-74.1)	771.6 (125.9-11481.5)
16	Cluster (9)	50.2 (14.8-169.5)	20.3 (4.0-78.9)	2810.3 (71.0-33327.3)
	Conventional (12)	14.8 (0.5-456.7)	20.7 (8.7-52.7)	1226.5 (391.2-6421.0)
52	Cluster (83)	34.1 (3.6-213.8)	25.2 (6.9-72.4)	2816.5 (274.9-20931.5)
	Conventional (86)	27.0 (2.1-352.5)	24.6 (8.7-83.2)	2183.2 (191.3-32900.3)

Dp, *D pteronyssinus*.

The geometric mean is expressed with its 95% CI.

efficacy of the conventional schedule was low. However, at T1', the cluster schedule led to no further reduction in asthma symptoms, whereas the conventional schedule led to a 45% decrease. Thus the overall results at T1' for decreases in asthma symptoms were similar in both groups. This finding is consistent with the results of an earlier study at our center.¹³

No significant reduction in the use of medication for asthma registered on diary cards was evident until after 1 year of treatment. This might be explained by the fact that in this study patients were allowed to use their initially prescribed medications to stabilize the disease both during the initial phase of immunotherapy and during T1 and T1'. This way, we approximated clinical practice as closely as possible and reduced the risk of adverse reactions. However, it is more difficult to detect variations in symptoms and use of rescue medication when the patient is clinically stable with appropriate pharmacologic treatment.

Mean PEF values recorded by patients did not differ between groups. Daily variations in PEF, considered a more robust parameter than mean PEF,^{31,35} were significantly reduced only in the cluster group and only at time T1'. At baseline, variability was small (about 7%) and could be considered within the normal range.³¹ However, a 7% variability has also been reported in patients with moderate-to-severe asthma who were clinically stable with pharmacologic treatment.³⁶ Our patients in the cluster group were graded initially as having a higher score for asthma symptoms. This might explain why immunotherapy reduced variability in PEF only in the cluster group. Longitudinal studies have shown that patients with asthma in whom the clinical expression of the disease was initially greater subsequently showed the

greatest reductions in PEF variability once the disease was controlled.³⁶

Numerous studies have analyzed the effect of immunotherapy on cutaneous reactivity to the allergen. On the whole, there was a continuous reduction in cutaneous sensitivity,² which in some studies was correlated to clinical improvements.³⁷

In this study we have seen a progressive reduction in cutaneous reactivity to the allergen, but the most interesting thing has been the different kinetics of changes in skin sensitivity with both treatment schedules. In previous immunotherapy studies, using semirush schedules or cluster schedules with pollens,¹⁰ mites,⁸ or hymenoptera venom,³⁸ a rapid reduction in cutaneous sensitivity to the allergen was observed. Reduction in specific cutaneous reactivity at a maintenance dose equivalent to that obtained with the conventional schedule was achieved with the same cluster schedule as the one used in our study.³⁹ Ewbank et al⁴⁰ compared 3 doses of cat immunotherapy administered by using a cluster schedule and observed a dose-dependent decrease in skin sensitivity on reaching the maintenance dose, which remained unchanged after a year of treatment.⁴¹

In our study there was already a skin reactivity reduction with the cluster schedule at the sixth week, when the maximum 25.3-BU dose was reached. Cutaneous sensitivity in the conventional group did not decrease with the low 0.85-BU dose at week 6 but did at week 12, with accumulated doses of 22.65 BU.

Therefore in this initial phase of treatment, we observed a reduction in skin reactivity to the allergen parallel to the immunotherapy dose administered. This sensitivity continued to decrease in the cluster group, even though

patients only received placebo from the sixth to the 12th week, which would seem to indicate a temporary delay between allergen administration and its effect on skin sensitivity. In week 18, both schedules' effects on cutaneous reactivity were the same.

The majority of studies have shown that in conventional immunotherapy with allergenic extracts, specific IgE serum concentration against the allergen initially increases in the phase of increased doses and then decreases to basal levels after a few months of treatment. Nevertheless, there are numerous individual variations in these specific IgE modifications.^{42,43} Some studies with nonconventional immunotherapy schedules were unable to demonstrate consistent changes in specific IgE levels.^{8,40,44} In contrast, Stevens et al¹⁵ showed that the specific IgE increase 2 months after administering a semirush pollen schedule was greater than that produced with the conventional schedule.

The contribution of specific IgG to clinical improvements is debatable. However, a change in IgG levels is a useful marker (and more consistent than IgE changes) of immunologic response to immunotherapy with allergens.⁴² A more notable increase has been recorded in levels of specific IgG₄ than in the other IgG subtypes in conventional,⁴⁵ rush,¹⁰ and semirush⁸ schedules, with a progressive increase in this immunoglobulin throughout immunotherapy, reaching higher levels in those patients receiving the longest treatment.⁴⁵ Recently, Mothes et al⁴⁶ have concluded that immunotherapy-induced specific allergen antibodies can have an inhibiting effect on immediate reactions mediated by IgE, which reduces histamine liberation caused by allergen-induced basophils. Moreover, these IgG antibodies can inhibit the presentation of allergens mediated by IgE to the T cells and eosinophils, reducing delayed reactions.⁴⁷

In the build-up phases of this study, the cluster schedule induced increases in the specific IgE, total specific IgG, and specific IgG₄ levels to the allergen that were similar to those resulting from conventional immunotherapy, although the former occurred more quickly because maintenance doses were reached in half the time.

After a significant increase, which coincided with maximum immunotherapy doses in the cluster group, specific IgG₄ levels continued to increase through the period of placebo administration (sixth through 12th weeks) and later during the maintenance period up to a year. This immunoglobulin also continued to increase in the conventional group up to a year of immunotherapy, at which time specific IgG₄ levels were similar in both groups. This dose-dependent increase of specific IgG₄ levels at maintenance, which remained after 1 year of immunotherapy, has also been observed in previous studies.^{40,41}

In our immunotherapy unit, systemic adverse reactions with conventional schedules occur in 4.8% of all patients and have been seen after 0.37% and 0.4% of all injections.^{48,49} These figures are similar to the rates reported by other groups who, like us, use mite extracts most frequently for immunotherapy.⁵⁰ The frequency of adverse

reactions with cluster schedules varies between studies, depending mainly on the type of allergen extract, previous knowledge of the optimum dose of allergen, and the time to reach the maintenance dose. The frequency (percentage of patients) of systemic adverse reactions with different schedules during the initial phase ranged from 5% to 65%.^{5,7,8,9,11,48,49} Møllerup et al⁶ compared 3 cluster schedules with different allergen extracts (both aqueous and depot) and found that tolerance was best with an 8-week schedule with an aluminum hydroxide-adsorbed depot extract. In a previous study our cluster schedule for mite allergy with an aqueous extract led to systemic adverse reactions in 3% of the patients (0.1% of the doses).¹³

In the present study we used the same cluster schedule in a double-blind design with a larger sample size and patients who were representative of our population of candidates for immunotherapy. We observed mild systemic adverse reactions during the incremental-dose phase in 3% of the patients who received the cluster doses (0.15% of all doses), in 4% of the patients who received conventional schedule doses (0.31% of all doses), and in none of the patients who received placebo. Our cluster schedule was therefore tolerated as well as the conventional schedule and better than other cluster schedules for mite allergy that used shorter initial phases to reach the maintenance dose sooner.^{5,8}

In conclusion, our results show that immunotherapy with a standardized extract of *D pteronyssinus* in a 6-week cluster program was tolerated as well as the conventional program. The cluster schedule had the advantages of halving the time to reach the effective dose and to attain clinical and immunologic improvement.

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