

Double-blind, placebo-controlled study of *Alternaria alternata* immunotherapy: Clinical efficacy and safety

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Allergen-specific immunotherapy (ASIT) with fungal extracts has been beset by safety and efficacy problems, which result mainly from qualitative and quantitative variations. Little has been published on the safety and efficacy of these extracts. The objective was to analyze the safety and efficacy of ASIT with an *Alternaria alternata* extract. A total of 28 patients were selected with rhinitis and/or bronchial asthma because of *Alternaria* allergy and monosensitization to molds. The patients were randomized to an active ASIT or placebo group, both groups on a conventional immunotherapy schedule (increasing weekly doses until maintenance dose and then monthly doses). Adverse reactions were classified with the European Academy of Allergology and Clinical Immunology system. Clinical efficacy was analyzed for a year with symptom/medication diary cards, peak expiratory flow (PEF) measures, clinical severity score, severity of symptoms (visual analog scale), subjective evaluation of treatment by the patient and the physician, and a quality of life questionnaire. Twenty-three patients completed the study; all reached the established maintenance dose with only two mild adverse reactions in the whole sample. Significant improvements were found after 6 months in respiratory symptoms in the active treatment group, and in all symptoms in both groups. PEF increased significantly in the active treatment group but not in the placebo group. The severity of asthma decreased in the active treatment group, and the severity of rhinitis decreased in both groups. Visual analog scale scores for severity of symptoms improved in all phases in the active treatment group, but only after 12 months in the placebo group. Physicians judged the disease course as significantly better in the active treatment group. ASIT with the *A. alternata* extract was safe, with clinical improvements after one year of treatment.

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Molds can cause allergic respiratory disease in the upper and lower airways in sensitized persons (1–8). The genera of molds that cause allergy most often are *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* (3, 7, 9) and the most frequently involved genera (especially in children) are *Cladosporium* in northern Europe, and *Alternaria* in the Mediterranean region (2, 3).

Recent studies have shown that the prevalence of mold allergy is greater than previously believed (1, 3, 9). The exact prevalence of allergic respiratory disease caused by molds is not known, but varies widely in different reports and especially across different populations (3, 9, 10). According to a multicenter European study in 1997, 9.5% of all patients suspected of having respiratory allergy were sensitized to *Alternaria*,

Cladosporium, or both, and the highest prevalence was found in Spain (20%) (2). Molds are currently considered the third most frequent cause of allergic respiratory disease after mites and pollens (9).

To date few controlled studies have been published on the results of allergen-specific immunotherapy (ASIT) with fungal extracts, probably because of problems that arise from the variability in antigenic potency between different strains of molds. Most studies concluded that treatment was effective (6, 11–16) but that fungal extracts were not as well tolerated as other allergen extracts (12, 13, 15–18). In the light of these results the Immunotherapy Subcommittee of the European Academy of Allergology and Clinical Immunology (EAACI) noted the need to perform controlled trials on safety and efficacy of ASIT with mold extracts (19).

An earlier study by our group described a high-quality allergen extract obtained from *Alternaria alternata*, which was biologically standardized and adapted to diagnostic and therapeutic purposes. The maximum tolerated dose for ASIT was determined and defined as that which produced no systemic adverse reactions (20). The aim of the present study was to analyze the efficacy and safety of ASIT with this extract in patients with rhinitis, bronchial asthma, or both who were monosensitized to *A. alternata*, using as maintenance dose the established maximum tolerated dose.

Methods

Patients

A total of 28 patients with rhinitis, bronchial asthma, or both due to *A. alternata* were selected on the basis of the following criteria:

- Positive skin prick tests with a commercial *A. alternata* extract (Bial-Aristegui) ($> 3 \times 3$ mm).
- Serum-specific IgE for *A. alternata* (CAP-System, Pharmacia, Uppsala, Sweden) ≥ 0.7 kU/l.
- Monosensitization to molds.
- Indication for ASIT (according EAACI Immunotherapy Subcommittee) (19).

Patients were excluded if they had contraindications for ASIT (19), had received ASIT previously, or could not attend regular appointments at the ASIT unit during the study period. All patients gave their informed consent to participate in the study; for patients who were minors at

Table 1. Clinical and demographic parameters

	Active group	Placebo group
n	14	14
Sex (M/F)	12/2	8/6
Age (mean $\pm$ SE)	12.85 $\pm$ 4.08	14.92 $\pm$ 6.47
Diagnosis (R + BA/R)	12/2	13/1
Severity rhinitis (mean $\pm$ SE)	7.71 $\pm$ 1.82	7.43 $\pm$ 2.21
Severity asthma (mean $\pm$ SE)	4.50 $\pm$ 1.24	4.46 $\pm$ 1.94

M, male; F, female; R, rhinitis; BA, bronchial asthma.

the time of the study, informed consent was obtained from their legal guardian.

The patients were randomized by means contingent assignment by groups of four patients. So, every patient was assigned to one of the two groups: active ASIT and placebo.

Clinical and demographic data for the patients are shown in Table 1.

Study phases

Four visits were scheduled for the recording and analysis of data: T0 (at the beginning of the study, before the start of ASIT), T1 (when the maintenance dose was reached, about 14 wk later T0), T2 (after 6-months of maintenance treatment) and T3 (after 12-months of maintenance treatment).

Extract

The allergen extract was obtained in the course of work carried before the study reported here, and consisted of a metabolic extract of *A. alternata* that had been biologically standardized, with a skin prick test (concentration of allergen eliciting a mean wheal diameter equal to 10 mg/ml histamine) of 2.09 mg/ml and an Alt a 1 content of 1.25 μ g/ml (20). The presence of the allergen was estimated by a monoclonal antibody (mAb)-based enzimoimmunoassay (21). The extract used here was from the same batch as in the previous study.

The placebo consisted of the excipient used to prepare the allergenic extract suspension. To ensure that double-blind, masked conditions were maintained, 0.01 mg/ml histamine was added to 10% of the doses of placebo.

Allergen-specific immunotherapy

A conventional subcutaneous ASIT schedule was used (Table 2). It consisted of an initial phase with increasing weekly doses until the maintenance dose was reached, i.e. the maximum

Table 2. Dose increment schedule

Week	Vial	Volumen (ml)	Dose (UBE)
1	1	0.1	1.67
2	1	0.2	3.34
3	1	0.4	6.68
4	2	0.8	13.36
5	2	0.1	16.7
6	2	0.2	33.4
7	2	0.4	66.8
8	2	0.8	133.8
9	3	0.1	167
10	3	0.2	334
11	3	0.4	668
12	3	0.6	1002
13	3	0.8	1336
14	3	1	1670

tolerated dose established in a previous study (1670 UBE, corresponding to 0.167 mg of lyophilized extract with an Alt a 1 content of 0.1 μ g) (20) or the highest dose possible if the maximum dose was not tolerated. The initial phase was followed by a period of monthly maintenance doses. Patients in both groups received the same number of doses of active treatment (*Alternaria* extract) or placebo (excipient or excipient + histamine).

Adverse reactions were classified according to the EAACI system (19).

Clinical assessment

- Symptoms/medication diary cards: each patient recorded symptoms and the medication needed daily during 4 wk in each phase of the study. Annotations were made on a specially designed card used successfully in previous studies (22). The severity of symptoms was assessed on a scale of 0–3 for five symptoms in rhinitis (itching, sneezing, congestion, nasal discharge and eye itching) and for three symptoms in asthma (cough, wheezing and fatigue). During the clinical evaluation periods, the patients were not allowed to use long-term self-medication, and as rescue medication they were permitted any antihistamine except astemizole, short-acting beta agonists or oral corticosteroids. The doses of medication were scored as follows: none 0; antihistamines 1; beta agonists 2; oral corticosteroids 3. At the end of the study, partial (symptoms and medication use) and combined scores (symptoms + medication) were calculated, along with the percentage of symptom-free, medication-free days for each patient in each study period. Patients with asthma were instructed to measure peak

expiratory flow (PEF) and to record it as the best of three tries twice a day (morning and evening before any bronchodilator was used). At the end of each period, mean values and the variability were calculated as described by Quackenboss et al. (23).

- Clinical severity score: it was evaluated at the start and at the end of the study on the basis of the patient's clinical status during the 3 months prior to the evaluation. The severity of asthma was evaluated with a combined scale of symptoms (0–5 points) used in earlier studies (22, 24), medication needed to control the disease (0–5 points) and respiratory function (1–3 points). The severity of rhinitis was evaluated as the combined score of symptoms (according to Meltzer: 0–12 points) (25) and medication use (0–2 points).
- Visual analog scale: at the start and at the end of each phase of the study, the patients evaluated disease status subjectively on a scale of 0 (no symptoms) to 10 (very severe symptoms) (26).
- Evaluation of treatment by patients: at the end of each phase of the study except T0, patients evaluated their status during the preceding month in comparison to the month before they started treatment. Patients scored their health status on a scale of 0 (much worse) to 4 (much better).
- Evaluation of treatment by the physician: at the end of the study, the physician evaluated the course of the disease throughout the study with the same 5-point scale as patients used to evaluate their health status (see above).
- Quality of life questionnaire: before the start of ASIT and at the end of the study, all patients completed a quality of life questionnaire validated for Spanish patients with rhinitis and with asthma (27).

Environmental allergen monitoring

Throughout the study period, spores were collected with a Burkard volumetric spore-trap (Burkard Manufacturing Co. Ltd, Hertford, UK) installed on the roof of our hospital. Spores were trapped onto adhesive tapes that were cut into sections representing one day. Each section was viewed by light microscopy and the number of trapped *Alternaria* spores was counted by experienced personnel. Exposure was defined as the mean daily concentration of spores per cubic meter of air.

Statistical analysis of data

The data were entered in a database of personal computers and filtered to avoid inconsistencies. Then the data were coded and derived variables were generated to validate the information with checks for internal consistency, missing data and outliers. All statistical analyses were performed with SPSS, v. 11 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were obtained and comparability of the two groups was confirmed. Then Mann–Whitney’s test was used for between-group comparisons, and Wilcoxon’s test was used to compare the results within each group for each phase of the study with the baseline values. Differences were considered statistically significant at $p < 0.05$.

Results

Of the 28 patients who began the study, 23 reached the maintenance dose with ASIT. The five patients who did not complete the study had asthma and were last to follow up for a variety of reasons. Two (both in the placebo group) were withdrawn because of unstable asthma, one (placebo group) dropped out of the study because of a change in place of residence, and two (one in the placebo group and one in the active treatment group) dropped out because of difficulties traveling to the hospital for appointments. For these five patients, we used the data recorded up until the time when their participation in the study ceased.

There were no differences between the active treatment and placebo groups in age or sex distribution or in diagnosis. No significant dif-

ferences were seen between groups in the efficacy variables measured at baseline.

Safety monitoring

All patients who completed the study attained the maintenance dose of 1670 UBE. A total of 711 doses were administered, 478 (67%) during the initial phase and the rest during the maintenance phase. Only two adverse reactions were recorded in two different patients (0.28% of all doses, 7.14% of all patients). Both reactions occurred in patients with asthma in the active treatment group. One reaction occurred during the initial phase with a dose of 6.68 UBE, and consisted of skin itching with no appreciable lesions immediately after injection of the allergen suspension (ISR-1). The itching subsided with oral antihistamines. This patient later attained the maintenance dose and completed the study with no further reactions. The other adverse reaction occurred during the maintenance phase in a patient, who had been on the maintenance dose for 6 months with no previous reactions. It consisted of rhinoconjunctivitis symptoms that appeared 60–90 min after administration of the dose (LSR-RC) and resolved spontaneously. Subsequently, this patient tolerated the maintenance dose well until the end of the study and experienced no further adverse reactions.

Clinical efficacy

The data for the variables are summarized in Table 3.

Table 3. Summary of values

	ACTIVE GROUP				PLACEBO GROUP			
	T0	T1	T2	T3	T0	T1	T2	T3
Symptoms								
L	0.47 ± 0.47	0.33 ± 0.31	0.31 ± 0.43	0.39 ± 0.49	0.30 ± 0.26	0.22 ± 0.16	0.26 ± 0.18	0.27 ± 0.25
O	0.33 ± 0.34	0.23 ± 0.27	0.13 ± 0.20	0.33 ± 0.47	0.13 ± 0.27	0.21 ± 0.37	0.06 ± 0.12	0.11 ± 0.18
N	0.67 ± 0.48	0.59 ± 0.45	0.60 ± 0.47	0.79 ± 0.54	0.65 ± 0.52	0.48 ± 0.46	0.25 ± 0.19	0.39 ± 0.29
T	0.50 ± 0.39	0.41 ± 0.32	0.38 ± 0.33	0.55 ± 0.49	0.43 ± 0.35	0.36 ± 0.30	0.30 ± 0.28	0.41 ± 0.40
Medication	0.37 ± 0.57	0.33 ± 0.42	0.48 ± 1.19	0.51 ± 1.19	1.77 ± 2.43	0.56 ± 0.67	0.39 ± 0.72	0.21 ± 0.36
Sympt + medic.	0.44 ± 0.42	0.37 ± 0.26	0.43 ± 0.56	0.53 ± 0.54	1.07 ± 1.23	0.43 ± 0.41	0.28 ± 0.36	0.23 ± 0.23
% Healthy days	16.26 ± 19.61	28.75 ± 32.80	24.81 ± 30.71	14.61 ± 33.06	28.15 ± 28.11	45.71 ± 36.49	48.66 ± 24.74	44.51 ± 1.21
Peak respiratory flow	362.67 ± 106.79	381.62 ± 108.58	398.96 ± 128.97	403.90 ± 128.72	351.97 ± 116.99	349.91 ± 100.02	369.36 ± 107.38	349.14 ± 99.78
Clinical severity								
BA	4.50 ± 1.24	ND	ND	3.18 ± 1.54	4.46 ± 1.94	ND	ND	3.78 ± 1.20
R	7.71 ± 1.82	ND	ND	4.62 ± 1.33	7.43 ± 2.21	ND	ND	5.20 ± 2.04
VAS	4.86 ± 2.63	4.00 ± 2.52	3.23 ± 2.71	3.23 ± 1.96	3.93 ± 2.46	3.60 ± 2.72	3.11 ± 2.20	1.60 ± 1.43
Patient evaluation	ND	3.85 ± 0.56	3.77 ± 0.60	3.69 ± 0.75	ND	3.50 ± 0.71	3.78 ± 0.44	4.00 ± 0.67
Doctor evaluation	ND	ND	ND	3.77 ± 0.44	ND	ND	ND	3.30 ± 0.48

Data are given as the mean ± s.d.

Symp + medic., combined score of symptoms and medication; VAS, visual analogue scale; L, lung; O, ocular; N, nasal; T, total; BA, bronchial asthma; R, rhinitis; ND, not determined.

- Symptoms/medication diary cards: Scores for all symptoms were low at the start of the study, with a mean score of 0.50 in the active treatment group and 0.36 in the placebo group. In the active treatment group, respiratory symptoms showed overall improvement, and the difference between groups was significant in T2 ($p < 0.05$). In the placebo group, there were no significant changes. We found no changes in ocular or nasal symptoms in either group. When the overall score for all symptoms was compared, we found significant improvements in both groups in T2 compared to T0 ($p < 0.05$), although the difference disappeared in T3. Scores at the start of the study were also low in both groups for medication use. There were no differences during the study in the active treatment group, whereas the need for medication decreased in the placebo group in T3 compared to T0 ($p < 0.05$). When symptoms and the need for medication were analyzed together, we noted a significant decrease in T2 in the active treatment group ($p < 0.05$) and in T3 in the placebo group ($p < 0.05$). There were no significant changes during the study in the percentage of symptom-free and medication-free days in either group. In the analysis of mean PEF we found significant improvements ($p < 0.05$) in all phases compared to T0 in the active treatment group. In the placebo group there were no significant changes in this variable (Fig. 1). Analysis of the variability in PEF showed that in the active treatment group there was a tendency toward a decrease in all phases, although the differences did not reach a statistical significance. However, in the placebo, variability in PEF increased in T1 and T2 compared to T0, although these changes also failed to reach significance.
- Clinical severity score (Fig. 2): In patients with asthma we noted a significant decrease in disease severity in the active treatment group ($p < 0.05$), but no change in severity in the placebo group. The severity of rhinitis decreased

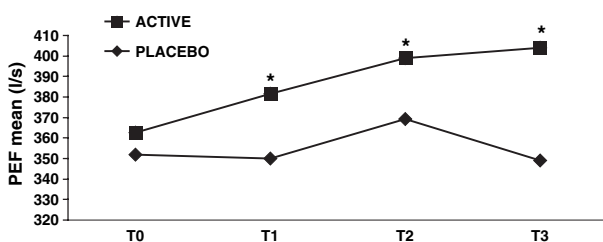


Fig. 1. Evolution of peak expiratory flow. * $p < 0.05$.

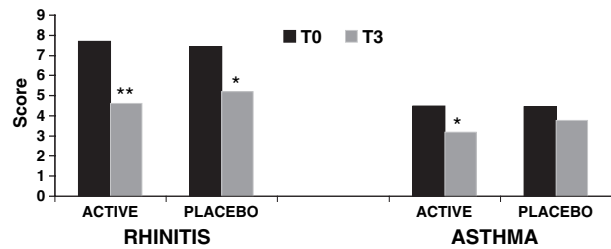


Fig. 2. Clinical severity score for rhinitis and asthma. * $p < 0.05$; ** $p < 0.01$.

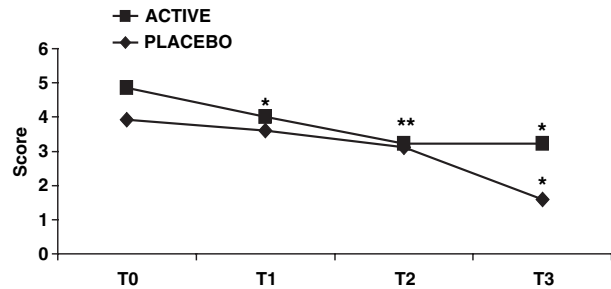


Fig. 3. Evolution of changes in the visual analog scale score. * $p < 0.05$; ** $p < 0.01$.

in both groups, although the decrease was greater in the active treatment group ($p < 0.01$) than in the placebo group ($p < 0.05$).

- Visual analog scale of symptoms (Fig. 3): Patients in the active treatment group reported significant improvements in their status in all phases (T1: $p < 0.05$; T2 $p < 0.01$; T3 $p < 0.05$). Patients in the placebo group also reported better health in all phases, although the improvement was significant only in T3 ($p < 0.05$).
- Patient's evaluation of treatment: We found no significant changes in any phase in either group.
- Physician's evaluation of treatment: According to the physicians' evaluations, the disease course was significantly better in the active treatment group than in the placebo group ($p < 0.05$).
- Quality of life questionnaire (Fig. 4): Among patients with asthma, overall quality of life improved in both groups, but the gains were not statistically significant. When different domains of the questionnaire were analyzed separately, a significant improvement in symptoms was found in the active treatment group ($p < 0.05$). Among patients with rhinitis we also found an improvement in overall quality of life in both groups, although the gains again failed to reach significance. When different domains of the questionnaire were

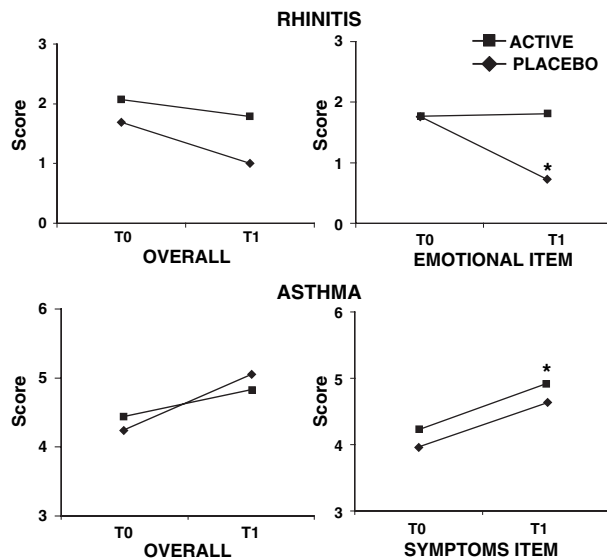


Fig. 4. Quality of life questionnaire (overall and statistically significant results for rhinitis and asthma). Note that in rhinitis to smaller score the quality is better and in asthma is the opposite.

analyzed separately, a significant improvement in emotional status was found in the placebo group ($p < 0.05$).

Environmental allergen monitoring

The highest spore counts for *Alternaria* were recorded during the months of May to October. It corresponded, mainly, to T2, which was performed between July and September. One month before, in June, the peak concentration, at 1632 spores/m³ of air was reached (Fig. 5).

Discussion

Few controlled studies of the efficacy and safety of ASIT with fungal extracts have been published (11–18, 28). Here we studied the efficacy and safety of an *A. alternata* extract that was biologically standardized in the course of previous work.

Although the calculated sample size called for the inclusion of 36 patients, recruitment problems led to a final sample size of only 28 patients. Two patients were withdrawn because their symptoms were unstable probably because long-term medication was not allowed during the evaluation period. Both patients had asthma and belonged to the placebo group. Problems with patient selection arose from the criterion of including individuals who had been sensitized only to mold (a necessary criterion in our opinion to ensure

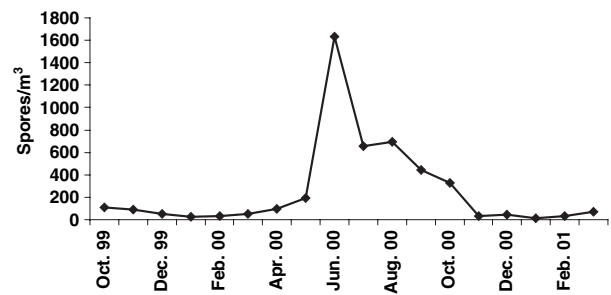


Fig. 5. *Alternaria* spores/m³ during the study period.

that the findings were specific for this group of patients) and from the high incidence of mono-sensitization in children. These circumstances, which meant that data were available for a smaller number of patients than we had anticipated, probably limited the power of our statistical tests.

Mean age of the patients was close to 14 yr, with a range of 7–29 yr. This young age range reflects the higher incidence of sensitization to mold at early ages (4, 9, 10, 29), as well as the criterion of limiting our study to patients sensitized only to mold. Most of our patients had asthma, and only three patients had exclusively nasal symptoms. Similar problems with recruitment were reported by other authors, who noted that the highest incidence of asthma occurs at earlier ages than for allergic rhinitis in general (30), as well as the association between sensitization to *Alternaria* and a diagnosis of asthma (5, 6, 8, 9).

All our patients reached the pre-established maintenance dose. This contrasts with our earlier experience with other allergens (31) and with *Alternaria* (28), and is also at variance with the findings of others who studied fungal extracts (12, 13). We believe the difference may have arisen because the maintenance dose used for our patients was calculated on the basis of an earlier study, in which we determined the maximum tolerated dose (20). As noted, this dose was defined as the highest dose that produced no systemic reactions. Although this criterion was perhaps too conservative, it was based on our experience with *Alternaria* immunotherapy (28), which indicated poor tolerance as has been reported by most authors (12, 13, 15–18). Unsurprisingly, this dose was far below the dose we had identified as the maximum dose; this probably explains why the maintenance dose was reached by 100% of the patients in our trial.

The same reasons could explain that tolerance of the extract was excellent, with only two mild adverse reactions. Both occurred in patients in

the active treatment group, and both patients were able to remain on their normal schedule. These figures are similar to or slightly lower than those obtained in our earlier studies with other extracts (31), and are much lower than those we obtained in earlier work with a different *Alternaria* extract (28). In other trials with fungal ASIT, few reactions were reported (11, 14), although most studies noted the poor tolerance of this type of ASIT (12, 13, 15–18).

We used several parameters to evaluate clinical efficacy, as there is currently no single method that is universally accepted (19). Although respiratory symptoms improved in all phases in the active treatment group, the difference became statistically significant only after 6-month treatment. During this period symptoms in general improved significantly in both the placebo and active treatment groups. We do not know the reason for these similar results in both groups, as it cannot be attributed to a smaller environmental exposition in this period or to other known causes. These improvements ceased to be significant after 12-month treatment. The causes of the lack of significance at the end of the study may have been the low baseline scores; although the improvement was greater in the active treatment group, it failed to reach statistical significance. This result has been noted in other IT studies involving patients with mild to moderate disease, as ASIT is not indicated for patients with severe or unstable disease (19). Dreborg and colleagues, for example, found no changes in symptoms or need for medication after 10-month treatment (12). It should be recalled that in the present study patients were allowed to use only rescue medication to control their disease; consequently our group included patients with mild to moderate disease who were stable without long-term treatment.

Our findings with regard to medication use were inconclusive, probably for the same reasons (baseline scores were low). We have not found any reason to the decreasing of need for medication in placebo group in T3.

Our results for PEF were more conclusive, with significant improvements in all phases of the study in the active treatment group vs. no change in the placebo group. These improvements were important, as PEF is the only objective parameter recorded by patients themselves, which is not influenced by the perception that their disease is not serious. Moreover, the PEF values substantiate the subjective improvement recorded in bronchial symptoms in the active treatment group and are evidence of the efficacy of treatment for asthma.

Visual analog scales to rate health status have been shown to be a reliable parameter thanks to their correlation with actual clinical improvement and with other parameters of efficacy (32). We found statistically significant improvements in all phases of the study in the active treatment group, and after 12 months in the placebo group.

Our findings with the quality of life questionnaire were inconclusive, probably because baseline scores were quite high, indicating that the patients' quality of life was scarcely altered by their disease and thus unlikely to show much further improvement with treatment.

In overall terms, the parameters we used to evaluate clinical efficacy suggest that patient-dependent data (symptoms/medication card, patient's evaluation of treatment, quality of life questionnaire, and to a lesser extent the visual analog scale) indicated for both groups that their disease was mild and had little effect on their quality of life. Consequently, little further clinical improvement (or at least improvements that could be considered statistically significant) could be expected to appear. In contrast, the most objective parameter that our patients recorded (PEF) and the parameters evaluated by the physician (although it should be remembered that this was a double-blind trial) were indicative of moderately severe disease that improved during the study in the active treatment group and showed less improvement in the placebo group. The lack of agreement between the patients' and physicians' perception of severity and control of respiratory disease has been noted by others (33, 34), and may have several causes. One possible cause worth noting here is the use of a different language, low expectations for improvement on the patient's part, and the perception that the disease is not serious.

A low level of exposure to the offending allergen cannot be adduced, as the *Alternaria* spore counts (especially during the summer) were high, in agreement with observations by other authors (3, 4, 12). Spore concentrations during the summer were far over the threshold of 100 spores/m³ reported to provoke symptoms in *Alternaria*-sensitized patients (3).

The present study shows that IT with our *A. alternata* extract in patients with rhinitis, bronchial asthma, or both is a safe treatment with a rate of adverse reactions similar to other standardized non-fungal extracts. The treatment tested here was effective in improving respiratory symptoms and function. In contrast, it did not appear to be so effective in the treatment of rhinoconjunctivitis or in reducing the need for medication after 1 yr of treatment.

Some authors have pointed out that the clinical documentation of the efficacy of ASIT is affected by the duration of the treatment, amount of allergen administered, and the criteria for patient selection (35). So, we suspect that if the maintenance dose were increased slightly to a point where tolerance remained acceptable, clinical efficacy would be greater. As an alternative, administering the maintenance dose tested here for a longer period of time may also increase the effectiveness of the extract in terms of efficacy and safety. This aspect is in agreement with the fact that negative or poor results were reported with ASIT for perennial allergens in short-term studies whereas much more favorable outcomes were obtained in studies lasting more than 18 month (36).

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