
Sublingual immunotherapy for *Alternaria*-induced allergic rhinitis: a randomized placebo-controlled trial

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Background: Respiratory allergy due to *Alternaria* is a relevant clinical problem, and specific immunotherapy may represent a viable treatment option. Sublingual immunotherapy (SLIT) is safe and effective, but data for *Alternaria* are lacking.

Objective: To assess the efficacy of standardized SLIT in patients sensitized to *Alternaria* in a randomized, prospective, double-blind, placebo-controlled trial.

Methods: Patients with rhinitis with or without intermittent asthma and ascertained allergy to *Alternaria* were enrolled. After a baseline season, SLIT or matched placebo was given for 10 months. Symptoms and rescue medication intake were recorded on diary cards between June and October. Skin prick testing was performed and specific IgE, IgG4, and precipitin levels were measured at baseline and at the end of the study.

Results: Twenty-seven patients (age range, 14–42 years) were randomized, and 26 completed the study. The baseline characteristics were homogeneous in the 2 groups. After treatment, patients receiving SLIT had a significant improvement in symptoms and a reduction in medication intake vs placebo and vs the run-in season, whereas no change was seen in the placebo group. Skin prick test reactivity significantly decreased only in the SLIT group. No change was seen in specific IgG4 levels in the 2 groups, whereas Alt a 1 specific IgE levels significantly increased in the active group. One patient in the active group reported oral itching and conjunctivitis at the beginning of treatment.

Conclusion: SLIT seems effective and safe and may represent a valuable therapeutic option in respiratory allergy due to *Alternaria*.

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INTRODUCTION

Respiratory allergy to fungi is a worldwide problem because molds may grow everywhere and release their allergenic components in the environment; however, fungal allergy is one of the less investigated aspects of respiratory allergy. Although inferior to that of more common allergens, the prevalence of sensitization to fungi is probably higher than previously believed.^{1,2} Among fungi, *Alternaria alternata* is one of the more frequently responsible for sensitization and symptoms. In a multicenter European survey,³ the rate of sensitization to *Alternaria* was reported to range from 3% to 20%.

A alternata is ubiquitous outdoors and indoors and has a high rate of spore germination with consequent release of its

antigenic components.⁴ The main antigens are Alt a 1, a 29-kD dimer that reacts with serum IgE in more than 80% of sensitized patients,^{5,6} and Alt a 2, which is recognized by approximately 15% of sera.⁷ The highest concentration of spores is usually found in summer and fall, when *Alternaria* provokes symptoms of asthma and rhinitis. In this context, as happens for other aeroallergens, specific immunotherapy (SIT) can represent an important therapeutic option. Indeed, there are few clinical studies of injection SIT with *Alternaria*,^{8–11} and the extracts are still not fully standardized and characterized.¹² In addition, injection SIT with *Alternaria* has been reported to be associated with a high rate of adverse events.¹³ After more than 20 years of clinical use and controlled trials,¹⁴ sublingual immunotherapy (SLIT) is now accepted as a viable alternative to the subcutaneous route and is largely used in Europe and in many other countries worldwide. The clinical efficacy of SLIT is well established, particularly in rhinitis, where confirmatory meta-analyses are also available.^{15,16} In addition, SLIT displays a favorable safety profile¹⁷ so that it could represent an attractive option to treat *Alternaria* allergy. Based on these considerations, we designed a randomized controlled trial to evaluate the clinical efficacy, the immunologic effects, and the safety of SLIT with an *A alternata* extract in patients with respiratory allergy.

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METHODS

Study Design

This study was designed as a randomized, double-blind, placebo-controlled, 2 parallel groups trial. Patients fulfilling the inclusion criteria had the baseline variables recorded during a run-in season (June-October) and then were randomized to receive either *Alternaria* SLIT or matched placebo, in addition to rescue medications. The randomization (according to a computer-generated list) was performed in January, and the immunotherapy lasted until the next October, for a total of 10 months. Clinical scores, rescue medication use, and adverse events were recorded in diary cards. Specific IgE, IgG4, and precipitins were assayed at the beginning and at the end of the study. Counts of outdoor *Alternaria* spores were also obtained. All the patients (or their parents) provided written informed consent, and the trial was approved by the ethical committee of Rimini Hospital.

Patients and Diagnosis

Adolescents and adults of both sexes were enrolled. The inclusion criteria were (1) moderate/severe persistent rhinitis (with or without intermittent asthma) lasting at least 3 years and occurring only during the summer-fall months and (2) sensitization to *A alternata*, confirmed by positive skin prick test (SPT) reactions (>3 mm) or CAP-RAST assay results (>0.35 kU/L). The exclusion criteria were persistent asthma, morphologic abnormalities of the nose (polyps, septal deviation, or turbinate hypertrophy), sensitization to perennial allergens that could interfere with *Alternaria* (ie, *Parietaria* and house dust mite) or to pollens whose season overlaps with that of *Alternaria* (ie, mugwort and ragweed), chronic obstructive pulmonary disease, previous courses of SIT for *Alternaria*, long-term treatments with systemic corticosteroids, malignancies, and systemic immunologic disorders. Pregnant or lactating women were excluded as well. Sensitization to cat or dog dander was admitted if the patient did not keep the pet at home. Owing to the strict inclusion criteria, the enrollment was extended to more than 1 year, keeping the blinding active until the last patient completed the study.

The diagnosis of rhinitis was clinical, based on Allergic Rhinitis and its Impact on Asthma criteria.¹⁸ The diagnosis of asthma was based on the Global Initiative on Asthma criteria.¹⁹ All SPTs were performed with a panel of standardized extracts (Stallergenes, Milan, Italy), including *Alternaria*, grass, *Parietaria*, mugwort, ragweed, birch, cypress, house dust mite, *Cladosporium*, and cat and dog dander. Positive (histamine, 0.1%) and negative (saline) controls were also applied. The SPTs were conducted and read according to current guidelines.²⁰

Immunotherapy and Concomitant Treatments

The SLIT was a glycerinated extract in drops (Anallergo, Firenze, Italy) standardized in radioallergosorbent test (RAST) units (RU) and prepared according to Good Manufacturing Practice.²¹ The extract was prepared in 3 vials at different concentrations (100, 1,000, and 10,000 RU/mL) and

had to be taken in the morning, kept under the tongue for 2 minutes, then swallowed. The build-up phase lasted 15 days, starting with 1 drop from the 100-RU vial and increasing by 1 drop daily, up to 5 drops. This was repeated with the other 2 concentrations until the dose of 5 drops from the 10,000-RU vial was reached. The maintenance dose (5 drops, 10,000 RU) was taken on alternate days for 10 months. The content of major allergen Alt a 1 was 1.5 µg/mL in the 10,000-RU vial. The cumulative dose per patient was approximately 60 µg of Alt a 1. Patients were carefully instructed on the use of SLIT and received written instructions on the possible adverse events and their management. A contact physician was always available at the center. Patients were randomized to 1 of the 2 treatments according to a computer-generated list, and the blinding was maintained until the last patient completed the study. The placebo was indistinguishable by taste and aspect from the active SLIT. Adherence was assessed by measuring the remaining volume of the extract in returned vials and, by subtraction, the actually consumed volume. Adherence was expressed as the percentage of the actual vs expected consumption.

All the patients were allowed to use the following medications: cetirizine (10 mg, 1 tablet daily), nasal cromolyn (2 puffs 4 times a day), and inhaled salbutamol (2–4 puffs on demand). Short courses of systemic corticosteroids (betamethasone, 0.5 mg for 3–4 days) were permitted in the case of very severe symptoms. Topical corticosteroid therapy was not allowed.

Diary Cards for Clinical Scores

Patients were instructed to record daily the presence and severity of symptoms during the *Alternaria* season, from June to October. Nasal obstruction, rhinorrhea, itching, and sneezing were scored from 0 (absent) to 3 (severe). The same score was used for asthma symptoms (cough, wheeze, and chest tightness), if present, and for conjunctivitis symptoms (gritty eyes and redness). A total score for June through October was calculated for statistical analysis. The patients also recorded on the same diary card the intake of the medications described previously herein. The following scores were arbitrarily assigned to each medication: cromolyn (4 puffs 2 times a day), salbutamol (2–4 puffs), and cetirizine (1 tablet) = 1 point and oral betamethasone (1 tablet) = 2 points. Starting from randomization, the patients also had to report on a separate diary card the occurrence of any adverse event they judged to be related to SLIT.

Immunologic Variables

Alternaria specific IgE and IgG4 levels were measured at the beginning and at the end of the study using an immunoenzymatic assay (ImmunoCAP, Phadia AB, Uppsala, Sweden), as were Alt a 1 specific IgE and IgG4. Results are expressed, according to the calibration curve, in kilounits per liter for IgE and in milligrams per liter for IgG4. *Alternaria* specific IgG precipitins were assayed using an immunoenzymatic test (EnzyDex Acti-Tip System; Dexall Biomedical, Gaithersburg, Maryland). The measurement is achieved using a spec-

trometer, and the results are expressed as percentage of absorbance compared with a reference standard, where less than 15% is considered negative. The skin reactivity to *Alternaria* was assessed at the beginning and at the end of the study by means of SPT using the arithmetic mean of the major wheal diameter and its orthogonal.

Statistical Analysis

The total symptom score during the *Alternaria* season was the primary outcome measure, and the secondary outcomes were drug intake scores, combined symptom + medication score, and immunologic variables. The statistical evaluation was performed on the per-protocol population. The χ^2 test and the *t* test were used to compare sex and age between the 2 groups. Symptom and medication scores, immunologic variables, and SPTs were analyzed using the *t* test for paired or unpaired samples. All the tests were 2-tailed, and the limit of significance was set at $P < .05$. A total sample size of 24 patients would have had 90% power to detect a difference of 20% in the symptom score, assuming a standard deviation of 100 and using a level of significance of $P < .05$ in 2-sided testing. Owing to an expected 10% dropout rate, the sample was increased to 27 participants.

RESULTS

Twenty-seven patients were enrolled (15 active and 12 placebo), and 26 completed the study. The only dropout, in the placebo group, was due to consent withdrawal immediately after randomization. All the active patients received a cumulative dose of approximately 60 μg of Alt a 1. The adherence to SLIT, evaluated by measuring the volume of extract in the

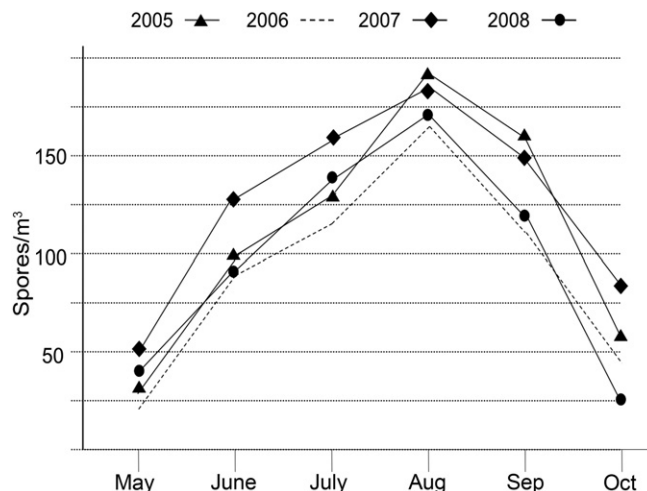


Figure 1. *Alternaria* spore counts in the geographic region where the study was conducted.

returned vials, was 85% to 95% in the 2 groups. The 2 groups were homogeneous at enrollment for clinical and demographic characteristics (Table 1). Eighteen patients recorded the baseline values in 2006 and terminated the study in 2007, whereas the remaining participants started in 2007 and ended in 2008. Nonetheless, the allergen load was stable and comparable through the years, with the spores present in summer and fall months (Fig 1). In addition, the patients were homogeneously distributed for clinical characteristics and demography between the 2 years of enrollment.

The mean (SD) symptom scores were significantly reduced in the active group compared with the placebo group at the end of treatment (182 [67] vs 315 [115], $P = .02$). Similarly, in the active group there was a significant improvement in mean (SD) symptom scores vs the baseline season (182 \pm 67 vs 421 [102], $P < .001$), whereas no change was seen in the placebo group (Fig 2A). Concerning the medication scores, a significant reduction was seen in the active group vs run-in

Table 1. Characteristics of the 2 Groups at Baseline

	SLIT group (n = 15)	Placebo group (n = 12)	P value
Age, mean (SD), y	18.8 (7.2)	24 (8.8)	0.5 ^a
Age range, y	16–42	14–44	NA
Sex, M/F, No.	8/7	7/5	0.6 ^b
Duration of rhinitis, mean (SD), y	4.4 (1.3)	5.25 (1.4)	0.09 ^a
Rhinitis, No.	10	8	0.8 ^b
Rhinitis + asthma, No.	2	2	0.8 ^b
Rhinoconjunctivitis, No.	3	2	0.7 ^b
Monosensitized, No.	2	2	0.9 ^b
Other sensitizations, No.			>0.5 ^b
Grass	8	7	
Olive	2	1	
Birch	0	1	
Cypress	3	2	
Ragweed	0	0	
Mugwort	0	0	
Cat	0	1	

Abbreviations: NA, not applicable; NS, not significant; SLIT, sublingual immunotherapy.

^a By *t* test.

^b By χ^2 test.

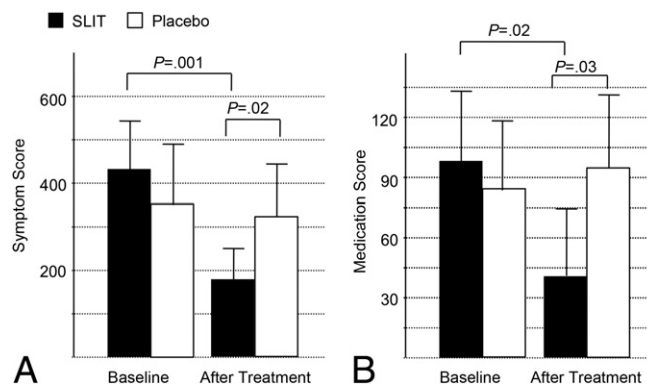


Figure 2. Mean symptom (A) and medication (B) scores in the sublingual immunotherapy (SLIT) and placebo groups. The significant differences (*t* test) are reported above the bars. Error bars represent SD.

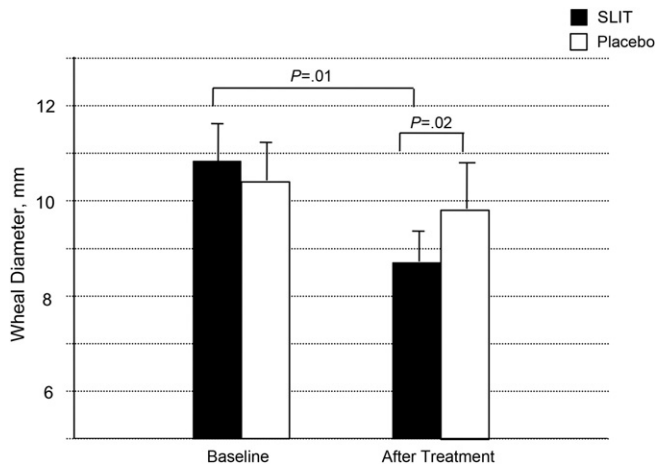


Figure 3. Mean wheal diameter of the *Alternaria* skin prick test reaction in the sublingual immunotherapy (SLIT) and placebo groups. The significant differences (*t* test) are reported above the bars. Error bars represent SD.

($P = .02$) and vs the placebo group (Fig 2B). Also, the combined symptom + drug intake scores were significantly reduced after treatment in the active group vs the placebo group (231 [113] vs 414 [173], $P = .01$) and vs baseline (231 [113] vs 586 [154], $P < .001$). No change was seen in the placebo group. The mean improvement in the symptom + drug intake score in the active group compared with the placebo group was 38%. The mean (SD) diameter of the wheal induced by *Alternaria* extract was decreased vs baseline in the SLIT group (10.9 [3.4] vs 8.7 [3] mm) but not in the placebo group (10.2 [3.8] vs 9.7 [3.5] mm) (Fig 3).

No significant intragroup or intergroup differences could be detected in *Alternaria* specific and Alt a 1 specific IgG4 levels owing to the great data dispersion. On the contrary, there was a significant increase in *A alternata* specific and Alt a 1 specific IgE levels in the SLIT group vs baseline and vs the placebo group ($P = .02$ for all) (Fig 4). Finally, there was a decrease in the mean (SD) precipitin levels only in the active group at the end of treatment vs baseline (9.5 [5.1] vs 4.6 [1.8]), but this did not achieve significance ($P = .09$).

The treatment was well tolerated. Only 1 patient in the SLIT group reported oral itching and conjunctivitis during the build-up. These adverse effects were mild and spontaneously disappeared in a few days.

DISCUSSION

A alternata is one of the most important fungal allergenic sources. Although epidemiologic data on the burden of sensitization to *Alternaria* are fragmentary and incomplete, there is convincing evidence that it is relevant in a not negligible fraction of allergic patients. A European survey³ found a prevalence of sensitization to *Alternaria* in up to 20% of allergic patients, and a similar survey limited to Italy²² reported a prevalence of 1.3% to 29%, although approximately only 2% of patients were monosensitized. Because of the

overall poor knowledge about this type of allergy, immunotherapy to *Alternaria* has not been adequately developed²³ compared, for example, with immunotherapy to mites, grass, or trees. In fact, there are only a few clinical trials, 4 of which were performed with injection immunotherapy^{8,10,11,24} and 1 comparing SLIT and the subcutaneous route⁹ but without a placebo group. Similar to the earlier studies, the most recent trial²⁴ reported positive clinical results, thus suggesting that SIT would be a suitable approach for *Alternaria* allergy. The introduction and validation of SLIT have provided a new therapeutic opportunity, corroborated by the good safety profile of SLIT itself.

Based on this background, we undertook a trial to assess the clinical effects of *Alternaria* SLIT in patients with rhinoconjunctivitis. The results favored SLIT, with a significant reduction of symptom and drug intake scores only in the active group. Of note, the improvement in the combined symptom + medication score was approximately 38% vs the placebo group, and this fulfills the recommendations of the World Allergy Organization.²⁵ The cumulative dose (60 μg of Alt a 1 = 6 μg per month) could be considered low compared with the doses used for other aeroallergens, but it is 60 times greater than that used with subcutaneous immunotherapy.²⁴ There was an increase in the Alt a 1 specific and *A alternata* specific IgE levels. This fact testifies to some immunologic effect of the treatment. On the other hand, the increase in specific IgE is actually in contrast with the decrease in symptoms and skin reactivity, but this phenomenon is also usually observed with injection immunotherapy. No change in IgG4 was seen, and this fact is not surprising because other studies^{26,27} reported that the IgG4 modifications with SLIT are often marginal or not relevant. In this regard, it must be remembered that immunologic variables are only surrogate markers and cannot replace the clinical evaluation.²⁵ This means that the absence of detectable changes in serum immunoglobulins does not invalidate the positive clinical results. In the past, it was reported that precipitins could be associated with a higher rate of adverse reactions,^{28,29}

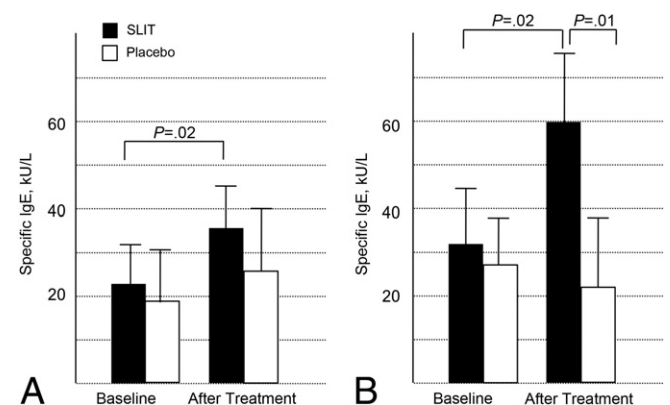


Figure 4. Mean *Alternaria* (A) and Alt a 1 (B) specific IgE levels in the sublingual immunotherapy (SLIT) and placebo groups. The significant differences (*t* test) are reported above the bars. Error bars represent SD.

although there was no convincing proof of this phenomenon. For this reason, we also assayed precipitins, which displayed a nonsignificant reduction. This remains of uncertain interpretation, also owing to the absence of relevant adverse effects of SLIT. As expected, the treatment was safe and well tolerated, and the only reported adverse event was mild and self-resolving.

The main robustness of the trial is the strict inclusion criteria, established to warrant a causal relationship between exposure and symptoms. This implied the exclusion of patients sensitized to perennial allergens or to pollens with seasons that overlap with that of *Alternaria*. The counterpart of this was the difficulty encountered in recruiting patients. On the other hand, the trial by Tabar et al²⁴ included 30 patients, which is in line with our figures. A second possible weakness is that only 1 maintenance dose, empirically established, was used. In principle, a dose-ranging study would be necessary to explore whether the efficacy can be improved.

Despite the mentioned limitations, this is the first double-blind randomized trial of SLIT for *Alternaria* allergy. The main clinical results suggest that this approach would benefit carefully selected patients when *Alternaria* is clearly identified as the major cause of rhinitis or asthma symptoms.

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