

Original article

Randomized controlled open study of sublingual immunotherapy for respiratory allergy in real-life: clinical efficacy and more

Background: Some aspects of sublingual immunotherapy (SLIT) still need to be addressed: magnitude of the clinical efficacy, effect on the bronchial hyperreactivity adherence to treatment, preventive effect. We attempted to clarify these points in a randomized open, controlled, two parallel group study in a real-life setting.

Methods: Five hundred and eleven patients with allergic rhinitis with or without intermittent asthma were randomized to drugs only or drugs + SLIT (rate 2 : 3) for 3 years. The clinical score (symptoms + drug intake) was measured each year during the allergen exposure. Pulmonary function test, methacholine challenge and skin tests were performed at the beginning and at the end of the study. Adherence to treatment was assessed by measuring the consumed extract.

Results: Three hundred and nineteen patients received SLIT and 192 drugs only. Dropouts were 15% in the SLIT group and 12% in the controls. There was a significant improvement of clinical scores in the SLIT group: baseline 147 ± 3.3 , first year 72.9 ± 1.3 , second year 68.3 ± 1.8 , third year 54.7 ± 2.8 ($P < 0.0001$ vs baseline). Control group: baseline 138 ± 2.3 , first year 124.1 ± 3.7 , second year 111 ± 3.3 , third year 121 ± 3.8 ($P = \text{NS}$). Only four patients reported systemic itching. Adherence was $>80\%$ in 72% and $>60\%$ in 18% of patients. The number of patients with a positive MCh challenge decreased significantly after 3 years only in the SLIT group. New skin sensitizations appeared in 38% of the controls and in 5.9% of the SLIT patients ($P = 0.01$).

Conclusion: Sublingual immunotherapy approximately halved the clinical scores and significantly reduced the bronchial hyperreactivity. Similarly to subcutaneous immunotherapy, SLIT displayed a preventive effect on the onset of new skin sensitizations. The adherence rate was quantitatively satisfactory.

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Allergen immunotherapy (IT), together with allergen avoidance and drug therapy, is a cornerstone in the management of respiratory allergy (1). Immunotherapy is capable of modifying the immunological response to the offending allergens at the earliest stages, so that clinical symptoms are progressively improved. The traditional subcutaneous route is well established since several decades and its efficacy has been confirmed in numerous trials (2). The possible risk of severe (even fatal) adverse events (3) encouraged the search for safer noninjection approaches for administering IT (4). During the last 15 years, the sublingual route [sublingual immunotherapy (SLIT)] was extensively investigated, and many controlled trials confirmed its efficacy in respiratory allergy (5, 6). In addition, both clinical trials and postmarketing surveillance studies confirmed the optimal safety profile in both adults and children (7–9). Based on these results, the most recent official document, the ARIA guideline (10), validated the clinical use of SLIT also in paediatric patients.

At present, some concerns still exist about SLIT. First, the overall number of patients studied in the controlled trials is considered small; secondly, there is no information about the adherence to the treatment (that is self-administered); thirdly, it is not clear if the magnitude of the efficacy of SLIT, can be considered clinically relevant (11). In addition, subcutaneous IT displayed a preventive effect on both the occurrence of new sensitizations (12) and the onset of asthma (13), but such evidence still lacks for SLIT.

SLIT has been available in Italy (and other Mediterranean countries, such as Spain and France) for more than 10 years; therefore a solid clinical experience is available in the field and a great number of patients currently receive SLIT. Thus, we studied, in a randomized fashion, a large population of patients with respiratory allergy (rhinitis and/or asthma) because of common allergens. After a baseline clinical evaluation, patients were randomized to receive SLIT or drug treatment only.

The clinical efficacy and safety of SLIT were the primary endpoints. Taking advantage of the large population, this study could also elucidate some of the controversial aspects: adherence to treatment, prevention of new sensitizations, effect on bronchial hyperresponsiveness and age-efficacy relationship.

Patients and methods

Overall study design

The study was randomized, open and controlled, with two groups of allergic patients receiving SLIT plus drugs or drugs only for 3 years. The patients were evaluated in an observation period of 1 year and then in a 3-year course of SLIT, by assessing the clinical scores during the exposure to the causal allergen. Skin prick test and pulmonary function test (PFT) with methacholine (MCh) challenge were performed at baseline and after 3 years. A dropout analysis and a quantitative assessment of the adherence to treatment were also carried out. The study design is summarized in Fig. 1.

Patients and diagnosis

Outpatients seen at the Allergy Unit, Cuasso al Monte Hospital (Varese, Italy), were enrolled. Inclusion criteria were:

- Clinical history of respiratory allergy at least in the past 2 years. Clinical criteria included recurrent episodes of wheezing, cough or chest tightness (diurnal or nocturnal) and typical rhinitis symptoms (sneezing, itching, rhinorrhea, congestion) with or without eye itching and redness.
- Skin prick test positivity to one or more of the common inhalant allergens, but moulds and pet dander.
- Age between 15 and 65 years.
- Intermittent asthma according to GINA criteria (14), and with a FEV_1 within normal limits (i.e. >79% of predicted value).

Patients who had received previous courses of IT, with anatomical abnormalities of the upper respiratory tract or suffering from malignancies or systemic immunological disorders were excluded from this study.

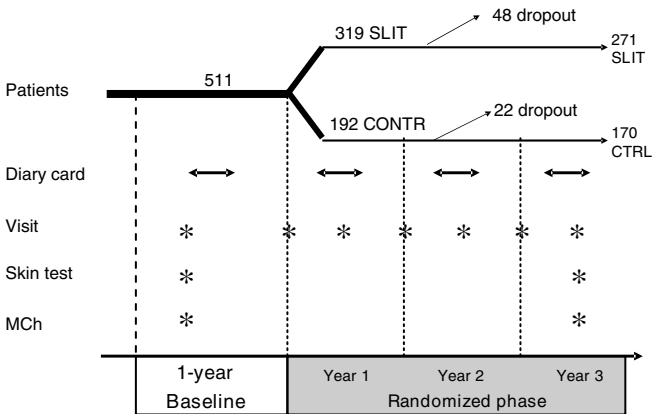


Figure 1. Study design. MCh, methacholine challenge.

The patients were followed up for 1 year with a standard pharmacological treatment, then they were randomized, following a computer-generated list, to receive SLIT or drug therapy alone (control group).

Skin test and PFT

Prick tests were performed according to international guidelines (15) with commercial standardized extracts (Alk Abello, Lainate, Milan, Italy) of the following allergens: house dust mites, grasses, parietaria, cat dander, dog dander, birch, olive, ragweed, mugwort, *Alternaria* and *Cladosporium*. Tests were carried out on the forearm, using positive (histamine 1%) and negative (saline) controls for comparison. The results were expressed as the mean of the major diameter of the wheal plus its orthogonal. Reactions > 5 mm were considered positive.

Pulmonary function tests were performed by a computerized spirometer (Masterlab Yaeger, Wurtzburg, Germany). The MCh challenges were carried out when the patients were asymptomatic and free of medications (inhaled and oral) for at least 15 days, and they were performed in each patient in the same period of the year. A dosimeter (Yaeger), activated by the inhalatory effort was used and increasing doses of MCh were administered, starting from 125 to 1200 µg. The test was considered positive (MCh+) if the dose causing a fall of 20% of FEV_1 was equal or < 1200 µg (16, 17).

Specific IT and pharmacological treatment

SLIT was prescribed only for the clinically relevant allergen, based on clinical history and skin positivity. In a few patients with multiple sensitizations, the main causal allergen was not immediately clear. In those cases, a nasal provocation test was carried out, and the allergen eliciting symptoms at the lowest doses was chosen (18). For ethical reasons (expected better outcome of SLIT) the randomization was set at 3 : 2 in favour of SLIT.

The SLIT (Anallergo, Florence, Italy) was prepared as a glycerinated solution to be administered as sublingual drops in the morning, after the patient had fasted. The patients were carefully instructed by the physician about the self-administration, and detailed written instructions were provided. All the extracts were standardized through combined radioallergosorbent test (RAST)-inhibition and bioequivalence method: the potency was expressed as RAST-Units/ml (RU/ml). The build-up phase, of about 50 days, involved the administration of the extract at progressively increasing concentrations (100, 300, 1000, 3000 RU/ml). In the maintenance phase, five drops from the 10 000 RU/ml vial were given three times a week. The SLIT was administered continuously for approximately of 3 years. In those patients receiving pollen IT a dose reduction by one-third was performed in the pollen season.

The mean cumulative dose per year was about 390 mcg Der p 1/ Der p 2, 70 mcg Phl p 1 and Par j 1 and 100 mcg Bet v 1. This corresponds to about 10 times the dose administered in a corresponding subcutaneous course with the same product.

The following medications were allowed: nasal cromolyn, oral antihistamines (loratadine or cetirizine 10 mg, 1 tablet/day), intra-nasal corticosteroid (beclometasone dipropionate 1 puff b.i.d.), inhaled albuterol (100 mcg two to three puff on demand).

Clinical diary card

Patients were instructed to fill a diary card during the periods of significant exposure to their causal allergens. The diaries were

recorded in: November to February for mites, March and April for birch, May and June for grasses and parietaria, July–October for ragweed and mugwort. The following symptoms were considered: nasal itching, sneezing, rhinorrhea, obstruction, cough, wheezing, and conjunctival itching/redness. Each symptom was scored as 0 = absent, 1 = mild, 2 = moderate, 3 = severe. In addition, each dose of each rescue drug was scored as 1. For each patient, the monthly sum of drugs + symptoms was obtained, the mean sum of the months in each period of observation (2–4 months depending on the allergen) was used for statistical analysis.

Safety and adherence

Patients were required to record on a proper diary card, at each dose, any local or systemic adverse event possibly related to SLIT administration and the time of onset. For convenience, the reactions were subdivided into: systemic (asthma, rhinitis, urticaria, angioedema, generalized itching, diarrhoea, vomiting) and local (oral itching/swelling, oedema of the tongue). Any other suspected adverse event had to be described. The patients were instructed to contact the centre when they needed medical advice.

Adherence was assessed by measuring with a pipette the remaining volume of extract in the returned vials and comparing it with the expected amount of extract consumed during a given period of treatment. The volume of the extract actually consumed by each patient was expressed as a percentage of the expected consumption. Using this method, adherence was graded as: poor (<40%); insufficient (<60%); good (<80 and >60%); excellent (>80%).

Statistical analysis

Comparisons between frequencies have been tested by Pearson chi-squared, whilst the Mann–Whitney *U*-test was performed to verify differences between the two groups (19, 20) both regarding the mean patients' age and the symptom score at the four timepoints. The probability level has been computed using a complete randomization method (permutation or exact test; P_{exact}) or by a Monte Carlo (21) simulation based on a 10 000 sampled tables ($P_{\text{Monte Carlo}}$) when computation was not possible. The type I error (α) has been set at the 0.01 level. The means (age, symptom scores, etc.) have been reported with the standard error of mean ($\pm$ SE). The General Linear Model (GLM) for repeated measures (22) was used to assess the effect of age (< or $\geq$ 18 years) and gender as random factors on symptom scores and their variations. All the statistical analyses have been computed using the Statistical Package for Social Sciences ver. 10.05 (SPSS®, SPSS Inc., Chicago, IL).

Results

Five hundred and eleven patients completed the baseline assessment (1-year observation) and were randomized (through a computer-generated list) to receive SLIT (319 patients) or drug therapy alone (192 patients). The two groups were homogenous for all demographic characteristics but sex. The clinical and demographic characteristics of the patients are summarized in Table 1.

Each patient received SLIT only for one allergen as follows: 166 mites, 89 grasses, 56 birch, six parietaria and two mugwort.

Table 1. Demographic and clinical data at baseline (randomization)

	SLIT	Control	Test	d.f.	$P_{\text{Monte Carlo}}$
Patients	319	192			
Mean age	22.8	21.5	$U = 22\ 296.5$		0.567
Age Range	5–60	5–58	$W = 59\ 152.5$		0.567
Sex-ratio (M/F)	1.29 (180/139)	1.70 (121/71)	$\chi^2 = 5.593$	1	0.014
Rhinitis alone	128 (40.1%)	72 (37.5%)	$\chi^2 = 0.938$	1	0.333
Rhinitis + asthma	191 (59.9%)	120 (62.5%)			0.333
Sensitized to HDM	166 (52.0%)	98 (51.0%)	$\chi^2 = 0.127$	1	0.741
Sensitized to pollens	153 (48.0%)	94 (49.0%)			0.741

U and *W*, values of the Mann–Whitney test; χ^2 , chi square estimate; d.f., degree of freedom of the chi-square test; $P_{\text{Monte Carlo}}$, *P*-values using the Monte Carlo estimation procedure.

Drop-outs and safety

During the 3 years of the controlled study 70 patients dropped-out: 48 patients (15%) in the SLIT group and 22 patients (12%) in the control group. The overall difference between the two groups was not significant ($\chi^2 = 4.05$; d.f. = 1; $P_{\text{Monte Carlo}} = 0.051$). Noteworthy, 14 of 22 (63.6%) control patients vs nine of 48 (18.7%) SLIT patients dropped out because of intolerable worsening of symptoms ($\chi^2 = 29.096$; d.f. = 1; $P_{\text{Monte Carlo}} < 0.001$), requiring more aggressive treatment including systemic corticosteroids. The five dropouts because of adverse events in the SLIT group were: three oral itching, one asthma and one abdominal pain. Thus, 271 patients of the SLIT group and 170 patients of the control group could be analysed at the end of the 3-year period of observation (Fig. 2).

During the 3 years of SLIT treatment, four of 271 patients (1.5%), reported one episode of generalized itching (without apparent skin lesions) within 30 min after taking the dose. These four adverse events appeared during the maintenance phase and self-resolved without therapy in <2 h. Those patients were instructed to temporarily halve the dose, then to gradually increase it again. This intervention allowed tolerating the maximum dosage.

Clinical scores

The monthly clinical score differed significantly at each time point, including baseline, as shown in Fig. 3. The symptom scores significantly improved only in the SLIT group, where they approximately halved since the first year (baseline 147 ± 3.3 , first year 72.9 ± 1.3 , second year 68.3 ± 1.8 , third year 54.7 ± 2.8 ; $P < 0.0001$ vs baseline). No change was seen in the control group (baseline 138 ± 2.3 , first year 124.1 ± 3.7 , second year 111 ± 3.3 , third year 121 ± 3.8 ; $P = \text{NS}$ at all times). Also the inter-group comparisons showed a significant difference at the all time points (Fig. 3). The results of the GLM for repeated measures showed that the proportion of reduction of clinical scores because of treatment was significantly different in the 3-year period (GLM

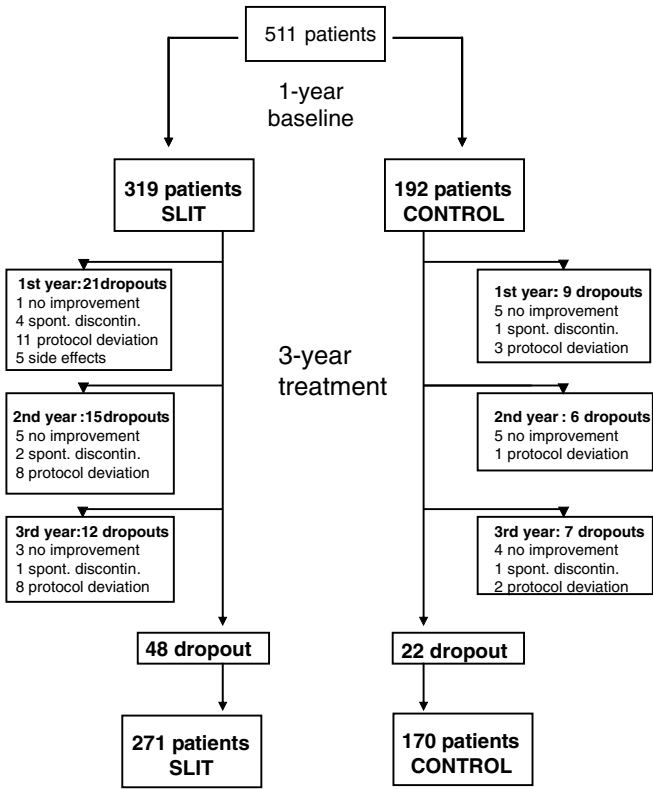


Figure 2. Study flowchart and dropout analysis.

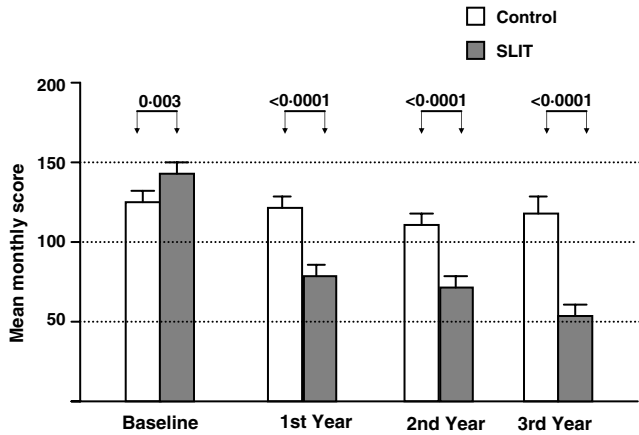


Figure 3. Mean monthly symptom scores in SLIT and control patients at baseline, after 1, 2 and 3 years of treatment. Above the bars are reported the *P*-values for the Mann–Whitney test (MonteCarlo Method).

within-subjects effects: $F = 237.077$; d.f. = 3; $P < 0.001$). Interestingly, in the subgroup of patients with age < 18 years the reduction of symptom scores was significantly more pronounced than in patients aged ≥ 18 years (Fig. 4), as assessed by the GLM analysis (between subjects effect: $F = 212.183$, d.f. = 1; $P = 0.002$). No difference in clinical efficacy among allergens could be detected.

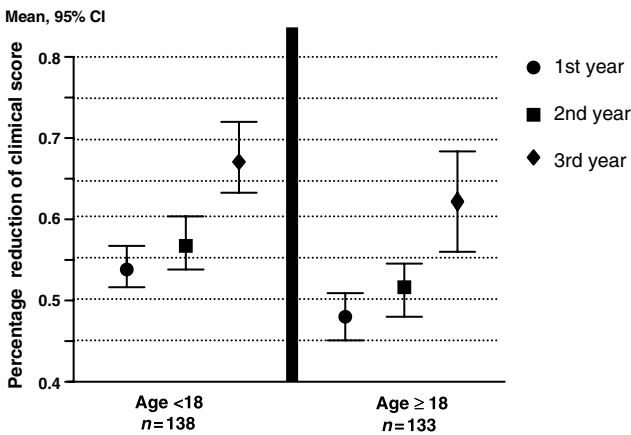


Figure 4. Clinical improvement in each year of treatment (expressed as the proportion of reduction from the baseline scores) in paediatric and adult patients.

Adherence

The adherence to the prescribed SLIT treatments (independent of the allergen extract used) over the 3-year period resulted as follows: excellent ($> 80\%$) in 195 of 271 (72%) patients, good (from 60 to 80%) in 49 of 271 (18%) cases, and poor or insufficient in 27 of 271 (10%) of patients.

Pulmonary function test and methacholine challenge

The pulmonary function remained within the normal range ($FEV_1 > 80\%$) at the interim visits and at the final visit in all patients and no case of persistent asthma (needing for stable therapy with inhaled steroids) appeared. There was a significant evolution of the MCh challenge results (Table 2). In the SLIT group, there was a significant reduction of MCh positive cases, from 160 of 271 patients at baseline (either rhinitics and asthmatics) to 63 of 271 at the third year ($\chi^2 = 143.572$; d.f. = 1; $P_{\text{Monte Carlo}} < 0.001$), whereas no significant reduction was observed in the control group where the MCh+ patients were 106 of 170 at baseline and 95 of 170 at the third year ($\chi^2 = 3.032$; d.f. = 1; $P_{\text{Monte Carlo}} = 0.082$).

Table 2. Evolution of disease and bronchial hyperreactivity. Absolute number of patients and percentage in parentheses

	SLIT			Controls		
	Before	After	P	Before	After	P
Pure rhinitis (MCh-)	111 (40.9)	208 (76.7)	<0.001	64 (37.6)	75 (44.1)	NS
Hyperreactivity (MCh+)	160 (59.1)	63 (23.3)		106 (62.4)	95 (55.9)	

P, *P*-value for the Pearson chi-square test.

Skin sensitizations

At the end of the treatment period, one or more new skin sensitizations with respect of those existing at the time of randomization appeared in 16 of 271 (5.9%) patients of the SLIT group and in 64 of 170 (38%) patients of the control group ($\chi^2 = 70.885$; d.f. = 1; $P_{\text{Exact}} < 0.001$).

Discussion

The present guidelines, based on rigorous experimental studies, have accepted SLIT as a suitable treatment, and a recent meta-analysis study (6), further confirmed its efficacy in allergic rhinitis. The published clinical trials involved a limited number of patients, because of the need for very selected and homogeneous subjects. The strict selection allowed to study some specific aspects, such as the immunological effects at cellular and humoral level (23–25), but necessarily limited the clinical information. For instance, the magnitude of the clinical effect was reported to range between 20 and 60%, and the prevention on the onset of new sensitizations could not be assessed. In fact, this latter effect could be demonstrated for subcutaneous IT in retrospective studies, involving hundreds of patients (26, 27). Moreover, the available trials do not provide information on the effect of SLIT on bronchial hyperreactivity, and the adherence, which is a major concern, was never quantitatively assessed (5, 28).

Studying a large number of patients in a real situation can provide a more reliable quantitative evaluation of the effectiveness of the treatment, and allow to better define other clinical aspects, such as the effect on the onset of new sensitizations. These are the reasons why we evaluated the outcome of SLIT in a relatively unselected population and in a real-life setting. In our study the clinical scores showed an improvement of about 50% vs baseline values, since the first year of treatment, despite the scores were significantly higher in the SLIT group at baseline. Also, It is important to notice that the clinical effect of SLIT was greater in young patients than in adults, thus providing a quantitative support to the believe that IT does better in younger.

Another important finding was the significant modification of the nonspecific bronchial hyperresponsiveness to MCh, which is considered as a distinctive feature of asthma (29). This is in agreement with other observations made with both SLIT (30) and injection IT (31) in smallest

groups. The prevention of the onset of new sensitizations suggests that SLIT, similarly to the subcutaneous route, acts as a biological response modifier. This fact fits with the recently demonstrated long-lasting effect of SLIT after its discontinuation (32). In the controlled trials so far available, the adherence to treatment was generically reported as 'satisfactory', but no systematic assessment was indeed performed. Our quantitative data probably represent a more realistic evaluation of the adherence to SLIT, that did not differ from that reported for drugs (33). Of course, measuring the volume of the remaining extract does not warrant that the patients really took the vaccine, but this approach obviously avoids an overestimation of the adherence: if the remaining volume of the vaccine is higher than expected we can be assured that the patient has not fraudly discharged the extract.

It can be argued that in the present study no immunological parameter [e.g. allergen specific immunoglobulin (Ig)G or IgE, specific reactivity, skin reactivity] was investigated, but is also true that the clinical improvement is the only outcome available to define the efficacy of IT. The so-called 'paraclinical' measures can be considered as an adjunct, but cannot replace the clinical assessment. Another possible flaw of the present study is the open design. We decided to have a large number of subjects in order to clearly assess the clinical parameters, and keeping more than 500 patients in a 3-year double-blind study is difficult to make. Indeed, the patients knew that a new treatment was added, and therefore a placebo effect, that may account for up to 30% of the measured outcome (34), could be expected. Nevertheless, it must be considered that in our study the change in clinical scores was > 50% and it cannot be explained as a simple psychological effect. In addition, the change in the number of skin positivities and MCh reactivity are unlike to be affected by the placebo effect.

In conclusion, the above results further confirm the clinical efficacy and effectiveness of SLIT, even if evaluated in a real-life setting. In addition, SLIT was globally associated with an optimal adherence rate and proved capable of preventing the onset of new sensitizations.

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