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Long-Lasting Effects of Sublingual Immunotherapy for House Dust Mites in Allergic Rhinitis with Bronchial Hyperreactivity: A Long-Term (13-Year) Retrospective Study in Real Life

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Key Words
Bronchial reactivity · Rhinitis · Sublingual immunotherapy

Abstract

Background: Subcutaneous immunotherapy for respiratory allergy has shown a long lasting efficacy after its discontinuation, whereas evidence in the case of sublingual immunotherapy (SLIT) is weak. This retrospective study evaluates whether SLIT exerts a long-lasting effect and whether it relates to its duration. ***Methods:*** Sixty-five patients allergic to mite and positive to methacholine challenge 13 years ago were studied. Twelve (control group, SLIT 0) were treated for 4 years only with standard pharmacological therapy (SPT), while 53 received SLIT and SPT. Among these, four groups were identified according to SLIT duration. Fifteen patients were treated for 1 year (SLIT 1), 10 for 2 (SLIT 2), 14 for 3 (SLIT 3) and 14 for 4 years (SLIT 4). Clinical parameters (symptom monthly score, SMS), bronchial reactivity and FEV_1 were evaluated in 1992 (run-in), 1993 (baseline) and every 2 years from 1997 to 2005. ***Results:*** Two to 3 years after the treatment ended, a positive effect on SMS, but not methacholine challenge and FEV_1, was seen in the SLIT groups versus SLIT 0. At this time interval an effect on methacholine challenge was also seen in SLIT 3. After 7–8 years a significant difference was seen for SMS, i.e., it was significantly better in SLIT 4 than in the other groups, while bronchial reactivity was still improved in SLIT 1, 3 and 4 only after 5–6 years. ***Conclusions:*** The effects of a 4-year SLIT on clinical parameters but not bronchial reactivity and FEV_1 last 7–8 years after its discontinuation. SLIT shorter than 4 years yields proportionally less impressive results.

Introduction

Since the publication of the WHO Position Paper [1], specific immunotherapy (SIT) is considered to be the only treatment that can modify the natural course of allergic diseases. It is now also clear that SIT cannot be considered as a 'desensitizing therapy'. It has been shown that allergic persons with an atopic constitution, based on a genetic predisposition, produce specific IgE antibodies as a reaction to environmental allergens. They are unable to develop a spontaneous tolerance or anergy. Those who are born allergic, remain allergic. SIT, through the application of high quantities of allergens, helps allergic patients to develop such state of tolerance.

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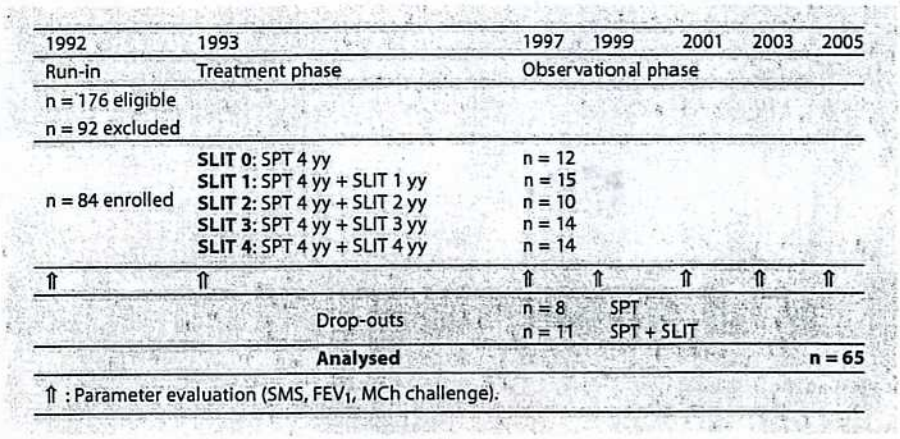

Fig. 1. Study design of the 13-year retrospective study of long-term effects of SLIT in patients submitted only to SPT, and 1, 2, 3 or 4 years of SLIT plus SPT at the Cuasso al Monte Hospital (Varese, Italy).

SIT, which has recently been called 'antiallergic vaccination' [1], can therefore not be considered as an immunization in the true sense of the word (i.e., an active immunoprophylaxis aimed at the creation of a specific state of immunity against an infective agent). It rather constitutes an immunomodulation, which induces a lymphocyte tolerance against the allergen, by means of a shift from T_H2 to T_H1/T_H0 or, as has recently been suggested, by increasing the activity of T-regulatory cells [2].

We cannot expect a long-term immunity from SIT, as it is the case with viral agents (e.g., measles), but we can find many controlled trials in the literature that show an effectiveness of SIT injections, both for rhinitis [3–7] and asthma [8–10], long after cessation of 3- to 5-year therapeutic cycles.

Sublingual SIT (SLIT) has been well established as a valid therapeutic option and recognized by guidelines and meta-analyses [11, 12], on the basis of numerous controlled double-blinded placebo-controlled studies [13–20]. Safety is the strong point of SLIT. This is documented by both revisions of clinical studies [21] and postmarketing analyses [22–24], which underline its absolute safety profile. One of the criticisms that has always been brought against SLIT is that the patients' adherence to the therapy is uncertain, because it is self-administered. A recent systematic real-life study has shown that therapy adherence is very high (over 95%), independent of the patients' socioeconomic characteristics [25]. A randomized, controlled study that involved more than 500 patients has furthermore demonstrated that SLIT can prevent new sensibilizations as well as subcutaneous SIT [26]. The only double blind, double-dummy study available in the literature that directly compares the clinical effectiveness of SLIT and subcutaneous SIT has shown no significant differences between the two methods of administration [27], but resulted in a better tolerance profile for SLIT. It has finally been observed that SLIT is not only effective against rhinitis, but also against asthma and conjunctivitis [28]. This confirms that its immunologic effect is systemic and can, as has been pointed out before, most likely be attributed to the downregulation of T_H2 lymphocyte function [29].

SLIT has been introduced recently in the therapeutic practice. So far, only few studies on its effect on asthma prevention in rhinitis patients [30] and on its long-lasting effects (over 5 years) [22, 31] are available. In the following, we therefore report the results of a retrospective observational study on the clinical long-term effects of SLIT in adult patients with monohypersensitivity to *Dermatophagoides*, with allergic rhinitis and associated bronchial hyperreactivity (BHR).

Material and Methods

Overall Design

The study (run-in, treatment and observational period) was carried out from 1992 to 2005 in the allergological outpatient's department directed by the first author (M. Marogna).

One hundred seventy-seven patients (nonsmokers, no job-related asthmatic risk), monosensitized to *Dermatophagoides*, suffering from allergic oculorhinitis and BHR, were initially screened.

After a 1-year run-in period (from 1993 on) (fig. 1), all patients were treated with the standard pharmacological treatment (SPT) for at least 4 years. SLIT was suggested to patients that did not respond adequately to SPT (n = 84; i.e., decreasing of symptom scores in February 1993 <50% symptom scores in February 1992). Symptom scores in February 2002 were computed after a pharmacological wash-out of at least 2 weeks.

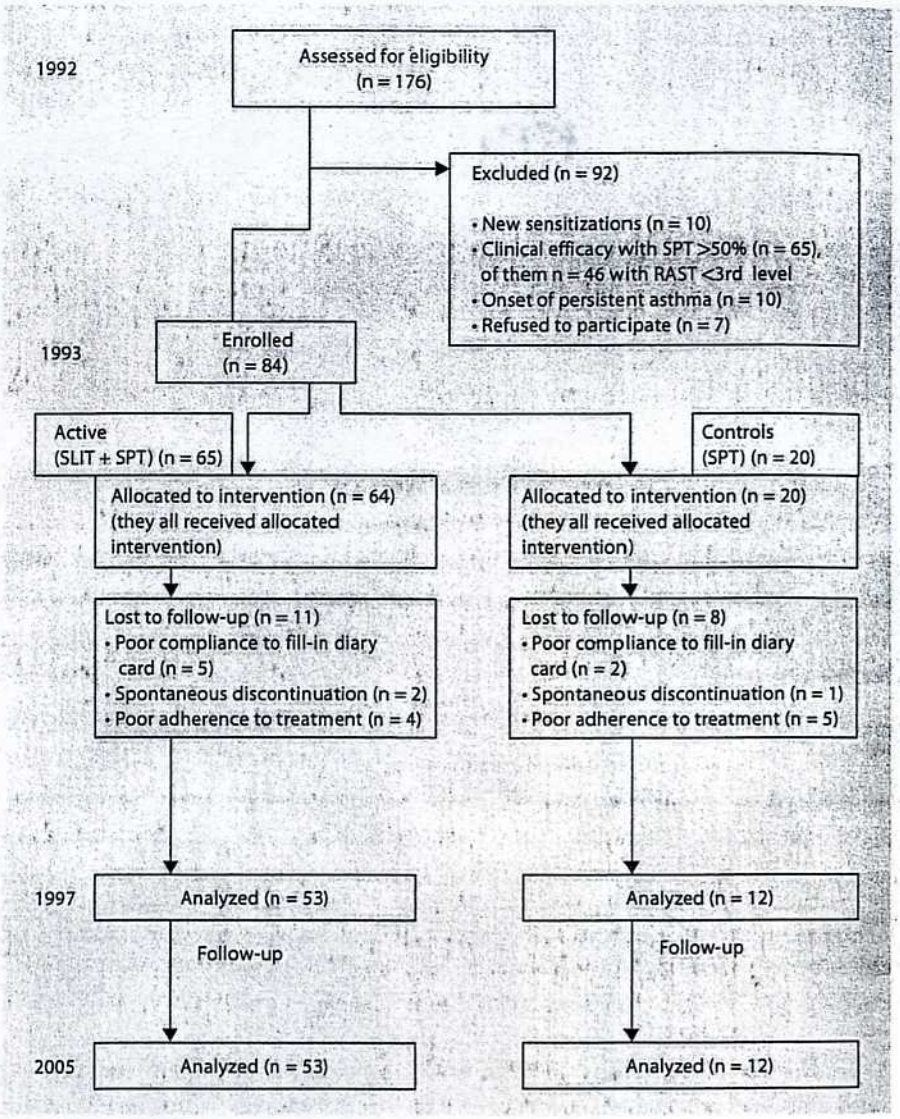

Fig. 2. Diagram showing the flow of participants through each stage of the retrospective trial.

Sixty-four out of 176 SPT low-responder patients were submitted to SLIT and contemporary SPT, while 20 patients were treated only with SPT (control group, SLIT 0). At the end of the observational period (2005), 53 patients were grouped on the basis of the duration of SLIT: SLIT 1 (4-year SPT and 1-year SLIT); SLIT 2 (4-year SPT and 2-year SLIT); SLIT 3 (4-year SPT and 3-year SLIT); SLIT 4 (4-year SPT and 4-year SLIT) (fig. 1).

Inclusion criteria were the following: (1) clinical history of at least 2 years of respiratory allergy to *Dermatophagoides*. Clinical criteria included sporadic episodes of wheezing, cough or sense of thoracic tightness (day or night), as well as the typical symptoms of rhinitis (nasal itching, sneezing, rhinorrhea, nasal congestion) with or without conjunctival itching and reddening, during the period September to February, (2) positivity to skin prick test (>5 mm) only for *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*; (3) age between 18 and 65 years, (4) BHR during the observation period with or without intermittent asthma, according to the GINA criteria, FEV_1 within the normal range (i.e. >79% of the expected value), and (5) minimum class III RAST positivity to *D. pteronyssinus* and *D. farinae*.

Patients that were sensitized for other common antigens or had persistent asthma in accordance with GINA criteria, patients with anatomical anomalies of the upper respiratory tract, with neoplastic and/or autoimmune diseases and those patients that had already been treated with SIT were excluded from the study. Patients with a low adherence to the treatment were excluded from the analysis, and the drop-outs (n = 11 in the SLIT group and 8 in the control group) were subsequently excluded from the analysis (fig. 2).

Clinical parameters such as the symptom mean monthly score (SMS), the FEV_1, and the methacholine (MCh) challenge were evaluated (see below) at run-in (1992), at baseline (1993), and every 2 years from 1997 on (fig. 1).

The SPT for all patients lasted for 4 years and was applied during the autumn-winter period (from September to February). It consisted of an oral antihistamine (cetirizine or loratadine 10 mg/day orally). In the case of a worsening of rhinitis and/or asthma, patients could receive additional salbutamol inhalations (100 μg, 1–2 puffs, when needed) and nasal corticosteroids (beclomethasone dipropionate 100 μg, 2 puffs b.i.d. per nostril). During the observational period, following the active treatment phase, patients used these medications (antihistamines, nasal steroids and topical β_2-stimulants) only when needed.

Clinical Evaluation

During the entire treatment phase, patients underwent medical control visits about once a year (every 10–14 months, always at the end of the period of maximum exposure to mite antigens, i.e., between September and February). After the treatment phase, medical checkups were carried out every 2 years, during the same time period.

Skin Prick Test/RAST

In accordance with the most recent guidelines [32], skin tests were carried out with a set of standard allergen extracts (ALK-Abellò, Milan, Italy), which included: *D. pteronyssinus* and *D. farinae*, grass, birch, olive, dog and cat epithelia, *Ambrosia*, *Artemisia absinthium*, *Parietaria*, *Alternaria* and *Cladosporium*. Skin tests were done at the run-in, at the beginning and end of the treatment phase and, during the following observation phase, every 2 years, always during the autumn-winter period (fig. 1). RASTs (CAP System, Pharmacia, Sweden) were carried out at the run-in. Only patients with monosensitivity to *Dermatophagoides* with at least a class III RAST positivity were included in the study design.

Pulmonary Function Test and MCh Challenge

Pulmonary function test, according to international guidelines [33], were performed by a computerized spirometer (Masterlab, Jaeger, Würzburg, Germany). MCh challenges were carried out when the patients had been asymptomatic and free of medications (inhaled and oral) for at least 12 h, and they were performed in each patient at the same time of year (February). A dosimeter (Jaeger) activated by the inhalatory effort was used to give increasing doses of MCh, from 30 to 1,290 μg. The dose of MCh causing a fall of 20% in FEV_1 was defined as PD_{20}. The test was considered positive (MCh+) if the dose causing a fall of 20% in FEV_1 was equal or <1,200 μg. Only the patients with an MCh-positive challenge were enrolled in the study.

Pulmonary function test and MCh challenge were performed at run-in, at the beginning and the end of the treatment phase and every 2 years during the observational period (fig. 1).

Treatments

Oromucosal specific hyposensitizing treatment (LAIS®, Lofarma SpA, Milan, Italy) involved the administration of a *Dermatophagoides* antigen mixture (*D. pteronyssinus* 50%, *D. farinae* 50%) contained in orally soluble tablets (allergoid SLIT), at the following dosages (titrated in allergenic units, AU): 25, 100, 300 and 1,000. The monomeric allergen is obtained through chemical modification of native allergens with alcaline cyanate (carbamylation of amino groups). The extract is standardized by RAST inhibition in comparison to an internal standard [34].

The treatment with allergoid SLIT began with an initial, 14-week dose increase phase, during which each dose was administered 3 times a week, according to a schedule provided by the manufacturer. This was followed by a maintenance phase, in which the maximum tolerated dose (which for all patients turned out to be 1,000 AU) was administered once a week. The mean cumulative annual dose per patient during the maintenance phase was about 60,000 AU.

All patients received detailed instructions and written information about the rules and procedures of self-administration. All participants also received appropriate treatment with antiallergic pharmaceuticals (cetirizine or loratadine; 10 mg/day), which were to be used continuously and regularly during the autumn-winter period. In addition, depending on the symptoms, patients were allowed to take salbutamol inhalations (1–2 puffs, 100 μg per puff) and intranasal corticosteroids (beclomethasone dipropionate, 2 puffs per nostril, b.i.d.; 400 μg/day).

Clinical Diary Card and Symptom Scores

All patients were instructed to fill in a daily diary card during the period of maximum antigen exposure (September to February), reporting the following symptoms: nasal itching, sneezing, runny nose, nasal obstruction, cough, wheezing, conjunctival reddening or itching. Each symptom had to be scored according to severity, using the scale: 0 = absent, 1 = slight, 2 = moderate, 3 = severe. In addition, each administered dose unit of the medications that were allowed (when needed) was assigned a point value of 1 (salbutamol 1 puff, nasal steroid 2 puffs per nostril). In the end, for use in subsequent statistical analyses, these points were used to calculate for each patient a mean monthly score for clinical symptoms and pharmaceutical usage. These data were collected at the run-in, the beginning and end of the treatment phase and every 2 years during the 9-year observational period (fig. 1).

Statistical Analysis

Sex ratio at baseline was compared by the Pearson χ^2 test, whilst the Kruskal-Wallis test for independent groups was performed to verify differences between the control and SLIT groups [35], both regarding the mean patient age and clinical parameters (FEV_1, SMS, MCh).

Clinical parameters were checked every 2 years after the end of treatment, and tests for differences were done between successive time steps (TS) of 2 years (2–3, 4–5, 6–7, 8–9 years from the end of treatment). Changes in SMS and FEV_1 after n TS from the end of the SLIT treatment in the five groups of differential SLIT duration were tested by the Wilcoxon test for paired samples [36].

The McNemar change test for dichotomous paired data was used to test MCh positivity change from baseline [35] to 2–3 years from the end of treatment, and from 2–3 years after the treatment to 2 years later (4–5 years from SLIT).

Multiple comparisons between treatments were performed by the Mann-Whitney test [35]. To take into account the multiple comparisons we corrected the α level using the Dunn-Šidák significance level correction method: $\alpha' = 1 - (1 - \alpha)^{1/k}$, where k is the number of comparisons [37]. As our purpose was to compare the four groups of SLIT treatment, hence k = 6, and subsequently $\alpha'_{0.05} = 0.0085$, $\alpha'_{0.01} = 0.00167$, and $\alpha'_{0.001} = 0.000167$.

Table 1. Demographic (age) and clinical parameters (FEV_1, MCh, and symptom scores) at baseline (1993) in monosensitized (house dust mites) patients not treated (SPT), or submitted to SLIT 1, SLIT 2, SLIT 3 or SLIT 4 at the Cuasso al Monte Hospital (Varese, Italy)

		SLIT 0	SLIT 1	SLIT 2	SLIT 3	SLIT 4	χ^2	d.f.	P_{MC}
Age	Mean	25.65	26.90	26.14	25.07	25.50	0.598	4	0.966
	SEM	1.24	1.66	1.87	1.49	1.79			
	Max	35	40	41	36	38			
	Min	18	18	18	18	18			
	n	20	21	14	15	14			
FEV_1	Mean	101.20	100.00	100.64	98.93	99.64	2.953	4	0.577
	SEM	1.162	1.199	1.781	1.755	1.443			
	Max	117	111	114	116	110			
	Min	94	92	91	91	93			
	n	20	21	14	15	14			
MCh	Mode	2	2	2	3	3	1.348	4	0.860
	IQ	1	1	1	1.25	2			
	n	20	21	14	15	14			
SMS	Mean	334.40	344.67	341.29	324.47	336.86	0.946	4	0.923
	SEM	11.56	14.05	17.70	16.25	17.46			
	Max	416	480	448	449	462			
	Min	240	248	246	241	246			
	n	20	21	14	15	14			

Mean values, SEM, minimum, maximum and sample sizes (n) are reported for age, FEV_1, and SMS, while mode and interquartile distance (IQ) are reported for sensitivity to MCh. Kruskal-Wallis test results are reported along with the Monte Carlo probability estimate (P_{MC}).

The probability level was computed using a complete randomization method (permutation or exact test; P_{Exact}) or by a Monte Carlo simulation based on a 10,000-sample table (P_{MC}) [38] when computation was not possible. The means (age, symptom scores, etc.) were reported with the standard error of mean (±SEM).

All the statistical analyses have been computed using the Statistical Package for Social Sciences ver. 12.01 (SPSS®).

Results

At baseline (1993), age, sex ratio, and clinical parameters (FEV_1, MCh and symptom scores) did not significantly differ in SLIT 0 and SLIT groups (table 1).

The effect of the allergoid SLIT treatment on SMS (fig. 3a) was the most evident still being significantly present for all groups both after 2–3 years and also after 4–5 years (same test results for both 1997 and 1999; SLIT 1, $z = -3.408$, $P_{Exact(1\ tail)} < 0.001$; SLIT 2, $z = -2.191$, $P_{Exact(1\ tail)} = 0.027$; SLIT 3, $z = -3.296$, $P_{Exact(1\ tail)} < 0.001$; SLIT 4, $z = -3.297$, $P_{Exact(1\ tail)} < 0.001$). This was even more consistent in the patients treated with the allergoid SLIT for 4 years, as a significant symptom decrease was observed again after 7–8 years from the end of treatment, while no significant variation was recorded in SMS for all groups ($P_{Exact(1\ tail)} > 0.050$; $z = -3.235$, $P_{Exact} < 0.001$).

As expected, SMS of SLIT 0 did not differ from baseline ($z = -0.078$, $P_{Exact} = 0.970$). SMS did not increase significantly when 2 more years passed with no SLIT treatment (for all groups, $P_{Exact(1\ tail)} > 0.050$).

Regardless of treatment duration, the SLIT effect on the increment of FEV_1 did not last longer than 2–3 years (for all groups, $P_{Exact} > 0.050$; fig. 3b). Further worsening of FEV_1 was detected after 8 years from the end of treatment in all groups, but for SLIT 1, and for 4 patients, as in the latter ones, a significant improvement was detected (SLIT 1_{2003}, $z = -1.681$, $P_{Exact} = 0.097$; SLIT 2_{2003}, $z = -2.117$, $P_{Exact} = 0.037$; SLIT 3_{2005}, $z = -2.836$, $P_{Exact} = 0.002$; SLIT 4_{2005}, $z = -1.999$, $P_{Exact} = 0.046$).

The effect of the allergoid SLIT on sensitivity to MCh after 2–3 years from the end of treatment was significantly detectable only for patients treated for at least 3 years (McNemar test: SLIT 3, $P_{Exact} < 0.001$; SLIT 4, $P_{Exact} < 0.001$). From 2–3 to 4–5 years after the end of treatment

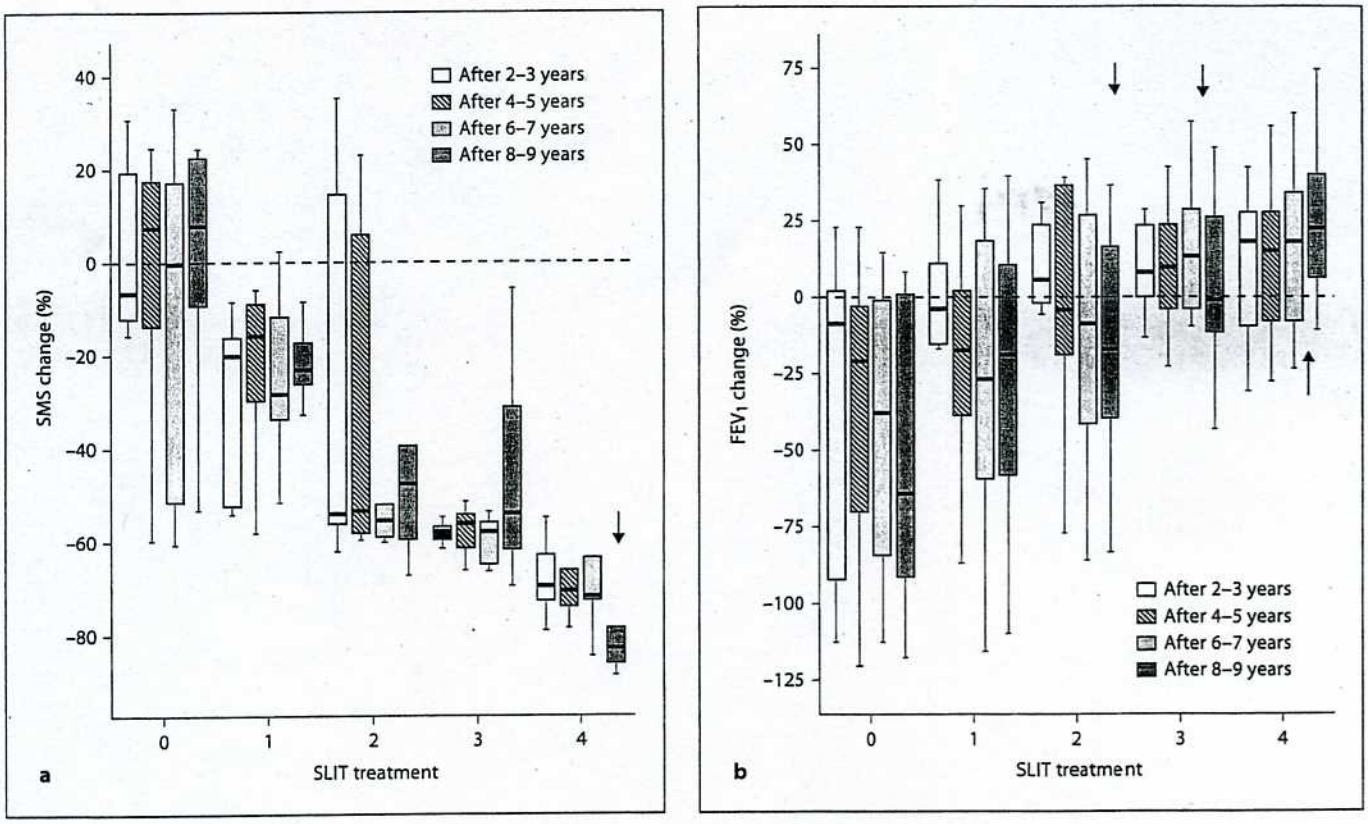

Fig. 3. Change of clinical parameters (**a** SMS and **b** FEV_1) after 2–3, 4–5, 6–7 and 8–9 years from the end of SLIT treatment in patients submitted only to SLIT 0, or to SLIT 1, SLIT 2, SLIT 3 or SLIT 4. Arrows indicate significant (by Wilcoxon test for paired samples) decrement (downward) or increment (upward) of clinical parameters in each treatment group after time steps of 2 years. Boxes represent the interquartile distance (1st quartile, lower box extreme; 3rd quartile, upper box extreme); thick line represents the 2nd quartile (median), while whiskers represent the extreme values.

no significant change (i.e., improvement or worsening of sensitivity to MCh) was detected ($P_{Exact} > 0.050$).

Comparing the four SLIT groups (fig. 4) after 5–6 years from the end of treatment, significant differences in occurrence of positivity to MCh were detected in SLIT 1 ($\chi^2 = 5.400$, d.f. = 1, $P_{Exact} = 0.035$), in SLIT 3 ($\chi^2 = 7.143$, d.f. = 1, $P_{Exact} = 0.013$), and SLIT 4 ($\chi^2 = 10.286$, d.f. = 1, $P_{Exact} = 0.002$), while no difference in the occurrence of positivity to MCh for SLIT 2 patients was observed ($\chi^2 = 0.400$, d.f. = 1, $P_{Exact} = 0.754$).

The effect of treatment duration was particularly clear comparing the clinical parameters (SMS and FEV_1) in patients that stopped the therapy (of different duration) at least 5 years before. In fact, both SMS and FEV_1 significantly differed in the four groups of treatment ($\chi^2 = 18.951$, d.f. = 3, $P_{MC} < 0.0012$, and $\chi^2 = 8.558$, d.f. = 3, $P_{MC} = 0.036$, respectively) (fig. 3a). SMS of patients treated with SLIT for 1 year significantly differed from SMS of patients treated for 3 years (U = 38,500, W = 143,500, $P_{Exact(1\ tail)\ corrected} < 0.010$), and from those treated for 4 years (U = 26,500, W = 131,500, $P_{Exact\ (1\ tail)\ corrected} < 0.001$; fig. 3a). Similarly, patients that were treated for 2 years (SLIT 2) differed significantly in their SMS from those treated for 4 years (U = 27,000, W = 132,000, $P_{Exact(1\ tail)\ corrected} < 0.050$), and even those treated for 3 years differed from those treated for 4 years (U = 40,500, W = 145,500, $P_{Exact\ (1\ tail)\ corrected} < 0.050$).

Conversely, as expected, FEV_1 were significantly greater in patients submitted to SLIT for 4 years than in patients treated with drugs alone or treated only for 1 or 3 years (U = 48,000, W = 168,000, $P_{Exact(1\ tail)\ corrected} < 0.050$, and U = 44,500, W = 149,500, $P_{Exact(1\ tail)\ corrected} < 0.050$; fig. 3b).

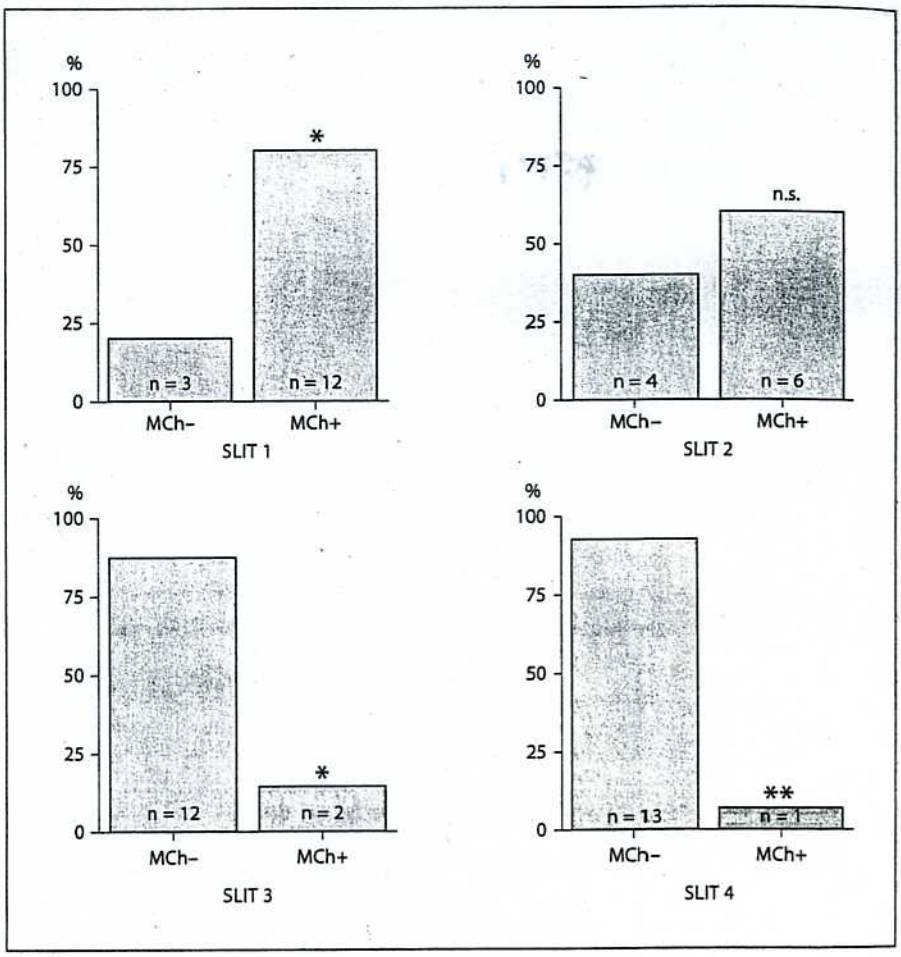

Fig. 4. Long-term effect of SLIT on responsiveness to MCh. Comparison of responsiveness to MCh (PD_{20} FEV_1 <1,200 μg) after 5–6 years from the end of treatment in patients submitted to SLIT 1, SLIT 2, SLIT 3 or SLIT 4. * $P_{Exact} < 0.050$; ** $P_{Exact} < 0.010$. n.s. = Not significant.

Discussion

SLIT is considered as a valuable alternative to subcutaneous immunotherapy for the treatment of respiratory allergy both in adults and in children [11]. Nevertheless, some aspects of SLIT have not yet been fully elucidated. Among these, one of the most intriguing and worth considering, also from an economic point of view, is its long-lasting efficacy after discontinuation.

The principal aim of this retrospective, open, drug therapy-controlled study was thus to investigate whether the clinical effects of SLIT, that in our case was a chemically modified allergoid SLIT, are maintained for some years after its end and, in addition to that, whether the extent of these is related to the duration of the same, whereas the clinical efficacy per se was assumed to be sufficiently demonstrated by previous placebo-controlled studies.

To do that, we based our observations on three distinct parameters: the clinical benefits, i.e., the SMS, the variations of BHR evaluated by the MCh challenge and the changes of FEV_1.

The benefit of SLIT in terms of an increment of FEV_1 disappeared immediately, already being no longer observable 2–3 years after the end of treatment. Yet, a worsening of this is likely to occur, already after 1 year, if no SLIT is administered at all.

Similarly, a 1-year SLIT has quite a short-term effect both on SMS reduction and BHR improvement: they in fact already disappeared at the 2nd year after the end of treatment.

On the other hand, conversely from FEV_1, a 2- to 3-year SLIT therapy is sufficient to maintain the benefits both on SMS reduction and BHR even for 5–6 years, but not for longer periods. It is noteworthy to stress that both the FEV_1 and BHR evaluations have been performed every year in the same month (February), in order to avoid possible variations in bronchial reactivity due to variations of the environmental allergen charge.

Essentially, our results showed that only a 4-year treatment is capable to guarantee a long-term duration – even up to 7–8 years – of clinical benefits, i.e., on SMS decrement, after it had ended. In a certain sense, our results are not very different from those obtained by Di Rienzo et al. [22] and indeed confirm them, but in this case only a 4- to 5-year SLIT duration was considered while the effects of shorter treatments were not evaluated. Also in the observational study done by Andri and Falagiani [39] the clinical improvement of 4–5 years after stopping local nasal immunotherapy (different from SLIT but similarly consisting of transmucosal administration) was significant: 54.6% of the 22 patients studied were almost free of symptoms, whereas 27.3% complained of only minor symptoms.

In contrast, our data, though retrospective, clearly indicate that the long-lasting effect of SLIT is strictly related to the length of treatment. In fact, neither clinical benefits nor effects on BHR and even less on FEV_1 were observed after 5–6 years when SLIT shorter than 2–3 years was evaluated, while a significant clinical efficacy was still present after 7–8 years from SLIT discontinuation when therapies of at least 4 years were considered.

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