SUBLINGUAL IMMUNOTHERAPY IN THE CONTEST OF A CLINICAL PRACTICE IMPROVEMENT PROGRAM IN THE ALLERGOLOGICAL SETTING : RESULTS OF A LONG TERM OBSERVATIONAL STUDY

M. Marogna MD [1], **A. Massolo** [2]

Summary

The aim of this study was to assess the efficacy and safety of sublingual immunotherapy (SLIT) in a Clinical Practice Improvement (CPI) program carried out in allergology.
The study was conducted between 1992 and 2001 using an observational type methodology in line with standard clinical practice. The program consisted of 4 basic steps :

- setting up of a decision-making tree ;
- standardization of main diagnostic and therapeutic aspects ;
- data collection ;
- definition and evaluation of main clinical endpoints.

Study patients were screened among 1508 patients with pollen and/or dust mite respiratory allergy, 350 of which, one year after having experienced a pharmacological treatment failure, were administered immunotherapy by injective (n = 111) or alternative route (n = 239). For each one of the three immunotherapy treatment groups (nasal, SLIT or injective) there was a control group of patients who, despite their poor response to pharmacological treatment, continued with pharmacological therapy alone (n = 314 in total; 68, 192 and 54 respectively). The observation of 130 SLIT patients, 106 of which were treated for at least 36 months, towards the control group evidenced that such therapy, apart from resulting efficient and particularly safe, has an unfailing protective effect against the development of asthma and new allergic sensitizations.

Key-words : Respiratory Allergy - Sublingual Immunotherapy - Clinical Practice Improvement Program.

INTRODUCTION

Today, respiratory allergopathies are always more often chronic and featured by a remarkable multiplicity of pathogenic factors ("infection", "environment", etc.).

The picture, often discouraging, that appears to any Reference Specialized Allergology Center shows poorly compliant patients, prone to self-medication and/or alternative medicine, in whom even specific immunotherapy (SIT) would fail unless an appropriate support, consisting of an educational program and clinical examinations at short intervals, is provided.

These observations, along with the availability of new, non injective antiallergic vaccines and an always deeper knowledge of patient immunoallergic profile, induced us, in the early nineties, to set up a decision-making algorithm for SIT prescription and follow-up. The aim of this tool was to allow a tailored selection of the vaccine type on the basis of every single patient clinical and functional features (figure 1).

Therefore, three groups of patients, other than full responders to standard pharmacological treatment, to whom SIT could be proposed, were identified. Group 1 consisted of patients with pure rhinitis, free from any functional affection of the lower airways and complaining mild oculorhinitis (R) symptoms. Group 2 consisted of patients with moderate to severe oculorhinitis symptoms and possible concomitant bronchial hyperreactivity (BHR) and/or intermittent asthma (R/A). Group 3 consisted of patients with persistent, mild to moderate asthma, either associated or not to moderate to severe oculorhinitis and BHR (A/R). When defining the degree of severity for asthma and rhinitis, indications included in the most recent guidelines on this subject (1, 2) were taken in due consideration. R, R/A and

[1] Rehabilitative Pneumology Unit Cuasso al Monte P.O. Fondazione Macchi Hospital - Varese (Italy).
[2] Section of Behavioural Ecology, Ethology and Wildlife Management, Dept. Environmental Sciences, Siena University, Via P.A. Mattioli 4, I-53100 Italy.

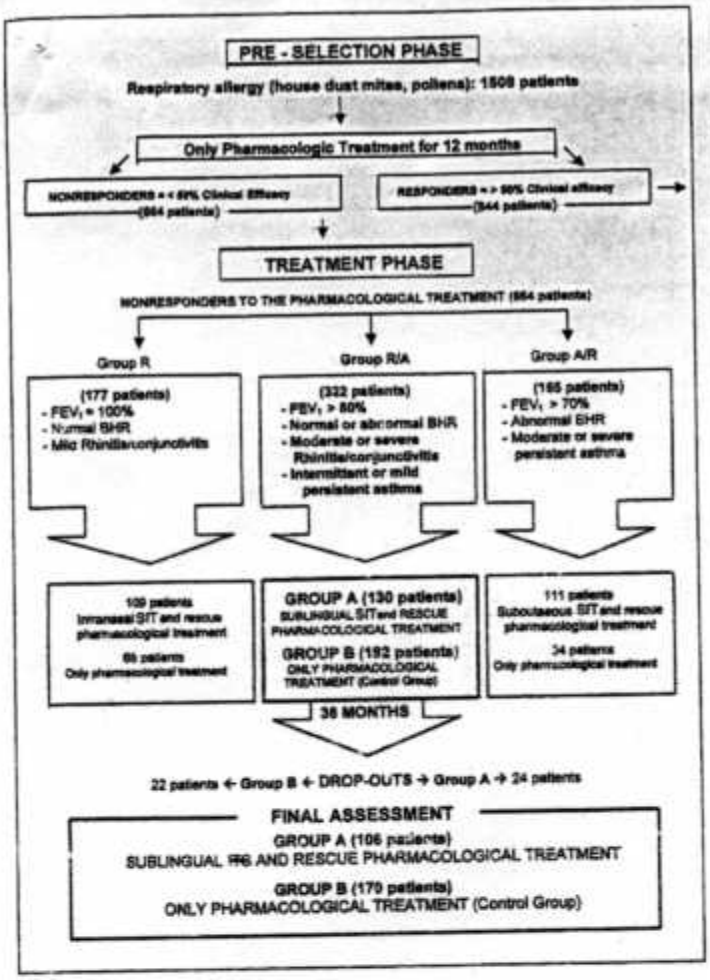

Figure 1 : Decisional tree.

A/R patients were assigned to intranasal (INIT), sublingual (SLIT) and subcutaneous (SIT) SIT, respectively. In every single group, a subset of patients acting as a control, who had spontaneously decided to continue standard pharmacological treatment despite its poor efficacy, were identified (see also Section "Patients").

The double aim of this study, as far as SLIT patients only were concerned, was :

- to ascertain the validity of such a practical clinical approach to patients with respiratory allergopathy ;
- to compare SLIT with pharmacological treatment and assess whether SLIT, conducted for at least 36 months in patients other than full responders to standard pharmacological treatment.

Could :

- improve moderate to severe rhinoconjunctivitis and/or intermittent asthma to a statistically significant extent ;
- correct BHR ;
- prevent BHR, asthma and the development of any new sensitization. The implementation of a Clinical Practice Improvement (CPI) Program (3 ,4, 5) constitutes an innovative approach for the allergological practice. Through an appropriate patient evaluation program during every phase of the diagnostic and therapeutic course, following needs should potentially be met :
  - standardization of patient selection and treatment methods ;
  - uniformity of collection and analysis instruments as far as data relevant to areas having a significant clinical and prognostic relevance (clinical endpoints) are concerned ;
  - performance of observational studies on a large number of cases and possible adoption of corrective methods on a both diagnostic and therapeutic level.

The need to operate in an observational context depends on a low degree of adaptation of controlled, randomized clinical studies to practical medicine, which experimental semplifications are not easily extrapolated to (6, 7). On this respect, attention should be given to the problem posed by compliance that is often hard to be assessed in daily clinical practice (8). In addition, there are some specific issues of allergological practice that cannot be either misunderstood or undervalued. Among them, attention should be given to :

- the substantial multidisciplinary nature of allergological practice ;
- the need for a tailored approach to subject in order to optimize many complex therapeutic actions ;
- the partial lack of objective and standardized instruments to evaluate patients ;
- the need for long term (minimum 3 years) clinical trials.

Therefore, the implementation of an instrument such as a CPI program, using an observational methodology, would make possible to assess efficacy and tolerability of medications used and meet some of the needs mentioned above at the same time.

MATERIALS AND METHODS

The study was conducted between 1992 and 2001, using an observational methodology. Such decision is related to following motivations :

- better adherence to clinical practice reality ;
- evaluation, on a larger number of cases, of main diagnostic and therapeutic endpoints ;
- feasibility of a study in a pretty large population and with a minimum duration of 36 months.

The fundamental steps of this process were :

- setting up a decision-making algorithm for the selection of patients to be administered SIT ;

- definition of clinical endpoints ;
- setting up of diagnosis standardization criteria, treatment assignment and clinical endpoint evaluation ;
- statistical evaluation of results and introduction of corrective measures, if any, in the diagnostic and therapeutic routine.

PATIENTS

All subjects included in the study and treated with SIT were outpatients affected by a respiratory allergopathy. They were divided in three groups keeping into account both prevailing clinical features, as previously described, and, more specifically, the intended type of SIT :

- group R (n = 109) was given INIT ;
- group R/A (n = 130) was given SLIT ;
- group A/R (n = 111) was given SIT.

For each one of the three immunotherapy treatment groups (INIT, SLIT or SIT) there was a control group of patients who, despite they were not particularly responsive to pharmacological treatment, continued the pharmacological therapy alone (n = 314 in total : 68, 192 and 54, respectively). Therefore, the R/A group, which this paper refers to, consisted of two subgroups: group A, consisting of 130 patients treated with SLIT (code LAIS, Lofarma S.p.A., Milan, Italy) and group B (control), consisting of 192 patients treated with drugs only. Fifty-five out of the 130 patients who were initially administered SLIT (mean age 19,5 years; 50M and 56F) had a dust mite allergy, while the remaining 75 were affected by pollen allergy. Among these latter, 43 patients suffered from a grass allergy and 32 from a birch tree allergy. The 106 patients who continued the treatment for at least 3 years were treated as follows : 44 patients treated with Lais® *Dermatophagoides*, 38 patients treated with Lais, grass and 32 patients treated with Lais® *birch tree*. In particular, the most relevant aspects of the decision-making tree used are the following :

- patients were given sublingual SIT after a pharmacological treatment of at least one year, missing a completely satisfactory response to medications (i.e. under 50%) ;
- the assignment to one SIT rather than another was defined on the basis of a multivariance analysis, which took into consideration prevailing pathology (oculorhinitis and/or asthma), patient medical history and some study parameters (FEV1 and aspecific BHR study) ;
- the control group, treated with drugs alone, consisted of patients having the same features as subjects who had been given SIT, willing to continue with medications, although efficacy was not as satisfactory as it should be.

All patients were followed, as far as main clinical variables were concerned, throughout a "window period" of 2-3-4 months, corresponding to the period of maximum exposure to the specific allergen in the geographic area concerned (9).

Before being enrolled in the study, all patients had to undergo following diagnostic tests :

➢ skin prick test (SPT), in accordance with most recent guidelines on this subject (10) ;

➢ exhaustive respiratory function tests (RFT) associated to Methacoline test (MCh), in accordance with the most recent guidelines on this subject (11, 12). In particular, the following should be remarked :

- the detection of specific Resistances to, and Conductances of, the air flow (Raw and Sgaw) was performed with the steady volume plethysmographic method, while spirometric parameters were detected with a modified Fleisch tube pneumotachographic method (both methods have been adopted by MASTERLAB-JAEGER integrated diagnostics) ;
- bronchial challenge with MCh was performed with Metacolina Lofarma® 1%, using a steady pressure dosimeter, a 0.1% MCh solution and a dispensing time of 0.69 seconds = 30 microliters per puff = 30 micrograms of methacoline per puff, resulting into a cumulative dose-response curve : 30-60-120-240-390-690 micrograms. The MCh test was performed in those cases where patient medical history evidenced asthma-equivalent symptoms (cough).

Exclusion criteria applicable to this study program were :

- age under 5 and over 60 years ;
- multiple sensitization with allergens significantly interfering with the evaluation of selected endpoints (other perennial allergens other than mites or pollens included in the same "window-period" of observation/evaluation) ;
- FEV_1 < 70% of theoretical value ;
- severe asthma ;
- previous and/or protracted treatment with corticosteroids ;
- absolute or SIT-related contraindications such as : pregnancy, use of beta-blockers, cardiopulmonary diseases, autoimmune diseases, neurologic disorders, primary or secondary immunodeficiency.

SIT administration was carried out in accordance with the most recent Position Paper on this matter (16, 17). In particular, with regard to allergenic extracts used for therapeutic purposes, features of products used are reported hereafter. Oromucosal SIT (code LAIS® Lofarma S.p.A., Milan, Italy) consisted of the administration of allergens chemically modified with alcaline cyanate (aminogroups carbamilation) with the aim of achieving monomeric allergoids, as described by Mistrello *et al.* in 1996 (13), titrated in Allergenic Units (A.U.) and incorporated into orosoluble tablets. The Allergenic Unit is a

biological unit defined as 1/40 the average nasal challenge dose in a wide group of allergic volunteers (as described by Giannarini *et al.* in 1998) (14). Treatment required an initial dosage increase period of 14 weeks, during which every dose had to be taken thrice a week in accordance with the standard plan set up by the manufacturer. After that period, a maintenance phase, where the maximum tolerated dose (that was 1000 A.U. for all patients) had to be taken once a week, followed. The yearly average dose during the maintenance period was approximated to 60000 A.U.

CLINICAL ENDPOINTS

Five clinical endpoints were selected. All of the selected endpoints and relevant rating scales could be grouped in either substantially positive or substantially negative final opinions. Patients were clinically examined once every six months.

■ **Treatment Clinical Efficacy :** this endpoint was evaluated by means of a monthly diary in the "window-period", taking into consideration following items : nasal itch, rhinorrhea, sneezing, nasal obstruction, lacrimation, cephalalgia, cough, dyspnea. Each symptom was evaluated in accordance with the following scale : 0 = absent, 1 = mild, 2 = intense, 3 = very intense. At the end of the observation period, data were standardized on the basis of the following symptom rating scale :

- *insufficient response* : < 25% (=0) ;
- *poor response* : 25-<50% (=1) ;
- *good response* : 50-<75% (=2) ;
- *very good response* : >75% (=3).

■ **Drug consumption :** this endpoint was evaluated by means of a specific monthly diary in the "window-period" of observation. In order to standardize the therapeutic approach, following multistep pharmacological strategy, applied to all study patients with symptoms limited to the first airways (nasal and conjunctival mucosa), was used :

➢ ***1st step :*** chronic administration of cromones (or analogous medications) ;
➢ ***2nd step :*** local or systemic long-acting antihistamines ;
➢ ***3rd step :*** topic corticosteroids ;
➢ ***4th step :*** systemic corticosteroids.

For patients with symptoms concerning the lower airways (intermittent asthma), nothing but short-acting beta mimetics were used in case of need. At the end of the observation period, data were categorized on the basis of the following rating scale :

- insufficient response : continuative use of systemic cortisone-based drugs for more than 5 days, possibly associated to other therapies (=0) ;
- poor response : continuative use of antihistamines, topical or systemic steroids or beta-agonists for less than 5 days (=1) ;
- good response : non continuative use of antihistamines or topic steroids or beta-agonists for more than 7 days (=2) ;
- very good response : only cromones and/or antihistamines for a maximum of 7 days (=3).

Tolerability/Safety : this endpoint was evaluated by means of a patient monthly diary and specific interview at every control visit by using the following scale :

- *insufficient* : systemic reactions (=0) ;
- *poor* : moderate to severe local reactions and need for medical treatment (=1) ;
- *good* : mild local reactions and no need for medical treatment (=2) ;
- *very good* : no reaction (=3).

Also for the group receiving pharmacological treatment only, a 4 point scale (ranging between 0 and 3) with the same items (unsatisfactory, fair, good, very good), depending on patient opinion, was used.

■ **Compliance :** this endpoint was evaluated by means of a specific diary and checking whether residual vaccine met the expected quantity. Compliance was expressed according to following classification :

- *insufficient* : <40% (=0) ;
- *poor* : 40 - <60% (=1) ;
- *good* : 60 - <80% (=2) ;
- *very good* : >80% (=3).

■ **Respiratory function (RFT) and bronchial hyperreactivity study :** this endpoint was evaluated through spirometry with pneumotachographic method and bronchial smooth muscle tone study with plethysmographic method before and after MCh challenge test. At the beginning and at the end of the observation period, results were categorized on the basis of following criteria :

- normal ;
- BHR ;
- asthma.

Symptoms were assessed through time and an opinion in terms of improvement (normalization) or worsening (arousal of BHR/asthma) of the respiratory function picture.

STATISTICAL ANALYSIS

In accordance with main international references on this subject, it was decided to carry out an evaluation of the outcomes from the observational study after 36 months of treatment (16, 17). In order to assess treatment efficacy in

terms of frequency of cases where a consistent response to the different therapeutic treatments was experienced, a series of tests based on Pearson's chi-square method (Siegel&Castellan 1988) were conducted. The level of significance was calculated with the help of complete randomization (permutation or exact test). This technique allows an exact estimation of the level of significance, thus the maximum power of the associated test is assured thereby, at least as much as in classic parametric or non parametric tests. The strength and power of permutation tests are assured by comparing observed distributions with exact distributions of sample under analysis (calculated by means of *ad hoc* permutations), avoiding any comparison with theoretical distribution (Good 2000). In order to guarantee a higher safety of test results, the value of first type error was fixed to *alpha* = 0,01. In case of multiple comparisons, if any, the levels of significance were corrected by using the Dunn-Sidak method. Such method prescribes that the new level of significance is fixed to *alpha'* = 1-(*1-alpha*) raised to 1/k, where key is the number of comparisons that have been carried out and *alpha* is the reference value (0.01) (Sokal and Rohlf 1995) (27, 28,29). The limit of significance was fixed to $p = o < 0.01$.

For the analysis of data relevant to main demographic parameters, non parametric descriptive analysis was used. As far as the evaluation of symptoms, drug consumption, tolerability and compliance data are concerned, scores obtained towards observed improvement were considered, along with the number of patients falling in each one of the following four opinion categories: insufficient = 0, poor = 1, good = 2, very good = 3. After that, patients were grouped in two further categories: patients with a substantially negative opinion (insufficient or poor) and patients with a substantially positive opinion (good or very good). Data were subsequently compared with those of the relevant control group, using the methods of analysis mentioned above. As far as the analysis of data relevant to the respiratory function study was concerned, scores obtained at the beginning and at the end of the observation period were compared to each other and the outcome was evaluated as improvement or worsening between treatment groups. As far as the analysis of data relevant to the arousal of new forms of sensitization between treatment groups was concerned, same methods of analysis mentioned above were used.

RESULTS

DROP-OUT ANALYSIS

In the course of the 36 months of observation, 24 out of 130 patients who had received SLIT (18%) dropped out from the study. The reasons for dropping out were the following :

- no improvement meeting expectations : 5 patients ;
- lack of compliance : 3 patients ;
- patient refusal, missing any obvious justification : 5 patients ;
- poor co-operation with study design : 4 patients ;
- reasons not related to treatment (moving out, etc.) : 2 patients ;
- contraindications arising in the course of the study (1 case of pregnancy) : 1 patient ;
- environment reclamation associated to a marked improvement of symptoms : 1 patient ;
- improvement exceeding expectations : 2 patients ;
- finally, 1 patient did never start the treatment as he did not understand its usefulness.

In the control group, under pharmacological treatment, 22 drop-out cases (corresponding to the 11%) were recorded for the following reasons :

- no improvement meeting expectations : 14 patients ;
- lack of compliance : 4 patients ;
- patient refusal, missing any obvious justification : 2 patients ;
- poor co-operation with study design : 2 patients.

ASSESSMENT OF EFFICACY

The analysis of data after 36 months of observation demonstrated a statistically significant response diffe-

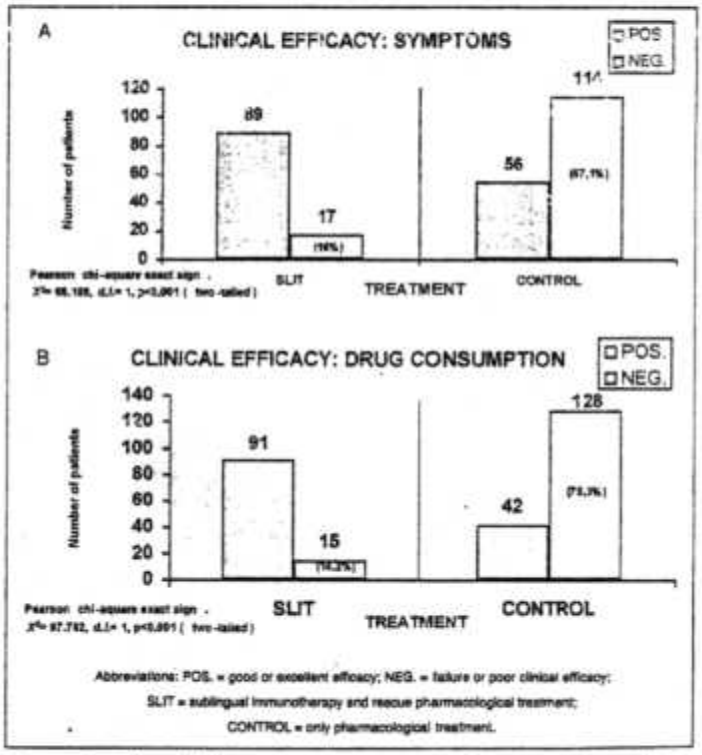

Figure 2 : Clinical efficacy : patient's assessment of symptoms (a) and drug intake (b) after 36 months of treatment.

rence ($p < 0.001$) between the group of patients treated with SLIT and the control group. In addition, SIT efficacy is supported by the concordance between data relevant to symptom reduction and data relevant to drug consumption (figure 2).

Globally, 89 out of 106 patients treated with SLIT for three years (84%) showed a significant clinical improvement. On the contrary, efficacy appeared definitely lower in the pharmacological treatment group: only 56 out of 170 patients who continued pharmacological treatment for at least 36 months could be defined responders thereto (32.9%). Accordingly, drug consumption was dramatically cut down in patients treated with SLIT (in 91 out of 106 patients, corresponding to 85.8%), while it was still high in the control group (128 out of 170 patients, corresponding to 75.3%).

ASSESSMENT OF TOLERABILITY/SAFETY AND COMPLIANCE

In the SLIT group, no serious adverse reaction was reported and no patient dropped out from the follow-up program because of unwanted reactions. Only two cases of diffused itch, regressed after administration of antihistamines, were reported. The two patients in question, however, completed the 3-year treatment period. The difference, in terms of side effects, between the SLIT group and the control group was at the limit of statistical significance ($p = 0.012$) (figure 3). The analysis of data relevant to compliance shows a compliance exceeding 90% in almost all SLIT patients, towards an approximate value of 75% in patients under pharmacological treatment, with a statistically significant difference between the two groups ($p < 0.001$) (figure 3).

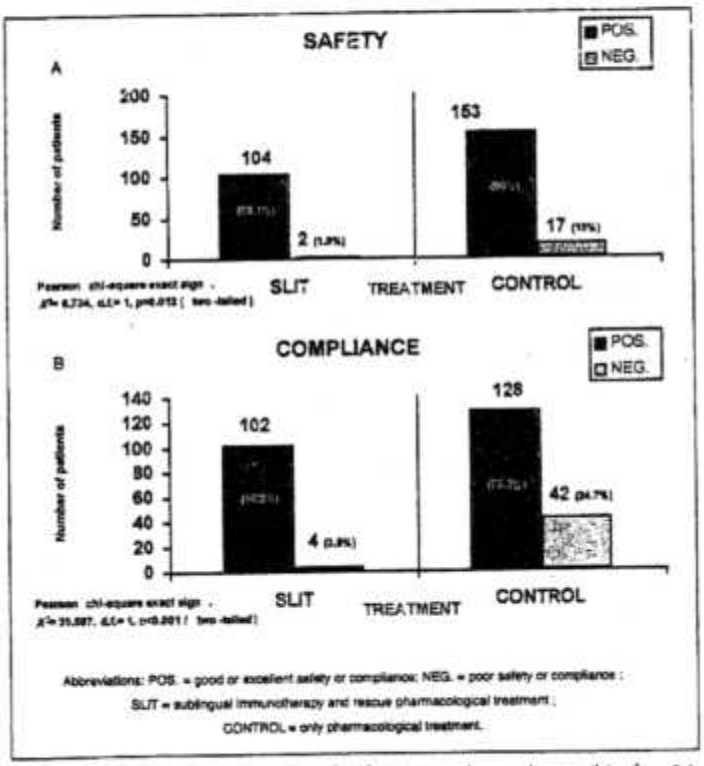

Figure 3 : Patient's assessment of safety (a) and compliance (b) after 36 months of treatment.

ASSESSMENT OF BHR

In total, the number of SLIT patients with a RFT normal result during treatment was almost double ; the number of patients affected by asthma fell down from 34% to 5% and the number of subjects with BHR changed from 21% to 6% (table 1). Therefore, the involvement of the small airways was reduced in such way that it could be observed only in 1/5 of patients showing an initial functional impairment. On the other hand, no significant variation was observed in the control group. SLIT capability to prevent the "allergy walking" from rhinitis to asthma, in comparison with pharmacological treatment alone, is obvious even under a statistical point of view. In fact, no development of pure rhinitis, either to BHR ($p < 0.001$) or asthma ($p < 0.001$) could be observed. BHR was significantly corrected in both hyperreactive patients ($p = 0.002$) and in those suffering from (Class 1) intermittent asthma, in whom a significant normalization of the bronchial smooth muscle tone and of bronchial permeability index ($p = 0.001$) was seen (table 2).

ASSESSMENT OF THE APPEARANCE OF NEW FORMS OF SENSITIZATION

During the three years of observation, a progressively increasing number of group B patients developed new forms of allergic sensitization, while group A patients proved to be significantly more protected ($p < 0.001$) (table 3).

DISCUSSION AND CONCLUSIONS

The aim of this study was to assess the efficacy and safety of SIT, with particular regard to sublingual immunotherapy, in a Clinical Practice Improvement program in allergology. The fundamental steps of this process were: setting up a decision-making tree, using an observational method adhering to standard clinical practice, defining a control group, measuring specific endpoints and evaluating results under a statistical point of view, in accordance with a previously defined pattern. The decision to operate in window periods was determined by the need for homogenizing cases as far as exposition to allergens was concerned, while

allowing the observation of all selected endpoints. The selection of control cases, receiving standard pharmacological treatment alone as preferred by patients, meets some extremely practical needs. Study cases are clinically significant, even if the lack of randomization and analysis of confounding factors does not allow any generalization or extrapolation to all allergic patients (18). The decision to operate in an observational context was mandatory, as study main goal was to set up some investigation instruments applicable to daily clinical reality, and also because the study provided a minimum observation period of 36 months. On the other hand, it is acknowledged that long-term observation required by studies conducted in allergic patients makes much more difficult to perform large size randomized clinical studies. The percentage of patients receiving SIT is higher than that encountered in standard clinical practice. A possible explanation

TREATMENT	PERIOD	NORMALITY	ASTHMA	HYPERREACTIVITY
SLIT	BEFORE	n = 48 (% = 46)	n = 36 (% =34)	n = 22 (% =21)
	AFTER	n = 95 (% = 89)	n = 5 (% = 5)	n = 6 (% = 6)
CONTROL	BEFORE	n = 64 (% = 38)	n = 55 (% = 32)	n = 51 (% = 30)
	AFTER	n = 75 (% = 44)	n = 51 (%= 30)	n = 44 (% = 25)

Table 1 : Responsiveness to functional tests.
Abbreviations :
SLIT = sublingual immunotherapy and rescue pharmacological treatment.
CONTROL = only pharmacological treatment.
n = number of patients.

From	to	SLIT		CONTROL		X^2	df	p value (two tailed)
		n	%	n	%			
RHINITIS	N	48	100.0	33	51.5	32.148	1	p < 0.001
	H	0	0.0	14	21.9	12.000	1	p = 0.001
	A	0	0.0	17	26.6	15.132	1	p < 0.001
	Sub-total	48	100	64	100	-	-	-
HYPER-REACTIVITY	N	19	86.4	23	45.1	10.712	1	p = 0.002
	H	2	9.1	18	35.3	5.305	1	NS (p = 0.024)
	A	1	4.5	10	19.6	2.725	1	NS (p = 0.156)
	Sub-total	22	100	51	100	-	-	-
ASTHMA	N	28	77.8	19	34.6	16.284	1	p < 0.001
	H	4	11.1	12	21.8	1.721	1	NS (p = 0.263)
	A	4	11.1	24	43.6	10.806	1	p = 0.001
	Sub-total	36	100	55	100	-	-	-

Table 2 : Responsiveness to functional tests after 36 months of treatment.
Abbreviations :
SLIT = sublingual immunotherapy and rescue pharmacological treatment.
CONTROL = only pharmacological treatment ; X^2 = Chi square test.
df = degrees of freedom ; N = normality ; H = hyperreactivity ; A = asthma.
n = number; NS = not statistically significant.

BASELINE	n	New sensitisations		X^2	df	p value (two tailed)
		n	%			
SLIT	106	3	2.8	47.021	2	p < 0.001
CONTROL	170	64	37.6			

Table 3 : Frequency of new sensitisations with the two treatments.
Abbreviations :
SLIT = sublingual immunotherapy and rescue pharmacological treatment.
CONTROL = only pharmacological treatment.
n = number of patients; X^2 = Chi square test; df = degrees of freedom.

for that could be the following: patients calling at specialized allergology centers have often experienced a diagnostic and therapeutic path that is not always in line with most recent scientific achievements. Therefore, case histories are selected ones, but it should be remembered that the higher is difficulty, the higher is clinical significance under a practical point of view. Given all these premises, the results of this observational study confirm data on sublingual SIT efficacy and tolerability available in the literature (19, 20, 21, 22). The study of respiratory function shows an evident protective action of SIT administered by sublingual route and seems in line with most recent experimental evidences (23, 24). In addition, the analysis of data relevant to compliance shows that the setting up of an appropriate patient follow-up program from a specialized center, although keeping into account methodological restrictions imposed by the study, with particular regard to pharmacological treatment evaluation, is capable to bring compliance up to optimal levels. In particular, compliance to SIT appears definitely high if compared with other experiences reported in the literature (25, 26).

In conclusion, the results of this study suggest that : 1) SIT should be started as precociously as possible, also in patients with no apparent involvement of the small airways. In fact, it was seen that it has a protective action against the development of rhinitis into asthma and the appearance of new forms of sensitization. 2) It is possible to hypothesize a broader use of SIT also in patients with a poor compliance to pharmacological treatment.

REFERENCES

1. Canonica G.-W., Holgate S.-T. (Eds.). - The impact of allergic rhinitis on quality of life and other airways diseases. *Allergy* 1998;53 (41 Suppl):1-30.
2. NHLBI/WHO-Workshop report. - Global strategy for asthma management and prevention. National Heart, Lung and Blood Institute, Publication n° 95-3569, Jan 1995.
3. Horn S.-D. - CPI (clinical practice improvement) : improving quality and decreasing cost in managed. *Med Interface* 1995;8:60,70.
4. Katon W. - Improvement of outcomes in chronic illness. *Arch. Family Med.* 2000;9:709.
5. Curry S.-J. - Organizational interventions to encourage guidelines implementation. *Chest* 2000;118:40.
6. Concato J., Shah N., Horwitz R.-I. - Randomized, controlled trials, observational studies, and their hierarchy of research designs. *N Engl J Med* 2000;342:1887.
7. Benson K., Hartz A.-J. - A comparison of observational studies and randomized, controlled trials. *N Engl J Med* 2000;342:1878.
8. Bender B., Milgram H., Rand C. - Non adherence in asthmatic patients : is there a solution to the problem ? *Ann. Allergy Asthma Immunol.* 1997;79:177.
9. SIAIC. - Memorandum su : diagnostica delle allergopatie. Bollettino della Società Italiana di Allergologia e Immunologia Clinica 1992;4:9.
10. Dreborg S., Frew A. (Eds.) - EAACI Subcommitee on Allergen Standardization and Skin Tests. Position Paper : allergen Standardization and Skin Tests. *Allergy* 1993;48 (Suppl.):48-82.
11. Sterk P.-J., Fabbri L.-M., Quanjer Ph.-H. *et al.* - Airway responsiveness standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Official Statement of the European Respiratory Society. *Eur. Resp. J.* 1993;6(Suppl.):53-83.
12. Magyar P., Dervaderies M., Toth A., Lantos A. - Inhalation of hypertonic potassium chloride solution: a specific bronchial challenge for asthma. *Giorn It Mal Tor* 1983;37:29.
13. Mistrello G., Brenna O., Roncarolo D., Zanoni D., Gentili M., Falagiani P. - Monomeric chemically modified allergens: immunologic and physicochemical characteristics. *Allergy* 1996;51:8-15.
14. Giannarini L., Maggi E. - Decrease of allergen-specific T-cell response induced by local nasal immunotherapy. *Clin Exp Allergy* 1998;28:404-12.
15. Marogna M., Tiri A., Riva G. - Clinical practice improvement program for immunotherapy of respiratory allergic diseases. *Intern J Immunopath Pharm* 2001;14:93-101.
16. Malling H-J., Weeke B. - Immunotherapy position paper. *Allergy* 1993;48(Suppl):14.
17. WHO position paper. - Allergen immunotherapy : therapeutic vaccines for allergic disease. *Allergy* 1998:53:1.
18. Horwitz R.-I., Viscoli C.-M., Clemens J.-D., Sadock R.-T. - Developing improved observational methods for evaluating therapeutic effectiveness. *Am J Med* 1990;89:630.
19. Passalacqua G., Canonica G.-W. - Alternative route for allergen specific immunotherapy. *J Invest Allergol Clin Immunol* 1996;6:81.
20. Tari M.-G., Mancino M., Monti G. - Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to the house mite. A double-bind study. *Allergol Immunopathol* 1990;18:277.
21. Giovane A.-L., Bardare M., Passalacqua G., Canonica G.-W. - A three-year double-blind placebo-controlled study with specific oral immunotherapy to dermatophagoides: evidence of safety and efficacy in pediatric patients. *Clin Exp Allergy* 1993;24:53.
22. Caffarelli C., Sensi L-G, Marcucci F., Cavagni G. - Preseasonal local allergoid immunotherapy to grass pollen in children: a double-blind, placebo-controlled, randomized trial. *Allergy* 2000;55:1142.
23. Clavel R., Bousquet J., André C. - Clinical efficacy of sublingual swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five grass pollen extract in rhinitis. *Allergy* 1996;53:493.
24. Bousquet J., André C., Scheiman P. - Double blind placebo controlled sublingual immunotherapy in house dust mite asthma. *J All Clin Immunol* 1997;89:572.
25. Rhodes B.-J. - Patient dropouts before completion of optimal dose, multiple allergen immunotherapy. *Ann. Allergy Asthma Immunol* 1999;82:281.
26. Lower T., Henry J., Mandik L., Janosky J., Jr.Friday G.-A. - Compliance with allergen immunotherapy. *Ann. Allergy* 1993;70 480.
27. Good P. - Permutation tests : a practical guide to resampling methods for testing hypoteses. 2rd Ed. Springer Verlag. 2000;270 pp.
28. Siegel S., Castellan N.-J. - Nonparametric statistics for the behavioral sciences. Second Edition. McGraw Hill Book Company, New York 1998;477 pp.
29. Sokal R.-R., Rohlf F.-J. - Biometry. The principles and practice of statistics in biological research. Third Edition. W.H.Freeman&C., New York, USA 1995.