

CLINICAL DATA AND INFLAMMATION PARAMETERS IN PATIENTS WITH CYPRESS ALLERGY TREATED WITH SUBLINGUAL SWALLOW THERAPY AND SUBCUTANEOUS IMMUNOTHERAPY

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The clinical efficacy of immunotherapy, either by high dose sublingual-swallow therapy (SLIT) or subcutaneous immunotherapy (SCIT), has been demonstrated in patients with pollinosis but few studies have been carried out analysing differences in these treatments in terms of an improvement of clinical and allergic phlogosis parameters. The aim of this double-blind placebo-controlled study is to investigate the efficacy of high dose SLIT and SCIT using a purified standardized *Juniperus ashei* extract in a population of allergic patients monosensitized to cypress. Forty patients with cypress-allergic rhino conjunctivitis were administered therapeutic or placebo SLIT or SCIT for 12 months. Laboratory parameters were studied, namely the eosinophil cationic protein (ECP) level in nasal lavage and in serum, as well as the number of eosinophils (EOS) in peripheral blood and in nasal lavage and the level of eosinophil chemotactic activity (ECA). These parameters were correlated with clinical symptoms, evaluated by means of the clinical symptoms score (CSS). After SCIT and SLIT the levels of ECP and ECA were reduced in nasal lavage. We also observed a significant reduction in the values of ECP in serum in the patients treated with SLIT. EOS were unchanged in peripheral blood, but significantly reduced in nasal lavage. These data were in accordance with the improvement of clinical symptoms, supported by the close correlation between CSS and laboratory parameters. Our data confirm a clinical improvement correlated with a decline in inflammation parameters after one year of immunotherapy, supporting the hypothesis that treatment with a major allergen of cypress is able to change the course of allergic rhinitis.

Epidemiological data have demonstrated an increased prevalence of cypress allergy among patients in recent years in Italy (1-2) and in other European countries (3-5). The clinical features are characterized by a higher prevalence of conjunctivitis and rhinitis during the pollen season, with a lower

percentage of asthma (2, 6), thus resembling the symptoms of “recurrent” viral infections and causing undervaluation of this pollinosis (7).

Following reports of the clinical efficacy of immunotherapy, we conducted a double-blind placebo-controlled study, treating allergic patients

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monosensitized to cypress with sublingual-swallow immunotherapy (SLIT) or with subcutaneous immunotherapy (SCIT) using a standardized, aluminium hydroxide-adsorbed *J. ashei* extract, or placebo administered in the same way. Some allergic inflammation parameters were controlled. In particular, we monitored the eosinophil levels in peripheral blood and nasal lavage, the levels of ECP in serum and nasal lavage and the local effectiveness of the eosinophil chemotactic activity (ECA). The purpose of this double-blind, placebo-controlled study is to evaluate the clinical course of the pollinosis after immunotherapy and to determine whether there are differences during the course of SCIT or SLIT, in terms of allergic inflammation parameters and clinical features.

MATERIALS AND METHODS

Subjects

The study population consisted of 40 patients (23 females and 17 males; mean age 39 ± 2.4 years, range 18-55 years) with allergic rhinoconjunctivitis, monosensitized to cypress, and 10 controls, with no allergic symptoms (Table I). All patients were non-smokers and were not in treatment with immunotherapy, corticosteroids or beta-blockers. The sensitization was confirmed by a positive skin prick test (SPT). Other exclusion criteria were any contraindications to the specific immunotherapy, according to the guidelines of the European Academy of Allergology and Clinical Immunology (EAACI). The 10 controls (7 females and 3 males; mean age 38 ± 1.9 years, range 20-50 years) were considered healthy donors and were negative to SPT with the same allergens. All patients admitted to the study reported symptoms of rhinoconjunctivitis strictly correlated with the cypress pollen season (January-March).

This double-blind placebo-controlled study was conducted by randomly assigning SLIT, SCIT or placebo to each patient using a computer-generated code. In the resulting scheme: SLIT was administered to 10 patients, SCIT to 10 and placebo to the other 20 patients. See Table I for the clinical data on the randomized patients.

The allergological evaluation was performed by skin prick tests (SPT, Antony, Stallergènes) and RAST (Pharmacia, Uppsala, Sweden). Blood samples were drawn from all subjects before and after 1 year of treatment. All patients underwent a nasal cytological examination, as well as analysis of nasal lavage. This relatively non-invasive method, well tolerated by patients, is a valid tool for the assessment of inflammation of the

upper respiratory tract. Laboratory tests were performed to ascertain the values of EOS, ECP and ECA.

Informed consent to take part in the study was obtained from all participants.

Allergen Extracts and treatment

The active treatment was given with extract of standardized *Juniperus ashei*, administered as a glycerosaline solution (SLIT, StaloralR; Stallergènes Sa, Antony, France) or adsorbed onto aluminium hydroxide phosphate (SCIT, Phostal; Stallergènes Sa, Antony, France). The allergen extracts were standardized by RAST inhibition, isoelectric focusing and immunoblotting. The biological activity was evaluated by SPT, and the Index of Reactivity (IR) indicated the potency of the pollen extract. The Jun a 1 major allergen content was $76 \mu\text{g/ml}$ of the 100 IR allergen extract; the daily dose in maintenance treatment was $228 \mu\text{g Jun a 1}$.

The placebo preparations were prepared with sugar for sublingual administration and with histamine dihydrochloride adsorbed onto aluminium hydroxide for the injection. In this way, the placebo had the same appearance and taste as the SLIT and SCIT.

The sublingual treatment was self-administered by each patient at home. The drops of extract were held under the tongue for 2 minutes before swallowing. The induction phase, lasting 30 days, was followed by a maintenance phase lasting 11 months. The solution was dosed at 300 IR/ml (equivalent to 30 as the power of histamine), administered three times a week (8 drops, in the maintenance phase).

The subcutaneous treatment, administered in hospital, included a 12-week induction phase with weekly injections and a maintenance phase lasting 9 months, with monthly administrations. The extract was prepared in four concentrations (0.1, 1, 10 and 100 IR/ml). The treatment was administered from February 2006 to February 2007; patients completed CSS at the beginning and end of each treatment. The patients were kept under observation for 30 minutes after each injection.

All patients showed good tolerance of both SCIT and SLIT, and no side effects were observed during the treatment. For this reason, we were able to register high patient compliance.

Cypress Pollen Count

In our region cypress pollinosis occurs in winter, even though it varies from species to species. It generally starts in January and ends in February for cypress arizonica and in March or April for cypress sempervirens. Maximum pollination is normally registered from mid-February to mid-March, in accordance with the progressive global warming. The trend of the cypress pollen count in Apulia

for this period, kindly supplied by the Italian Association of Aerobiology (AIA), Monitoring Center of Brindisi 1, reveals increased fluctuations in the atmosphere of daily counts during the main pollen season (about 264/m³ of air), reaching the largest amount at the end of February (about 450/m³ of air).

Skin prick test (SPT)

A positive control (histamine, 10 mg/l) and a negative control (glycerinated saline) with a standard panel of allergens including *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, cat, grass pollen, *Alternaria alternata*, *Parietaria officinalis*, olive, *Artemisia vulgaris*, *Cupressus sempervirens* and *Cupressus arizonica* (Stallergenènes, Antony, France) were administered and interpreted in accordance with the European Academy of Allergology and Clinical Immunology (EAACI) Subcommittee on Allergen Standardization and Skin Tests. The response is considered positive if the diameter of the wheal is more than 3 mm larger than the one provoked by the negative control (8).

Clinical symptoms score (CSS) and rescue medication

For the clinical symptoms score, before and during the pollen season the patients attributed a daily score to their rhinitis symptoms, i.e., nasal obstruction, sneezing, nasal itching, and running nose, on a scale of four: 0: no symptoms; 1: slight symptoms; 2: moderate symptoms; 3: severe symptoms, disturbing their social life and work. The total ranged from 0 to 12, i.e. the maximum score for the four symptom types, as reported on the case sheet. During the treatment, rescue medications were allowed to control allergic symptoms: oral antihistamines, nasal and inhaled corticosteroids, chromoglycate for eye symptoms.

Specific Ig E

Specific serum IgE titres for *Juniperus* were measured and evaluated according to the manufacturer's instructions, as the CAP system (Pharmacia, Uppsala, Sweden). Values are calculated as kU/l, and considered as follows: <0.35 – negative; between 0.35 and 0.7 – low; between 0.7 and 3.5 – moderate; between 3.5 and 17.5 – high; between 17.5 and 50 – very high; >50 – extremely high.

Nasal lavage (NL)

The method described by Naclerio et al. was used (9). NL fluid was taken before and after 12 months of treatment. The subjects were asked to retain 3 ml of 0.9% physiological solution at 36°C in one nostril for about 10 s, and the operation was then repeated in the other nasal cavity. The lavage fluid was sealed in test tubes and centrifuged at 300 g for 8 min. To inactivate proteolytic enzymes, 5 mmol/l ethylenediaminetetraacetic acid and

0.5 mmol/l phenylmethyl-sulfonyl fluoride were added to the test tubes. The liquid and cellular phases were separated, and the supernatant was stored for subsequent analyses.

ECP

The ECP levels in the serum and nasal fluid were assessed by the fluoroenzymatic immunoassay (FEIA) with the Pharmacia CAP System (Uppsala, Sweden). In serum, the standard curve ranges between 2 and 200 mg/l; in accordance with the parameters established by the manufacturer, normal levels are thus in the range 2–16 mg/l (10). By contrast, in NL, we obtained values ranging between 25.1 and 71.9 mg/l before treatment.

Eosinophil isolation

EOS were obtained from peripheral blood and prepared by Sher & Glover's method (11). Briefly, after EOS isolation with 6% dextran (MW 40 000) (Pharmacia, Sweden), the cells were washed thrice in phosphate-buffered saline (PBS) (pH 7.5, 0.2 M), resuspended in 9.5 ml PBS, and supplemented with carbonyl iron (General Airline and Film Corporation, Linden, NJ, USA). After incubation for 10 min at 37°C under mild shaking, the cells were placed on ice for a few minutes. Cells with ingested carbonyl iron particles were then removed with a magnet. The purity of the EOS population ranged from 85% to 95%, as detected by staining with yellow eosin according to Durham & Kay's method (12).

Chemotaxis

Chemotaxis was performed by a modified Boyden chamber technique, as described by Snyderman et al (13). Briefly, 0.2 ml of cell suspension were placed in the upper compartment of a Boyden chamber (Neuroprobe, Bethesda, MD, USA), and 0.2 ml of NL, as chemotactic stimulus, diluted 1:4 in Hanks' balanced salt solution (HBSS) (GIBCO, Grand Island, NY, USA), were used to fill the lower compartment. A 5 mm pore polycarbonate filter, approximately 120 mm thick, separated the chambers. Buffer alone was used as control. The chambers were incubated for 90 min at 37°C in humidified air containing 5% CO₂. The filters were then removed, washed in saline, fixed in ethanol, and mounted on glass slides. The cells were counted at 10 mm intervals from the original monolayer to the lower surface, in 5–10 high-powered fields (hpF).

ECA in NL

The ECAs were measured in the nasal lavage as described by Hakanson et al. (14). The samples were diluted 1:4 in HBSS. The chemotactic activity was measured by the method described above. The ECA were

calculated in units by assessing the maximum migration obtained with the chosen dilution and subtracting the migration toward PBS (random migration) (15). Only in eight patients were the ECA measured in the NL. Samples were taken in these patients both during the season and post-season. All evaluations were made in triplicate.

Blood EOS count

Blood EOS were counted by the UNOPETTE Test (Becton Dickinson, NJ, USA) on peripheral blood anticoagulated with EDTA. Results are expressed as EOS/mm³.

Nasal cytology

The nasal lavage fluid from each patient was collected and centrifuged, and the pellets were then mounted onto slides. The specimens were then spread, fixed in ethanol, and stained using Papanicolaou's method. The slides were examined under oil immersion by light microscopy at $\times 400$ magnification. The number of EOS was calculated as a percentage of 400 cell counts. Each test was performed in triplicate (16).

Statistics

To compare data of atopic Cypress-sensitive patients and normal donors, we used the non-parametric Kruskal-Wallis test. To analyze data in each group, the "sign test" was used.

Values of $p < 0.05$ were considered significant. Statistical data were evaluated by SAS software.

RESULTS

Inflammation levels in nasal lavage and in serum. Correlation with clinical parameters

The sensitization to cupressaceae was revealed in all patients by positive SPT. As shown in Table II, the level of ECP in the nasal lavage was significantly decreased in patients treated with both SLIT and SCIT, comparing the values obtained at time 1 with those measured at time 0. In the patients treated with placebo, both as SCIT or SLIT, the levels of ECP did not vary. At the same time, the values of ECP in allergic patients were significantly higher than those in the healthy donors considered as controls ($p < 0.001$). It should be noted that the comparison between the active groups and control was performed at time 0.

There were some differences in the levels of ECP in the serum. In fact, in the patients treated with SLIT a significant reduction was shown, while in the patients treated with SCIT there was no statistically significant change in the serum ECP

Table I. *Clinical data of randomized patients.*

	TREATMENT		TREATMENT	
	SCIT	PLACEBO/SCIT	SLIT	PLACEBO/SLIT
Number of patients	10	10	10	10
Female	7	6	6	4
Male	3	4	4	6
Mean age (years)	38.2	39.3	40.1	39.8
Mean duration of rhinitis (years)	2	1.8	2.9	2.1
Juniperus-specific IgE (mean), kU/l	15.3	18.2	13.1	12.4
Allergic Family history(%)	29	35	37	31

values. As shown in Table II, in the patients taking SLIT or SCIT placebo, the ECP serum values were unchanged and significantly higher than the values in healthy donors ($p<0.001$). With regard to IgE levels, mean pre-treatment serum levels were not significantly different between patients assigned to SLIT, SCIT or placebo (Table I). A mild increase in the levels was obtained after 1 year of therapy, in patients taking active SLIT or SCIT. By contrast, no significant differences were found in patients taking placebo (data not shown).

The reduction of the ECP levels in the nasal lavage was correlated with the improvements of the clinical symptoms. In this light, the laboratory data are in accordance with the clinical improvement, supporting the strict correlation between clinical aspects and inflammation parameters. In fact, patients treated with SCIT (Fig. 1A) show very moderate symptoms, exceeding a score of 2.5 only in two cases and in three cases of patients treated with SLIT (Fig. 1B). By contrast, in patients taking placebo either as SCIT or SLIT, the CSS evaluation showed no difference from the values obtained before treatment (Fig. 1, C and D).

EOS count

The situation was completely different with regard to the number of EOS in the peripheral blood, which showed no significant variations in the three groups of patients, whereas the number of EOS in nasal lavage was decreased in the patients treated with either SLIT or SCIT but not in patients taking SLIT or SCIT placebo, as compared to the basal values (time 0). In healthy donors the number of EOS was significantly lower ($p<0.001$), see Table II.

Levels of ECA

With particular reference to the ECA, our results (Fig. 2, A and B) demonstrate that the levels of ECA were significantly decreased in patients treated with therapeutic SLIT and SCIT, but unchanged in the patients taking placebo, as either SLIT or SCIT (Fig. 2, C and D).

DISCUSSION

In literature, few studies have been conducted on specific immunotherapy to cypress using extracts of

Table II. Eosinophil count and ECP levels in nasal lavage and in serum.

PARAMETER	SCIT group (10 patients)	SLIT group (10 patients)	PLACEBO SCIT group (10 patients)	PLACEBO SLIT group (10 patients)	CONTROLS (10 subjects)
EOS/mm³ in Peripheral blood	$T_0: 185.5 \pm 35^a$	$T_0: 168.2 \pm 31^b$	$T_0: 200.3 \pm 37^c$	$T_0: 193.1 \pm 44^d$	159 ± 52^e
	$T_1: 203.4 \pm 32$	$T_1: 178.3 \pm 44$	$T_1: 170.2 \pm 45$	$T_1: 220.2 \pm 39$	
	$p_{T_0/T_1}: NS$	$p_{T_0/T_1}: NS$	$p_{T_0/T_1}: NS$	$p_{T_0/T_1}: NS$	
	a vs e: NS	b vs e: NS	c vs e: NS	d vs e: NS	
EOS in NL (%) (mean$\pm$SD)	$T_0: 31 \pm 5$	$T_0: 27 \pm 8$	$T_0: 30 \pm 3$	$T_0: 35 \pm 4$	2.2 ± 1
	$T_1: 10 \pm 3$	$T_1: 16 \pm 5$	$T_1: 28 \pm 4$	$T_1: 32 \pm 5$	
	$p_{T_0/T_1} < 0.001$	$p_{T_0/T_1} < 0.001$	$p_{T_0/T_1}: NS$	$p_{T_0/T_1}: NS$	
	a vs e < 0.001	b vs e < 0.001	c vs e < 0.001	d vs e < 0.001	
ECP in serum (ug/ml) (mean$\pm$SD)	$T_0: 29.3 \pm 7.1$	$T_0: 27.6 \pm 8.1$	$T_0: 25.4 \pm 3.3$	$T_0: 26.7 \pm 4.5$	8.7 ± 2.3
	$T_1: 26.8 \pm 5.5$	$T_1: 16.4 \pm 2.2$	$T_1: 26.6 \pm 2.5$	$T_1: 24.6 \pm 3.7$	
	$p_{T_0/T_1}: NS$	$p_{T_0/T_1} < 0.001$	$p_{T_0/T_1}: NS$	$p_{T_0/T_1}: NS$	
	a vs e < 0.001	b vs e < 0.001	c vs e < 0.001	d vs e < 0.001	
ECP in NL (ug/ml) (mean$\pm$SD)	$T_0: 50.3 \pm 8.7$	$T_0: 44.6 \pm 10.5$	$T_0: 52.2 \pm 13.3$	$T_0: 57.3 \pm 10.1$	8.5 ± 1.7
	$T_1: 23.4 \pm 6.4$	$T_1: 20.6 \pm 5.5$	$T_1: 46.5 \pm 15.9$	$T_1: 53.7 \pm 12.1$	
	$p_{T_0/T_1} < 0.001$	$p_{T_0/T_1} < 0.001$	$p_{T_0/T_1}: NS$	$p_{T_0/T_1}: NS$	
	a vs e < 0.001	b vs e < 0.001	c vs e < 0.001	d vs e < 0.001	

T_0 : basal condition

T_1 : after 12 months of treatment

NS: not significant

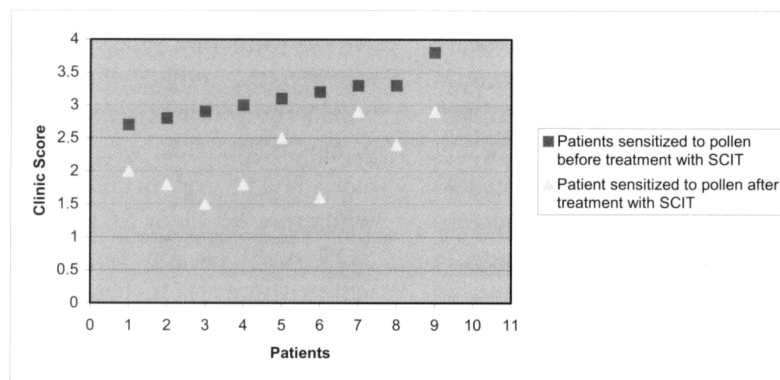


Fig. 1. A. Values of CSS in patients treated with SCIT

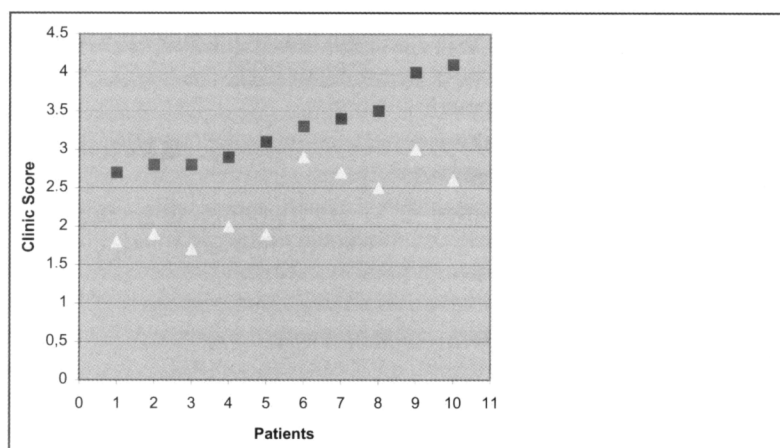


Fig. 1. B. Values of CSS in patients treated with SLIT.

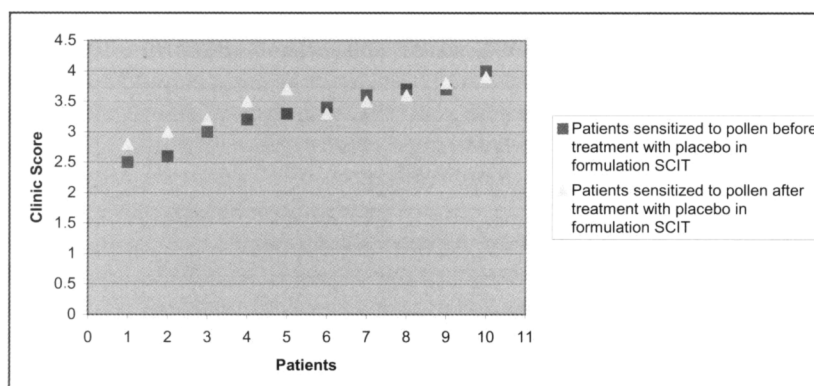


Fig. 1. C. Values of CSS in patients treated with placebo- SCIT.

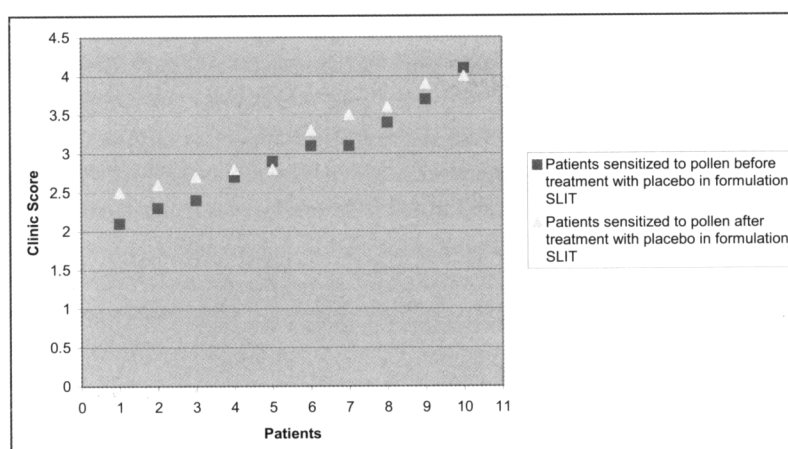


Fig. 1. D. Values of CSS in patients treated with placebo- SLIT

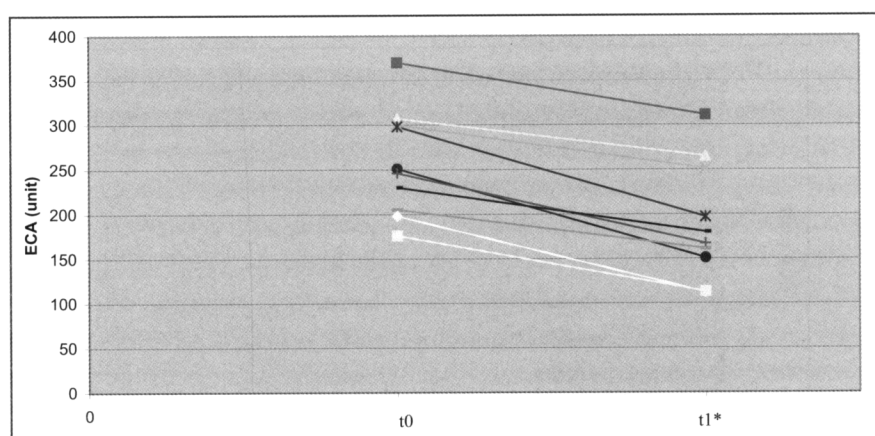


Fig. 2. A. ECA in NL of patients treated with SCIT.

t0: before treatment; t1: after treatment; * $p < 0.01$ in comparison with t0

standardized allergens. In fact, this type of extract has only recently become available (17). In a study conducted by Miller et al. (18), two groups of 17 patients were analyzed, the first treated with SCIT of *Juniperus ashei* and the second with placebo. After three months of treatment there was no significant variation between the 2 groups in terms of rhinitis symptoms and asthma. This phenomenon is probably due to the dosage used, that was relatively low (1,000 PNUs) and to the fact that both the patients treated with SCIT and the control group had undergone SCIT in previous years. Nevertheless, Pence et al. (19) obtained the same results in patients submitted to SCIT, even though a higher dosage was used (3,000 PNUs). Using the same allergens, Fling et

al. (20) observed an increase in immunoglobulins G (IgG) and IgG4 in the serum and a reduction of the cutaneous reactivity to SPT. However, after 3 months of treatment these Authors did not observe any improvement of the symptoms nor significant immunological variation. Comparing a group of patients monosensitized to cypress with a group of polysensitized patients, Parker et al. (21) showed a significant reduction of the clinical symptoms, of the cutaneous reactivity and an increase in the IgG1 and IgG4 levels in the 2 groups of patients after treatment. Another study, published in 2007 by Vervloet et al. (22), examined the safety and the effectiveness of ultra-rapid SLIT with standardized *Juniperus ashei* extract in patients with cypress

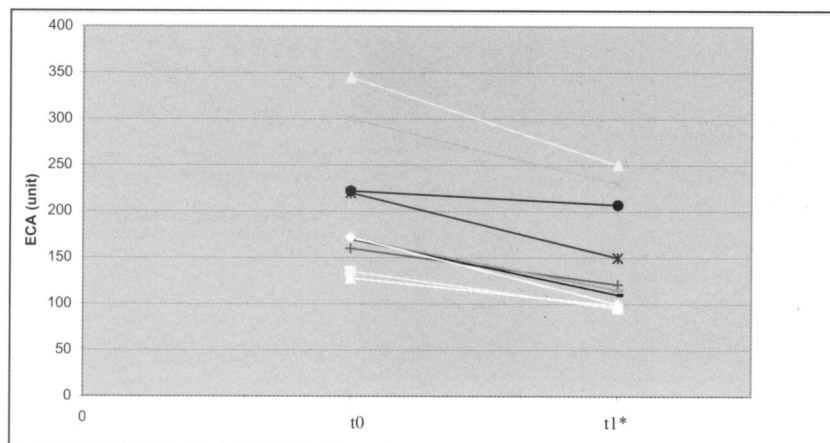


Fig. 2. B. ECA in NL of patients treated with SLIT.

t0: before treatment; t1: after treatment; * $p < 0.001$ in comparison with t0

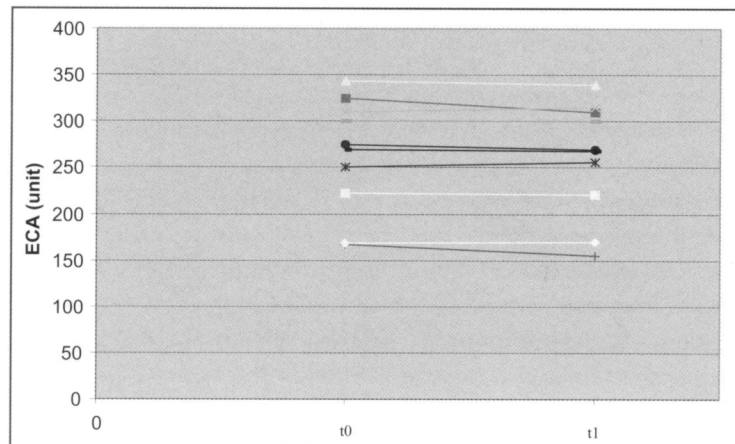


Fig. 2. C. ECA in NL of patients treated with placebo SCIT.

t0: before treatment; t1: after treatment

rhinoconjunctivitis. The improvement of the symptoms was not significant in the pollen season, but a significant difference was observed between the subjects treated with immunotherapy and those treated with placebo in terms of the consumption of drugs, including nasal corticosteroids. The latest study, published in 2007 by Charpin et al. (23), evaluated the effectiveness of SLIT with standardized *Juniperus ashei* extract in patients affected by seasonal allergic rhinoconjunctivitis to pollens of native cypress (*C. sempervirens* and *C. arizonica*). The results demonstrated a significant reduction of the ocular symptoms in the first year

(41%) and second year (63%) of treatment in the treated patients in comparison to the placebo group. Notably, the rhinitis symptoms were particularly improved by the second year. The immunological effectiveness of this treatment was underlined by the variation of the levels of specific antibodies: a reduced seasonal increase of the IgE was recorded in the treated group, and an increased level of IgG4.

The originality of these last two studies lies in the use of *Juniperus ashei* extract, present in the USA, used in both the SLIT and SCIT for the treatment of allergy to various kinds of cypress (European, American and Japanese). This extract has a superior

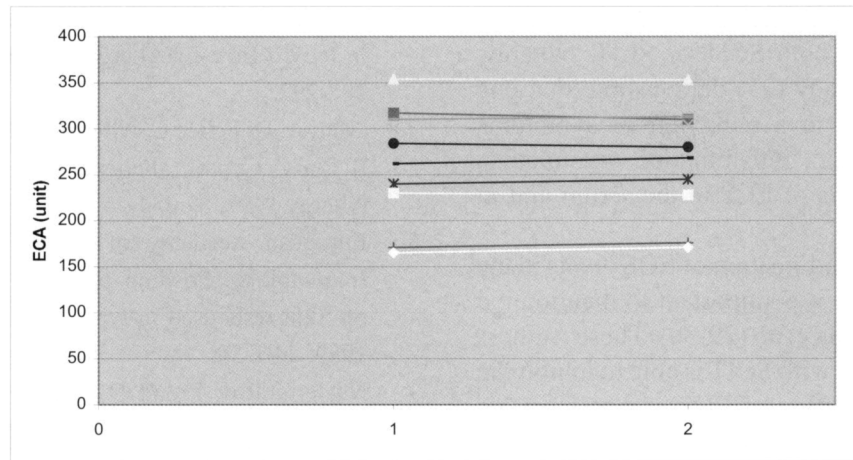


Fig. 2. D. ECA in NL of patients treated with placebo SLIT.
t0: before treatment; *t1*: after treatment

allergenic activity, in fact it results 20-fold superior to *C. Sempervirens* and 11-fold superior to *C. arizonica*. The effectiveness of the therapy could therefore be due to a different biological power of a single major cypress allergen, like *Juniperus ashei* (22- 23). Confirming these literature data, our study underlines that the employment of Immuno-therapy specific (ITS) with *Juniperus ashei* extract is an effective treatment. This contrasts with other studies in which the ITS for cypress was conducted with extracts of *C. Sempervirens* and *arizonica*, which seemed to be less effective.

The present data show that after one year of treatment with SCIT or SLIT, the ECP and ECA at nasal level were significantly reduced in comparison to those in patients taking placebo, while significant variations of the ECP in the serum were obtained only in the patients treated with SCIT. Thus, our data show that evaluation of the ECP at nasal level is a more sensitive parameter of allergic inflammation in comparison to ECP in the serum (24), and represents a marker that correlates with the clinical symptoms (25-26).

At the same time, the number of EOS at the nasal level was significantly reduced in comparison to the control group. By contrast, the number of EOS in the peripheral blood showed no variations in the three groups of subjects. In a previous paper (25) we showed that the number of peripheral EOS is not a sensitive marker to evaluate the “late phase”

of allergic rhinitis inflammation. The evaluation of ECP at the nasal level and in the serum reflects a mechanism of EOS activation and, consequently, represents a reliable marker of inflammation in allergic rhinitis. Evaluation of these parameters is thus a valid method for monitoring the course of therapy. In addition, our data show a strict correlation between the clinical improvement in patients and a reduction in the inflammation parameters. The ECP level in the serum was decreased only in patients treated with SLIT. These data could be correlated to activation in patients treated with SLIT of T lymphocytes with regulatory activity, responsible for secretion of lymphokines with eosinophil chemotactic activity.

Evaluating the effectiveness of therapy in allergic patients, we found that, as compared to the evaluations in serum, the inflammation parameters at the nasal level during allergic rhinitis show the best correlation with the clinical symptoms (25), underlining the importance of inflammation parameters in monitoring the clinical course of seasonal allergy.

The reduced production of ECP at the nasal level could be considered a marker of therapeutic effectiveness and seems to represent a valid method for monitoring the course of therapy. In fact, our data show that the level of ECP and the number of EOS in the nasal lavage were both reduced during SCIT or SLIT therapy, and that this reduction

corresponded to a significant clinical efficacy during the administration of both SCIT or SLIT. Notably, the data in another study (27) demonstrate that one year of SLIT results in a reduction of symptoms and of some allergic inflammation parameters, especially of the levels of ECP in the serum and in nasal secretion.

As to the lack of modification of EOS levels in the peripheral blood (28), it is important to mention the data reported by Nagata et al. (29-30). These Authors showed that treatment with SCIT is able to inhibit the transendothelial migration of EOS and to suppress the production of antigen-dependent ECA by the mononuclear peripheral cells. As a consequence, there is a reduction in EOS accumulation at the level of the nasal mucosa, and in the release of further chemotactic factors that can contribute to maintain the inflammation.

In conclusion, our data confirm that specific immunotherapy with the extract of *Juniperus ashei* can be used both as SCIT and SLIT, and that the inflammation parameters considered can be utilized to assess the clinical effectiveness of the treatment.

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