

Immunotherapy with an Extract of *Olea europaea* Quantified in Mass Units

Evaluation of the Safety and Efficacy after One Year of Treatment

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Summary. Sensitization to olive pollen is a frequent cause of rhinoconjunctivitis (RC) and bronchial asthma (BA) in the region of Jaén (southern Spain), where this allergen reaches atmospheric levels of almost 7000 grains/m³ during pollen season (May and June) and produces high morbidity. Specific immunotherapy (SIT) has proven very efficient in allergic RC and BA caused by grass pollen. Considering the availability of a biologically standardized extract of *Olea europaea*, with its major allergen quantified in mass units, we decided to investigate SIT with this extract in a group of rhinitic and/or asthmatic patients monosensitized to olive. We studied tolerance, safety, and efficacy by comparison of the active group (subjected to SIT) with a control group that did not receive SIT. A hyposensitizing dose of *Olea europaea* extract was administered preseasonally, establishing a maintenance dose 3.8 times higher than those administered in conventional treatments. Eighty-three percent of the patients reached the proposed maximal dose of 75 BU, equivalent to 45 µg Ole e 1, with a rate of 0.8% of systemic reactions. A significant decrease in cutaneous ($p < 0.001$) and bronchial ($p < 0.001$) reactivity was observed in the active group, but not in the control group. Also, a decrease in specific IgE and an increase in IgG₁ and IgG₄ were found in the group of patients treated with SIT. Regarding clinical evolution, the active group, but not the control group, experienced a clear statistically significant improvement both in nasal ($p < 0.05$) and bronchial ($p < 0.05$) symptoms, in addition to a significant decrease in the consumption of antihistamines ($p < 0.05$) and β_2 -agonists ($p < 0.01$). In conclusion, SIT with olive extract proved to be safe and efficacious for the treatment of asthma and rhinitis caused by this allergen.

Keywords: asthma, clinical evaluation, efficacy, immunotherapy, mass units, *Olea europaea*, safety

Introduction

Specific immunotherapy (SIT) is described as a safe and efficacious treatment, as long as the following conditions are met:

- 1) demonstration of an IgE-mediated hypersensitivity mechanism;
- 2) when sensitization to the identified allergen plays an important role in the development of symptoms and the severity of the disease;
- 3) availability of high-quality and biologically standardized allergen extracts.

There are numerous studies, many of them double-blind placebo-controlled ones, that show the efficacy of SIT in the treatment of both allergic rhinitis [1–10] and asthma

caused by sensitization to allergens released by pollen [11–14].

Olive pollen seems to have a lower allergenic potency than grass pollen [15], necessary levels being above 400 grains/m³ for patients to present symptoms [16]. Notwithstanding, it is a highly sensitizing allergen, as shown by the high frequency of positive results found in skin tests in regions of extensive olive culture [17]. The capacity of that allergen released by pollen to produce rhinitis and/or asthma in the exposed population through an IgE-mediated mechanism is amply documented in the European Mediterranean areas [18–22]. So, although the exposure period of patients to that allergen is short (about 6 weeks), its capacity to produce severe symptoms in sensitized patients is notorious [23, 24].

In some areas of Mediterranean Europe the maximal olive pollen grain counts do not exceed 4000 grains/m³

[25, 26]. However, in the province of Jaén (southern Spain), which has the most extensive olive culture in all Spain, there is a higher allergenic pressure, where daily peaks can reach 7000 grains/m³ [27] during pollen season. It has been shown that the prevalence of sensitization to olive in that area reaches 84.5% of patients [28], of which 14.3% were monosensitized. The same study showed that olive allergen induces bronchial asthma in 60% of the sensitized patients, more frequently in younger ages.

Another study performed with over 4000 allergic patients from all Spain showed that 47% of patients with rhinoconjunctivitis and 51% of the asthmatics were sensitized to olive [29], which demonstrates the importance of sensitization to this allergen in our country.

The above considerations prompted us to study the tolerance, safety, and efficacy of SIT, being at present the only ethological treatment available, in monosensitized patients to olive.

Material and Methods

Patients

A total of 46 patients of both sexes with a clinical history of rhinitis and/or asthma caused by sensitization to *Olea europaea* with at least 2 years of evolution were enrolled. Patients had to be between 6 and 40 years old, have positive skin-prick tests to *Olea*, and a specific IgE to that allergen class 2 or higher (Pharmacia CAP). Those patients who had received previous immunotherapy, who were sensitized to other allergens, or those in whom immunotherapy was relatively or absolutely contraindicated according to the criteria established by the Immunotherapy Subcommittee of the EAACI [30] were not included in the study.

Study Design

The study was randomized, open, controlled, and with parallel groups. Patients were assigned to either group through a simple randomization procedure. Patients treated with preseasonal immunotherapy from September to April composed the active group and patients receiving symptomatic treatment but not immunotherapy composed the control group.

The study was performed with the approval of the Ethics Committee of the Hospital and according to legislation in force [31]. All patients gave their informed consent to participate in the study.

Patients were controlled three times: T₀ (before immunotherapy), T_M (second 14-day dose after reaching the maintenance dose), and T_F (after the pollen season, 1 year after the start of the study). All parameters evaluated in each pollen season are specified in Table 1.

Table 1. Control times and parameters evaluated

Control time	Parameter
T ₀	SPT, CPT, sBPT, <i>in vitro</i> , diary cards
T _M	SPT, CPT, <i>in vitro</i>
T _F	SPT, CPT, sBPT, <i>in vitro</i> , diary cards

SPT (Skin Prick Test), CPT (Conjunctival Provocation Test), sBPT (specific Bronchial Provocation Test), *in vitro* (specific IgE, IgG1 and IgG4 to *Olea europaea*)

Material

The same extract was employed throughout the study, both for diagnosis and for treatment. The extract of *Olea europaea* used was biologically standardized [32] and had its major allergen (Ole e 1) quantified in mass units, according to the methodology described by the manufacturer (ALK-ABELLÓ, S.A.) [33, 34]. The total allergenic activity was expressed in BU/ml and the content of major allergen Ole e 1 was measured in µg/ml: 100 BU contained 60 µg of Ole e 1.

The extracts for prick-tests were prepared at four concentrations: 500, 100, 20, and 4 BU/ml. Histamine chloride (10 mg/ml) and sodium chloride (0.9%) solutions were included as positive and negative controls, respectively. The same person performed all prick-tests at the same time of the day.

The conjunctival provocation test was performed according to the method described by Möller et al. [35]. The material used was lyophilized at 300 BU/vial, each series was reconstituted the day the test was performed, and five-fold dilutions (from 150 to 0.24 BU/ml) were prepared. The specific bronchial provocation test was performed according to the method described by Cockcroft [36]. Identical lyophilized material as conjunctival provocation test was used, and 10-fold dilutions were prepared (from 150 to 0.015 BU/ml). In both cases, the same person always performed the tests, and prior to performing the test, it was verified that patients had not taken any medication that could alter the results.

The extract of *Olea europaea* used for treatment was adsorbed to aluminium hydroxide gel (PANGRAMIN® DEPOT). The treatment was delivered in two packages: *initiation treatment* and *maintenance treatment*. In the first case, the package contained the standard three vials with increasing concentrations. Vial 1 with 0.25 BU/ml (0.15 µg/ml of Ole e 1); vial 2 with 2.5 BU/ml (1.5 µg/ml of Ole e 1); vial 3 with 25 BU/ml (15 µg/ml of Ole e 1). An additional vial 4 was included with 250 BU/ml (150 µg/ml of Ole e 1). The *maintenance treatment* package consisted of a vial 3 or 4, according to the tolerance shown by the patient during the build-up phase.

The *initiation treatment* was administered in weekly doses until the maintenance dose was reached, previously established in 0.3 ml of vial 4 (75 BU, corresponding to 45 µg Ole e 1). After reaching that dose or the established

dose in each case depending on the tolerance shown by the patient, it was administered twice with a 2-week interval and from then, each 21 days until the beginning of the pollen season, when it was discontinued.

All doses of SIT were administered in the Immunotherapy Unit of the Hospital. Before and after each dose, the clinical status of each patient was evaluated according to the instructions set in the Position Paper on Immunotherapy published by the EAACI [30], evaluating if the patient's condition allowed for the administration of the treatment. The peak expiratory flow (PEF) was measured before the administration of each dose, 30 minutes and 6 hours after.

In vitro Measurements

Serum samples were obtained from each patient at the three control times established in the study (T_0 , T_M , and T_F), and specific IgE, IgG₁, and IgG₄ to *Olea europaea* were measured by immunoassay, using specific antihuman immunoglobulin monoclonal antibodies produced by ALK-ABELLÓ, S.A. [37–39]. All serum samples obtained from each patient at the three controlled times were analyzed simultaneously. In these assays, the extract of *Olea europaea* was adsorbed to wells of microtiter plates COSTAR EIA/RIA (Costar Corporation, Cambridge, MA, USA), and the wells were sequentially incubated with serum samples (conveniently diluted for each assay) and with the anti-immunoglobulin monoclonal antibodies labeled with peroxidase. The enzymatic reaction was developed with ortho-phenylenediamine (Merck, Darmstadt, Germany) and was stopped with 2 N HCl. The absorbances were read at 492 nm, using a reference filter at 630 nm with a microplate reader (Model Emax, Molecular Devices, Sunnyvale, CA, USA). The levels of specific immunoglobulins were established by comparison with calibrated reference sera against *Lolium perenne* in the case of IgE and against *Phleum pratense* for the IgG subclasses. The results are expressed in arbitrary units per milliliter (AU/ml).

Clinical Evaluation

In order to evaluate clinical efficacy, all patients were provided with a diary card to register symptoms and medication consumption during the pollen season, from 1 of May to 30 of June during two consecutive years. The evaluation of nasal, conjunctival, and asthmatic symptoms was performed according to the following score: from 0 (lack of symptoms), 1 (mild: symptoms present but not bothersome), 2 (moderate: symptoms interfering with comfort but not disabling), to 3 (severe: symptoms causing discomfort and disabling).

Medication was quantified according to the following

criteria: antazoline (eye drops, 2 points/application), azelastine (nasal inhaler, 3 points/application), terbutaline (inhaler, 6 points/application), and cetirizine (tablets, 6 points/tablet).

The severity of asthma was evaluated before and after the treatment, according to the classification of the International Consensus Report on Diagnosis and Treatment of Asthma [40].

Olea europaea pollen count was performed by means of a Burkard volumetric spore trap placed 25 m high on a central building in the city of Jaen.

Safety Evaluation

Registration of adverse reactions was performed according to the classification established by the Position Paper published by the Subcommittee of Immunotherapy of the EAACI [30].

Statistical Analysis

The statistical analysis of the data was performed with the BMDP Statistical Software. Clinical and demographic parameters were compared with the Student's *t*-test (quantitative variables) and Fisher's exact test or χ^2 (qualitative variables). The results of the provocation tests, both conjunctival and bronchial were compared with the Mann-Whitney test (intergroup) and the Wilcoxon test (intragroup). The same tests were applied for the *in vitro* parameters in which, in addition, the evolution at the three time points was analyzed by ANOVA. Symptom and medication scores were analyzed with non-parametric tests and asthma severity with χ^2 tests (intergroup) or McNemar tests (intragroup). The modifications in cutaneous sensitivity were analyzed by a parallel line assay (AIASA CRS PLA) [41], calculating the cutaneous tolerance index (CTI), defined as the factor by which the concentration of the extract has to be multiplied after immunotherapy to obtain the same weal size than before the start of the study.

Results

No dropouts occurred, and all 46 patients initially included (23 in the Active Group and 23 in the Control Group) completed the study. Their characteristics are given in Table 2.

Immunotherapy

The total number of doses administered was 501, of which 88% corresponded to the build-up phase. The mean dura-

Table 2. Characteristics of the patients

	Active (n = 23)	Control (n = 23)
Demography		
Age (years)	13 [10–28]	24 [11–32]
Sex (m/f)	10/13	7/16
Asthma severity (mild/moderate/severe)		
	7/9/7	2/10/4
Diary cards		
Symptom score	26.7 [15.0–36.5]	22.2 [16.8–40.4]
Medication score	78.0 [40.9–108.0]	73.7 [21.5–144.5]

Data are medians [IQR] except for sex and asthma severity (absolute frequency)

tion of the treatment was 185 ± 2.5 days, in which the total cumulative dose reached was 483 ± 35 BU (equivalent to 289 ± 21 mg of Ole e 1). The mean number of doses administered to each patient was 21.8 ± 0.4 , with a mean maximal dose administered of 65.3 ± 4.6 BU (39.2 ± 2.8 mg of Ole e 1). Some 83% of the patients reached the maximal dose foreseen in the protocol (75 BU, corresponding to 45 mg of Ole e 1).

A total of 15 adverse reactions to SIT occurred in 7 patients, which represents 3% of the total administered doses. All reactions appeared in the build-up phase. Eleven of them (2.2% of total doses) were local, 9 local reactions were immediate, and 2 were delayed. Treatment (oral antihistamines and local cold) was required in only 8 cases. It is important to point out that 82% of the local reactions occurred during the administration of the additional vial 4, with the maximal concentration.

Systemic reactions (SR) occurred on four occasions, which represents a 0.8% of the total administered doses. All of these reactions appeared in 2 patients and were delayed; three were classified as mild (2 rhinoconjunctivitis and 1 rhinoconjunctivitis plus cough), and 1 was moderate (dyspnea and a fall of PEF of 10%). Only the last one required treatment (antihistamines and β_2 -agonists). None of the SR were preceded by a local reaction.

Evaluation of Clinical Efficacy

Symptoms

When nasal, ocular, and pulmonary symptoms were analyzed together, a significant ($p < 0.001$) decrease was found in the active group but not in the control group. When each group of symptoms was analyzed independently, we observed a significant decrease ($p < 0.05$) of nasal symptoms only in the active group. When we considered pulmonary symptoms (cough, sibilances, wheezing and shortness of breath), we found significant differ-

Table 3. Changes in symptom and medication scores

	Active T ₀	T _F	p value†	Control T ₀	T _F	p value§
Symptoms						
Nasal	44.5 [26.2–64.7]	33.0 [21.0–57.5]	0.023	55.0 [32.0–77.7]	44.0 [25.5–66.5]	0.836
Cough	35.5 [14.0–53.7]	14.0 [8.5–30.0]	0.002	29.0 [13.2–49.7]	34.0 [13.0–51.5]	0.844
Sibilances	14.5 [0.7–22.2]	5.0 [0.0–10.5]	0.002	3.0 [0.0–26.5]	9.0 [1.5–20.5]	0.286
Wheezing	23.0 [18.7–44.0]	10 [2.5–22.5]	0.005	19.0 [6.2–33.7]	15.0 [1.5–20.5]	0.140
Shortness of breath	0.0 [0.0–22.2]	0.0 [0.0–0.0]	0.033	1.5 [0.0–20.2]	0.0 [0.0–1.0]	0.164
Conjunctivitis	12.5 [5.2–15.5]	0.0 [0.0–0.0]	<0.001	25.0 [6.0–57.7]	0.0 [0.0–5.0]	0.009
Total	26.7 [15.0–36.5]	15 [8.3–20.4]	<0.001	22.2 [16.8–40.4]	19.7 [10.4–24.7]	0.115
Medication						
Ocular	7.5 [0.0–15.5]	6.0 [0.0–22.5]	0.570	7.5 [0.0–22.5]	8.0 [0.0–24.5]	0.916
Nasal	20.0 [7.5–40.2]	14.0 [1.0–40.5]	0.295	19.5 [2.0–53.0]	34.0 [7.5–47.5]	0.836
β ₂ agonists	14.0 [8.0–30.0]	11.0 [1.5–21.0]	0.009	9.5 [0.8–28.2]	10.0 [1.5–21.0]	0.700
Antihistamines	19.0 [8.0–30.0]	5.0 [0.0–18.5]	0.023	19.0 [5.5–36.2]	12.0 [0.0–24.5]	0.112
Total	78.0 [40.9–109]	45.0 [15.7–82.6]	0.002	73.7 [21.5–145]	62.7 [30.9–99.2]	0.569

Data are medians [IQR]. §p-values correspond to the change from T₀ to T_F (Wilcoxon test)

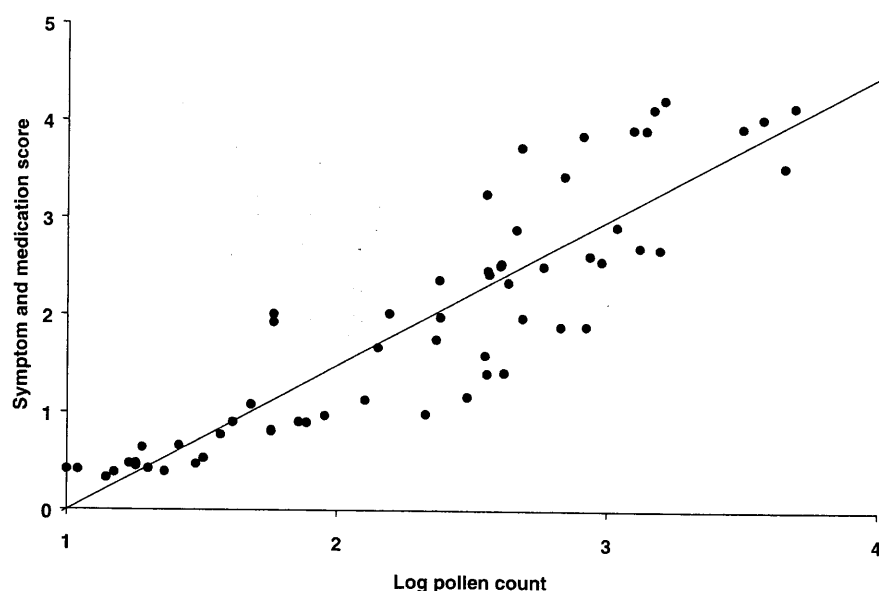


Figure 1. Regression analysis from symptom and medication scores and pollen counts (expressed as logarithm of grain/m³)

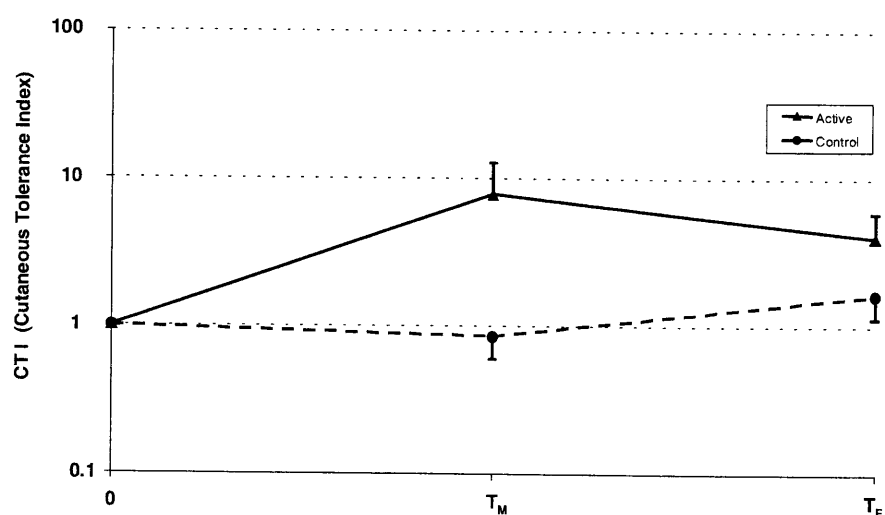


Figure 2. Evolution of immediate cutaneous response (SPT) in the active and control group

ences only in the active group, as shown in Table 3. When all asthmatic symptoms were globally evaluated, a significant decrease ($p < 0.001$) was found in that group of patients but not in the control group. Ocular symptoms were the only ones that decreased significantly in both groups.

Medication

The global score referred to the drug consumption decreased significantly in the group of patients receiving immunotherapy ($p < 0.01$), but not in the control group. Analyzing each drug individually, we found no difference in any of the two groups of patients in the use of nasal and ocular medication. However, a significant decrease occurred in the active group in relation to the consumption of β_2 -agonists and antihistamines ($p < 0.01$ and $p < 0.05$,

respectively) that did not occur in the group of patients that did not receive immunotherapy (Table 3).

Symptom and medication scores were significantly correlated with pollen counts during the first pollen season, before treatment (Spearman rank correlation coefficient = 0.927, $p < 0.0001$) (Figure 1). Pollen counts were higher in this first pollen season (AUC = 38634 grains) than in the second pollen season (AUC = 22417 grains).

The Evolution of Asthmatic Disease

An improvement in the severity of the disease was found in the active group ($p < 0.001$), but not in the control group. When both groups were compared, no differences were found at T_0 , while at the end of the treatment the difference was significant in favor of the active group ($p < 0.001$).

Table 4. *In vivo* and *in vitro* parameters

	Active		Control	
	T ₀	T _M	T ₀	T _F
SPT (mm²)				
500 BU/ml	55.0 [43.0–76.0]	27.5 [21.5–39.5]	75.7 [63.7–94.0]	65.0 [50.5–83.5]
100 BU/ml	31.5 [25.5–43.0]	13.0 [7.5–16.5]	41.0 [28.2–59.1]	30.5 [20.5–44.0]
20 BU/ml	0.0 [15.5–27.0]	6.5 [3.5–9.0]	25.0 [14.0–36.1]	19.0 [13.5–30.5]
4 BU/ml	8.0 [7.0–14.0]	2.5 [1.0–6.1]	10.5 [6.9–16.0]	8.0 [3.5–9.5]
CPT (BU/ml)	30.0 [6.0–30.0]	30.0 [6.0–30.0]	6.0 [6.0–30.0]	6.0 [6.0–30.0]
sBPT (BU/ml)	11.5 [5.0–50.0]	–	14.8 [6.0–92.2]	14.3 [9.0–70.6]
Specific Igs (AU/ml)				
IgE	12.1 [5.6–45.4]	2.3 [1.3–11.1]	12.1 [2.8–38.0]	9.6 [2.4–42.0]
IgG1	1350 [534–4775]	15115 [7416–26218]	623 [484–3710]	719 [347–2455]
IgG4	172 [33–344]	874 [247–1630]	106 [24–286]	94 [26–239]

Data are medians [IQR]

Skin Tests

The active group experienced a decrease of 7.8 times in skin sensitivity ($p < 0.01$) when the maintenance dose was reached (T_M) and a decrease of 4 times ($p < 0.01$) a year after the start of the study (T_F). In contrast, patients of the control group did not show any change in skin sensitivity at T_M, showing a small decrease (1.6 times, $p < 0.05$) at the end of the study (see Figure 2).

Comparing both groups, the cutaneous sensitivity of the control group is 13.4 times higher in T_M and 4.4 times higher in T_F compared to the active group ($p < 0.01$).

Conjunctival Provocation Test (CPT)

No significant differences in the conjunctival sensitivity of the patients was found neither in the control nor in the active group. However, upon comparing both groups we detected a higher tolerance in the active group when the maintenance dose was reached ($p < 0.05$), which was not kept at the end of the study.

Specific Bronchial Provocation Test (sBPT)

An important decrease in bronchial sensitivity was found in the active group, but not in the control group. In the first case, the mean dose required in T₀ to induce a fall in FEV₁ equal or above 20% (PC₂₀) was 9.4 BU/ml, while in T_F the mean PC₂₀ value rises to 50.2 BU/ml ($p < 0.001$). In the control group, the mean PC₂₀ value in T₀ was 18.7 BU/ml and 21 BU/ml at T_F.

In vitro Parameters

Regarding specific IgE, a significant decrease in the active group was found both at T_M as well as at T_F with relation to T₀ ($p < 0.01$), which was not the case in the control group. The values of specific IgG₁ and IgG₄ rise at T_M and T_F significantly in relation to T₀ ($p < 0.01$). The results are shown in Table 4.

When both groups of patients were compared, significant differences were found in their evolution ($p < 0.05$ for IgE and IgG₁ and $p < 0.001$ for IgG₄), both at T_M as well as at T_F.

Discussion

The good results obtained in our study are of special relevance, first, because it was performed in a region in southern Spain where patients are exposed to very high pollen levels, much more than other European regions;

and second, because of the important sensitizing capacity of the olive, in relation to the tolerance and safety of SIT as well as to its efficacy.

We obtained an identical rate of total adverse reactions and systemic reactions as other authors did in a similar study with monosensitized patients, using the same extract of *Olea europaea* and same administration schedule, although the study mentioned was performed in a different region of Spain with lower allergenic pressure [42].

The apparent lack of influence of the allergenic pressure on the tolerance to the SIT shown by the patients would justify additional studies to clarify this point. The rate of side effects found is within the tolerance range (0.05–3.2% of SR) found in a review of immunotherapy clinical trials performed with allergens released by pollen and published by Steward [43]. However, our side effects figures are slightly above those found by various authors in their daily clinical practice in the administration of immunotherapy with standardized extracts [44, 45, 46]. This is probably because of the fact that, in a clinical study, the registration of incidences occurring during the administration of the treatment is more meticulous.

As to *in vivo* parameters, both the skin tests and the bronchial provocation tests proved their validity for the monitoring of SIT. An important decrease in sensitivity was found in the active group, although no correlation was found between those parameters, the symptom and medication score. In the case of the conjunctival provocation test, we think that the lack of significance in the decrease of sensitivity is because of the short time interval in which patients received SIT. A longer course of SIT may be needed to discover significant differences with this test.

Significant differences in the levels of measured immunoglobulins (IgE, IgG₁, and IgG₄) have been found in the active group but not in the control group. It is noteworthy to point out that variations found in the levels of the analyzed IgG subclasses in the active group correspond with the usual pattern found by other authors [47], while specific IgE in the group of patients treated with SIT falls below the initial values. This contrasts with the evolution of that parameter normally found in patients treated with SIT [48], although a similar behavior is found in some publications [26], though no variation of this parameter is observed after 1 year of SIT treatment with olive allergen in others [49].

Most remarkable among the results obtained is, without any doubt, the clinical evolution in response to the SIT. This aspect already described by Aas [50], who established the priority of that response over the modification of any other parameter (*in vivo* and *in vitro*), remains prevailing today. Also, the European Academy of Allergy and Clinical Immunology established clinical evaluation as the only method that really reflects the clinical efficacy of SIT [30].

In our study, diary cards for the control of symptoms and medications and the evaluation of the severity of asthma

were employed to measure the efficacy of SIT. With respect to symptom score, we found in the active group with respect to the former year a global improvement that is not found in the control group. If we analyze the different symptoms scored (nasal, conjunctival, cough, dyspnea and sibilances) individually, we find a significant improvement of all of them in the active group. Clinical improvement could hypothetically be ascribed to the lower pollen levels observed in the second year with respect to the previous year. However, no decrease of thoracic or nasal symptoms was observed in the control group, so that the clinical improvement found can be attributed to SIT. Lower levels of pollen during the second year could explain the lack of differences found between both groups.

Differences in the need of symptomatic treatment and in the total medication used (β_2 -agonists and oral antihistamines) between the two pollen seasons were found in the active group, but not in the control group. However, no differences were found in the use of ocular and nasal medication in either groups, even though the symptoms decreased.

The good results obtained in the clinical evaluation (decrease of symptoms, reduction in medication consumption, and reduced severity of asthmatic disease) after a year of SIT with olive extract, as well as the fact that the rate of adverse reactions is similar, both in severity and frequency, to that found with SIT with other standardized extracts, endorse SIT with olive extract as being highly efficacious and safe for the treatment of rhinitis and asthma caused by that allergen.

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