Double-blind, placebo-controlled study of immunotherapy with *Parietaria judaica*: Clinical efficacy and tolerance

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Summary. Allergy to *Parietaria* causes significant morbidity in most Mediterranean areas.

The aim of this study is to investigate the efficacy and tolerance of *Parietaria* depot extract at 25 BU/mL (1.5 μg/mL Par j 1).

We performed a multicenter double-blind, placebo-controlled study in rhinitic patients with/without asthma, sensitized to *Parietaria*. 42 patients followed 20-month immunotherapy. Clinical efficacy was based on symptom and medication scores and the percentage of healthy days (days without symptoms or medication). Severity of asthma/rhinitis scales, visual analogue scale, evaluation of the treatment by doctors and patients, immediate and delayed cutaneous response and quality of life questionnaires were also studied.

The active group showed a sustained decrease in symptoms (p = 0.008), medication (p = 0.009) and both (p = 0.001), and an increase in healthy days (p = 0.001) throughout the study, with a threefold increase of healthy days and almost a three time reduction in medication only after one year of treatment. Asthma and rhinitis severity scales also decreased after immunotherapy, and blinded clinical evaluation by physicians confirmed efficacy in 85% and 77% of the active patients. Patient's self-evaluation returned similar results. None of these changes were observed with placebo.

Immediate cutaneous response was significantly reduced at the maintenance phase in the active group and remained reduced throughout the study. Late-phase response after intradermal testing also showed a statistical decrease in actively treated patients.

Immunotherapy was well tolerated and every systemic reaction reported was mild.

In *conclusion*, immunotherapy with *Parietaria* 25 BU/mL is an effective and safe treatment for patients with respiratory allergies.

Key words: *Parietaria*, immunotherapy, rhinitis, allergens, clinical efficacy, tolerance.

Introduction

Allergy to *Parietaria judaica's* pollen is an important condition deserving attention from both allergists and health authorities. *Parietaria judaica* is a wild plant widespread throughout the Mediterranean area [1-6] with a long pollen season that stretches from February to November. Allergic symptoms are more intense during spring and ameliorate during the rest of the pollen season. *Parietaria*'s pollen causes an elevated need for anti-allergic medication among affected patients and, as a result, a decrease in their quality of life [7-9].

Prevalence of sensitization to *Parietaria judaica's* pollen is very high in the Mediterranean areas, reaching 41.4% among allergic patients on the Spanish Mediterranean coast [10] and 63.4% in central Italy [11], and rises to as much as 80% in pollen-allergic patients [11] or allergic patients from the Italian southernmost regions (Sicily) [12].

The development of safe and effective treatments for specific IgE-mediated allergy has been a long pursued objective. Specific immunotherapy (SIT), which is the most accepted method for treating allergic respiratory diseases, constitutes the only etiologic treatment able to modify the immune response and change the natural course of the disease [13-15].

It is known that clinical efficacy of subcutaneous SIT is related to the dose administered [13, 16-18], higher doses being more efficacious. However, clinical studies of immunotherapy with *Parietaria judaica* have shown very poor tolerance compared with other allergens, and this has limited the maximum dose achievable to about one tenth of the doses used in SIT with other pollens [19]. Recently, a dose-scale pilot study [20] using a conventional build-up schedule and the same mass-unit standardized extract as in the present study, showed the tolerability of a dose ten times higher than previously published treatments with a low rate of systemic reactions (0.35%). It can be concluded that SIT with a less aggressive schedule and using biologically standardized *Parietaria judaica* extract quantified in mass units can improve tolerance and allow for higher maintenance doses.

We are presenting the results of a double-blind placebo-controlled clinical study for the efficacy and tolerance of high doses of injectable SIT with a *Parietaria judaica* extract quantified in mass units.

Material and methods

Patients

57 rhinitic patients, aged 15-55 years and sensitized to *Parietaria judaica* with or without mild seasonal asthma, were selected by clinical history, positive cutaneous tests and specific IgE. None had received SIT with *Parietaria* in the previous 2 years. Absence of evident sensitization to any other aeroallergens and of relative and absolute contraindications to SIT as outlined by the European Guidelines [13] was required.

Study design

The study was conducted by the Allergy departments of 3 Spanish hospitals according to ICH Guidelines with the approval of the local Ethics Committees and the Spanish Regulatory Authorities. All patients were informed and gave their written consent to participate in the study.

In December 1998, the patients entered the double-blind placebo-controlled study for a period of 20 months of immunotherapy. They were randomly assigned 28 and 29 to the Active (AG) and Placebo (PG) groups respectively.

Efficacy evaluations were carried out at the following control times: T_{99} (first pollen season before immunotherapy); T_0 (just before starting immunotherapy); T_3 (first maintenance dose); T_{00} (pollen season 2000); T_{12} (after one year of immunotherapy) and T_{01} (pollen season 2001). In order to determine the value of SIT in the management of patients allergic to *Parietaria* pollen we assessed their daily symptoms and use of medication as the principal efficacy outcome. Rhinitis and asthma severity scales were also employed, as they are commonly accepted clinical indexes. The impact of the treatment on the subjective opinion of both doctors and patients, and the impairment caused by the disease on the quality of life of the patients were measured by general and disease specific health related quality of life questionnaires. Objective measurements included the assessment of tolerance, and the evaluation of changes in target organ sensitivity. These were revealed by conjunctival provocation tests (CPT), quantitative skin prick tests (SPT), for early cutaneous response, and intracutaneous tests (ICT), for delayed cutaneous response. Detailed information of the tests run and parameters measured at every control time is included in Figure 1.

Immunotherapy

An extract of *Parietaria judaica*, that was biologically standardized [21] and had its major allergen (Par j 1) quantified in mass units, according to the methodology of the manufacturer (ALK-ABELLÓ, S.A.) [22], was used throughout the study for both *in vivo* tests (SPT, ICT and CPT) and treatments.

The total allergenic activity was expressed in BU/mL and the content of major allergen Par j 1 was measured in μg/mL; 25 BU/mL contained 1.5 μg/mL of Par j 1.

The extract of *Parietaria judaica* used for treatment was adsorbed onto aluminium hydroxide gel (Pangramin ® Depot). The initiation treatment was presented in three vials with ten-fold increasing concentrations from 0.25 BU/mL (0.015 μg/mL Par j 1) to 25 BU/mL (1.5 μg/mL Par j 1). The maintenance treatment package contained only the vial with the maximum concentration. Placebo vials also contained aluminum hydroxide and coincided in appearance with the active. For further blinding, 30% of vials contained histamine at different concentrations: vial 1 (0.05 μg/mL), vial 2 (0.5 μg/mL) and vial 3 (5 μg/mL).

Administration schedule is presented in Table 2. All doses of SIT were administered at hospitals. Before and
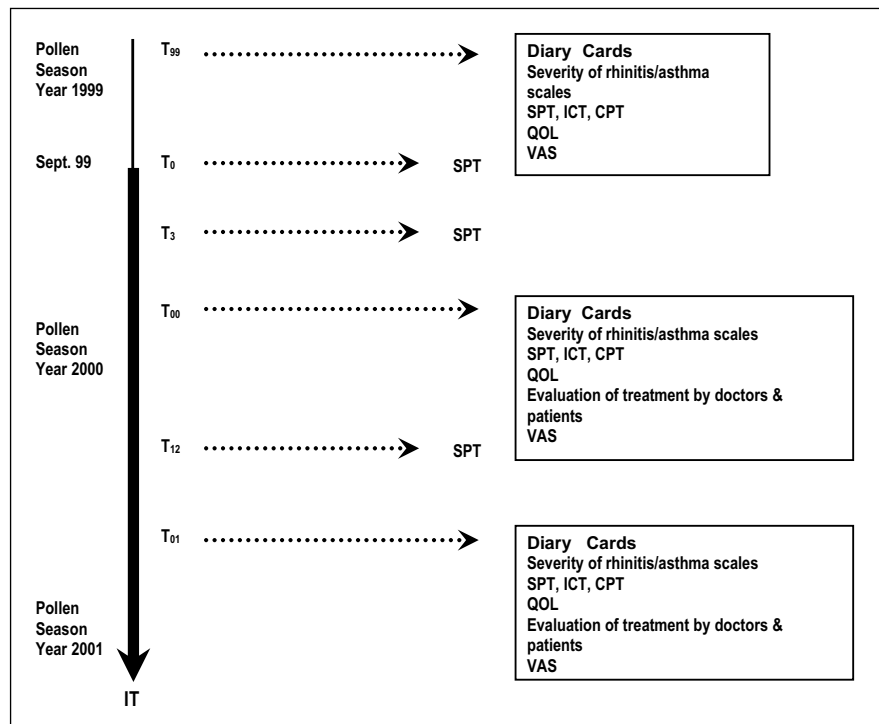
Figure 1. SPT = Skin Prick Test; ICT = Intracutaneous Test; CPT = Conjunctival Provocation Test; QOL = Questionnaire of Quality of Life; IT = Immunotherapy.

after each dose, the clinical status of each patient was determined following the instructions described in EAACI's Position Paper [13]. The peak expiratory flow (PEF) was measured before, 30 minutes and 6 hours after the administration.

Tolerance

Tolerance was monitored throughout the study dose by dose. Patients remained under close medical supervision for 30 minutes after each injection.

Any local symptom onset from the time of the administration until 48 hours later were registered. Only the immediate (within the first 30 minutes) with a wheal diameter greater than 5 cm, and the delayed (from 30 minutes to 48 hours) with an induration greater than 10 cm, were classified as adverse local reactions.

All immediate and delayed systemic reactions appearing after SIT administrations were registered, as well as the time of onset, duration, requirement of treatment and treatment administration, and the causal interdependence with SIT. Reactions were classified and graded according to EAACI's Position Paper [13].

Symptom and medication scores

The presence of allergic symptoms and the need for rescue medication were the primary outcome measurements. Patients recorded their daily symptom and medication scores in a diary card for 8 weeks during three consecutive pollen seasons (1999, 2000 and 2001). Separate scores for nasal (itching, sneezing, rhinorrhea and nasal obstruction), conjunctival (any ocular distress) and bronchial symptoms (coughing, dyspnea and wheezing) were recorded. Symptom scores were established in a 0 to 3 scale (absent, mild, moderate and severe, respectively).

Medication scores were assigned as follows: oral antihistamines: 1, short acting inhaled β_2 agonists: 2 and oral corticoids: 3. Two patients in the active group were prescribed long acting β_2 agonists during the 1999 season and each use was scored with 2 points.

For each patient and control time, the percentage of healthy days (days not requiring medication and without symptoms) was calculated.

Pollen counts for *Urticaceae, Parietaria's* family, were monitored in the geographical areas of the study

Table 1. Characteristics of the patients.

	Active group	Placebo group
N° of patients	28	29
Age (y)*	36.4 ± 11.0	33.0 ± 9.7
Onset of disease (y)*	9.0 ± 8.6	6.8 ± 6.9
Sex		
Male/ Female	11/17 (39.3/60.7%)	15/14 (51.7/48.3%)
Allergic symptoms		
Rhinoconjunctivitis	28 (100%)	29 (100%)
Asthma	13 (46.4%)	10 (34.5%)
Severity of Rhinitis#		
Mild	1 (3.6%)	3 (10.3%)
Moderate	27 (96.4%)	19 (65.5%)
Severe	0 (0%)	7 (24.1%)
Severity of Asthma##		
Mild	11 (84.6%)	10 (100%)
Moderate	2 (15.4%)	0 (0%)
Severe	0 (0%)	0 (0%)

* Mean ± standard deviation; # All parameters were not significant except Severity of Rhinitis (p=0.009); ## In asthmatic patients.

from February to July of the studied years (1999, 2000 and 2001) with a Burkard volumetric collector, and expressed as grains/m^3. Allergen exposure by each patient was calculated as the area under the curve (AUC) corresponding to the exact period in which each patient filled out the dairy card. Thus, we were able to compare the theoretical exposure received by patients of the active and placebo groups.

Assessment of the severity of rhinitis and asthma scales

Severity of rhinitis and asthma were evaluated by the investigators at T_{99}, T_{00} and T_{01}. Severity of rhinitis was quantified using Meltzer's scale [23], which separates four different symptoms: itching and sneezing, nasal congestion, nasal secretion and postnasal dripping. Each one of the symptoms is rated from 0 to 3 and the addition of all the scores yields the value of severity.

Severity of asthma was assessed by the investigators using a modified version of Olaguibel's clinical severity score [24] based on symptoms, medication level and respiratory function. Symptom level is based on Aas' clinical classification [25]. Medication level ranged from 1 to 5 and respiratory function was classified from 1 to 3 over spirometry results. Severity of asthma was summarized in a total score resulting from the addition of the symptom, medication and respiratory function scores.

In vivo tests

Cutaneous parameters: Skin prick test (SPT) and Intracutaneous tests (ICT)

SPT was performed with four five-fold dilutions of *P. judaica* extract containing 500, 100, 20 and 4 BU/ml. Histamine HCI 10 mg/ml and a 0.9% sodium chloride solution were used as positive and negative controls. All solutions were double-tested on the volar surface of the forearm. The same person at each centre performed all prick tests during the same period of the day. Recorded wheal areas were measured by planimetry, changes evaluated by Parallel Line Assay (PLA) [26] and expressed using the Cutaneous Torelance Index (CTI), which indicates the difference in allergen concentration needed to elicit the same skin response. Any medication that could affect the cutaneous response was discontinued beforehand [27].

Delayed cutaneous response was assessed by ICT, injecting 0.02 ml of *P. judaica* extract (0.39 BU/ml) in the volar surface of the forearm. Late responses were recorded as the mean diameter of the swelling at 6, 24 and 48 hours.

SPT and ICT were performed at the times specified in Figure 1.

Conjunctival provocation test (CPT)

The extract was kept freeze-dried and immediately reconstituted in 3 mL sterile, distilled water (final concentration of 100 BU/mL) before use. Dilutions of 20, 4, 0.8 and 0.16 BU/mL were prepared. The method described by Möller [28] and Dreborg [29] was followed and a positive result obtained when pruritus with conjunctival injection appeared simultaneously. Medication that could affect the conjunctival response was suspended [27]. CPT was performed at the times specified in Figure 1.

Rhinitis and Asthma quality of life (RQLQ/ AQLQ) and SF-36 health survey questionnaires

At each pollen season visit (T_{99}, T_{00}, T_{01}), patients completed a general quality of life questionnaire, the validated translation of the SF-36 health survey questionnaire related to their disease, the Spanish versions of the RQLQ [31] and/or AQLQ [32].

Evaluation of treatment by doctors and patients

At the end of 2000 and 2001 pollen seasons, doctors and patients evaluated the efficacy of the treatment compared to the basal situation by means of the following scale: much worse [1], worse [2], the same [3], better [4], much better [5]. Patients also evaluated the status of their disease using a visual analogue scale (VAS) [33].

Statistical analysis

All parameters were analyzed with BMDP Statistical software. Physician and patient evaluations were analyzed by Mann-Whitney and Wilcoxon tests. For all other variables, intra-group comparisons were evaluated by paired Student's t-test and by ANOVA for repeated measures when more than two control times were involved (overall evolution). Inter-group comparisons were analyzed by Student's t-test and by ANCOVA for repeated measures, with one grouping factor and one within factor using the basal measure as covariate constant across trial when more than two control times were involved. Statistical analysis of SPT was performed by means of PLA [26]. P values lower than 0.05 were considered statistically significant and two-sided tests were used.

Results

Patients

At inclusion, the active and the placebo groups were comparable for all variables except rhinitis severity

Table 2. Schedule of treatment.

Week	Vial	Injected volume (mL)	Biological activity (BU)	Par j 1 (μg)
			Dose	
1	1	0.1	0.025	0.0015
2	1	0.2	0.05	0.003
3	1	0.4	0.1	0.006
4	1	0.8	0.2	0.012
5	2	0.1	0.25	0.015
6	2	0.2	9.5	0.03
7	2	0.4	1	0.06
8	2	0.8	2	0.12
9	3	0.1	2.5	0.15
10	3	0.2	5	0.3
11	3	0.4	10	0.6
12	3	0.6	15	0.9
13	3	0.8	20	1.2
15	3	0.8	20	1.2
17	3	0.8	20	1.2
Monthly	3	0.8	20	1.2

(Table 1). Five patients abandoned the study before the beginning of the treatment and a further eight afterwards for personal reasons not related to the treatment. Two more patients were withdrawn from the study, one in the PG for a recurrent disease not related to the treatment and one in the AG for pregnancy (Figure 2).

Immunotherapy

The active treatment group received a total of 803 injections (384 in the build-up period and 419 during maintenance), while the placebo patients received 724 injections (348 and 376, respectively). The mean number of injections for each patient was 29.3 ± 6.2. All patients in the active group reached the predetermined maintenance dose of 20 BU although in one case the dose was reduced to 15 BU due to nasal and bronchial symptoms at the second maintenance administration.

Tolerance

Twenty-five adverse reactions were detected, representing 1.6% of doses (2.6% in AG and 0.6% in PG), of which 5 were local (LR) and 20 systemic (SR). All LR occurring in the AG: 3 were immediate and 2 delayed; 3 appeared during build-up and 2 during maintenance. All LRs resolved spontaneously without treatment.

With regard to SR, 16 were in the AG and 4 in the PG, 17 appearing during the build-up period and 3 during maintenance. All were delayed and classified as mild. 10 were confined to the upper respiratory tract (7 AG/3 PG) and described as rhinitis, rhinoconjunctivitis, sneezing, rhinorrhea and/or nasal congestion; 4 affected the lower respiratory tract (all in AG) described as cough, dyspnea, wheezing and/or chest tightness, 4 were cutaneous symptoms (AG) described as pruritus or urticaria and 2 were unspecific manifestations (1 AG/ 1 PG) described as cephalea or fever. No case of anaphylactic shock was observed. Fifty-five percent of SR did not require any treatment. Antihistamines were administered in 8 occasions and ß2 agonists just once.
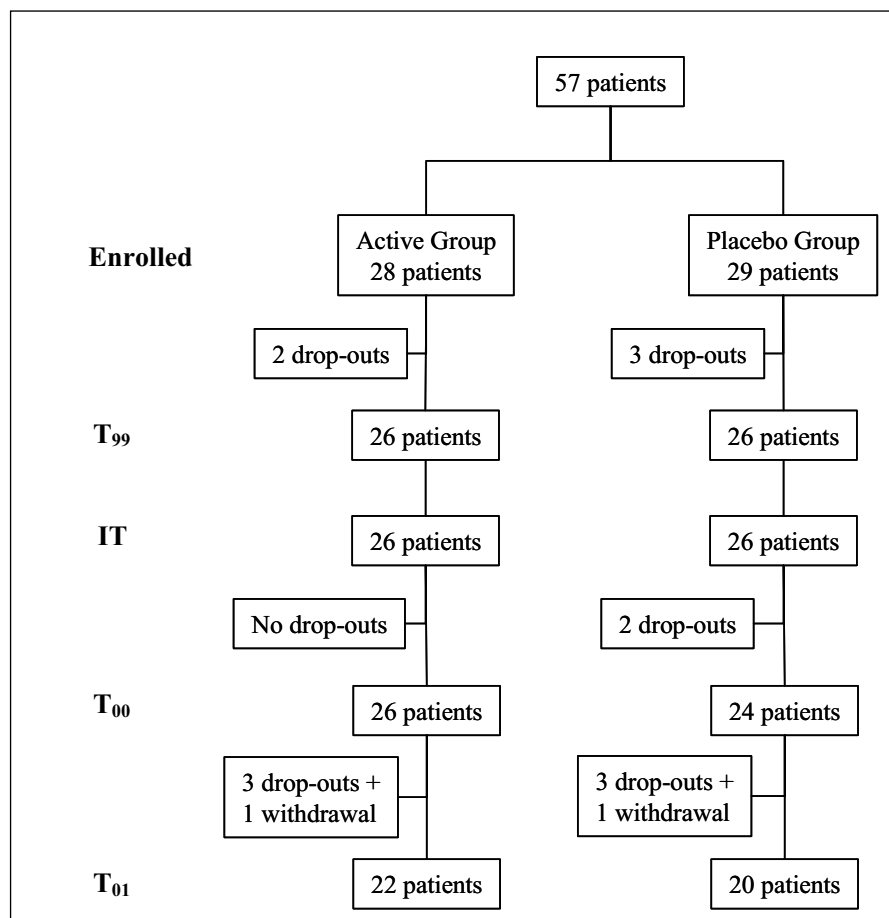

Figure 2. Trial profile

Enrolled — **Active Group** 28 patients / **Placebo Group** 29 patients

57 patients

2 drop-outs / 3 drop-outs

T_{99} — 26 patients / 26 patients

IT — 26 patients / 26 patients

No drop-outs / 2 drop-outs

T_{00} — 26 patients / 24 patients

3 drop-outs + 1 withdrawal / 3 drop-outs + 1 withdrawal

T_{01} — 22 patients / 20 patients

Symptom and medication scores

The mean scores of symptoms and medication, the combined scores of symptoms and medication (S&M) and the percentage of healthy days are presented in Table 3. At T_{99} there were no differences in the scores between AG and PG. At T_{00} symptoms in the active group had been significantly reduced to a 64.6% of T_{99} (p=0.001), medication score to a 34.1% (p=0.033) and S&M score to 51.2% (p=0.002). No significant changes were observed in the placebo group. At T_{01} AG maintained the benefits shown at T_{00} and again, no significant changes were observed in PG. Consistently, there was a significant increase in healthy days in the AG during T_{00}, representing a 302.5% (p=0.0002) of the value registered at T_{99}. At T_{01} was 262.7% (p=0.004). Differences in the placebo treated patients were not statistically significant at either control time.

Overall evolution (T_{99}-T_{00}-T_{01}) of AG for all total parameters was statistically significant by ANOVA, while no changes were observed in PG. The comparison of evolutions of the treatment groups by ANCOVA confirms the significant differences between groups.

The level of allergen exposure received by patients of both groups, AG and PG, was comparable at all three seasons. However, pollen counts differed among seasons, as shown by the average of patients' exposure (AUC) between March and July, being 1103 grains/m3 in 1999, 1288 grains/m^3 in 2000, and 574 grains/m^3 in 2001.

Assessment of the severity of rhinitis and asthma scales

In assessing the severity of the allergic disease, both groups were comparable before starting SIT regarding asthma (p=0.296) but not rhinitis (p=0.049).

AG experienced a decrease in the rhinitis score of 20.2% from T_{99} (p=0.045) at the end of the immunotherapy. Severity of rhinitis in PG group was significantly higher than AG at every control time. A significant decrease was also registered in asthma scores, with a reduction of 15.0% (p=0.015). PG scores were never different from T_{99}.

In vivo tests

Immediate skin test was significantly reduced in AG when patients reached the maximum SIT dose and this reduction was maintained during the rest of the
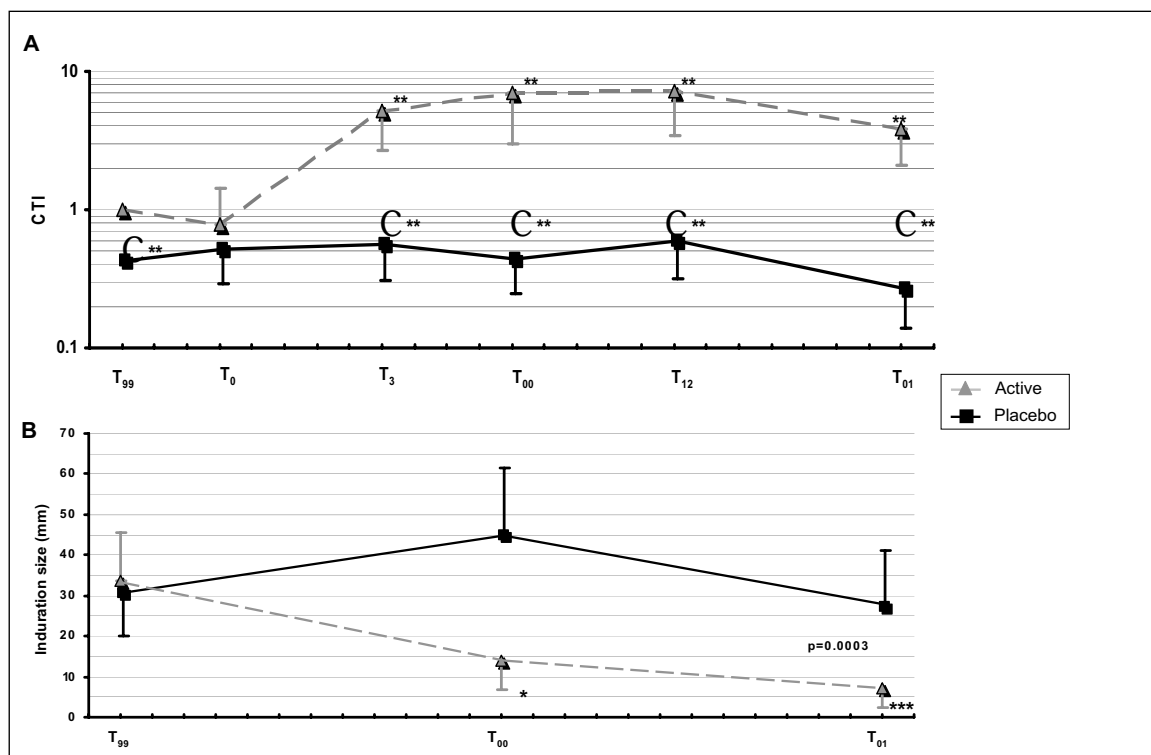

Figure 3. Evolution of the immediate and delayed skin reactivity.
A: Evolution of immediate skin reactivity (CTI: Cutaneous Tolerance Index, ratio of the concentration of the extract provoking the same skin response). B: Evolution of the delayed skin response (Intracutaneous test).
Active group in dashed-grey and Placebo group in black. Levels of statistical significance: *p<0.05, **p<0.01, ***p<0.001

Table 3. Clinical efficacy: Symptoms and medication scores.

| | ACTIVE | | | | | PLACEBO | | | | | |
| | | | | p-value | | | | | p-value | | Active vs## |
	T_{99}	T_{00}	T_{01}	T_{99} vs T_{00} *	Overall#	T_{99}	T_{00}	T_{01}	T_{99} vs T_{00} *	Overall#	
SYMPTOMS											
Bronchial	0.37±0.43	0.23±0.31	0.25±0.27	0.0353	n.s	0.29±0.23	0.22±0.25	0.45±0.65	n.s	n.s	n.s
Nasal	0.76±0.41	0.50±0.37	0.55±0.39	0.0008	0.0083	1.00±0.46	0.94±0.60	0.91±0.63	n.s	n.s	n.s
Ocular	0.52±0.42	0.36±0.38	0.39±0.45	0.0413	n.s	0.63±0.60	0.81±0.70	0.69±0.66	n.s	n.s	0.0480
TOTAL	0.64±0.36	0.41±0.33	0.44±0.32	0.0010	0.0076	0.84±0.46	0.84±0.55	0.80±0.54	n.s	n.s	0.0186
MEDICATION											
Bronchial	0.53±1.52	0.12±0.52	0.02±0.08	n.s	0.0811	0.07±0.22	0.02±0.07	0.03±0.12	n.s	n.s	n.s
Nasal	0.48±0.41	0.23±0.29	0.32±0.40	0.0007	0.0172	0.67±0.74	0.62±0.76	0.62±0.84	n.s	n.s	n.s
Ocular	0.30±0.32	0.14±0.24	0.26±0.34	0.0083	0.0388	0.53±0.72	0.39±0.60	0.64±1.08	n.s	n.s	n.s
TOTAL	1.00±1.48	0.34±0.59	0.35±0.47	0.0333	0.0092	0.73±0.84	0.65±0.77	0.92±1.73	n.s	n.s	0.0390
SYMPTOMS AND MEDICATION											
Bronchial	1.00±1.44	0.36±0.47	0.26±0.27	n.s	n.s	0.37±0.35	0.23±0.27	0.50±0.71	n.s	n.s	n.s
Nasal	1.00±0.42	0.62±0.42	0.72±0.43	0.0000	0.0007	1.33±0.62	1.25±0.69	1.22±0.81	n.s	n.s	0.0274
Ocular	0.67±0.43	0.42±0.43	0.52±0.47	0.0042	0.0291	0.89±0.75	1.00±0.77	1.01±1.00	n.s	n.s	0.0281
TOTAL	1.14±0.93	0.59±0.42	0.62±0.37	0.0023	0.0011	1.21±0.60	1.16±0.64	1.27±1.03	n.s	n.s	0.0007
HEALTHY DAYS											
Bronchial	45.0±33.3	62.1±34.7	65.8±36.2	0.0491	0.0300	52.3±31.3	67.4±36.3	62.8±47.8	n.s	n.s	n.s
Nasal	16.5±20.7	38.6±30.4	34.4±35.0	0.0021	0.0080	8.5±16.9	15.3±28.0	20.0±25.9	n.s	n.s	n.s
Ocular	46.6±30.2	66.6±30.4	56.8±34.2	0.0028	0.0230	44.0±31.6	36.8±36.7	40.8±34.1	n.s	n.s	0.0265
TOTAL	11.8±13.9	35.7±29.9	31.0±33.7	0.0002	0.0010	8.3±17.0	12.4±27.3	18.8±25.0	n.s	n.s	0.0138

Data shown are mean ± standard deviation. * Paired student's t test; # ANOVA for repeated measures; ## Difference in evolution (ANCOVA for repeated measures).

treatment. Patients in the PG did not show variations in the immediate skin reactivity. The difference in immediate skin sensitivity (CTI) at the end of the treatment was 13.9 (95%CI between 7.1 and 31.3). Figure 3 shows the evolution of immediate skin response.

Delayed cutaneous response after an immediate wheal and flare response peaked at 6h and continued for 48 h. AG response was significantly reduced by 46% at T_{00} (p=0.27) and continued to 80% at T_{01} (p=0.001), as seen in Figure 3. Group evolutions were markedly different (p<0.001). Responses at 24 and 48 hours were also significantly different (p=0.012 and p=0.013, respectively). No statistically significant differences were found in the CPT results.

Rhinitis and asthma quality of life (RQLQ/AQLQ) and SF-36 health survey questionnaires

The RQLQ results in the AG improved significantly in respect of the placebo group (p=0.007) although no statistically significant difference was found in the AQLQ and SF-36 results between both groups.

Evaluation of treatment by doctors and patients

According to the clinician's criteria, 84.6% of AG at T_{00} and 77.3% at T_{01} underwent a significant clinical improvement (p<0.001 at T_{00} and p=0.003 at T_{01}; compared to PG). The same situation was observed in the patients' self-evaluation as 84.7% of treated patients at T_{00} and 72.7% at T_{01} experienced a significant clinical improvement (p<0.001 at T_{00} and p=0.030 at T_{01}). No significant changes were observed in the PG. Before immunotherapy, the VAS showed no differences between groups. After 9 and 18 months of immunotherapy (T_{00} and T_{01} respectively), patients of the AG perceived an improvement in their allergic disease that was significant when compared to the PG (p=0.008).

Discussion

Studies of injected immunotherapy with *Parietaria* following a double-blind placebo-control design, although few [5, 8, 34, 35], have all shown clinical efficacy. One of them [8] has been performed with a native alum-adsorbed allergen extract while in the other three, chemically modified extracts, allergoids, were employed. The fundamental difference between the present study and the previous ones is the method of standardization of the allergen extract used for SIT. For this study we used a native alum-adsorbed *Parietaria* extract quantified in terms of major allergen content (Par j 1) by monoclonal antibodies.

In our opinion, this study has three characteristics that represent an improvement over those previously published: Firstly, it includes a larger sample of patients. Secondly, SIT was administered during a longer period (symptoms and medication scores were controlled during three pollen seasons) and thirdly, it is the only study in which the major allergen of the extract, Par j 1, was quantified.

The WHO position paper on Specific Immunotherapy [15] concludes that high doses of allergen are required for an efficacious immunotherapy. But when compared to other allergens, the immunotherapy with *Parietaria* is far less tolerated. In a study comparing tolerance to different allergens, the maximum doses of *Parietaria* tolerated by patients were, on average, 25 times lower than those reached with grass pollen and 10 times lower than those reached with mites [19]. It is worth saying that the *Parietaria* extract used in that study was the same used by us, but without major allergen quantification. Later, in an open study using the same Par j 1 quantified extract as in our present study, García-Villalmanzo [20] proved that patients could reach a 10 times higher maintenance dose (0.53 µg on average), while maintaining a good tolerance and obtaining substantial modifications in their immunological parameters (increase in specific IgG and IgG1 and IgG4 subclasses and decrease in cutaneous sensitivity to *Parietaria*).

In our study, all patients reached the maximum dose expected (1.2 µg of Par j) 2.3 times higher than in the previous one without any immediate systemic reaction, and with only a mild delayed systemic reaction in 1.99% of the patients.

The better tolerance achieved could be explained by the treatment schedule. While in the Basomba study [19] SIT was administered according to a rush schedule (two days up-dosing), in our study the patients followed a conventional weekly administration (12 weeks up-dosing).

Neither systemic nor local reactions caused withdrawal of SIT. Despite the length of the study, it is noteworthy that once the treatment started, only 8 patients dropped out and two more had to be withdrawn because of concomitant disease and pregnancy. All SRs were mild and antihistamines were administered very rarely; β_2-agonists were required on just one occasion. As with previous reports [36-39], the rate of SRs was clearly higher during the dose escalation compared to the maintenance phase.

The percentage reduction in allergic symptoms and antiallergic medications is scientifically accepted as the main measurement of clinical efficacy [13, 40, 41]. In the two years of SIT with *Parietaria*, symptoms and medication combined scores were significantly reduced,

almost halved. Particularly important in both seasons was the reduction of the need for medication, with scores close to three times lower than in T_{99}. It is not possible to compare the benefit of our product with the extracts used for SIT in other published studies because none expresses the results of diary cards as percentages of reduction.

IT with *Parietaria* preferentially improved rhinitis as evidenced by the more notable reduction in nasal and ocular symptom scores, the decrease in the severity of rhinitis scale and the increase of the specific quality of life questionnaire RQLQ. Basal differences in severity of rhinitis between active and placebo groups were compensated in the ANCOVA analysis [42]. Notwithstanding this, asthma symptoms were also ameliorated; bronchial medication showed the highest decrease in the diary cards and the severity of asthma was reduced at the end of the immunotherapy.

We found that asthmatic patients in our study started off from an over-average quality of life [43]. It is then reasonable to assume that benefits of the immunotherapy could not surpass their already high basal. In turn, the difference in RQLQ went beyond the cut-off of 0.5 for clinical significance. As far as we know, there are no other published immunotherapy studies assessing the change in QoL for comparison.

Physicians' and patients' evaluations confirmed the clinical efficacy of the treatment, since they found high clinical efficacy at T_{00} and T_{01}.

A decrease in delayed skin measurement has been proposed as an early marker of SIT response [44]. The reduction of cutaneous response (CTI) assessed in our study was 5.1, while in previously reported studies following the same procedure [20], a maximum of 2.2. was observed. The importance of the magnitude of this change regarding clinical efficacy remains uninvestigated.

According to Malling's proposed a classification of clinical efficacy sustained on S&M scores [45] specific immunotherapy with *Parietaria* has shown consistently moderate efficacy during two consecutive seasons, by combined S&M scores, and high efficacy when considering changes in need for medication. In conclusion, *Parietaria judaica* 25 BU/mL (1.5 µg/mL Par j 1) proved to be both clinically efficacious and safe, as judged on a significant decrease in symptoms and medication scores, and a significant increase in the percentage of healthy days, none occurring in the placebo group. Future studies should be carried out in order to evaluate the long-term effect and the mechanism of action of the injected SIT.

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