ORIGINAL ARTICLE

Asthma and Rhinitis

Intradermal *Phleum pratense* allergoid immunotherapy. Double-blind, randomized, placebo-controlled trial

Francisco Javier Sola Martínez[1] | Ruth María Barranco Jiménez[2,3] |
Cristina Martín García[4] | Carlos Senent Sánchez[5] | Carlos Blanco Guerra[6] |
Montserrat Fernández-Rivas[3,7] | Arantza Vega Castro[3,8] | Ignacio Dávila González[3,9,10] |
Antonio Carbonell Martínez[11] | Carmen Panizo Bravo[12] | Elisa Gómez Torrijos[13] |
David Rodríguez Gil[14] | Ricardo Palacios Peláez[14]

[1]Hospital Universitario Ramón y Cajal, Madrid, Spain

[2]Hospital Universitario 12 de Octubre, Madrid, Spain

[3]RETIC ARADyAL, Instituto de Salud Carlos III, Madrid, Spain

[4]Hospital Virgen de la Concha, Zamora, Spain

[5]Hospital Virgen del Valle, Toledo, Spain

[6]Hospital Universitario de La Princesa, Instituto de investigación Sanitaria Princesa, Madrid, Spain

[7]Hospital Clínico San Carlos, Universidad Complutense, IdISSC, Madrid, Spain

[8]Hospital Universitario de Guadalajara, Guadalajara, Spain

[9]Hospital Universitario de Salamanca, Salamanca, Spain

[10]Department of Biomedical and Diagnostic Sciences, Faculty of Medicine, University of Salamanca, Salamanca, Spain

[11]Hospital General Universitario Reina Sofía, Murcia, Spain

[12]Hospital Virgen del Prado, Talavera de la Reina, Spain

[13]Hospital Universitario de Ciudad Real, Ciudad Real, Spain

[14]Diater Laboratorios S.A, Leganés, Madrid, Spain

Correspondence
David Rodríguez Gil, Diater Laboratorios S.A. Avenida Gregorio Peces Barba n°2, Leganés, Madrid 28918, Spain.
Email: d.rodriguez@diater.com

Abstract

Background: In allergology, the intradermal approach is generally used to establish an aetiological diagnosis, with limited experience in specific allergen immunotherapy.

Objective: To evaluate the efficacy and safety of immunotherapy with an allergen extract of glutaraldehyde-polymerized *Phleum pratense*, administered intradermally, in patients with rhinoconjunctivitis sensitized to grass pollen.

Methods: Multicentre, randomized, double-blind, placebo-controlled clinical trial in patients from 12 to 65 years of age with rhinitis or rhinoconjunctivitis, with or without asthma, due to grass pollen allergy. Patients were divided into three groups and received a total of six doses in a weekly interval, of either placebo; 0.03 or 0.06 μg of protein per dose of *P pratense* allergoid. The primary objective was to evaluate the combined symptoms and medication consumption score (CSMS). The secondary objectives were symptoms and medication, tolerance to the conjunctival provocation test, specific IgE and IgG4 antibodies and the safety profile according to the WAO scale.

Results: The dose of 0.06 μg of protein proved to be effective versus the placebo by significantly reducing CSMS and increasing tolerance to the allergenic extract in the conjunctival provocation test, after the first pollen season. This group showed a significant reduction in specific IgE after the second pollen season relative to the baseline. There were no variations in IgG4 levels. Only one grade 2 systemic reaction was recorded.

Conclusion & Clinical Relevance: Intradermal immunotherapy with *P pratense* allergoid has been shown to be effective and safe, reducing CSMS, increasing tolerance to the conjunctival provocation test and reducing IgE levels.

1 | INTRODUCTION

Allergic rhinitis affects 500 million people worldwide, and its prevalence continues to increase in many cities.[1] This is a heavy burden for healthcare resources and is associated with significant direct and indirect costs such as work absenteeism and decreased productivity.[2]

Allergen immunotherapy is the only treatment capable of changing the natural course of allergic diseases and has a long-term effect even after discontinuing the treatment.[3-7] Conventional immunotherapy involves the administration of high doses of the allergen during 3-5 years, by numerous subcutaneous injections or daily in the case of sublingual administration. Although both routes of administration have shown efficacy against rhinoconjunctivitis induced by sensitization to grass pollen,[8] subcutaneous administration is associated with a risk of systemic reaction, while sublingual administration requires daily doses that lead to a lack of adherence to the treatment.[9]

The skin acts as a fundamental barrier against the exterior, enabling the individual's immune system to interact, which encompasses, among other components, the mononuclear phagocytic series comprising macrophages, Langerhans cells and dendritic cells, forming an important link between innate and acquired immunity, representing an organ regularly used in the administration of vaccines[10]; choosing the administration route that ensures the most effective capture and presentation of antigens by presenter cells (APCs) in the population and subpopulations of T cells responsible for specific immunological responses seems to be crucial.[11]

The dermis, largely comprising connective tissue, houses a large number of T cells (CD4+ and CD8+) that practically doubles the total population of blood,[12] as well as macrophages and dermal dendritic cells. This fact justifies the intradermal (ID) administration of vaccines in active immunization.[13] The ID administration has shown the ability to generate humoral immune responses, equivalent to those obtained by subcutaneous (SC) or intramuscular administration (IM), but using lower doses of antigen.[14] Dendritic cells (DCs) express class I and II antigen-presenting molecules of the major histocompatibility complex, and T cells can be activated via C-type lectin receptors (CLRs) and Toll-like receptors TLRs. In this way, the DCs regulate and polarize the response of the subpopulations of T and B cells.[15]

Intradermal immunotherapy with allergens was first used in 1926 by Phillips.[16] Subsequently, he expanded his study in 1933,[17] showing favourable results in more than 90% of the patients treated. The hypothesis of using the intradermal route is based on the potential reduction of IgE production, the increase in IgG and the polarization of the immune response to the Th1 pathway, due to the effective stimulation of the DCs that reside in the dermis. This was verified in murine models, using ovalbumin as an immunogen in the absence of adjuvant.[18] Similar results have been described in humans using pollen allergen extracts from *Phleum pratense*, administered intradermally at low doses,[19] and, more recently, with mite extracts.[20] The WHO recommends the ID instead of the IM administration, as in the case of the rabies vaccine.[21]

We have previously conducted two phase II clinical trials with an allergoid of *P pratense* administered intradermally (EudraCT 2014-004429-42 and 2012-003319-79). In the latter, the dose of 0.03 μg protein was determined as that produced a negative result in the intradermal skin test with *P pratense* (largest papule diameter 2.9 mm), 15 minutes after administration.

The main objective of the present research was to study the efficacy of a polymerized *Phleum pratense* vaccine administered intradermally, at different doses, by means of combined symptom and medication scoring. Six doses of the product under investigation were administered pre-seasonally during two consecutive pollen seasons. The data obtained were compared with the placebo group. As secondary objectives, we proposed to study the safety of the intradermal route for the administration of immunotherapy with allergoids, the local tolerance of the allergen by the patient through conjunctival provocation test and the study of the variations produced in the levels of immunoglobulins before and after each cycle of immunotherapy.

2 | METHODS

2.1 | Trial design

A multicentre, randomized, double-blind, parallel-group placebo-controlled clinical trial of intradermal immunotherapy (IDIT) with two different doses of a polymerized extract of *P pratense* (Laboratorios Diater SA) in patients with allergic rhinoconjunctivitis or rhinitis to grass pollen was designed. The administration was carried out on a pre-seasonal basis. Randomization was carried out by the sponsor (Laboratorios Diater SA) in blocks of 6 for each participating hospital. The treatments were assigned on a 1:1:1 basis, so each block of six contained two high-dose treatments, two low-dose treatments and two placebos. The trial was authorized by the Clinical Research Ethics Committee, Hospital Universitario Ramón y Cajal, Spain and the Spanish Agency of Medicines and Medical Devices with EudraCT 2014-000429-18 (registered at https://www.clinicaltrialsregister.eu/). One hundred and fifty-seven patients were recruited from 11 Spanish hospitals. The participating hospitals were Hospital Universitario Ramón y Cajal (Madrid), Hospital Universitario 12 de Octubre (Madrid), Hospital Virgen de la Concha (Zamora), Hospital Virgen del Valle (Toledo), Hospital Universitario de La Princesa (Madrid), Hospital Clínico San Carlos (Madrid), Hospital Universitario de Guadalajara, Hospital Universitario de Salamanca, Hospital General Universitario Reina Sofía (Murcia), Hospital Virgen del Prado (Talavera de la Reina), Hospital Universitario de Ciudad Real. The

trial began in 2014 and finished in 2018, including a follow-up during two consecutive seasons for each patient enrolled. The treatment schedule and trial design are described in Figure 1. At 12 months, the placebo group was incorporated into the group receiving the highest dose (Placebo-high dose). The trial was designed in accordance with European Medicines Agency Guidelines on the Production and Control of Allergens,[22] Clinical Development in Immunotherapy with Allergens[23] and Good Clinical Practice.[24]

2.2 | Patients

Patients, who had signed the informed consent, aged between 14 and 65 were recruited, with a medical history of rhinitis or rhinoconjunctivitis with or without mild or moderate asthma due to exposure to grass pollen. Patient sensitization was shown using skin prick tests with *P pratense* extracts (5HEP_D, Laboratorios Diater SA, Madrid, Spain) and a wheal diameter of ≥3 mm. Specific IgE to *P pratense* and Phl p 1 was measured using the ImmunoCAP System (Thermo Fisher Scientific), and a cut-off ≥3.5 kU/L was required. Patients polysensitized to other pollens were included, whenever they did not interfere with the recording of symptoms and medication. Patients sensitized to perennial allergens were excluded.

We recruited 157 patients, of whom 148 received at least one dose (ITT). Patients were assigned as follows: 53 to placebo, 42 to low dose (0.03 µg protein/dose) and 53 to high dose (0.06 µg protein/dose; Figure 2).

2.3 | Immunotherapy with *Phleum pratense* polymerized with glutaraldehyde

The *P pratense* extract was polymerized with glutaraldehyde[25,26] and stabilized by lyophilization in single-dose vials with mannitol as a cryoprotectant (10 mg/mL) at concentrations of 0.3 µg protein/mL (low dose) and 0.6 µg protein/mL (high dose; Laboratorios Diater

SA). Physiological saline was used as the diluent. No aluminium hydroxide or any other excipient was used. The placebo had the same composition as the treatments, except for the polymerized allergen. The maximum volume of intradermal administration was 0.1 mL. Intradermal administration was performed by the Mantoux method, using G28 needles.

One weekly dose was administered pre-seasonally during five consecutive weeks between November to March, to complete a total of six administrations, all at the maximum concentration, without scale-up phase. The treatment was administered for two consecutive years.

2.4 | Trial endpoints

The main study endpoint was the combined symptom and medication score (CSMS). Symptoms and medication were recorded by patients every day during the pollen season during two consecutive pollen seasons.[23] The recorded nasal symptoms were nasal congestion, pruritus, mucus production and sneezing; a score based on symptom severity was used: 0 = no symptoms, 1 = mild, 2 = moderate and 3 = severe. The recorded ocular symptoms were hyperaemia, pruritus and tearing; an identical severity score was used. The score of every symptom was added up, resulting in a score ranging from 0 to 21. The result was then divided by the number of symptoms evaluated, resulting in a final symptom score ranging from 0 to 3. All patients had access to identical rescue medication when necessary. The patient had to use the medication sequentially until the symptoms were controlled, starting with loratadine and finally using deflazacort. The medication was scored depending on the drug and the doses used: 0 = no rescue medication; 1-4 eye drops (nedocromil); 3-6 = systemic antihistamine (loratadine); 2-8 = nasal corticosteroids (budesonide); 3-6 = oral corticosteroids (deflazacort), resulting in a score ranging from 0 to 24. This result was normalized to a scale of 0-3 by dividing the result obtained by 24. Subsequently, to calculate the
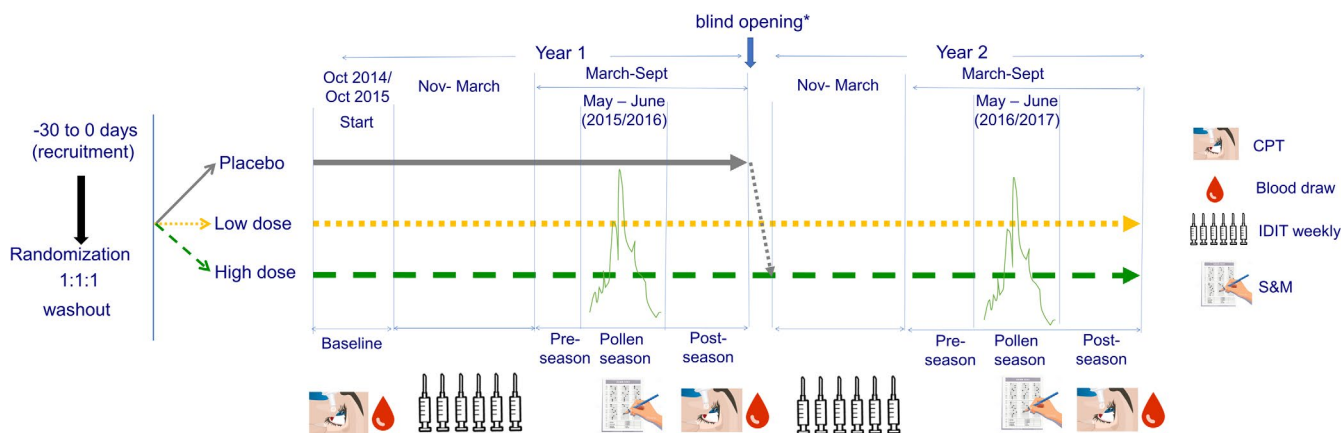

FIGURE 1 Study design. Low dose: 0.03 µg protein of polymerized *Phleum pratense* per dose. High dose: 0.06 µg protein of polymerized *P pratense* per dose. *Patients with placebo treatment were assigned to high-dose group. CPT: Conjunctival Provocation Test. IDIT, Intradermal Immunotherapy; S&M, symptoms and medication consumption recording

mean symptoms recorded and the mean medication consumed, both on a scale of 0-3, and dividing the sum of these by two.

Secondary endpoints included symptoms and medication consumption separately, symptom and medication-free days during the pollen season, concentration of allergen tolerated in conjunctival provocation test,[27] serum *P pratense*, Phl p 1 and Phl p 5 specific IgE and IgG_4 (ImmunoCAP ThermoFisher Scientific) determined at baseline and after each pollen season. The beginning of the pollen season has been defined as the first day with a count of at least 20 grains/m³ for three days. And the end of the pollen season was defined as the last day with a count of at least 20 grains/m³ for three days.

Safety was assessed by describing all reported adverse events (AE), classified according to the MedDRA dictionary. Adverse drug reactions (ADR) were graded according to World Allergy Organization (WAO) criteria.[28]

2.5 | Statistical analysis

To calculate the number of patients required, it was hypothesized that patients assigned to active medication groups should have a reduction ≥20% in the CSMS compared with placebo, according to the WAO recommendation, for a statistical power of 90%, a 95% confidence interval and a dropout rate of 20% of patients recruited. For the main and secondary endpoints, groups were compared using a two-tailed *t* test to evaluate differences between both active groups and placebo. The paired *t* test was used to make within-group comparisons throughout the trial. In the safety section, the prevalence of AEs was tested using a contingency Table and a two-tailed chi-square test. The confidence interval was 95% for all tests. Data management and graphical representations were made using the SAS Statistical software (version 9.4; SAS Institute, Inc).

3 | RESULTS

Out of the 157 patients recruited, 148 were included in the ITT population, with a mean age of 32 years (13-59). Of them, 45.9% were men; at enrolment, 10.1% of patients were under 18 years of age. Most patients had moderate nasal and ocular symptoms, 68% and 66%, respectively. Ten patients from the ITT population were lost to follow-up during the first year of treatment. Of the 138 patients who completed the first year of trial, 29 could not be included for analysis of main endpoint, 27 because they did not have fulfilled CSMS or because it was incomplete or illegible. Two protocol violations were
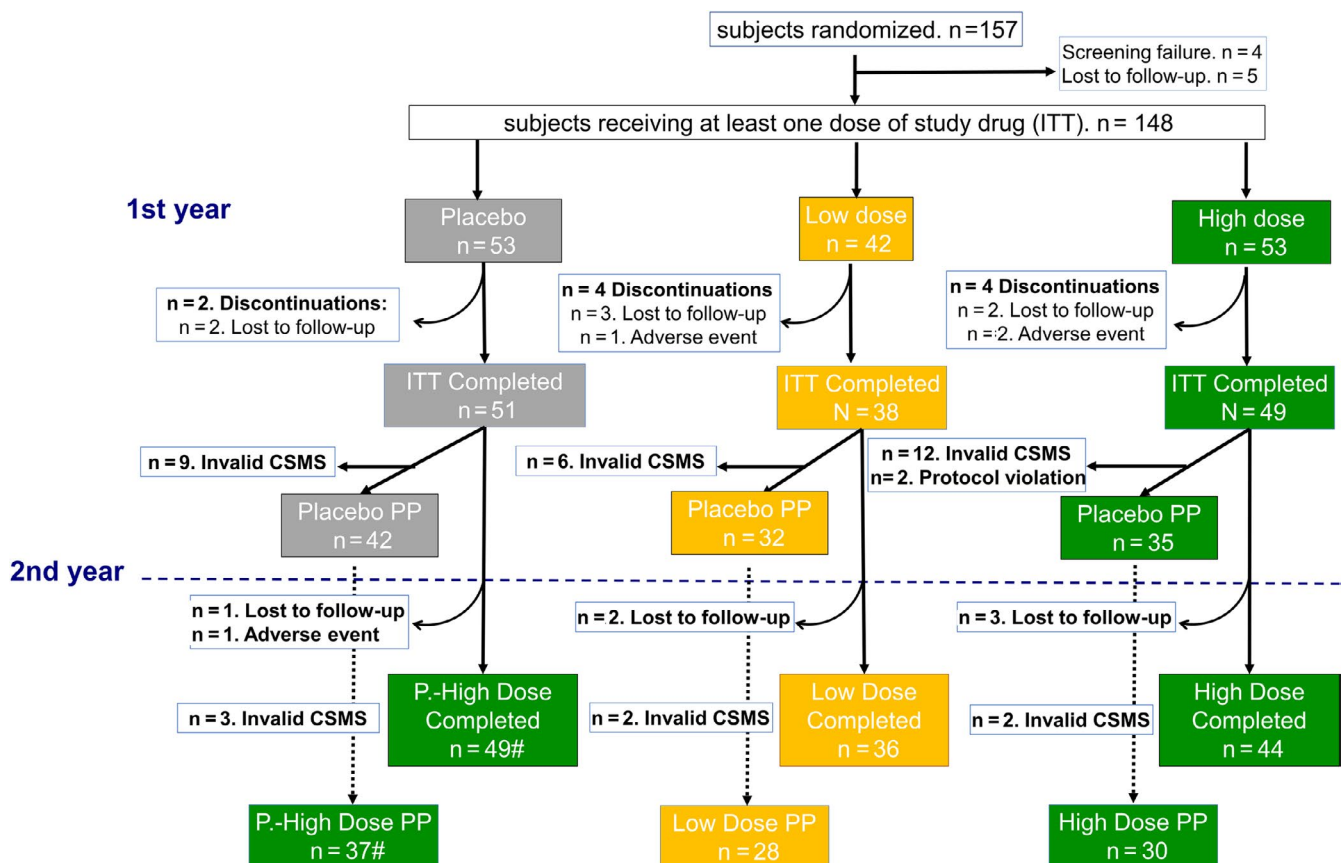

FIGURE 2 Flow diagram of subject disposition. #: These patients received high-dose treatment. *Protocol violation unrelated to the CSMS fulfilment

TABLE 1 Demographic data and allergological anamnesis of the ITT (Intention to treat) and PP (per-protocol) populations

Item	ITT population				PP population			
	Placebo	Low dose	High dose	Total ITT	Placebo	Low dose	High dose	Total PP
n	53	42	53	148	42	32	35	109
Age (range)	32 (14-52)	34 (16-55)	31 (13-59)	32 (13-59)	32 (13-56)	33 (16-55)	31 (13-56)	32 (13-56)
Male %	39.6%	50%	49.1%	45.9%	38%	46.9%	51.4%	45%
Under 18%	9.4%	7.1%	13.2%	10.1%	9.5%	9.4%	14.3%	11%
IgE *Phleum pratense*	30.3 (28.7)	22 (22.2)	29.9 (29.3)	27.4 (26.7)	30.6 (30.1)	22.3 (23.5)	29.2 (27.2)	27.4 (26.9)
IgE Phl p 1	23.8 (24.1)	16.9 (18.1)	21.6 (21.5)	20.8 (21.2)	25.1 (25.7)	17.9 (19.2)	18.7 (16.1)	20.6 (20.3)
IgE Phl p 5	12 (22.2)	7.1 (13.5)	14.4 (23.5)	11.2 (19.7)	11.6 (22.3)	6.41 (11.8)	15.4 (24.8)	11.1 (19.6)
Nasal sympt								
Mild	11%	10%	17%	13%	10%	9%	16%	12%
Moderate	71%	76%	57%	68%	73%	83%	64%	73%
Severe	17%	14%	26%	19%	17%	9%	20%	15%
Ocular sympt								
Mild	17%	17%	26%	20%	10%	17%	24%	17%
Moderate	77%	72%	49%	66%	83%	78%	64%	76%
Severe	6%	10%	26%	14%	7%	4%	12%	8%

also recorded. For this reason, it was not possible to analyse the main endpoint in the ITT population. Therefore, the main endpoint was evaluated in the per-protocol (PP) population of 109 patients (38% male) distributed as follows; placebo: (n = 42), low dose (n = 32), high dose (n = 35), without differences at baseline. (Figure 2) There were no differences in the three groups between the ITT and PP populations (Table 1).

During the first pollen season, the mean CSMS of the high-dose group (0.36 μg of cumulative dose protein) was 23% lower than in the placebo group (P = .02) and 28% lower than in the low-dose group (0.18 μg cumulative dose protein) (P = .017; see Figure 3). There were no differences between the low-dose and the placebo groups. Before the second pollen season, the patients that had received placebo group during the previous year were assigned to high-dose treatment. During the second pollen season, CSMS reduced by 31% (P < .001) for these patients compared with the previous year. In the case of patients treated with low doses, the mean decrease of the CSMS was 22% (P = .008). While
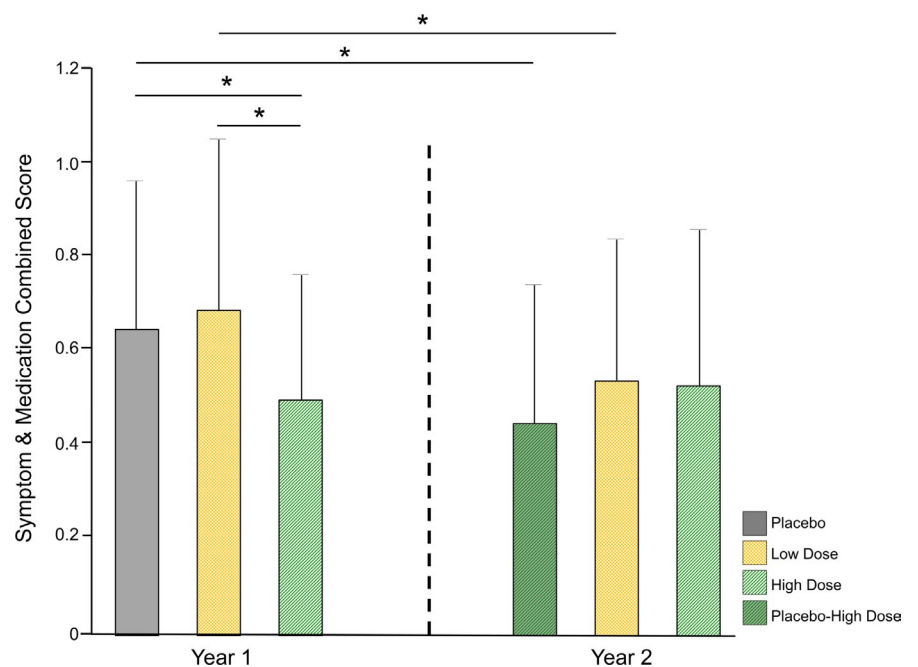
FIGURE 3 Bar plot of the means of the combined scores of the main endpoint after one and two years of immunotherapy. *P < .05
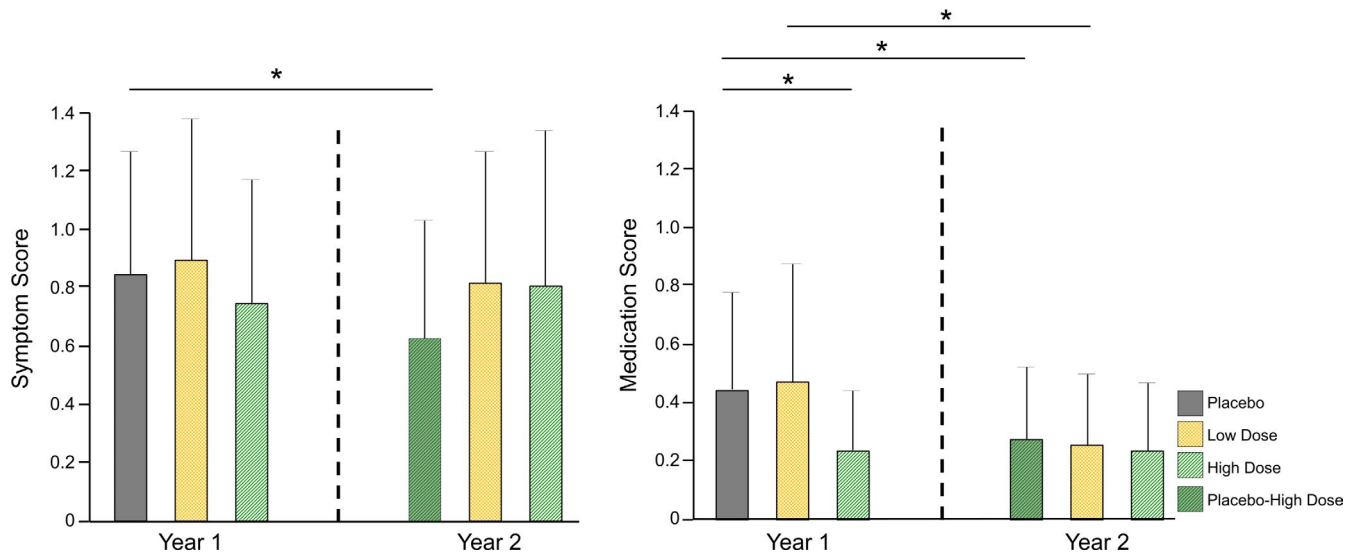
FIGURE 4 Bar chart of the score obtained for symptoms and medication separately after one and two years of immunotherapy. *P < .05

the group treated with high doses showed no differences from the previous year. In this group, 50% of patients decreased their CSMS the second year, 68% in the low-dose group and 73% in the group that went from placebo to a high dose.

By analysing the symptoms and consumption of medications separately (Figure 4), the high-dose group reduced its medication consumption by 47% (P = .002) compared to placebo, and by 50% (P = .006) compared to the low-dose group. The symptom score in the high-dose group decreased 12% compared to the placebo group and 17% compared to the low-dose group. In both cases, it was not significant. During the second pollen season, the placebo group that was treated with the high dose reduced their symptoms by 26% (P = .016) and the use of medications by 38% (P = .01) compared to the previous pollen season. In the low-dose group, symptoms

were reduced by 8% (P > .05) and the use of medications by 46% (P = .001).

During the first pollen season, the high-dose group presented 20% of the symptom-free days and 56% of the medication-free days, the low-dose group presented 16% of symptom-free days and 37% of medication-free days, while the placebo group had 12% of symptom-free days and 34% of medication-free days. During the second pollen season, all the groups increased their % of symptom-free days, by 27%, 21% and 36% for the high-dose, respectively, low-dose and placebo group is now treated with the high dose. Regarding the % of medication-free days, all the groups exceeded 50% of the days, with 59%, 57% and 54% for the high-dose, low-dose and placebo group now treated with a high dose, respectively.
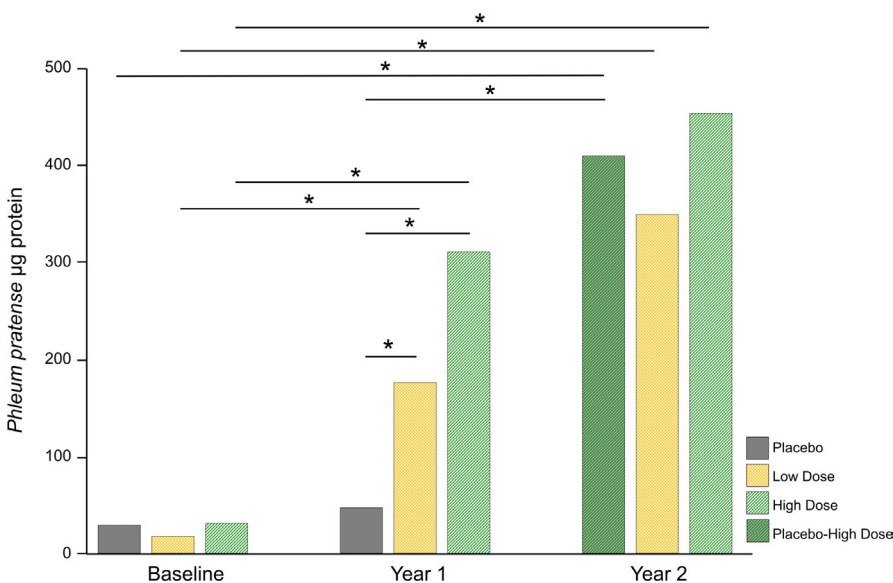

FIGURE 5 Micrograms of protein needed to produce a positive result in the conjunctival challenge test in the different trial groups at baseline, after the first year of treatment and after the second year of treatment. *P < .05
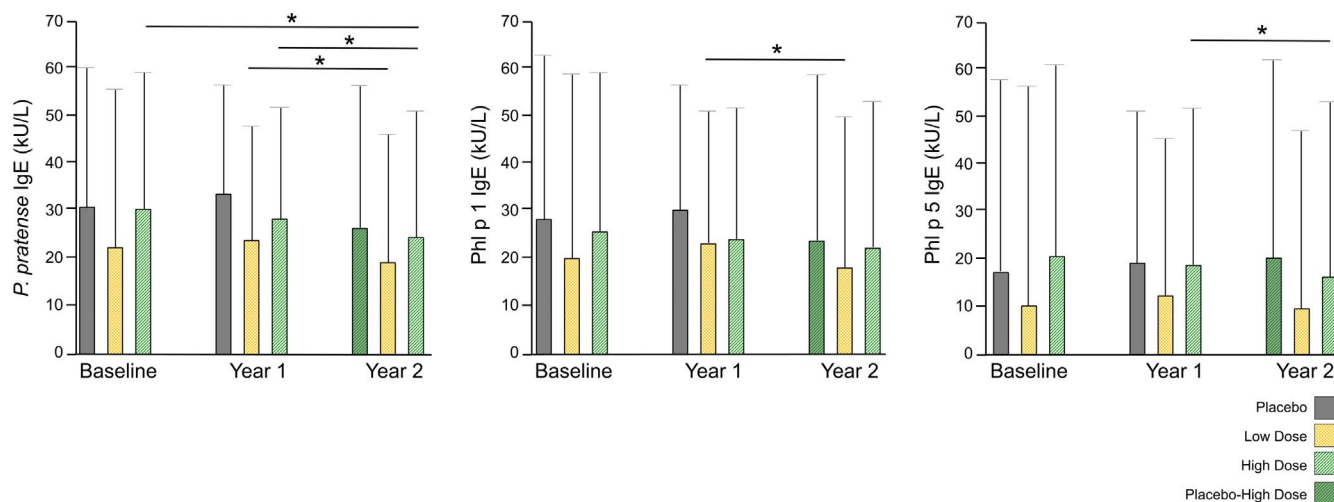
FIGURE 6 Specific IgE against *Phleum pratense*, Phl p 1 and Phl p 5 levels during immunotherapy. *P < .05

The secondary variables were analysed in the ITT population. At baseline, the conjunctival provocation tests showed similar results in the three groups: the mean concentration needed to induce a positive reaction ranged between 17 and 30 µg of protein/ml (*P* > .05; Figure 5). After the first pollen season, in the placebo group there was no variation in the concentration of *P pratense* tolerated, while in the active groups there were statistically significant differences in respect to baseline in the concentration needed to induce a positive conjunctival provocation test; the mean concentration able to induce a positive reaction was of 300 µg/mL in the high-dose group (*P* = .008), of 170 µg/mL in the low-dose group and of 45 µg/mL in the placebo group. After the second pollen season, all the groups showed an increase in the extract concentration needed to induce a positive provocation test, reaching 438 and 337 µg/mL in the high- and low-dose groups, respectively. The mean concentration was 395 µg/mL in the placebo group treated with the high dose; this was a significant increase from the previous year (*P* = .003).

The specific immunoglobulin levels against *P pratense*, Phl p 1 and Phl p 5 were monitored during the clinical trial. No differences were found between the groups at baseline. IgE levels against *P pratense*, Phl p 1 and Phl p 5 behaved similarly for the low-dose and high-dose groups, increasing after the first year of immunotherapy and decreasing below baseline levels after the second year. The only statistically significant variations were found in IgE levels versus Phleum pratense in the high-dose group, comparing the second year's values versus those of the first year and the baseline. A decrease in titre was also observed in the low-dose group in the second year comparison versus the first year. For IgE levels versus Phl p 1, only a statistically significant variation was found in the low-dose group in the comparison of the second year levels versus the first year. For IgE levels versus Phl p 5, a decrease in IgE levels for the high-dose group was found from the second year versus the first year (Figure 6). IgG and IgG4 levels did not increased in any group after the administration of immunotherapy.

Throughout the trial, 270 AEs were reported, including a serious AE not related to the study medication, consisting of mild grade urticaria. Of the 116 AE (43%) related to the study medication, 21 (18%) were reported as immediate local reactions, 52 (44.8%) as local delayed reactions, 25 (21.5%) as grade 1 systemic reactions and 2 (1.7%) as grade 2 systemic reactions. 14% of the AEs could not be classified because they could not complete the necessary information. In general, there were no significant differences between active groups compared with the placebo group in

TABLE 2 Ratio of ADRs per 100 injection visits according to group and year of treatment. *Systemic reactions graduation according to WAO. n (number of patients)

Type of ADR	Year 1			Year 2		
	Placebo (n = 53)	Low dose (n = 42)	High dose (n = 53)	Placebo-high dose (n = 51)	Low dose (n = 37)	High dose (n = 47)
Local immediate	0.3	1.2	0.6	1.3	4.1	0.7
Local delayed	0.6	0.8	1.9	6.3	3.6	5.3
Total local	0.9	2.0	2.5	7.6	7.7	6.0
Grade 1*	1.9	0.4	1.3	4	0.5	0.4
Grade 2*	0	0	0	0.7	0	0

the incidence of ADRs per 100 injections (Table 2). An increase in the incidence was observed during the second year, without differences between groups. Only one patient of the placebo-high dose group had two major systemic reactions of grade 1 according to WAO. In the rest of the patients, only local or grade 1 systemic reactions were registered. The most common presentation of local adverse reaction was the appearance of papules of up to 9 cm of area in the injection place´s that were resolved in a period of 24 hours spontaneously.

In a post hoc safety analysis, the local reactions recorded were re-evaluated and 75% of them were discarded, as 60% of them consisted of pruritus at the administration site and 15% of cases reported erythema, which spontaneously subsided within 24 hours. Both symptoms were considered typical for the ID administration of allergens.

4 | DISCUSSION

The results obtained in this clinical trial show that immunotherapy with the *P pratense* allergoid, administered intradermally during five consecutive weeks pre-seasonally with a dose of 0.06 μg of protein, significantly reduced the CSMS by 23% compared to the placebo, after only six administrations.

There are very few bibliographic references documenting the administration of intradermal immunotherapy with allergens[18,19,29-31] and results have been heterogeneous. This route of administration is generally used to the administration of vaccines such as BCG and influenza, with the advantage of its high immunogenicity due to the unique immunological characteristics of the dermis compared to the subcutaneous or muscle tissues, for example; in general terms, this enables a reduction in the volume and concentration of the antigen to obtain the same therapeutic effect.

The modification of allergenic proteins with glutaraldehyde, a cross-linking agent, leads to polymerization and transformation into high molecular weight molecules, above 100 kDa. In comparison with native allergenic proteins, the modified allergens retain their immunogenicity but have a reduced allergenicity, due to a reduction of IgE exposed epitopes, to the difficulty of IgE molecules to bridge IgE receptors, and to a reduction in the dissemination in the tissues.[25,26,32]

The data obtained in this clinical trial contrast with those obtained by other authors,[31] in which the ID administration of immunotherapy with a *P pratense* extract with a similar regimen and dose to that used in this study, worsened the nasal and eye symptoms of patients with active treatment compared to the placebo group. Nasal and eye symptoms were mildly reduced in our population, by 12% for the high dose. However, the reduction in medication was very significant, reaching 47% compared to the placebo; this is the main reason for the improvement in the primary endpoint. The difference in results could be due to the difference in the product used, as in our case we used an allergoid

that provides additional safety and a sustained antigen format without the need for an adjuvant. This improvement is translated into objective data, given that the high-dose group experienced five more symptom-free days and 13 medication-free days compared with the placebo group (*P* = .005) during the pollen season, and there is also an objective increase in the allergen conjunctival provocation test in active groups, data that can support the efficacy of immunotherapy treatment.

Efficacy was measured by the CSMS, according to the application guide.[23] Several problems have been detected in the use of this tool,[33] such as its subjectivity, since it consists of a self-evaluation by the patient. Due to this, we have seen that in the high dose it was sensitive enough to demonstrate a significant clinical improvement; however, in the low-dose group, the improvement was not statistically significant, which contradicts the results obtained in the conjunctival provocation test, objective test that measures the local tolerance to the allergen. Another problem was the exclusion of 27 patients, distributed in the three treatment groups, from the analysis of the main endpoint as a consequence of losing or not completing the CRF or providing an illegible CRF. This forced us to perform the analysis in the PP instead of in the ITT population, as recommended for this type of test. However, in our opinion, this does not invalidate the results obtained, since the effect of randomization has not been violated or biased in the PP population (Table 1), since the original proportion of patients per group was maintained. More studies are needed to evaluate the effect in a larger group and in a longer time period to verify the results.

The decrease in IgE against the *P pratense* extract is consistent with the hypothesis that use of the intradermal route for the administration of allergens enables a reduction in specific IgE[18,19,31] However, we did not find an increase in specific IgG antibodies against *P pratense* to correlate them with the clinical improvement found in the patients. One of the possible explanations for this is that immunoglobulin monitoring was not conducted at appropriate times, considering that intradermal immunotherapy is likely to produce immunological effects at different times than with, for instance, subcutaneous administration.[29] Due to the design of the clinical trial, the titration of IgE and IgG4 antibodies was done in visits after the conjunctival provocation test, a fact that can be conclusive for not having found correlation between both diagnostic tests, being in both cases secondary objectives of the clinical trial. The lack of correlation between the clinical improvement of patients added to the increased tolerance demonstrated in the conjunctival provocation test, and IgG4 levels need to be studied further in subsequent studies that demonstrate the pharmacodynamic mechanisms that lead to decreased symptoms and medication consumption when using IDIT, describing the exact timing of blocking antibody titration, which could not be demonstrated in this clinical trial.

Only one serious AE was registered that was considered by the investigators not being related to the treatment under study. No differences were found between the groups in the proportion of ADR reported by the patient per 100 injections. Only one patient registered systemic reactions higher than grade 1 according to WAO.

This suggests that the safety profile of the two active groups was similar to that of the placebo group, which represents a high safety profile of the product. As expected for intradermal immunotherapy treatment, patients in the immunotherapy group reported more local reactions than those treated with placebo. However, the rate of systemic reactions was slightly higher in the placebo group with respect to the two active groups. In the second year, there was an increase in the rate of local reactions of the three groups with respect to the first year, without differences between groups. No objective cause was found to explain this fact. The rate of systemic reactions was slightly higher in the placebo-high dose group compared with the other two active groups, they are differences between them.

One of the possible disadvantages of the intradermal administration of allergoids is the training and experience required by the healthcare professionals administering it, together with the sensation of pain and discomfort, greater than that found with other routes of administration.[34] Both aspects can be offset by using nano needles for intradermal administration, such as MicronJet600®, which ensures better dispersion of the antigen, are very easily used by personnel with little training and reduces the sensation of pain, starting with the absence of visible needles.[35]

Based on the data and experience acquired after carrying out this clinical trial, several advantages of the intradermal route in combination with an allergoid over traditional allergen administration routes such as subcutaneous and sublingual should be highlighted. These advantages are summarized in a shorter duration of treatment, only five weeks, with a lower number of doses, only six injections per year compared to the daily doses of sublingual administration or between 12 and 16 injections per year of the subcutaneous route. A high level of safety with only one patient suffering systemic Grade 2 reactions and a moderate number of local reactions similar to or less than those produced by the two usual routes of administration. In addition to these advantages, good efficacy results have been achieved, with a reduction in the dose of allergen of nearly one hundred and fifty times compared to the subcutaneous route and more than a thousand times compared to subcutaneous administration. Being the last added advantage of this injectable route of administration that does not need the addition of adjuvants such as aluminium hydroxide.

In summary, this is the first randomized, placebo-controlled clinical trial that has shown the efficacy and safety of allergen immunotherapy using an allergoid extract administered intradermally. There was a clear association between the increased dose of allergoid and greater clinical improvement, as shown by the CSMS, increase in tolerance to the conjunctival provocation test and reduction in IgE levels for high-dose group.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Arantza Vega Castro https://orcid.org/0000-0001-8675-0571
Elisa Gómez Torrijos https://orcid.org/0000-0002-4532-2498
David Rodríguez Gil https://orcid.org/0000-0001-5397-5766

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