



Tolerability and surrogate efficacy parameters of a polymerized depot mixture pollen extracts without dilutional effect

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Aim: To evaluate tolerability of subcutaneous immunotherapy, in a polymerized mixture (*Olea europaea*/*Phleum pratense*) depot presentation. **Patients & methods:** A total of 47 poly-allergic patients received: an abbreviated schedule with three injections at weekly intervals or a cluster schedule with two administrations in 1 day. Both treatments continued with 3 monthly maintenance administrations. **Results:** Two systemic reactions, (4.3%). One grade 0 and one grade I. No local reactions. Immunoglobulin levels, increased significantly at final visit versus baseline in sIgG and sIgG4; in both schedules and allergens, no significant changes in specific immunoglobulin E levels were detected. Cutaneous reactivity at final visit decreased significantly. **Conclusion:** Both administration schedules with polymerized mixture of *O. europaea*/*P. pratense*, presented an excellent tolerability profile and induced preliminary efficacy changes.

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Polysensitization is a major concern for the population with respiratory allergic disease [1] that, according to the medical literature, has increased over the time [2–6]. It is estimated that more than 70% of patients with allergic symptoms are polysensitized, having increased from figures around 27.5–31% to others up to 73.5% [7]. Moreover, polysensitization, more frequent in adults than in children, has been suggested to be a risk factor for subsequent development of allergic asthma and patients with greater number of sensitizations are associated with present more severe rhinoconjunctivitis symptoms and more impaired quality of life [8,9].

Aeroallergen polysensitization could have four different causes [10]: multiple sensitization with different allergens, but only one as primary or clinically relevant sensitization; poly-allergy when two or more allergens elicit in patient the clinical allergy response; sensitization to panallergens (profilins, polcalcins, tropomyosins, lipid transfer proteins. . .) if the patient generates the same specific immunoglobulin E (sIgE) to multiple allergens with common structural features, that are present in different sources, even unrelated ones and polyreactivity, simultaneous multiple sensitization with different allergen extracts in allergy tests as a result of different sIgE cross-sensitization, not necessarily due to homology of allergen structures. Molecular diagnostics [8,11] and nasal or conjunctival provocation challenges [12,13] are very helpful in the recognition of genuine sensitizations and the management of polysensitized patients.

Once poly-allergy has been diagnosed, the next challenge is to decide on patient treatment strategy. Allergen-specific immunotherapy is the unique allergy treatment that presents a disease modifying effect and induces tolerance

to the antigen [14,15], having the potential to provide long-term benefits after treatment has ended [16,17]. According to recent EAACI guidelines on allergen immunotherapy [18], for poly-allergic patients to homologous allergens (e.g, two grass pollens), a single extract with the most representative allergen of the homologous group or a mixture of the two homologous allergen extracts is recommended. When allergens do not belong to the same homologous group, (e.g, grass and olive pollens), the recommendation is to choose the clinically most important allergen extract or to administer two separate vaccines with each allergen extract for allergy immunotherapy treatment (AIT). When two separate preparations are selected for AIT, parallel administrations are suggested, with doses given, with interval of 30–60 min at different locations.

Allergen mixtures show limitations, including dilutional effect, potential allergen degradation as a consequence of enzymatic activity of some allergens and the difficulties of demonstrating evidence based efficacy of a high number of allergen combinations and/or different products [18].

However, a new polymerized depot mixture (100/100) with *Olea europaea* and *Phleum pratense* pollen extract, without dilutional effect since both allergens are administered up to complete dose, has been developed for subcutaneous immunotherapy by ROXALL Medicina España S.A., based on the following rational. Polysensitization is a prevalent problem in the Mediterranean area in Europe being grass and olive pollen cosensitization very common in countries like Spain, Italy and Greece [19]. It is expected the lack of proteolytic activity between pollens of different species as oleaceae and grass [20]. Additionally, the used allergenic extracts have been chemically modified by adding glutaraldehyde, forming large macromolecules hiding and disrupting the epitopes, which bind the specific IgEs and thus reducing their allergenicity but maintaining their immunomodulatory capacity. The result is a treatment with increased safety enabled to administer the complete dose of each allergenic source avoiding the dilutional effect during the mixture [21]. To get it, the final administered product must be elaborated from double concentrated bulks, to obtain two allergen sources mixture, maintaining the same administration volume of 0.5 ml.

Based on all of these, the current randomized controlled trial was designed with the aim of evaluating the safety and tolerability of subcutaneous immunotherapy, in a polymerized mixture (100/100) depot presentation with *O. europaea* and *P. pratense* pollen extract and to evidence surrogate efficacy changes in immunoglobulin titers and cutaneous reactivity.

Patients & methods

Study design & ethical considerations

This was a randomized, open, noncontrolled, multicenter, with parallel groups, clinical trial conducted at five sites in the southern of Spain, where cosensitization to grass and olive pollen is an important cause of respiratory allergy. The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference of Harmonization (ICH) guideline on good clinical practice, and was approved by relevant ethics committees and the Spanish regulatory authorities. All patients provided written informed consent to participate. The trial was registered on European Union clinical trial register under identification number 2015-001911-11.

Patients

The study population comprised adult subjects aged 18–60 years with a clinical history of *O. europaea*/*P. pratense* pollen-induced allergic seasonal rhinoconjunctivitis with or without mild asthma for at least 2 years prior to the study. Patients had to have a skin prick test result of ≥ 3 mm diameter against *O. europaea* and *P. pratense*, and a positive sIgE titer against *O. europaea* and *P. pratense* (levels of ≥ 0.7 kUA/l) determined by ImmunoCAP® (Thermo Scientific, Uppsala, Sweden). In addition, poly-allergy to both extracts was confirmed with component resolved diagnosis, in 77% of the patients, but it was not mandatory to report different allergens tested or their results. Unfortunately, one of the sites had not available in daily routine the component resolved diagnosis at the moment of study performing.

Subjects were excluded if they had received immunotherapy against *O. europaea* and/or *P. pratense*, or a cross-reactive allergen, in the 5 years prior to the study, or other concomitant AIT. Despite being well controlled, patients with moderate to severe asthma, or a forced expiratory volume in 1 s of $<70\%$, were ineligible. Patients were also excluded if they met any of the following criteria: a history of anaphylaxis; chronic urticaria; moderate to severe atopic dermatitis; immunological, cardiac, renal or hepatic diseases; current treatment with immunosuppressants, anti-IgE, tricyclic antidepressants, psychotropic drugs, β -blockers or angiotensin-converting enzyme inhibitors; women who were pregnant or breast-feeding.

Study interventions

The trial was initiated after the olive tree and grass pollen seasons of 2015. Following national and international recommendations, Allergovac® Poliplus injections containing a polymerized depot mixture (100/100) of *O. europaea* and *P. pratense* (ROXALL Medicina España S.A.) were administered by trained nurses under supervision of qualified allergologist in immunotherapy units [22].

Being a tolerability and safety study, there was not a placebo group. Patients were randomized in a ratio (1:1) to receive either an up dosing schedule of three increasing doses (volumes 0.1, 0.3 and 0.5 ml) on a weekly basis (abbreviated schedule) or a build-up phase of two injections (volumes 0.2 and 0.3 ml) on the same day separated by 30 min (cluster schedule). Taking into account that the product contained two pollen extracts at complete doses, it was decided to establish two different schedules – one more conservative and the other faster – to assess their tolerability and association with adverse reactions.

The preparation included two vials labeled with number 3, containing 2000 treatment polymerized units/ml, each one. The concentration in vial 3 of major allergen Ole e 1 was 11.28 µg/ml, and the major allergens Phl p 1 and Phl p 5 were 2.20 and 1.92 µg/ml, respectively. These values, are close to other major allergen quantifications, published for subcutaneous treatments used in European countries [23].

During the subsequent 12-week maintenance phase, patients received three maintenance doses (volumes 0.5 ml) at intervals of 4 weeks ($\pm$ 1 week).

Outcome measures

The main objective was to assess the tolerability and safety of the vaccine along the study but specially in the build-up phase. Therefore, the incidence of adverse drug reactions (ADRs), throughout the study was recorded. To get this aim, it was collected any adverse event after each vaccine administration; in the immunotherapy unit, during the first 30 min after injection considering it as immediate event, or in the patients' diaries for the 48 h after dose administration, considering it as delayed event, out of the health center. Later, all noxious and nonintended responses to any dose of investigational product administered, were classified as ADRs, when they were related to vaccine under study or adverse events if they would not be related by investigators.

Likewise, ADRs were classified as local (LR, reactions taking place at the injection site), or systemic (SR, generalized signs/symptoms taking place away from the injection site). All LRs were recorded regardless of size, considering clinically significant immediate LR those with wheal reaction $\geq$ 5 cm, while clinically significant delayed LR those $\geq$ 10 cm). LRs consisted of diffuse inflammation, redness, erythema, local painfulness, pruritus or reaction in injection site (when two or more local symptoms took place simultaneously). SRs were classified following the European Academy of Allergy and Clinical Immunology (EAACI) guidelines [22]. The use of this classification was agreed with all investigators at the moment of designing the study protocol. Later on, both LRs and SRs were also classified by Medical Dictionary for Regulatory Activities Version 18.1.

Skin prick testing was performed using four increasing concentrations of *O. europaea* and *P. pratense* extracts (100, 1000, 10,000 and 100,000 DBU/ml) as well as positive (histamine 10 mg/ml) and negative (saline) controls. Total 10,000 DBU/ml is the diagnostic unit used by ROXALL, and is equivalent to the concentration of allergen eliciting a wheal equal to histamine HCl 10 mg/ml. The change in cutaneous reactivity (wheal area in mm²) from baseline to the final visit was assessed.

Specific immunoglobulin levels (IgE, IgG and IgG₄) against *O. europaea* and *P. pratense* whole extracts were measured in patients' serum samples obtained at baseline and final visit by enzyme-linked immuno sorbent assay technique [24]. Samples were frozen and sent to ROXALL central laboratory for bioanalysis in accordance with Good Laboratory Practices.

Statistical methods

Tolerability, safety and descriptive analyses were performed using the safety population (patients who received at least one dose of treatment). Efficacy statistical analyses were performed using the intention-to-treat, (ITT) population (patients who met all inclusion/exclusion criteria received at least one dose of treatment, and had available data on efficacy variables) and the per-protocol population (patients who met previous criterial and moreover achieved their target maintenance dose, and completed the study without any protocol deviations).

For descriptive statistics, the categorical variables were described by absolute and relative frequencies. For the description of the continuous variables, the mean and the standard deviation were used.

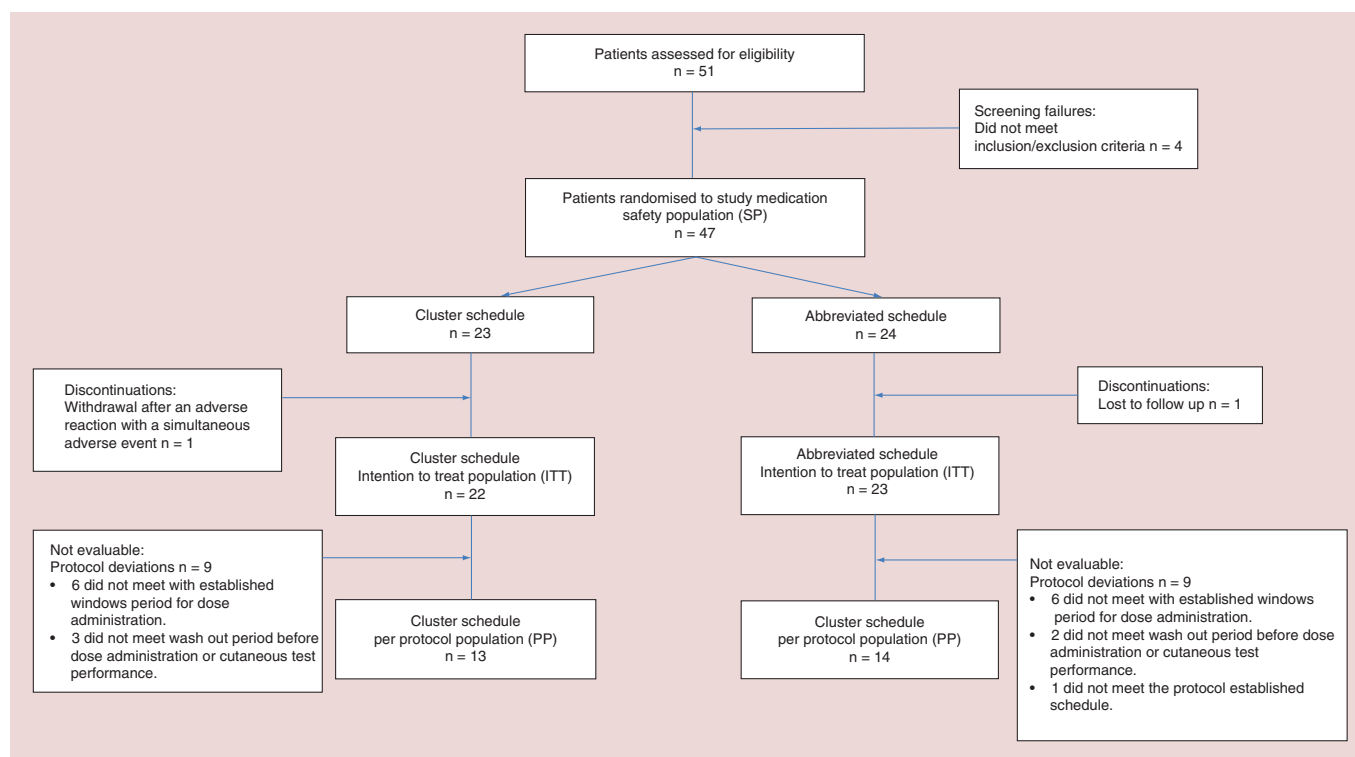


Figure 1. Patient disposition *Olea europaea*–*Phleum pratense*.

Differences between up dosing groups for demographic parameters were performed in basal visit using Fisher exact test or Wilcoxon test for independent groups.

Changes in immunoglobulin levels and skin prick test values, from baseline to final visit, were evaluated using the Wilcoxon nonparametric test for paired samples. In all statistical tests, a bilateral statistical significance level of 0.05 was applied. Statistical analyses were conducted using the Statistical Package Software version 9.3.

A formal sample size calculation was performed considering a percentage of SR 1.5% [25]. Establishing a CI of 95% with a precision of ± 3.5 and assuming a 5% of drop outs, the number of patients to be included in the study to provide adequate data on the primary end point was 50.

Results

In Spain usually, pollen season of *O. europaea* and *P. pratense* overlapped from April to June. A total of 51 subjects were enrolled out of the pollen season of both pollens described previously from October 2015 to March 2016. Only four of them were screening failure, since it was allowed the inclusion of patients with immunological and skin results of 6 months previous to the inclusion of the patient in the study. Then, 47 were randomized to receive AIT vaccine and analyzed. Based on a rigorous clinical history and allergy diagnosis, cosensitization and poly-allergy to *O. europaea* and *P. pratense* was confirmed so that investigators recommended the treatment with a vaccine of *O. europaea*/*P. pratense* (100/100). One patient in the cluster schedule withdrew from the study because of an adverse reaction grade 0, and a simultaneous adverse event described as respiratory infection. Another patient in abbreviated schedule was lost to follow-up without any relation to medication in study. According to the definition of study populations, these two patients, receiving only the first dose were analyzed in the safety population, provided that they did not have any final data for the ITT analysis. After the exclusion of the screening failures and discontinuations, the ITT analysis comprised 45 patients. A further 18 patients were excluded mainly as a result of protocol deviations, leaving 27 patients in the PP population. Patient's disposition is shown in Figure 1. The subjects' baseline demographic and clinical characteristics are presented in (Table 1).

Table 1. Patients baseline clinical characteristics (n = 47) (safety population).

Baseline characteristics	Cluster schedule	Abbreviated schedule
Age (years), mean $\pm$ SD	28.16 $\pm$ 9.33	33.98 $\pm$ 9.82
Women, n (%)	14 (60.87)	14 (58.33)
Rhinitis ARIA classification [26]		
Intermittent mild n (%)	0 (0)	1 (4.17)
Persistent mild n (%)	1 (4.35)	2 (8.33)
Intermittent moderate-severe n (%)	1 (4.35)	0 (0)
Persistent moderate-severe n (%)	21 (91.30)	21 (87.50)
Main concomitant illness		
Asthma, n (%)	16 (69.57)	13 (54.17)
Time from diagnostic (years), mean $\pm$ SD	8.96 $\pm$ 4.85	8.57 $\pm$ 5.90
IgE <i>Olea europaea</i> CAP class, n (%)		
2	0 (0)	2 (8.33)
3	11 (47.83)	6 (25.00)
4	4 (17.39)	6 (25.00)
5	6 (26.09)	5 (20.83)
6	2 (8.70)	
IgE <i>Phleum pratense</i> CAP class, n (%)		
2	4 (17.39)	4 (16.67)
3	6 (26.09)	7 (29.17)
4	6 (26.09)	4 (16.67)
5	3 (13.04)	6 (25.00)
6	4 (17.39)	3 (12.50)

ARIA: Allergic Rhinitis and its Impact on Asthma; n: Number of patients, SD: Standard deviation, only statistical significance was found for age, Wilcoxon test $p = 0.0254$; SP: Safety population.

Tolerability & safety

In safety study population, 74 adverse events were described, being only 2 (2.7%), related to study medication. In addition, 23 patients (46.8%) reported at least one adverse event, being the most frequent ones, headache (22 events), alterations in the musculoskeletal system (20 events) and respiratory tract infections (five events). Most of these events, (97.3%) were nonserious and mild in severity, and only three (3.3%) were moderate but no medication was needed to solve them. All patients recovered from adverse events, but one with anxiety was not resolved at the end of the trial.

During the study period, only two systemic reactions and no local reactions were recorded; one grade 0, described as general discomfort with dizziness during maintenance phase and a grade I reaction, described as rhinoconjunctivitis during the build-up phase. These two reactions represent 4.3% of patients, and 0.79% of the vaccine administrations. Both ADRs were of mild intensity and took place with the cluster, one day schedule. Symptomatic treatment was not required in either of them (Table 2).

All patients recovered of the systemic adverse reactions without any symptomatic treatment. One patient withdrew from the study due to the presence of an adverse drug reaction grade 0 and a simultaneously adverse event (respiratory tract infection). None of the patients failed to reach the maintenance dose established in the study protocol due to adverse reactions. Dose adjustments due to ADRs were not needed.

Schedule tolerability was evaluated between two treatment groups and no differences were found (Table 3). No clinically relevant changes in safety laboratory values were observed following treatment in any patients.

Immunoglobulin levels

Mean changes in immunoglobulin levels against *O. europaea* and *P. pratense* between baseline and final visit for the ITT population are described in Figure 2 and 3. Statistically significant increases in serum specific IgG and IgG4 levels at final visit were observed compared with basal visit (both $p < 0.001$; Wilcoxon test). Serum specific IgE levels slightly increased at final visit versus basal visit, without achieving statistical significance ($p = 0.2086$; Wilcoxon test to *O. europaea* and $p = 0.4270$; Wilcoxon test to *P. pratense*). No statistical differences were found

Table 2. Summary of adverse drug reactions (n = 47) (safety population).

	AR (n)	n (%)
Treatment-related adverse events	2	2 (4.3)
Severity		
Mild	2	2 (4.3)
Moderate	0	0 (0)
Severe	0	0 (0)
Change in treatment schedule		
None	2	2 (4.3)
Withdrawal due to adverse reactions		
Discontinuation	1	1 (2.1)
Schedule		
Cluster	2	2 (4.3)
Abbreviated	0	0 (0)
Phase		
Initiation	1	1 (2.1)
Maintenance	1	1 (2.1)
Classification according EAACI		
LR	0	0 (0)
SR	2	2 (4.3)
Grade 0/nonspecific	1	1 (2.1)
Grade I (rhinoconjunctivitis)	1	1 (2.1)
Treatment for recovery		
None	2	2 (4.3)

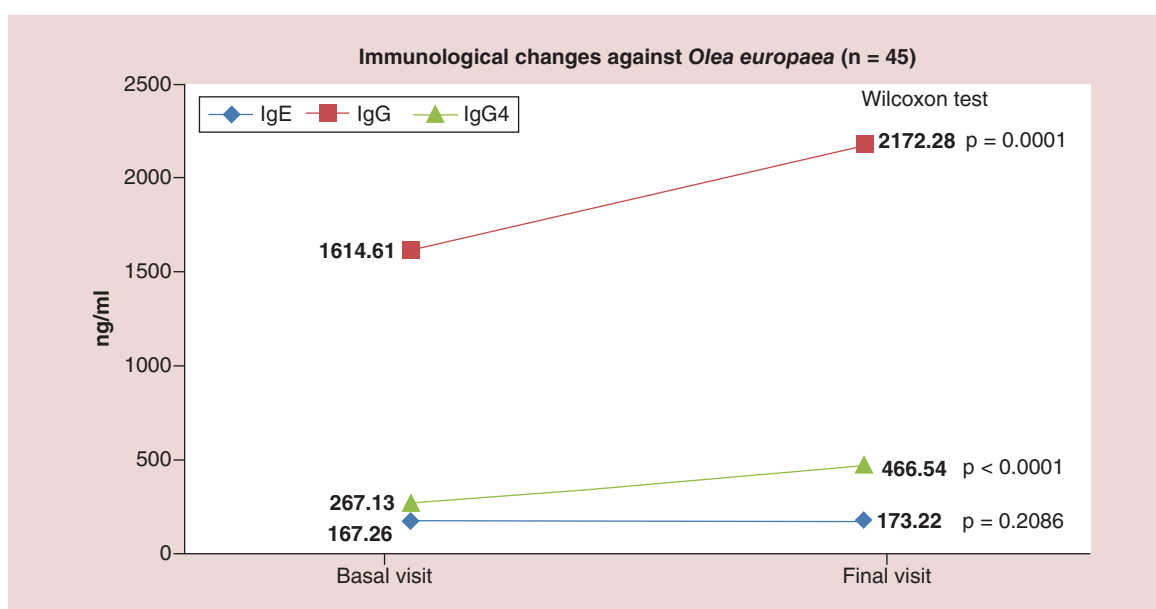
AR: Adverse reactions; EAACI: European Academy of Allergy and Clinical Immunology; n: number of patients; SP: Safety population; LR: Local reaction; SR: Systemic reaction.

Table 3. Tolerability schedule comparison (n = 47) (safety population).

ADRs per administration	Both schedules	Cluster schedule	Abbreviated schedule	p-value
Yes n (%)	2 (0.79)	2 (1.77)	0 (0)	p > 0.05**
No n (%)	250 (99.21)	111 (98.23)	139 (100)	

** Fisher exact test.

ADR: Adverse drug reactions; n: Number of patients; SP: Safety population; n (%), number and percentage of subjects.

**Figure 2. Change in mean immunoglobulin levels at final visit versus baseline to *Olea europaea*.**

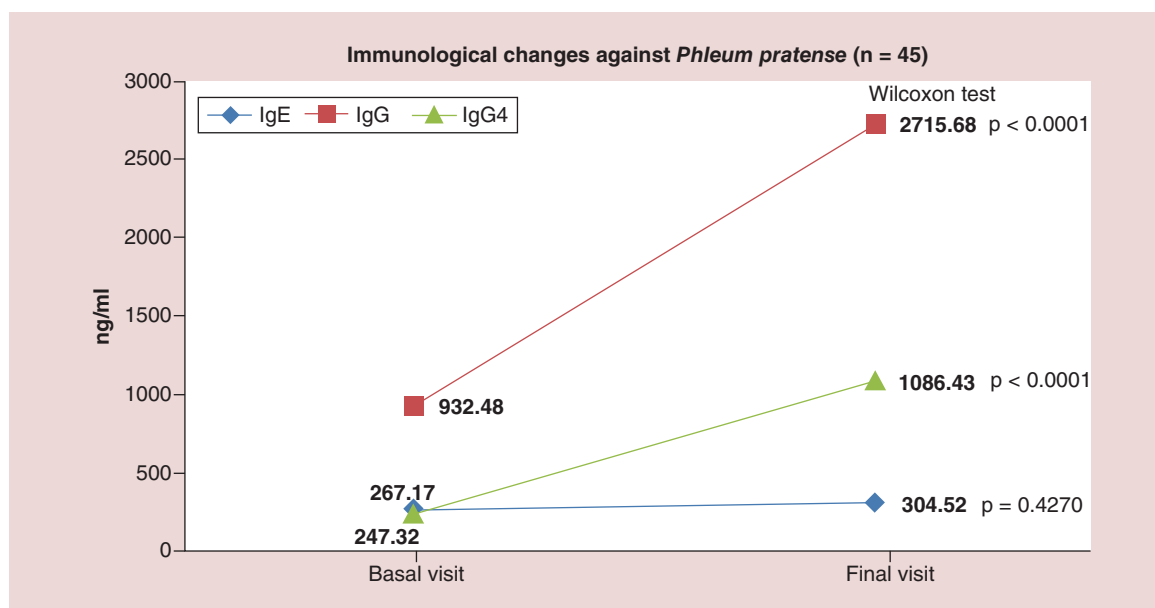


Figure 3. Change in mean immunoglobulin levels at final visit versus baseline to *Phleum pratense*.

between two schedule groups, in immunoglobulin titers, against *O. europaea* and *P. pratense* at final visit ($p > 0.05$; Mann-Whitney test).

Cutaneous reactivity

A dose-response skin prick test was performed with four increasing concentrations (vials 1–4). The results showed that cutaneous reactivity to both allergens decreased at the final visit compared with baseline values for ITT population. Mean values of wheal area in mm^2 was significantly reduced at the final visit compared with baseline using each one of the four tested vials Figure 4 & 5. Moreover, a comparison in wheal area reduction per treatment group was performed. Although the wheal area decreases against the larger *O. europaea* with the cluster schedule group than with the abbreviated schedule group, no statistical significance was achieved with the vials tested.

On the other hand, wheal area decreases against *P. pratense* had no correspondence with the schedule groups. In some cases, decreases were bigger with cluster schedule group and in others with abbreviated schedule group. No statistical differences were found between two schedule groups in this case.

Discussion

Polysensitization in patients with allergic respiratory diseases is a growing problem in Europa [1], particularly in Mediterranean area countries [19]. In an up-to-date European guideline about AIT in polysensitized and poly-allergic patients [27], it is not recommended to mix two or more immunotherapy allergenic extracts if they will not belong to the same homologous group. Mixing different allergenic sources was allowed for poly-allergic patients only if the two allergens have significant clinical and quality of life impacts and both overlap in time. However, when 2-allergen immunotherapy is prescribed, should be preferably separate administrations of single-allergen formulations; specially for nonhomologous allergens. In the case of a mixture administration, it should be ensured that each of the components is still present at optimal concentration in order to reach the adequate efficacy.

In the ALLERMIX study [28], carried out among clinicians of several European countries, a description can be found about the most commonly diagnosed allergy diseases, the frequency of daily use of AIT by the physicians and different strategies to manage poly-allergy along Europe. In this clinical study, it was established that for 50% of participants the most frequent clinical approach, for the treatment poly-allergic patients, was to recommend a single AIT formulation. In this case, the single selected extract was responsible of the majority of the patient's clinical symptoms. Another 20% of the responders, preferred to administer a single AIT formulation in parallel. Finally, other 25% chose to treat patients with an allergenic extract's mixture, usually no more two or three allergens. The prescription of allergens mixture would be more frequent if the eliciting allergens were seasonal and overlapped

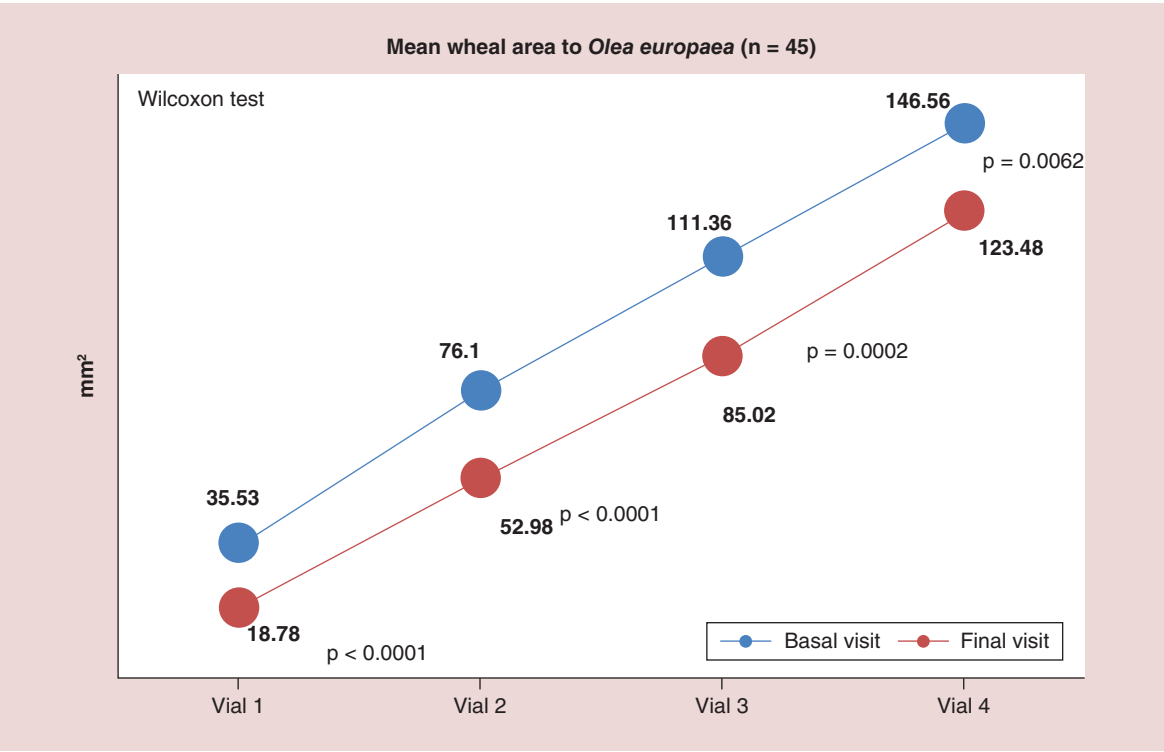


Figure 4. Change in mean wheal area at final visit versus baseline to *Olea europaea*.

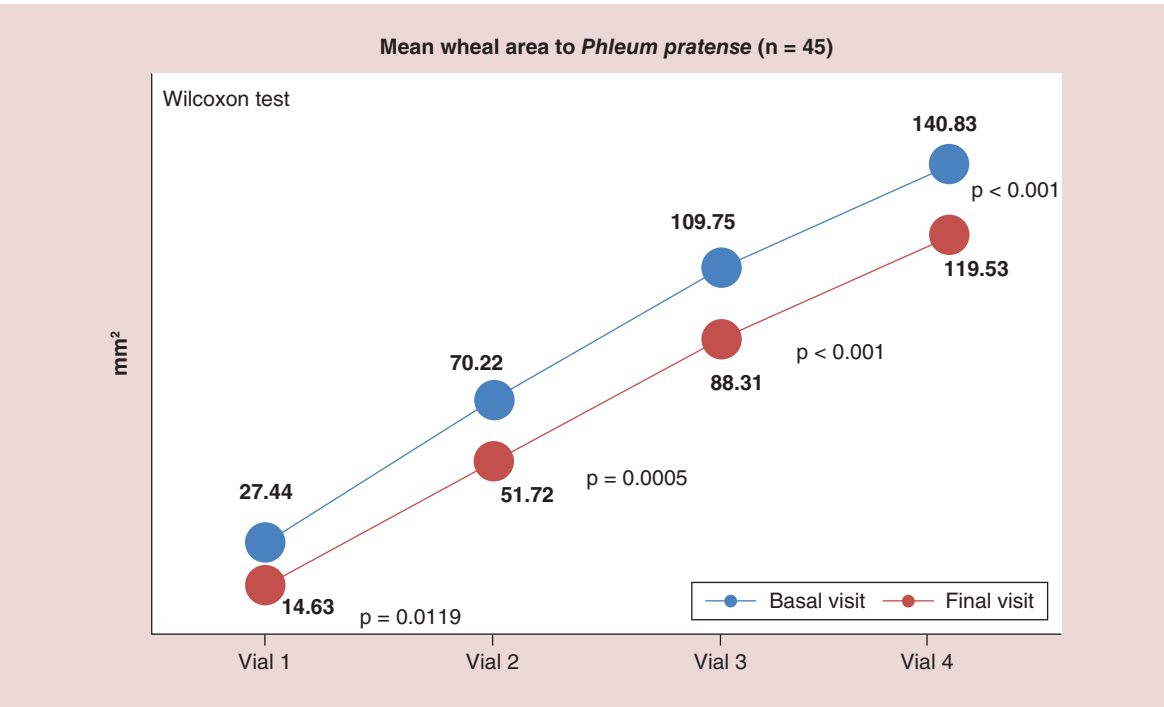


Figure 5. Change in mean wheal area at final visit versus baseline to *Phleum pratense*.

in the time. This fact was especially feasible in countries of southern of Europe, with *O. europaea* and *P. pratense*. When clinicians were asked about the recent recommendations of EAACI guidelines on immunotherapy [18], the majority answered to agree with them; for instance, to mix as few allergens as possible and to avoid mixing extracts that belong to different groups, seasonal and perennial allergens or seasonal allergens from no homologous groups. However, a considerable 35% of the participants, answered not to agree with this latter statement. Approximately, the 33% of participants preferred to prescribe an allergens mixture in nonequal proportions, although this is not in accordance with EAACI guidelines. The reasons to prescribe different proportions in an allergen mixture was based on correlations with symptoms intensity or skin testing results.

In line to Allergists' Consensus in Spain [19], AIT is claimed to be a first-line therapy option in patients sensitized to grass and olive pollen, if both allergens are clinically relevant, responsible of very bothersome symptoms and belong to nonhomologous groups and allergen degradation due to enzymatic activity is not expected. In addition, in the case of Allergovac Poliplus polymerized depot mixture (100/100) with *O. europaea* and *P. pratense*, there is no dilutional effect because both allergens are administered in a vaccine mixture up to complete dose. In the event *P. pratense*, this optimal dose was determined in a Phase II dose finding study [29]. On the contrary, in the case of *O. europaea* the dose was established by use. So far, mixing different allergenic sources in vaccines implied the dilution of the optimal dose of each source that was part of the preparation. With this new therapeutic option, the majority potential drawbacks of mixing allergens exposed in EAACI guidelines are avoided.

In addition, another inconvenience of mixing allergens is the lack of evidence supporting the efficacy of these mixtures in respiratory allergy. Therefore, the current study aim was to establish the tolerability profile of two schedules of the new AIT mixture vaccine, assessed as the incidence of ADRs. The safety and tolerability profiles of this vaccine based on results showed, can be considered fairly good. Local tolerability could be considered as excellent due to the lack of local reactions in the study. Additionally, regarding systemic safety and tolerability, it could be also described as very good because only two systemic reactions were recorded; one grade 0 described as discomfort and dizziness and one grade I defined as rhinoconjunctivitis. These reactions represent the 4.3% of patients, and 0.79% of the vaccine administrations. These percentages are in line with data published in other articles. In a publication about safety information from 6 controlled clinical trials, with house dust mites allergoid vaccines, the percentage of systemic reaction was 11.2 % of the included patients [30]. The figures in the same study, referenced to adverse reactions recorded in a 10 years post marketing period under daily practice conditions, were much lower (0.5%). In another phase IV prospective study with chemically modified allergen extracts, an incidence of systemic reactions of 1.31% per patient was described [25]. Usually in clinical trials, adverse reactions percentage used to be higher. On the other hand, it has to be taken in mind that current study evaluates the tolerability of a polymerized depot mixture (100/100) with *O. europaea* and *P. pratense*, meaning that, as primary endpoint, adverse reaction proportion is expected to be higher. In spite of administering complete doses of two extracts at the same time, the rate of reactions was in line with the mentioned published data.

The subcutaneous AIT mixture vaccine, induced early immunological responses confirmed by statistically significant increments of specific levels of sIgG and sIgG4 to *O. europaea* and *P. pratense*, after approximately 3 months of therapy. Similar results could be observed in another studies, where a rapid increased in sIgG and sIgG4 can be associated to the effect of blocking IgE-binding to allergens and B cell [31,32].

With regards to cutaneous reactivity to the causative allergens, a statistically significant reduction in immediate skin reactivity to the different concentrations of *O. europaea* and *P. pratense* extracts was observed, expressed as a decrease in mean wheal area, produced with each concentration tested. In some studies, these reductions have been seen to correlate with clinical efficacy [33]. In addition, other clinical trials showed similar reduction in skin reactivity after a short course of AIT with single allergen extract derived from *O. europaea* [31] and *P. pratense* [34].

As the main objective of this study was tolerability, the trial was designed as noncontrolled one, and this fact could have led to a certain degree of bias when interpreting the reported efficacy results. However, comparisons between two schedule groups may be done.

Another study limitation could be considered the fact of using an in force systemic reaction classification, currently less commonly used between researchers.

Conclusion

The results of this clinical trial show that treatment with polymerized depot mixture (100/100) with *O. europaea* and *P. pratense* extracts without dilutional effect, in abbreviated or cluster administration schedule, in patients with

allergic rhinoconjunctivitis, with or without asthma is a safe treatment, despite of being administered both allergens up to complete doses.

It also has for both extracts, encouraging subrogate efficacy signs in cutaneous reactivity and immunological responses. Taking into account all these results, this mixture vaccine could be a feasibly therapeutic option in poly-allergic patients due to sensitization to olive and grass pollens.

Future perspective

Polysensitization and poly-allergy is a big and amazing growing problem in Europe. In the next years this kind of patient will be one of the most frequent attended in the physician and allergologist officer. These patients will demand to develop and research new treatments: more effective, safer, cheaper and easier to compliance. In our opinion, the new polymerized depot mixture (100/100) with *O. europaea* and *P. pratense* pollen extract meets the majority of these features: this treatment is cheaper than received two separate vaccines for each allergenic extract, it presents an excellent safety profile and it is well tolerated, the adherence to only one treatment is better than two immunotherapy treatments and it shows good preliminary efficacy results that will must be confirmed in future large double blind placebo controlled studies. The good results of this study, could be very useful in the daily making treatment decision process of allergologist.

Summary points

- Polysensitization is a prevalent problem in the Mediterranean area in Europe being grass and olive pollen cosensitization very common in countries such as Spain, Italy and Greece.
- A new polymerized depot mixture (100/100) with *Olea europaea* and *Phleum pratense* pollen extract, without dilutional effect since both allergens are administered up to complete dose, has been developed for subcutaneous immunotherapy by ROXALL Medicina España S.A.
- Only two systemic reactions and no local reactions were recorded; one grade 0, described as general discomfort with dizziness during maintenance phase and a grade I reaction, described as rhinoconjunctivitis during the build-up phase.
- Statistically significant increases in serum specific IgG and IgG4 levels at final visit were observed compared with basal visit (both $p < 0.001$; Wilcoxon test).
- Mean values of wheal area in mm² were significantly reduced at the final visit compared with baseline using each one of the four tested vials.
- The results of this clinical trial show that polymerized depot mixture (100/100) with *O. europaea* and *P. pratense* without dilutional effect, in abbreviated or cluster administration schedule, has a good tolerability profile and induces significant immunological and cutaneous reactivity responses, in subjects suffering from allergic rhinoconjunctivitis with or without asthma, due to sensitization to olive and grass pollens.

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Financial & competing interests disclosure

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Ethical conduct of research

The study was conducted in accordance with the principles of the Declaration of Helsinki and the ICH guideline on good clinical practice, and was approved by relevant ethics committees and the Spanish regulatory authorities.

Data sharing statement

The authors certify that this manuscript reports original clinical trial data. Data reported in this manuscript will be available within the article or posted publicly at <https://www.clinicaltrialsregister.eu/>. Additional data from the study upon reasonable request to the corresponding author.

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