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Safety evaluation of a multiallergen immunotherapy treatment in polyallergic patients. APOLO observational study

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Aim: Assessment of safety, tolerability and changes in global clinical impression with an multiallergen immunotherapy treatment without dilutional effect in polyallergic patients. **Patients & methods:** This observational prospective study included patients with allergic rhinitis-rhinoconjunctivitis with or without asthma between 5 and 60 years old receiving immunotherapy treatment with a mixture of two allergenic sources. All adverse events were recorded. Global clinical impression, tolerability subjective assessment and satisfaction were also assessed. **Results:** 130 patients were analyzed. Nine clinically relevant local adverse reactions were reported in six patients (4.6%). Six systemic reactions (grades 0–I) occurred in four patients (3.1%). Patients improved significantly in their global clinical impression. Good tolerability subjective assessment and satisfaction values were also observed. **Conclusion:** This multiallergen immunotherapy treatment without dilutional effect can be considered as a potential therapeutic alternative for polyallergic patients suffering from allergic rhinitis.

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Allergic rhinitis (AR) represents a global healthcare problem affecting 10–20% of the total population, making AR the most prevalent chronic noncommunicable disease [1]. The allergic reaction extends beyond the nasal cavity, with conjunctivitis and asthma often being associated with AR [2]. Since it is often accompanied by ocular symptoms, it is common to use the terms ‘rhinitis’ or ‘rhinoconjunctivitis’ indistinctly [3]. Although AR is not a severe disease, it can result in considerable morbidity [4,5] and can cause a significant deterioration in patients’ quality of life (QoL) [6,7].

An important issue is the growing evidence that polysensitization, defined as the presence of more than one IgE-specific sensitization to unrelated allergen sources, is more prevalent than monosensitization [8,9]. This prevalence, in addition to being increased with age, has been shown to be a risk factor for the subsequent development of allergic diseases in general, and of allergic asthma in particular [10]. Polysensitization also affects the clinical expression of the disease; since it has been reported that polysensitized patients show more severe symptoms [10,11]. In addition, asthma is more likely to be associated with AR in polysensitized patients than in monosensitized patients [9].

However, a polysensitized patient does not necessarily have polyallergy, defined as a documented, causal relationship between exposure to two or more specific, sensitizing allergens and the subsequent occurrence of relevant clinical symptoms of allergy [10]. Once the polyallergy has been diagnosed, the subsequent challenge for physicians is to decide the most appropriate treatment strategy for these patients.

Many patients with AR rely on pharmacotherapy, but despite medication, a significant number of them continue to experience symptoms that impair their QoL [12]. Allergen immunotherapy (AIT) with the subcutaneous or sublingual administration of the causative allergen(s) is an additional treatment option, particularly for those patients with more troublesome disease, which remains inadequately controlled despite avoidance measures and regular pharmacotherapy [12,13]. AIT has also been shown to have a disease-modifying effect delivering long-term clinical benefits that may persist for years after discontinuation of treatment [12,14,15].

With the aim of reducing the inherent risks of subcutaneous immunotherapy (SCIT) treatments, polymerized extracts emerged, in which native extracts are modified chemically and have demonstrated their clinical effectiveness while keeping a low-risk profile in several studies conducted with different allergens [16–18].

A therapeutic option for polyallergic patients is the administration of AIT with a mixture of extracts. In fact, parallel, consecutive or mixed use of two products for AIT is recommended in polyallergic patients in whom two causal relevant allergens have a marked clinical and QoL impact [10,19,20]. Although multiallergen AIT treatment is a common practice in most allergists in the USA and 20–40% of AIT prescriptions in Europe [20], and that some clinical studies have clearly demonstrated their efficacy and safety, given the high number of possible combinations of allergens' mixtures and/or different marketed products currently available worldwide, not all of them have been sufficiently investigated [10–12].

The performance of observational studies is an adequate alternative to provide more evidence with these widely available products. In addition, these studies have proved to be a very useful instrument in obtaining new medicines information used under clinical practice conditions, which are not reproduced in clinical trials.

On the other hand, all these preparations with mixtures of allergens should use high-quality extracts, with scientific and well-standardized tests, as required by the World Allergy Organization [21].

Basic principles such as the possible cross-reactivity and the possible enzymatic degradation of allergens must be taken into account in multiallergen AIT preparations [9,22]. Moreover, mixing allergenic extracts from different sources may result in a dilution of individual allergenic components, which may not in any case be the optimum dose for the desired immunological effect. Avoiding this possible dilutional effect can be a critical aspect in maintaining the clinical efficacy of multiallergen AIT-based treatments. There is currently an increasing number of products for multiallergen AIT, where it is specified that, in its preparation, the dilutional effect is not present, also calling them in some cases, double-concentrated AIT products.

Following all these issues, a polymerized depot mixture with different pollens or mites extracts mixtures, and without dilutional effect since both allergens are administered up to complete dose (100/100), were developed for subcutaneous multiallergen AIT treatment in polyallergic patients by ROXALL Medicina España, S.A. The result is a treatment with an increased safety enabled to administer the complete dose of each allergenic source, thus avoiding the dilutional effect during the mixture [23]. In order to maintain this optimum final concentration of each allergen in the final administered product, it must be prepared from double-concentrated bulks, to obtain two allergen sources mixture, maintaining the same administration volume. One of the most frequently used extract combination of this product (*Olea/Pltheum pratense*) has been recently evaluated in a randomized clinical trial, showing a very good safety profile and also encouraging subrogate efficacy results either in cutaneous reactivity and immunological responses [24].

For all the aforementioned, nowadays both the diagnosis and treatment of the polyallergic patient are subjects of great controversy, and from which the scientific community demands greater evidence to validate the efficacy and safety of the different available AIT treatments based on multiple allergens.

The objectives of this observational study were the assessment of safety, tolerability and changes in patients' and physicians' global clinical impression with 'Allergovac Poliplus' multiallergen immunotherapy treatment, with two allergen mixtures (pollens or mites) in polyallergic patients with AR with or without asthma.

Materials & methods

This article was written following the Strengthening the Reporting of Observational studies in Epidemiology guidelines [25]. The study was a noninterventional prospective multicenter clinical study performed in 15 sites in Spain between December 2016 and September 2018. Patients were included in the study at a baseline visit in which patients' eligibility was checked and the informed consent was signed. Then, the patient started the treatment with the administration of the first dose of SCIT allergen mixture. The study was approved by Agencia Española de Medicamentos y Productos Sanitarios and all the involved regional competent authorities, and by an Institutional Review Board (CEIm Galicia) according to Spanish regulation, and other local Institutional Review Boards. In the

case of minors, two different specific informed consent forms were used, one for children between 5 and 11 years old, and another for children from 12 to 17 years old. In both cases, patient's parents or legal guardians' signature was also required.

Patient selection criteria

The assignment of a patient to a specific SCIT treatment with two pollens or mites mixture was not decided in advance by the study protocol, it was decided by the physician according to their usual clinical practice, and following the European Academy of Allergy and Clinical Immunology (EAACI) recommendations for the use of AIT with aeroallergens [12]. No intervention either diagnostic or of follow-up was applied to patients, other than the usual clinical practice.

Eligible patients were those aged between 5 and 60 years old with AR or rhinoconjunctivitis mediated by IgE, caused by sensitization to more than one allergenic source (pollens or mites) with or without mild/moderate associated asthma and susceptible to receive AIT treatment with a mixture of two allergens. No diagnostic procedures were established as a selection criteria for the studied participants. Only what diagnostic procedures were used, such as the skin prick test (SPT) or IgE determination, which resulted in the multiple-AIT treatment prescription, were recorded and described in detail in each case.

Pregnant or lactating women were not eligible, as they were not susceptible to receive treatment with immunotherapy, according to the usual clinical practice, as well as patients participating in other clinical studies. All patients were evaluated at baseline, and after 10 months of initiating treatment (after the completion of the first treatment package containing two vials each of 2.5 ml).

Treatments

Patients received SCIT treatment containing a mixture of two pollens or two mites (*Allergovac Poliplus*, ROXALL Medicina España S.A.) according to their sensitizations, clinical recommendations and summary of product characteristics (SmPC). It consists in a polymerized depot extracts mixture without dilutional effect, since both allergens are administered up to complete dose (100/100).

Patients received either an up dosing schedule of three increasing doses (0.1, 0.3 and 0.5 ml) on a weekly basis (abbreviated schedule) or a buildup phase of two injections (0.2 and 0.3 ml) on the same day both separated by 30 min (1-day schedule). In all cases, the maintenance dose consisted of 0.5 ml administered monthly. After finalizing the observation period of the study, patients remained with the maintenance treatment of 0.5 ml monthly, according to the usual clinical practice.

Outcomes measures

Safety

For safety assessment, any adverse event occurred during treatment and detected either by patient or by physician was recorded. Adverse reactions were defined as those adverse events classified by investigators as 'related', 'probably related' or 'possibly related' to AIT. A patient's diary was used for these purposes. The number, percentage and severity of reported adverse reactions were described, and assessed with reference to the total number of patients, and total number of administrations in the study.

Likewise, adverse reactions 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 ≥ 5 cm, and delayed LR those with ≥ 10 cm wheal. In the case of minor patients, clinically significant LR were those immediate LR with wheal reaction ≥ 3 cm, and those delayed LR those with ≥ 7 cm wheal. 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 EAACI guidelines [26]. Later on, both LRs and SRs were also classified by Medical Dictionary for Regulatory Activities (Version 18.1)

Changes in global clinical impression

Visual analog scale (VAS) was used to evaluate patients' and physicians' global clinical impression, at baseline and after having finalized the first treatment package (10 months). This scale ranged from 0 to 10, with 0 being the 'worst possible global clinical impression' and 10 being the 'best possible global clinical impression' including all

AR symptoms. Absolute change: Final visit value – Basal Visit value; and relative change: $([\text{Final visit} - \text{Basal visit}]/\text{Basal visit}) \times 100$ were calculated.

Tolerability subjective assessment

After treatment, patients' tolerability with received SCIT was subjectively assessed by the patient and by the physician using a VAS. This scale ranged from 0 representing that 'I have tolerated it very badly/patient has tolerated it very badly' to 10 representing 'I have tolerated it very well/patient has tolerated it very well'.

Satisfaction with AIT treatment

After treatment, patients' and physician's satisfaction with received AIT was also assessed using a VAS. This scale ranges from 0 to 10, with 10 being the highest degree of imaginable satisfaction, and 0 being the lowest degree of satisfaction that may exist.

Statistical analysis

Sample-size estimation was performed using the results of the study by Casanovas *et al.* in 2007 [27], in which the safety of polymerized and adsorbed into aluminum hydroxide allergenic extracts in a population of patients (both children and adults) sensitized to mites and/or pollens was evaluated. In this study, 1.57% of patients showed systemic adverse reactions. Taking into account that a sample size of 118 individuals provides an accuracy of $\pm 2.25\%$ to estimate this proportion of patients with systemic adverse reactions with a 95% CI, and assuming a 20% of losses, a total sample size of 148 patients was estimated to be recruited. For this estimation, Power Analysis and Sample Size software was used.

Safety (primary end point) and descriptive statistical analysis were performed considering every patient that received at least one dose of treatment. For efficacy analyses (secondary end points) patients fulfilling selection criteria that received the treatment and with data at final visit were considered.

The categorical variables were described by absolute and relative frequencies. For the description of the quantitative variables, the mean and the standard deviation were used. For the comparison of paired data, parametric tests (Student's *t*-test for paired data) or nonparametric tests (Wilcoxon) were used, according to the characteristics of the variables under study (normality of the variables, verified by the Shapiro Wilk test). For the qualitative variables, the Chi-square test was used to compare subgroups of patients. In all statistical tests, a bilateral statistical significance level of 0.05 was applied. All the statistical analyses of the data were carried out with the support of the statistical package SAS version 9.4.

Results

Descriptive data

A total of 152 patients from 15 study sites were recruited, and 130 of them could be included and analyzed. In the case of 22 patients, although they accepted to be treated with AIT and to participate in the study, they finally decided not to start AIT treatment, so they were study dropouts. 12 out of 15 study sites were distributed in the coastal area of Spain, and only three sites were located in the inland zone. Patients' mean age was 31.40 years (standard deviation = 13.33), with 20 of them under 18 years old (15.4%). 52.3% of patients were women. All baseline characteristics of patients are displayed on Table 1. In 53.1% of patients, both SPT and IgE determination against allergens were performed as diagnostic tests, while in 45.4% of cases, only SPT was performed. In the remaining 1.5%, IgE determination was performed as the unique diagnostic test.

Regarding the patients' sensitization profile, 35 patients were sensitized only to pollens, and 21 only to mites, with 21 of the total patients sensitized to pollens and mites concomitantly (Table 2). The remaining 53 patients presented other additional sensitizations to pollens and/or mites, including moulds, hymenoptera venom, epidermal derivatives, contactants, food and others.

According to the Allergic Rhinitis and its Impact on Asthma classification [4] for AR, most patients were classified as persistent and moderate/severe intensity AR (Table 3). With regard to Global Initiative for Asthma guidelines [28], 47.7% of patients presented associated asthma at study baseline, with 37.1% of them moderate asthma cases. The majority of patients reported the need to take symptomatic medication in the previous 12 months to the baseline visit (99.2%). In more than a half of cases, oral antihistamines were taken (52.3%), followed by topical nasal corticosteroids (22.1%).

Table 1. Patients' demographic and baseline characteristics.

Baseline characteristics	n = 130
Age (years), mean (SD)	31.40 (13.3)
Age categories, n (%)	
– 5–11 years	6 (4.6)
– 12–17 years	14 (10.8)
– ≥18 years	110 (84.6)
Gender, n (%)	
– Men	62 (47.7)
– Women	68 (52.3)
BMI, mean (SD)	24.70 (5.2)
Family allergic history, n (%)	56 (43.1)
Concomitant asthma, n (%)	62 (47.7)
Food allergy, n (%)	18 (13.8)
Atopic dermatitis, n (%)	13 (10.0)
Diagnosis, n (%)	
– Rhinitis	39 (30.0)
– Rhinconjunctivitis	91 (70.0)
Extract mixture composition, n (%)	
– Pollens	82 (63.1)
– Mites	48 (36.9)
Administration schedule, n (%)	
– 'Abbreviated'	17 (13.2)
– '1 Day'	122 (86.8)

SD: Standard deviation; BMI: Body mass index.

Table 2. Patients' sensitizations in frequencies and percentages.

Sensitization, n (%)	Additional sensitizations, [†] n (%)		Total, n (%)
	Yes	No	
Pollens	18 (13.8)	35 (26.9)	53 (40.7)
Mites	6 (4.6)	21 (16.2)	27 (20.8)
Pollens and mites	29 (22.3)	21 (16.2)	50 (38.5)
Total, n (%)	53 (40.7)	77 (59.2)	130 (100)

[†]Moulds, hymenoptera venom, epidermal derivatives, contactants, food and others.

Table 3. Patients' allergic rhinitis classification in frequencies and percentages (Allergic Rhinitis and its Impact on Asthma).

Type, n (%)	Intensity, n (%)		Total, n (%)
	Mild	Moderate/severe	
Intermittent	1 (0.8)	8 (6.2)	9 (6.9)
Persistent	9 (6.9)	112 (86.2)	121 (93.1)
Total, n (%)	10 (7.7)	120 (92.3)	130 (100)

Regarding SCIT composition, the majority of patients received treatment with a 2-pollen mixture – the most frequent combination within pollens mixtures – of grass and *Olea europaea* (Table 4). A '1 day' administration schedule was the most prescribed one (86.8%), in comparison with 'abbreviated' one (13.2%). A total of 1334 SCIT doses were administered in the study (1165 of them on '1 day' schedule).

Safety assessment

A total of 140 adverse events were reported in 28 patients. Of them, 133 were considered adverse reactions, and were reported as related, probably related or possibly related to AIT treatment. Thus, 25 patients (19.2%) had some adverse reaction during the study. None of the adverse reactions was serious, the majority of them (92.5%) classified as mild, thus only 7.5% were of moderate intensity. None of them had a severe intensity adverse reaction.

Table 4. Patients' distribution of allergen immunotherapy treatment compositions.

	n (%)
Composition	130 (100.0)
– Pollens	82 (63.1)
– Mites	48 (36.9)
Composition pollens (n = 82)	82 (100.0)
– Grasses + <i>Olea europaea</i>	48 (58.5)
– Grasses + <i>Cupressus arizonica</i>	8 (9.8)
– Grasses + <i>Parietaria judaica</i>	5 (6.1)
– <i>Cupressus arizonica</i> + <i>Olea europaea</i>	3 (3.7)
– <i>Cupressus arizonica</i> + <i>Parietaria judaica</i>	1 (1.2)
– <i>Olea europaea</i> + <i>Parietaria judaica</i>	3 (3.7)
– <i>Salsola kali</i> + Grasses	3 (3.7)
– <i>Salsola kali</i> + <i>Olea europaea</i>	7 (8.5)
– <i>Salsola kali</i> + <i>Parietaria judaica</i>	2 (2.4)
– <i>Platanus acerifolia</i> + Grasses	2 (2.4)
Composition mites (n = 48)	48 (100.0)
– <i>Dermatophagoides pteronyssinus</i> /D. <i>farinae</i> (DPT/DF mixture) + <i>Blomia tropicalis</i>	14 (29.2)
– D. <i>pteronyssinus</i> + <i>Lepidoglyphus destructor</i>	34 (70.8)

Table 5. Adverse reactions classification and distribution.

Adverse reactions	133 (100.0%)	Pollens 66 (49.6%), n (%)	Mites 67 (50.4%), n (%)
Severity			
– Serious	0 (0.0)	0 (0.0)	0 (0.0)
– Nonserious	133 (100.0)	66 (100.0)	67 (100.0)
Intensity			
– Mild	123 (92.5)	59 (89.4)	64 (95.5)
– Moderate	10 (7.5)	7 (10.6)	3 (4.5)
– Severe	0 (0.0)	0 (0.0)	0 (0.0)
Type of gradation			
Local and clinically relevant [†]	9 (7.1)	4 (6.1)	5 (7.5)
Systemic [†]	6 (4.5)	1 (1.5)	5 (7.5)
– Grade 0	3 (2.3)	0 (0)	3 (4.5)
– Grade I	3 (2.3)	1 (1.5)	2 (3.0)
– Grade II	0 (0.0)	0 (0.0)	0 (0.0)
– Grade III	0 (0.0)	0 (0.0)	0 (0.0)
– Grade IV	0 (0.0)	0 (0.0)	0 (0.0)
Patients with local adverse reactions clinically relevant[‡]	6 (4.6)	3 (3.7)	3 (6.25)
Patients with systemic adverse reactions	4 (3.1)	1 (1.2)	3 (6.25)

[†] EAACI classification 2014.

[‡] Reactions clinically relevant: Adults: immediate reactions >5 cm and delayed reactions >10 cm; Minors: immediate reactions >3 cm and delayed reactions >7 cm.

EAACI: European Academy of Allergy and Clinical Immunology.

Nine local reactions in the injection area, given in five patients (3.9% of patients), were clinically relevant (7.1% of the total 126 local reactions reported). Considering the systemic reactions, six were reported in four patients (3.1% of patients). Of them, three were classified as grade 0 (in two patients), and three as grade I (in two patients) (Table 5).

The grade 0 systemic reactions consisted of one in general discomfort (two reactions in the same patient) and the other in nonspecific symptoms. The three grade I systemic reactions were described as rhinitis (two of them in the same patient). There were no systemic reactions of grade higher than II (EAACI grading system) (Table 5).

While analyzing the results according to the type of treatments composition, for treatments with a mixture of two pollens, a total of 66 adverse reactions were described in 15 patients (18.3% of patients), with seven of them of moderate intensity (10.6%). Of the 65 local reactions reported, four of them were clinically relevant (6.1%) and occurred in three patients (4.6% of patients). One systemic reaction of grade I occurred in one patient (1.2% of patients).

Regarding treatments with a mixture of two mites, a total of 67 adverse reactions were described in 10 patients (20.8% of patients), with three of them of moderate intensity (4.6%). Of the 61 local reactions reported, five of them were clinically relevant (8.2%) and occurred in three patients (6.3% of patients). Five systemic reactions, three of grade 0 and two of grade I occurred in three patients (6.3% of patients).

Focusing in patients under 18 years old, four of them had any adverse reaction (20%). In total, 18 adverse reactions were reported, all of them being LR (12 immediate LR and six delayed LR). Only one LR was considered clinically relevant, as it was a delayed LR greater than 7 cm in diameter. No systemic reactions were reported in minor patients.

Taking into account the total number of doses administered (1334 doses), adverse reactions accounted for 9.97% of them (133 administrations), and systemic reactions for 0.45% of total received doses. Only 0.75% of doses caused some moderate intensity adverse reactions, all of them local adverse reactions. 24.1% of the adverse reactions occurred in the treatment initiation phase, and the rest (75.9%) in the maintenance phase. In the 17 patients who received treatment with the 'Abbreviated' schedule, there was only one patient who reported a single late local adverse reaction, and that was not clinically relevant. The rest of the reactions were reported in the '1 Day' administration schedule. All patients recovered within a few days. In relation to the actions taken, in 37 cases, concomitant medication was administered, in six cases the administration schedule was modified and in two cases the patient stopped the treatment.

Changes in global clinical impression

A total of 122 patients completed the study (two patients withdrew from the study due to adverse events, two patients due to own decision and four patients were lost to follow-up). Patients' mean values in VAS regarding their global clinical impression, improved from 4.43 (SD = 2.76) at baseline to 7.08 (SD = 1.88) at final study visit (mean difference 2.65 points; SD = 3.14) being a statistically significant difference ($p < 0.0001$). In the same assessment, from the physicians' perspective, very similar values were obtained improving VAS values from 4.67 (SD = 2.58) to 7.40 (SD = 1.76), with an absolute difference of 2.73 (SD = 2.85), and being also a statistically significant difference ($p < 0.0001$). Analyzing separately the groups of patients in treatment with pollens' or mites' mixtures, very similar results were obtained in both subgroups regarding patients' global clinical impression improvements (Table 6).

Tolerability subjective assessment

After AIT treatment, patients' tolerability with treatment was assessed using a VAS from both perspectives (patients and physicians). The mean values obtained in the VAS scale of tolerability at final visit were 8.44 (SD = 2.05; median 9.00) from the patient's point of view, and 8.56 (SD = 1.92; median 9.00) from physicians' point of view analyzing separately the groups of patients in treatment with pollens' or mites' mixtures, very similar results were obtained in both subgroups regarding tolerability (Table 7).

Satisfaction

After AIT treatment, patients' and physician's satisfaction with the treatment received was assessed using a VAS from both perspectives. Mean values of treatment satisfaction were 7.75 (SD = 2.01; median 8.00) and 7.82 (SD = 1.88; median 8.00) for patients and physicians, respectively. Analyzing separately the group of patients in treatment with pollens' or mites' mixtures, very similar results were obtained in both subgroups regarding satisfaction (Table 7).

Discussion

AIT is the only treatment option that can induce specific immune tolerance and has a long-term disease-modifying effect, inducing desensitization [12–15,26], and is nowadays considered the only etiological treatment of allergic diseases caused by inhalant allergens and Hymenoptera venom [12,26]. As recently evidenced, treatment with SCIT during 1 year significantly increases QoL in patients either monosensitized or polysensitized with AR, together with a significant positive impact on AR type and intensity, and a reduction in the percentage of patients showing

Table 6. Patients' and physicians' global clinical impression changes before and after multiallergen immunotherapy treatment.

	Global treatments (n = 122)				
	Basal visit	Final visit	Absolute change	Relative change (%) [†]	p-value [‡]
Physician					
Mean (SD)	4.67 (2.58)	7.40 (1.76)	2.73 (2.85)	100.11 (147.82)	p _{ABS} : <0.0001 p _{REL} : <0.0001
95% CI	(4.21; 5.13)	(7.08; 7.71)	(2.21; 3.24)	(72.92; 127.29)	
Median	4.00	8.00	3.00	56.73	
Patient					
Mean (SD)	4.43 (2.76)	7.08 (1.88)	2.65 (3.14)	110.28 (162.35)	p _{ABS} : <0.0001 p _{REL} : <0.0001
95% CI	(3.94; 4.93)	(6.74; 7.42)	(2.09; 3.21)	(80.29; 140.27)	
Median	4.00	7.00	2.50	63.64	
Pollens (n = 77)					
Physician					
Mean (SD)	4.51 (2.61)	7.51 (1.69)	3.01 (2.64)	106.89 (140.36)	p _{ABS} : <0.0001 p _{REL} : <0.0001
95% CI	(3.91; 5.10)	(7.13; 7.90)	(2.41; 3.60)	(74.14; 139.64)	
Median	4.00	8.00	3.00	60.00	
Patient					
Mean (SD)	4.38 (2.72)	7.09 (1.99)	2.71 (3.13)	102.64 (154.55)	p _{ABS} : <0.0001 p _{REL} : <0.0001
95% CI	(3.76; 5.00)	(6.64; 7.55)	(2.00; 3.42)	(66.32; 138.96)	
Median	4.00	7.50	2.50	66.67	
Mites (n = 45)					
Physician					
Mean (SD)	4.95 (2.53)	7.20 (1.88)	2.25 (3.16)	88.59 (160.75)	p _{ABS} : <0.0001 p _{REL} : <0.0001
95% CI	(4.19; 5.71)	(6.64; 7.76)	(1.30; 3.19)	(39.12; 138.06)	
Median	5.00	7.00	2.20	50.00	
Patient					
Mean (SD)	4.52 (2.86)	7.06 (1.69)	2.54 (3.17)	123.08 (175.76)	p _{ABS} : <0.0001 p _{REL} : <0.0001
95% CI	(3.66; 5.37)	(6.55; 7.56)	(1.59; 3.49)	(68.99; 177.17)	
Median	4.00	7.00	2.00	40.00	

Absolute change: (Final visit – Basal visit).
Relative change: ((Final visit – Basal visit)/Basal visit)*100.
NOTE: Scale from 0 (worst possible disease state) to 10 (best possible disease state).
[†]It was not possible to calculate the relative difference in those patients with values 0 at the initial visit.
[‡]Student's *t*-test for paired data in absolute difference and Wilcoxon test for paired data in relative difference (p_{ABS}: p-value in comparisons of absolute difference; p_{REL}: p-value in comparisons of relative difference).
SD: Standard deviation.

asthmatic symptoms after treatment [29]. Other randomized clinical trials also showed improvements in QoL in patients with AR treated with AIT [30,31].

A good safety profile was observed in the study, with the majority of adverse reactions local, and very few of them clinically relevant. Additionally, few systemic reactions were reported, none of them classified as serious.

Considering these results in relation to the treatment composition (two pollens or two mites), although a similar percentage of patients showed some adverse reaction, the treatments with a mixture of two mites showed somewhat higher percentages of patients with clinically relevant local reactions, and with systemic reactions. In both cases, the results were adequate for the safety profile of the treatments.

On the other hand, statistically significant improvements in Global Clinical Impression after 10 months of multiallergen AIT treatment were observed from both patients' and physicians' perspectives. This outcome measure was assessed by a VAS scale, according to publications where its use has been validated for a global evaluation of AR [32–34]. These improvements were observed in the overall studied patients, and also for the subgroups of

Table 7. Tolerability subjective assessment and satisfaction values after multiallergen immunotherapy treatment, by patients' and physicians' point of view.

	Global treatments (n = 122)	
	Tolerability subjective assessment [†]	Satisfaction [‡]
Patient		
Mean	8.44	7.75
95% CI	(8.07; 8.81)	(7.39; 8.11)
Physician		
Mean	8.56	7.82
95% CI	(8.21; 8.90)	(7.48; 8.15)
Pollens (n = 77)		
Patient		
Mean	8.58	7.95
95% CI	(8.20; 8.96)	(7.55; 8.35)
Physician		
Mean	8.76	7.97
95% CI	(8.44; 9.07)	(7.58; 8.36)
Mites (n = 45)		
Patient		
Mean	8.18	7.42
95% CI	(7.41; 8.94)	(6.72; 8.12)
Physician		
Mean	8.16	7.58
95% CI	(7.40; 8.92)	(6.95; 8.21)

[†]Scale from 0 (very poorly tolerated) to 10 (very well tolerated).

[‡]Scale from 0 (very dissatisfied) to 10 (very satisfied).

patients treated either with mites or with pollen mixtures. Also, high values in tolerability subjective assessment and satisfaction with the received treatment were observed, both for patients' and physicians' perspectives, and both for the overall and for the subgroups of patients with different treatments (mixtures of pollens or mites).

Limitations of this study are those of a noninterventional prospective, uncontrolled study in the real-life setting, such as unpredictable bias, confusion bias and selection bias. Another limitation of the study may be the fact that no biological confirmation of AIT treatment efficacy is available in the results, but this has not been possible to be performed due to the noninterventional design of the study.

It is well established that results from clinical trials are not always repeated in real-life clinical practice. An important fact to be taken into account is that the study was developed in the context of routine clinical practice, also commonly referred to as a real-life study, and follow-up results were obtained from patients who completed 10 months of multiallergen AIT treatment.

Although some multiallergen AIT clinical studies have clearly demonstrated its effectiveness, given the high number of possible combinations of allergens and/or different products currently used worldwide, all of them have not yet been sufficiently investigated [10,12]. However, multiallergen AIT treatment is a common practice in most allergists in the USA, and in 20–40% of AIT prescriptions in Europe [20], so it is necessary to have more scientific evidence for this type of treatment.

In some randomized clinical trials, the safety of immunotherapy treatments with allergen mixtures has also been previously assessed. In a study conducted by Alvarez-Cuesta *et al.* in 2005, patients with AR treated with a mixture of two pollens were evaluated. In this study, seven out of 53 patients experienced four local reactions, one of them in the active treatment group, and there were no systemic reactions among patients from the active group during the study [35]. In another randomized clinical trial performed by Pfaar *et al.* in 2013, mixed pollen extracts for immunotherapy in polysensitized patients were assessed. Local reactions occurred in 35.5% of patients in active treatment, and 13 systemic reactions occurred in 10 patients of the active group (5.7% of patients) [30].

Some well-designed, double-blind, placebo-controlled clinical trials have also evaluated the efficacy of treatment with multiple allergen formulations [36–39]. A review by Nelson identified 13 studies (published between 1965 and

2007) in which two or more allergens were administered simultaneously in SCIT (11 studies) or in SLIT (two studies) [37]. Nelson concluded that the sublingual or subcutaneous administration of two simultaneous extracts was effective, based on four studies that reported greater efficacy than placebo, or had the same efficacy as single AIT treatments. On the other hand, as concluded in a review by Demoly *et al.* in 2016 [10], all these preparations with allergen mixtures should contain high-quality extracts, with scientific and well-standardized tests, as required by the World Allergy Organization [21].

In relation to the latest, it is already known that when manufacturing mixtures of allergenic extracts for AIT purposes, some critical aspects, must be taken into account, such as the possible cross-reactivity of allergens, avoiding mixing those allergens, as well as the possible enzymatic degradation of allergens. Moreover, as stated by the last EAACI guidelines [12], in these cases it should also be ensured that the possible dilutional effect in the manufacturing process has been avoided, that is, each of the components is still present at optimal concentration in order to reach the adequate efficacy. Obviously, maintaining the optimal dose of each mixed extract to patients has to be a determining factor to achieve the same efficacy as the administration of two parallel treatments separately [22,40].

On the other hand, this consideration regarding the maintenance of the optimal dose in an allergen's mixture has a great impact on the treatment's safety question, because the maintenance of these doses produces a final preparation in which the final allergenic activity is the total sum of the activity of each component. This approach, which could be dangerous using native allergen extracts, can be achieved using polymerized extract and must be taken into account in the clinical development of these multiallergen AIT products.

In the case of *Allergovac Poliplus*, polymerized depot mixture (100/100) evaluated in this study, as previously stated, no dilutional effect is present, because both allergens are administered in a vaccine mixture up to complete doses. To the best of our knowledge, there are currently no published studies that clinically evaluate these types of products without dilutional effect, except for the above mentioned randomized clinical trial recently published by Quiralte *et al.* in 2019 [24], where the same commercial product *Allergovac Poliplus* was under study, but only with the *Olea europaea*/*Phleum pratense* combination. Results observed in this study regarding treatment safety and tolerability are in accordance with present study results, as a very good safety profile was observed only with two systemic reactions and no local reactions were reported (one grade 0 and one grade I). These two systemic reactions represented 4.3% of patients, and 0.79% of the total administrations, data that are very similar with those reported herein but involving other available allergen combinations including other pollens, as well as mite extracts combinations. On the other hand, significant increases of sIgG and sIg4, evidencing the changes in the patient immune system with induction of blocking IgG were observed, together with a significant reduction in average wheals elicited by either olive and grass pollen extracts, with every of the four concentrations tested, after 3 months of treatment.

In addition, AIT treatments based on polymerized extracts have been marketed and administered to patients for more than 20 years. The modification of natural allergens with glutaraldehyde [41,42], which produces extracts with a reduced IgE-binding capacity, while retaining T-cell reactivity, has proven to be safe and clinically effective [16,18] that allows the administration of high doses in a short period of time [43]. In addition, this modification significantly reduces the proteolytic activity of some extracts of native allergens, such as those derived from mites.

The product under study is a multiallergen AIT product, since it is composed of a mixture of two allergens, in which the extracts are present in the same concentration in which they would be present in the treatments separately (100% concentration). It is a 0.5% phenolated physiological saline solution extract composed of the combination of two allergenic extracts (pollens or mites), polymerized with glutaraldehyde and adsorbed onto 0.33% aluminum hydroxide. In its manufacture, all the principles and recommendations above mentioned to be taken into account are met.

Moreover, an important aspect to consider in the assessment of any therapeutic treatment is the adherence achieved by patients. Good adherence is a prerequisite for efficacy (regardless of the type of medication), and especially for AIT [44], so increasing the complexity of the treatment regimen will accentuate the importance of this parameter [45]. By the other hand, the different cost of two separate treatments versus a single multiallergen AIT treatment may also be a factor to take in consideration. Two parallel treatments of AIT will necessarily increase the cost of treatments, not only for the healthcare system but also for the patients themselves. Therefore, cost and adherence problems can sometimes mean that two parallel AIT treatments are not indicated in a particular patient, while a mixture of allergens in a single AIT treatment may be a more appropriate option in many cases.

Given all of the above, although more available clinical evidence is still necessary, multiallergen AIT treatments without dilutional effect have to be considered as a potential therapeutic alternative for polyallergic patients, with some considerable additional benefits, compared with AIT single treatments.

Conclusion

The results obtained in this observational study show a good safety profile for studied multiallergen treatment, with a low number of systemic adverse reactions, none of them serious. Significant improvements in global clinical impression after treatment were also observed. AIT treatment with *Allergovac Poliplus* with a mixture of two allergens without dilution effect can be considered as a potential therapeutic alternative for polyallergic patients suffering AR.

Summary points

- There is a growing evidence that polysensitization is more prevalent than monosensitization. This prevalence has been shown to be a risk factor for the subsequent development of allergic diseases in general, and of allergic asthma in particular.
- Once the polyallergy has been diagnosed, the subsequent challenge for physicians is to decide the most appropriate treatment strategy for these patients. A therapeutic option for polyallergic patients is the administration of multiallergen immunotherapy, containing a mixture of extracts.
- A polymerized depot mixture with different pollens or mites extracts mixtures, without dilutional effect, has been developed for subcutaneous multiallergen immunotherapy treatment by ROXALL Medicina España S.A.
- 25 patients (19.2%) had some adverse reaction and none of them was serious. Only 7.1% of local reactions were clinically relevant. Six systemic reactions were reported in four patients (3.1% of patients). Adverse reactions accounted for 10.6% of total administrations, with systemic reactions accounting for 0.45% of total received doses.
- Statistically significant improvements in global clinical impression were observed ($p < 0.01$), also in the subgroup of patients treated with pollens' or mites' mixtures, together with high values in tolerability subjective assessment and satisfaction reported from patients' and physicians' perspectives.
- Allergen immunotherapy treatment with *Allergovac poliplus* with a mixture of two allergens without dilution effect can be considered as a potential therapeutic alternative for polyallergic patients suffering allergic rhinitis.

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Ethical conduct of research

The study was approved by AEMPS (Agencia Española de Medicamentos y Productos Sanitarios) and all the involved regional competent authorities, and by an Institutional review board (IRB) (CEIm Galicia) according to Spanish regulation, and other local IRBs. The study was conducted in accordance with the principles of the Declaration of Helsinki and the ICH guideline on good clinical practices. Before participation, all patients gave their signed informed consent.

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