

Immunotherapy with depigmented glutaraldehyde-polymerized extracts: changes in quality of life

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Summary

Background Immunotherapy (IT) with modified allergens reduces allergic rhinitis (AR) symptoms and medications requirements. Improvement of quality of life (QOL) is a key point in the treatment of AR. The aim of this study was to provide evidence of changes related to the patient's QOL (well-being) induced by a modified (depigmented glutaraldehyde-polymerized) therapeutic vaccine and of its safety.

Material and Methods Fifty-three patients with a well-documented clinical history of seasonal AR sensitized to *Dactylis glomerata* and *Olea europaea* pollens were included in a randomized clinical trial. Twenty-five patients (Group-A) received a mixture of *D. glomerata* and *O. europaea* pollen extracts and 28 patients received placebo (group-C). Any adverse event was recorded and graded in accordance with EAACI guidelines. RQLQ was recorded before the treatment (pollen season 2000) and after 1 year of treatment (pollen season 2001). Dose-response skin prick test with each allergen extract was conducted at baseline and at the end of the study.

Results Each patient received 17 injections during this period. All patients completed the trial and no systemic adverse reactions were recorded. Symptom scores ($P < 0.001$) and medication requirements ($P < 0.001$) were significantly reduced in the IT group during the pollen season. This patient group also experienced greater and statistically significant improvement in overall RQLQ score and in five of the seven domains, all of them surpassing the threshold of 'minimal important difference' of 0.5 points.

Conclusions Results of this study provided evidence that IT with depigmented, glutaraldehyde-modified allergen extracts was well-tolerated and added beneficial effects to AR treatment in pollen allergic patients eliciting an improvement in QOL enough to justify a change in the patient's treatment.

Keywords pollen, polymerized vaccines, quality of life, RQLQ, safety, specific immunotherapy, therapeutic vaccine

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Introduction

Allergic rhinitis (AR) is a very common condition characterized by uncomfortable and subjectively irritating symptoms. They are more than a minor nuisance since symptoms of AR can decrease the physical, social and work activity, interpersonal relationships and general psychological well-being [1–4]. It has a substantial impact on patient's health-related quality of life (QOL). Unlike many other disorders whose treatment is focused on preventing death, the main goal of treatment of AR is to improve the patient's well-being [5, 6].

Immunotherapy (IT) is a specific therapy for AR and several reports have documented the safety and efficacy of this treatment [7]. Nonetheless, concerns over safety and the improvement degree related to the patient's QOL persist [7].

One of the most successful means of increasing safety has been to polymerize native allergens with glutaraldehyde. The removal of irrelevant low molecular components (i.e., flavonoids), which remain adsorbed to allergenic proteins, has improved the polymerization process [8]. This procedure has been shown to be effective and safe for therapeutic vaccines with several modified allergen extracts [9–12]. For clinical practice, clinicians need to know whether a statistically significant difference observed between two treatment groups also represents a clinically important difference. Although outcome measures (i.e., symptom score and medication usage) are all important indicators of AR, they do not provide evidence of the improvement degree related to the patient's well-being or QOL [13, 14] induced by the treatment. Therefore, it is important to have valid instruments to evaluate the overall impact of the rhinoconjunctivitis on patients' day-to-day life and whether it represents a clinically important difference. In this sense, RQLQ has shown strong discriminative (cross-sectional) and evaluative

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(longitudinal) measurement properties [15–17]. Furthermore, it can be used with confidence to measure rhinoconjunctivitis QOL in epidemiologic surveys, clinical trials and group patient monitoring. Efficacy of modified allergens has been evaluated in several reports, but today we do not know whether the improvement reported for this treatment is clinically important and if it represents a treatment able to improve the patients' QOL in the day-to-day life.

The aim of this study was to provide evidence of changes related to the patient's QOL (well-being) induced by a modified (depigmented and glutaraldehyde-polymerized) therapeutic vaccine and of its safety.

Material and methods

Patient population

Patients were recruited from the outpatient clinic of the Allergy Division of the 'Ramón & Cajal' University Hospital in Madrid (Spain). Fifty-three adult patients, ranging in age from 18 to 58 years, were included in the study. All patients met the following criteria: a clear clinical history of moderate and persistent rhinoconjunctivitis during the pollen season, follow-up for at least 2 years before included in the study; a positive skin-prick tests response to standardized grass (*Dactylis glomerata*) and *Olea europaea* pollen extracts (100 HEP/mL Laboratorios LETI, S.L.) and negative to a panel of 20 standardized allergens (*Cupressus arizonica*, *Platanus hybrida*, *Betula alba*, *Artemisia vulgaris*, *Parietaria judaica*, *Salsola kali*, *Chenopodium album*, *Taraxacum officinale*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Mucor mucedo*, *Penicillium notatum*, *Blata orientalis*, *Blatella germanica*, dog, cat and horse); a positive CAP (allergen-specific IgE) to *D. glomerata* and *O. europaea*.

Patient's characteristics are shown in Table 1. The exclusion criteria were those outlined in the WHO position paper on allergen IT [7]. Pregnancy were also considered as an exclusion criterion. No previous or ongoing IT of any form was administered.

The study was authorized by the Ethics Committee of the Hospital 'Ramón & Cajal' (Madrid), and the Spanish Health Authorities (Agencia Española del Medicamento). All patients gave their witnessed written consent to participate in the study.

Study design & patient assignment

The study was randomized, double-blind and placebo-controlled, and included two groups of patients. One group was treated with a mixture of two taxonomically non-related pollens in the same vaccine (group A) and the control group (group C) received placebo. The duration of the study was 3 years (2 years as a pretreatment monitoring period and 1 year as a treatment period – placebo or IT).

After the initial diagnostic tests, the patients underwent a pretreatment monitoring period of 2 years before beginning IT, for assessment of rhinoconjunctivitis and/or asthma severity (patient scoring of clinical symptoms and use of rescue medication) and inclusion criteria. Asthma was graded following GINA criteria. The patients were randomly

Table 1. Clinical data of the patients

	Immunotherapy	Placebo	
No. of patients	25	28	NS
Sex (M/F)	12/13	16/12	NS
Age (years)			
Mean	28	27	NS
Range	18–45	17–58	NS
Skin test (<i>Dactylis glomerata</i>)			
10 HEP	0.03	0.06	NS
Range	0.01–0.15	0.04–0.54	NS
Skin test (<i>Olea europaea</i>)			
10 HEP	0.39	0.27	NS
Range	0.20–0.72	0.18–0.98	NS
Asthmatic patients (n)	2	3	NS
Asthma severity	Mild	Mild	
RQLQ (pollen season 2000)			
Nasal symptoms	3.10	2.64	NS
Eye symptoms	1.65	2.21	NS
Non-hayfever symptoms	3.37	2.95	NS
Emotions	1.08	1.29	NS
Practical problems	1.53	1.93	NS
Sleep	1.70	1.61	NS
Activities	3.27	2.88	NS
Overall quality of life	2.67	2.22	NS

assigned to active treatment with IT (group A) or placebo treatment (group C). Both groups received pharmacologic treatment, if needed, consisting of oral antihistamines (Ebastine) and occasionally, and on demand, nasal corticosteroids (Budesonide). Asthmatic patients also received salbutamol on demand.

Patients, nurses and physicians were unaware of whether placebo or active treatment injections were being administered. At the end of the study, just before the statistical analysis was performed, the randomization codes were revealed.

Therapeutic vaccine and immunotherapy schedule

The allergen extract was a depigmented, polymerized extract supplied by Laboratorios LETI, S.L. (Tres Cantos, Madrid, Spain) and prepared as a biologically standardized extract. The extracts were prepared as follows. Briefly, native extracts were obtained extracting separately defatted pollen grains of *D. glomerata* and *O. europaea* in PBS followed by dialysis in 3 kDa flat-bed ultrafiltration membranes (Pellicon[®], Millipore, Madrid, Spain). The depigmentation step consisted of a controlled acid treatment to remove the remaining proportion of adsorbed pigments. The resulting depigmented allergen preparations were treated with glutaraldehyde [18], obtaining the depigmented-polymerized extract. Finally, the extract was adsorbed onto aluminum hydroxide for slow release.

SDS-PAGE electrophoresis was performed with native, depigmented and polymerized allergen extracts in order to monitor each step of the production process. The levels of Ole e 1, Dac g 2 and Dac g 1 and 5 content were determined by scanning densitometry [19]. The total allergenic potency was measured using a reverse-phase specific IgE binding inhibition ELISA [20]. The IgG binding activity was measured by ELISA-specific IgG inhibition assays [21]. The results were expressed as the quantity of freeze-dried extract needed to

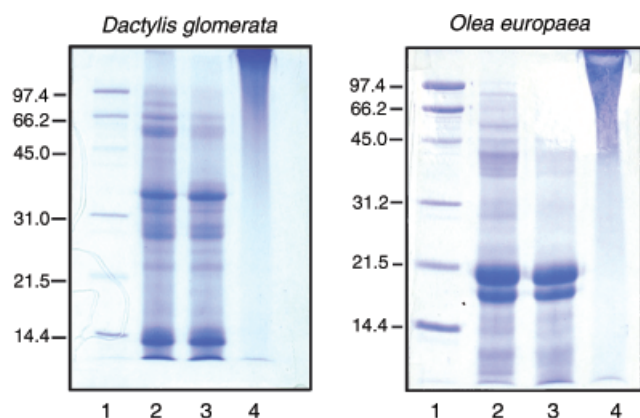


Fig. 1. SDS-PAGE electrophoresis pattern of the native (2), depigmented (3) and depigmented-polymerised (4) preparations of *Dactylis glomerata* and *Olea europaea*. Molecular Weight Markers (1).

Table 2. Immunotherapy schedule

Vial	mL	Day	<i>Dactylis glomerata</i>	<i>Olea europaea</i>
1	0.1	0	2.7	2.8
	0.3	7	8.1	8.4
	0.5	14	13.5	14.0
2	0.1	21	27.0	28.0
	0.3	28	81.0	84.0
	0.5	35	135.0	140.0
	–	–	–	–
	–	–	–	–
	0.5	Monthly	135.0	140.0

achieve 50% inhibition. The quantity to elicit 50% inhibition of the specific IgE binding for *D. glomerata* was 0.75 µg for the native unmodified extract and of 225 µg (300 times more) for the resulting polymer; and for *O. europaea* of 3.55 µg for the native unmodified extract and of 5325 µg (1500 times more) for the resulting polymer. Figure 1 shows the SDS-PAGE electrophoresis.

Two vials were prepared to be used as a therapeutic vaccine. Vial no. 2 contained 270 µg of *D. glomerata* and 280 µg of *O. europaea*, which were the result of depigmenting and polymerizing 100 HEP_L, corresponding to 750 µg of freeze-dried extract of *D. glomerata* (60 µg/mL Dac g 2, 20 µg/mL Dac g 1+5) and 3550 µg of freeze-dried extract of *O. europaea* (131 µg/mL Ole e 1). Vial no. 1 was a 10-fold dilution of vial no.2.

The placebo used was a series of dilutions of histamine phosphate solution [22] along with all the components of the vaccine (aluminium hydroxide, phenol and saline solution) with the exception of the modified allergens.

IT was performed by a trained technician in the presence of a physician, in a specialist unit. Patients were not pretreated with medication before each administration. IT administration schedule for active or placebo treatment was identical in both cases (Table 2). Once a maintenance dose was reached, it was repeated monthly. All patients remained under observation for 1 h after administration of the treatment, and local and systemic reactions (SR) were carefully checked. IT was not interrupted or decreased during the pollination period.

Diagnosis of allergy

Skin prick tests

Patients were skin tested with biologically standardized native extracts of each pollen on admission (baseline) and at the end of the trial. The skin prick tests (SPTs) were performed in duplicate in accordance with the prick-test technique on the volar surface of the forearm. Four 10-fold dilutions of freeze-dried extract of *D. glomerata* (100, 10, 1 and 0.1 HEP/mL) and *O. europaea* (100, 10, 1 and 0.1 HEP/mL) (Laboratorios LETI, S.L., Tres Cantos, Spain) were used throughout the trial. The extract was supplied freeze-dried and vacuum closed to be reconstituted just before use. Histamine HCl 10 mg/mL and glycerinated saline solution were used as positive and negative controls, respectively. Skin tests were done between 09:00 and 11:00 hours to avoid circadian variations. None of the patients was pretreated with drugs which could affect the performance of the test. Reactions were recorded after 15 min of application [23]. Weal areas were outlined with a fine tip marker and transferred, with transparent tape, to the corresponding sheet of a Case Report Form. The area of each weal was measured by planimetry using a Wacom palette (Wacom Technology Co., Vancouver, WA, USA) connected to the computer program MacDraft (Microspot USA, Inc. Boca-Raton, FL, USA). Parallel line assay was performed to evaluate changes in skin sensitivity. We used the median instead of the mean, because the latter is not influenced by extreme results [24, 25].

Allergen-specific immunoglobulin E

Serum specific IgE was measured by using the CAP System (Pharmacia Diagnostics, Uppsala, Sweden), with class 2 as the cut-off value.

Pollen counts

Daily pollen counts (grains per m³) were kindly supplied by the Aerobiology Committee of the Spanish Society of Allergy and Clinical Immunology. The counts were recorded according to a previously described technique [26, 27] using a Burkard volumetric spore-trap (Burkard Manufacturing Co., Rockmansword, Herst, UK). The pollen season was defined as the period of 2001 having 95% of the total yearly pollen grains, starting May 10th and ending June 15th.

Safety and efficacy assessments

Diary cards, symptom and medication scores

During the pollen season, patients recorded daily symptom scores, (nose, eyes and lungs). Nose (sneeze, blockage, and running), eye (itching, redness, and swelling) and chest (breathlessness, wheeze, chest tightness), were scored on a scale ranging from 0 to 3 (0 = none; 1 = slight, the symptom is clearly present but is not troublesome; 2 = moderate, the symptom is present, it is troublesome, but not disabling or insufferable; and 3 = severe, when the symptom is severe, disabling and/or insufferable). The daily total symptom score was calculated as the sum of all individual scores related to eye, nose and lungs. The intake of medication was recorded on diary cards and, was quantified according to Dreborg

et al. [28] in which the total number of tablets and puffs in 24 h made up the daily score.

Quality of life questionnaire

The standardized RQLQ developed by Juniper et al. [15] was used. RQLQ contains 28 questions which cover seven domains: activities (questions 1–3, maximum score 18), sleep (questions 4–6, maximum score 18), non-hayfever symptoms (questions 7–13, maximum score 42), practical problems (questions 14–16, maximum score 18), nasal symptoms (questions 17–20, maximum score 24), eye symptoms (questions 21–24, maximum score 24) and emotional (questions 25–28, maximum score 24). Patients rated each item on a scale of 0 (not troubled) to 6 (extremely troubled). The scores of the domains were expressed as the mean score for each item. The overall RQLQ was expressed as the mean of seven domain scores. Patients completed independently the questionnaire before the treatment (pollen season 2000) and after 1 year of treatment (pollen season 2001).

Consistent with previous analyses, a change in score greater than 0.5 on the RQLQ domain and overall scores was the critically meaningful 'minimal important difference' [5, 17, 29–33].

Local and systemic reactions

Thirty minutes after each injection, the patients were carefully evaluated for signs or symptoms of local or SR and the peak expiratory flow rate (PEFR) was also measured. After each injection a diary was filled for the next 2 days and measurements of swelling at site of injection, PEFR and drug consumption to alleviate symptoms were recorded (0, 30 min, 1, 2, 6, 12, 24 and 48 h). SRs were classified and graded according to the EAACI position paper [34].

Statistical analysis of the data

The software SPSS (SPSS Inc., USA) was used for statistical analysis. The value of the median and the interquartile (IQ) range was used [35] for descriptive statistics of symptom and medication scores and the duration and accumulated dose of IT. The value of the mean and 95% confidence intervals was used for pollen counts and for QOL (overall and each individual domains). Log-log regression in the bioassay of dose–response of *in vivo* SPTs was conducted [36] in order to calculate the individual allergen concentration needed after IT to achieve a skin reaction of the same size as 10 HEP before IT. Daily symptom and medication scores (area under the curve, AUC) during the pollination period [19, 35], RQLQ (overall and each domain), and the differences from the baseline in the individual 10 HEP were compared groupwise using the non-parametric Mann–Whitney's test.

The Wilcoxon's test was used to compare, into each group changes at baseline (pollen season 2000) and at the end of the trial (pollen season 2001).

Results

Patients

Of the 53 evaluated patients, 25 patients received IT and 28 placebo. Figure 2 gives an outline of the study design

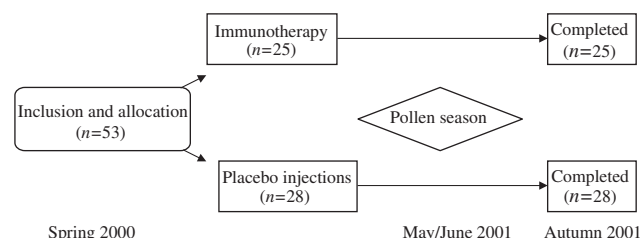


Fig. 2. Study flow chart 2000–2001. Timing of allocation, treatment, season and termination of study. Number of patients in parentheses. Assessment was performed in pollen season 2000 and 2001.

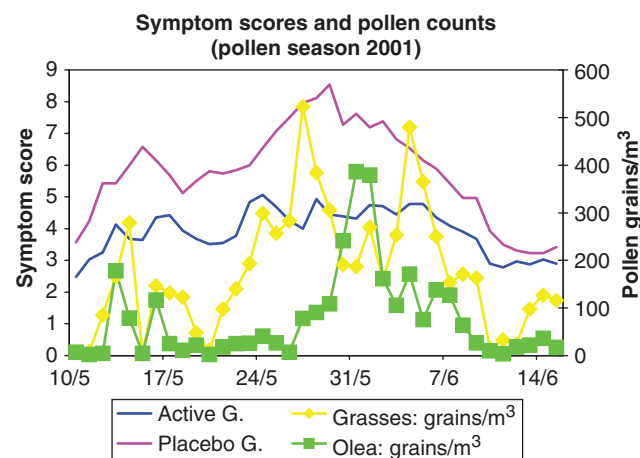


Fig. 3. Symptom scores and pollen counts (Pollen season 2001).

according to CONSORT rules [37]. At baseline, there were no statistically significant differences between the two groups in age, sex, duration of rhinoconjunctivitis, incidence of asthmatic patients, asthma severity, nasal symptoms, eye symptoms, non-hayfever symptoms, emotions, practical problems, sleep, activities and overall QOL.

Efficacy evaluation

Pollen count, symptom and medication scores The mean pollen counts (pollen season 2001) for grasses was 176 grains/m³ (95% CI: 135–218) and 77 (95% CI: 46–108) for *O. europaea*. Figure 3 shows pollen counts and symptom scores during the pollen season and Fig. 4 the pollen counts and the medication score. Table 3 shows the descriptive and comparative (Mann–Whitney) of the effects of IT on symptoms and medication use between the groups. The differences were statistically significant ($P < 0.001$), being both scores lower in the group treated with IT.

Skin prick tests Results are expressed as the amount of native (unmodified allergen) extract necessary to induce a weal equivalent to 10 HEP. At baseline, group A needed for *D. glomerata* a median of 0.03 mg (IQ range 0.01–0.15), and group C 0.06 (IQ range 0.04–0.54). After the period of treatment, the results were 0.21 mg (IQ range 0.07–0.38) for group A, and 0.07 (IQ range 0.03–0.50) for group C. For *O. europaea* the values at baseline, for groups A and C, respectively, were 0.39 mg (IQ range 0.20–0.72) and 0.27 (IQ range 0.18–0.98), being at the end 0.7 (IQ range 0.39–1.39) and 0.28 (IQ range 0.13–1.17). In the active group, seven

Table 3. Descriptive (median and interquartile range for symptom and medications scores, and mean and 95% confidence intervals for RQLQ) and comparative (Mann–Whitney *U*-test) statistics of the symptom and medication scores, and of the quality of life (overall and domains)

	Immunotherapy	Placebo	<i>P</i> -value
	Median (first and third quartiles)		
Symptom score	4.00 (3.52–4.45)	5.85 (4.96–6.81)	<0.0001
Medication score	1.17 (1.10–1.32)	1.67 (1.53–1.88)	<0.0001
Quality of life	Mean (95% confidence intervals)		
Activities	2.71 (2.24–3.17)	2.89 (2.30–3.48)	>0.05
Sleep	1.52 (1.23–1.81)	2.15 (1.67–2.64)	<0.05
Non-hayfever symptoms	1.32 (1.04–1.60)	2.08 (1.57–2.60)	<0.05
Practical problems	2.39 (1.90–2.88)	3.14 (2.59–3.70)	<0.05
Nasal symptoms	1.85 (1.48–2.22)	2.76 (2.18–3.34)	<0.05
Eye symptoms	1.24 (0.84–1.64)	2.19 (1.63–2.74)	<0.05
Emotions	1.17 (0.74–1.60)	1.15 (0.66–1.64)	>0.05
Overall quality of life	1.74 (1.50–1.98)	2.34 (1.87–2.81)	<0.05

Pollen season 2001.

Statistically significant values are in bold text.

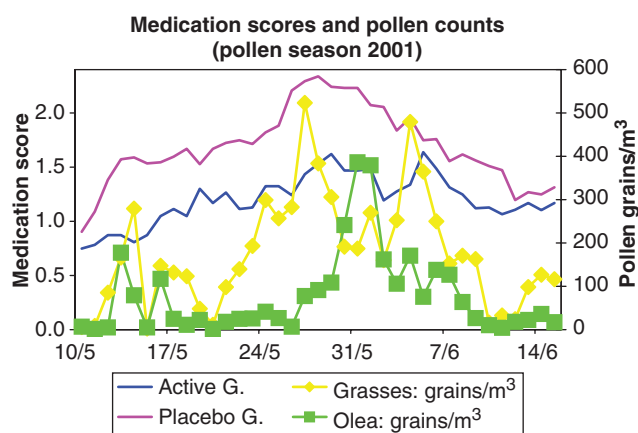


Fig. 4. Medication scores and pollen counts (Pollen season 2001).

times higher concentration of *D. glomerata* and 1.8 times more concentration of *O. europaea* after IT were needed to elicit the same size weal area than before IT. Changes in placebo group were smaller than in active group (1.2 for *D. glomerata* and 1.0 for *O. europaea*). The statistical analysis (Mann–Whitney) shows that between the two groups, and for each allergen tested, there are no significant differences at baseline ($P > 0.05$), whereas at the end the differences from baseline are significant ($P < 0.01$) between the two groups. When comparing the evolution of the skin reactivity from baseline to the end of the study, only the IT group showed significant differences ($P < 0.05$, Wilcoxon's test).

Quality of life questionnaire Before the treatment (pollen season 2000), there were no statistical differences ($P > 0.05$) for each of seven domains and in overall QOL between patient group treated with the modified allergen extract and the patient group that received placebo (Table 1). During the pollen season, IT-treated patients had significantly greater improvements both in overall RQLQ scores and in five of the seven HRQL domains (sleep, nonhayfever symptoms, practical problems, nasal symptoms and eye symptoms). These differences also reached, or surpassed, the proposed threshold

of clinical relevance (>0.5 U) for total score and the five domains, including practical problems. These results are summarized in Table 3.

Safety assessment

The dose increment schedule was shown in Table 2. After maintenance dose was reached (five weeks/six injections), injections were given monthly. The median time of treatment was 12 months. The median accumulated dose of *D. glomerata* was 1.48 mg (IQ range 1.44–2.83), and 1.66 mg (1.52–3.06) of *O. europaea*. All the patients reached the maximum dose planned.

IT was well tolerated by the patients. Seven patients experienced four local reactions (one active group and three placebo group) and there were no SR among patients from the active group during the study.

Compliance assessment

The overall adherence to treatment was excellent. All 53 patients (100%) who participated in this double-blind placebo-controlled study completed the treatment and reached the end of the trial.

Discussion

We have evaluated, for the first time, the benefits induced by modified therapeutic vaccines in the QOL of patients with AR. Results of this study provided evidence that IT with depigmented, glutaraldehyde-modified allergen extracts was a well-tolerated and effective treatment for AR in pollen allergic patients eliciting an improvement in QOL, which was so important to justify a change in the patient's treatment.

This study was designed as a prospective, randomized, double-blind and placebo-controlled study, which reinforces the results of the trial. The beneficial findings were based on consistent results, such as the improvement in symptom scores and medication requirements during the pollen season (Figs 3 and 4). These results are in agreement with previous reports of beneficial effects of IT with modified vaccines, as well as other depigmented modified extracts [9–12]. In addition, allergic patients of this study not only improved their symptoms and decreased medication requirements, but also the patients' QOL was improved. We should highlight that this improvement was found using a standardized QOL questionnaire. QOL questionnaires were introduced in the clinical evaluation of respiratory allergies by Juniper et al., in order to quantify the impact of symptoms under real life conditions and, afterwards, it was used to evaluate the efficacy of treatments in allergic diseases. We selected and based our study on the RQLQ, a disease-specific questionnaire, because it is a standardized and validated method. It has been demonstrated that RQLQ can be used with confidence to measure QOL in epidemiologic surveys, clinical trials, and patient monitoring. It has proven evidence of strong discriminative (reliability and cross-sectional construct validity) and evaluative (responsiveness and longitudinal validity) measurement properties [15–17]. Besides, disease-specific questionnaires offer better sensitivity to treatment effects although generic questionnaires allow for a compar-

ison of different disorders and patient population. An additional feature was that the 'minimal important difference' is also known, showing that mean changes in score from the RQLQ of greater than 0.5 are clinically important justifying a change in the patient's treatment [5, 33].

It is clearly stated in the literature [7] that there is a need of manuscripts studying the effects on QOL of therapeutic vaccines. Since then [7], two studies have analysed this issue: the Walker et al. paper [35] that showed statistically significant improvement in QOL and the Khinchi et al. manuscript [38] that showed no statistical difference in any of the nine concepts of the questionnaire. The first one used the RQLQ and the second one applied the SF-36 which is a generic questionnaire. Today, both the RQLQ and the SF-36 are used to document the impact of allergic rhinitis treatment on QOL [39]. In a comparison between the RQLQ and the SF-36, however, the RQLQ had strong evaluative and discriminative properties, whereas the SF-36 had acceptable discriminative properties but poor evaluative properties [40]. Our findings (RQLQ) are in agreement with those previously reported by Walker et al. [35], using an aqueous extract. Our results showed a statistically significant ($P < 0.05$) improvement in the overall QOL and in the domains of sleep, nonhayfever symptoms and nasal and eye symptoms, whereas non-significant differences were found in the domains of activities and emotions. Following the criterion reported by Juniper et al. this improvement would be enough to justify a change in the patient's treatment. We should stress that this improvement in QOL elicited by IT was found in spite of IT treated-patient group consumed fewer medications.

Other interesting feature of this study was the benefit found on using therapeutic vaccines based in a mixture of two taxonomically non-related pollen extracts in the same vaccine. Adkinson et al. [41] reported that multiple-allergen IT was not a beneficial treatment for allergic disease. Our results clearly disagree from those reported by Adkinson et al. [41], which might be because of: (i) allergic disease of our patients was not severe, (ii) patients did not need oral steroids to control symptoms and (iii) the allergen mixture was limited to two pollens. Although, results of this study clearly points out the benefit of IT with a mixture of two taxonomically non-related pollens, other studies should be performed in order to clarify what kind of patients might benefit of a mixture of allergen IT.

In summary, IT with depigmented and glutaraldehyde-modified allergen extracts was a well-tolerated and beneficial treatment for AR in pollen allergic patients eliciting an improvement in QOL so important to justify a change in the patient's treatment.

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CORRIGENDUM

Immunotherapy with depigmented glutaraldehyde-polymerized extracts: changes in quality of life

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In [1], on page 574, the text of the second paragraph has a mistake (in bold): “Two vials were prepared to be used as a therapeutic vaccine. Vial no. 2 contained 270 µg of *D. glomerata* and 280 µg of *O. europaea*, which were the result of depigmenting and polymerizing **100 HEP_L**, corresponding to 750 µg of freeze-dried extract of *D. glomerata* (60 µg/mL Dac g 2, 20 µg/mL Dac g 1+5) and 3550 µg of freeze-dried extract of *O. europaea* (131 µg/mL Ole e 1). Vial no. 1 was a 10-fold dilution of vial no. 2.”

The correct text is “Two vials were prepared to be used as a therapeutic vaccine. Vial no. 2 contained 270 µg of *D. glomerata* and 280 µg of *O. europaea*, which were the result of depigmenting and polymerizing **1000 HEP_L**, corresponding to 750 µg of freeze-dried extract of *D. glomerata* (60 µg/mL Dac g 2, 20 µg/mL Dac g 1+5) and 3550 µg of freeze-dried extract of *O. europaea* (131 µg/mL Ole e 1). Vial no. 1 was a 10-fold dilution of vial no.2.”

Reference

- 1 Álvarez-Cuesta E., Aragonese-Gilsanz E., Martín-García C., Berges-Gimeno P., González-Mancebo E., Cuesta-Herranz J. Immunotherapy with Depigmented Glutaraldehyde-Polymerized Extracts: Changes in Quality Of Life. Clin Exp Allergy 2005; 35:572–78.