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Table of Contents

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The role of allergoids in allergen immunotherapy: from injective to sublingual route

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Summary

Allergen immunotherapy (AIT) is aimed at inducing tolerance to allergens, such as pollens, dust mites or moulds, by administering increasing amounts of the causative allergen through subcutaneous or sublingual route. The evidence of efficacy of AIT is high, but the issue of safety, especially for the subcutaneous route, must be taken into account. The search for safer AIT products aimed at reducing the allergenicity, and thus adverse reactions, while maintaining the immunogenicity, that is essential for effectiveness, gave rise to the introduction of allergoids, which were conceived to fulfill these requirements. In the first allergoids glutaraldehyde or formaldehyde were used as cross-linking agent to polymerize allergens, this resulting in high molecular weight molecules (200,000 to 20,000,000 daltons) which were significantly less allergenic due to a decreased capacity to bridge IgE on its specific receptor, while maintaining the immunogenicity and thus the therapeutic efficacy. In recent years further agents, acting as adjuvants, were added to polymerized extracts. Moreover, a carbamylated monomeric allergoid was developed and, once adsorbed on calcium phosphate matrix, used by subcutaneous route. At the same time, in virtue of its peculiarities, such allergoid revealed particularly suitable for sublingual administration. A lot of clinical evidences show that it is well tolerated, largely safer and effective. Importantly, the higher safety of allergoids allows faster treatment schedules that favor patient compliance and, according to pharmaco-economic studies, they might be more cost-effective than other AIT options.

Background

Allergen immunotherapy (AIT) was introduced in 1911 by Noon and Freeman, with the provisional name of "desensitizing vaccine" (1). This treatment was aimed at reducing the reactivity to allergens, namely grass pollen, by subcutaneous administration of increasing amounts of the causative allergen but remained for decades merely empirical. The discovery of IgE antibodies in the 1960s (2) was crucial for the development of scientific knowledge on the mechanism of allergy, leading to a marked improvement in the diagnosis but also in the quality of allergen extracts for AIT (3). The introduction in the 1980s of immunotherapy products of high biological potency was a further step towards the quality improvement and the consequent reliability of AIT, but the issue of safety came to light. Reports of fatal reactions to subcutaneous immunotherapy from the UK (4) and the USA (5) were published, inducing to reappraise, especially in patients with allergic rhinitis, the feasibility of a treatment burdened by the risk of severe adverse reactions. Such an issue motivated the search for safer AIT products, intend-

ing to reduce the allergenicity, and thus adverse reactions while maintaining the immunogenicity that is essential to induce the immunological modification associated with effective AIT. The first approach to reach this goal was accomplished by introducing the allergoids, conceived to fulfill such the requirements, then followed by a dose reduction in co-administration of the allergen dosage concomitant to adjuvants, and by routes of administration different from the injective route.

The evolution of allergoids for subcutaneous immunotherapy

The first study on allergoids obtained by polymerization of allergens using glutaraldehyde as a cross-linking agent dates back to 1973 (6). Such chemical treatment resulted in high molecular weight molecules (200,000 to 20,000,000 daltons) which were significantly less allergenic due to a decreased capacity to bridge IgE on its specific receptor while maintaining the immunogenicity and thus the therapeutic efficacy. After 10 years of studies, Grammer et al. concluded that this approach was the most successful in providing a good balance of safety, efficacy and, and immunogenicity in multiple clinical trials (7). In Europe, the allergoids obtained by the treatment of the partially purified pollen extracts with formaldehyde were evaluated. In 1982 Puttonen et al. showed that the formaldehyde treatment resulted in a change of the net charge of proteins to the more acidic site, in a considerable reduction of the activities of naturally occurring enzymes of native allergen extracts, and the observation of only a trace of activity in the RAST inhibition assay (8). In the study by Bousquet et al. a lyophilized extract of grass pollen was dissolved in a phosphate buffer, adding formaldehyde to the solution to obtain a 10 mg/ml pollen extract. After incubation, the solution was dialyzed at +4" C to remove formaldehyde and lyophilized. The product was administered by a rush schedule and compared to SCIT with a common standardized grass extract. Both treatments were effective on grass induced rhinitis, more severe reactions were observed with the standardized extract, but also patients treated with the allergoid had SRs (9). The reduction but not abolition of SRs was also confirmed with other kinds of allergoids, such as the formalinized alum-absorbed allergoid. In a double-blind, placebo-controlled study on patients with grass-pollen allergy high doses of grass allergoid, corresponding to a cumulative pre-seasonal dosage of 46,050 protein nitrogen units (PNU), were administered, with only one systemic reaction. All patients were evaluated before and during the treatment by symptom-medication scores, specific nasal and skin reactivity, and immunological (specific IgE, IgG, IgG1 and IgG4 antibodies) parameters. The actively treated patients had significantly lower symptom-medication scores than placebo during the month of May and showed a significant decrease in specific skin and nasal reactivity, and a significant early increase in specific IgE, IgG, IgG1, and IgG4, with a subsequent decrease of IgE and IgG1 (10). A similar aluminum hydroxide-adsorbed depot allergen preparation produced by allergen modification by formaldehyde and titrated in therapeutic units (TU) was studied in a placebo-controlled trial on children with grass pollen-induced allergic rhinitis. Children in the immunotherapy group received 7 injections of grass pollen allergoid before grass pollen season and remained on maintenance treatment 27 months. Clinical and laboratory parameters were compared between the active and placebo-treated groups. After 1 year of immunotherapy, the rhino-conjunctivitis symptom-medication score was significantly lower in the immunotherapy group, and skin test reactivity and nasal reactivity to grass pollen were significantly decreased. Grass-specific IgG, IgG1 and IgG4 increased significantly already at the end of the s build-up therapy, while the seasonal increase in IgE was blunted by active treatment (11). A recent double-blind, placebo-controlled trial evaluated the dose-response relationship of the same allergoid preparation comparing a single species (*Phleum pratense*) and a multiple species mixture. Three doses of *P. pratense* allergoid (1800 TU, standard-dose 6000 TU and 18 000 TU) were compared with placebo and the marketed 6-grass pollen allergoid (6000 TU). The primary endpoint was the change in weal size in response to the intra-cutaneous testing before and after treatment, while secondary outcomes were the change in total nasal symptom score measured assessed in the allergen exposure chamber, the changes in *P. pratense*-specific IgG4 and the incidence of adverse events. All three doses of*the P. pratense*and the 6-grass pollen allergoid preparations were significantly superior to placebo for the primary endpoint, while no significant differences in the change in nasal scores were detected. The high-dose of*P. pratense*, when compared to the standard-dose, did not yield any additional significant benefit, but was associated with a slight increase in adverse reactions (12). Further allergoid preparations include the addition to polymerization (by glutaraldehyde or formaldehyde) of L-tyrosine and monophosphoryl lipid A, aluminum hydroxide.

Henmar et al. performed a direct comparison of three intact allergen extracts and four allergoids using IgE inhibition and basophil activation assays to measure the allergenicity, the human T cell proliferation and specific IgG-titres following mouse immunizations to assess immunogenicity of all products. The results showed important differences in both allergenicity and immunogenicity, that require specific documentation of clinical safety and efficacy for each product (13). As far as safety is concerned, the Paul-Ehrlich-Institute published a report on adverse drug reactions (ADRs) to injective immunotherapy from 1991 to 2000. ADRs to allergoids classified as serious were evaluated between 0.01% and 0.0005%, corresponding to one serious ADR in 10,000 to 200,000 injections. Although based only on absolute numbers, the hypothetical assumption regarding better

tolerance of the allergoids compared to native allergen preparations was not confirmed, while concerning delayed ADRs 75% of them were related to unmodified semi-depot preparations, and 25% were related to allergoids (14). In a recent review by Rajakulendran et al. on novel strategies for AIT, which analyzed the data from grass pollen allergoids currently available, the pharmaco-economic aspects were also considered. Based on the available studies, the authors concluded that allergoids, mainly based on their shorter schedules of administration, might be more cost-effective than other AIT options (15).

The development of allergoids for sublingual immunotherapy

A particular allergoid to be administered by sublingual route has been developed. and used for almost 30 years. The product used was a carbamylated monomeric allergoid, which is a chemically modified allergen obtained by substitution of ε-amino-groups of allergen lysine residues, which reduces IgE-binding activity while preserving immunogenicity. Initially this allergoid was used for subcutaneous route (16) once adsorbed into a matrix of calcium phosphate; at the same time the peculiarities (monomericity) of this allergoid made it particularly suitable for sublingual administration. The definition of monomeric derives from the selectivity of carbamylation, which does not concern the structural conformation, with no increase of the size of the allergen molecule as occurs with polymerization. The first double-blind, placebo-controlled trial on the efficacy of an allergoid administered by the sublingual route was published into Lancet as a demonstration of its originality. In patients with mite-induced rhinitis, active treatment resulting in significantly lower symptom scores and a significant decrease of the immune-mediated inflammatory response (17). The second trial evaluated the efficacy of sublingual tablets of monomeric allergoid obtained from grass pollen in children with rhinitis and asthma caused by grass pollen. Children receiving a preseasonal active treatment had a significant reduction of symptoms scores, particularly bronchial symptoms, and a decrease of nasal eosinophil cationic protein, with good tolerance to the allergoid (18). The safety in children was confirmed in subjects aged less than 5 years treated with either mite of grass pollen monomeric allergoids (19). A further safety study evaluated 105 patients (28 children and 77 adults) undergoing SLIT with a mite or grass pollen or Parietaria pollen by an ultra-rush schedule reaching the top dose in 20 minutes. Only one patient (0.9%) had an adverse reaction consisting of gastric pyrosis, with spontaneous recovery (20). Indeed, several other studies on the efficacy and safety of monomeric allergoids are available, which were analyzed in 2010 by Mösges et al., in a systematic review and meta-analysis. The global number of patients with allergic rhinitis included in these studies were 266 for grass pollen and 241 mite allergoid. The average improvement in symptom scores was 34% for grass pollen and 22% for mite allergoid in comparison with the placebo group, and the average improvement in medication scores was 49% and 24% for grass pollen and mite allergoid, respectively. Few side effects, with no systemic reactions, were reported in the trials (21). The most recent studies investigated the dose-dependence and dose-finding of monomeric allergoids. The first study evaluated the efficacy and safety of the dose of 1000 or 2000 allergy units (AU) in 34 mite allergic patients, using as primary outcome the change of the threshold of allergen concentration inducing a positive nasal provocation test. After 12 weeks all patients treated with 1000 AU and all but one treated with 2000 AU had an increase in the threshold dose inducing positive provocation tests. The rate of adverse reactions, all mild, was comparable with the two doses (22). In a randomized, double-blind, phase 2 study on 158 adult patients with grass pollen-induced rhinoconjunctivitis, four different doses, equal to 300, 600, 1000 and 2000 UA/day were administered. The rate of patients with no symptoms to conjunctival provocation test after treatment was 54.3, 47.6, 59.0 and 51.4%, respectively, suggesting 1000 UA/day as the optimal dose No serious adverse event was reported (23). However, in a 12-week double-blind, placebo-controlled dose-finding study on 131 patients with mite-induced rhino-conjunctivitis receiving the dose of 300, 1000, 2000. Or 3000 UA/day, the highest rate of treatment response, as assessed by the conjunctival provocation test, was observed with the 2000 UA/day (88.5%). An overall number of 20 treatment-related adverse events (all mild) were recorded (24). The positive clinical outcomes of the carbamylated monomeric allergoid are supported by immunological investigations, which disclosed that the mechanisms of action are those illustrated for AIT in general. In fact, SLIT with mite monomeric allergoid was shown to down-regulate allergen-specific IgE and to increase interferon-gamma- and interleukin (IL)-10 production, commonly associated with the development of allergen tolerance (25). The up-regulation of IL-10 was detected also during a short-term course (60 days) of SLIT with grass monomeric allergoid, along with allergen-specific T-cell proliferation and reduction of allergen-specific in vitro proliferation (26). In a study comparing two induction schedules of SLIT with mite monomeric allergoid of different duration (98 days vs. 16 days) the more rapid induction scheme was associated with a reduction in TNF-alpha and IL-4 at the end of induction (27).

For complete information of the reader, **table I** summarizes the main results of all the available studies on SLIT with carbamylated monomeric allergoid.

Table I *- Summary of the main results of all the available studies on SLIT with carbamylated monomeric allergoid.*

Allergen	Study	Study objective	Study design	No patient	Patology	Results
Lais Mites - Chemically Modified Allergen Extract of house dust mites (*Dermatophagoides pteronyssinus* 50%, *Dermatophagoides farinae* 50%)	*Pacor ML (1995) [30]*	Efficacy and safety	Open observational Study	14/-	Asthma of light or moderate degree	Before and after the treatment: · Reduction of the number and severity of asthma attacks (p<0.001) · Improving the expiratory peak flow (PEF) (p<0.001). · No side effects were observed and all patients concluded the study
	Passalacqua G (1998) [17]	Efficacy and safety	Randomised, placebo controlled, double-blind, parallel study	10 Active / 9 Placebo	Perennial rhinoconjunctivitis, at least for 2 years	Active vs Placebo: · Neutrophilic infiltration decreased (p=0·002). · Eosinophilic infiltration decreased before challenge (p=0·001). · ICAM-1 expression reduced before challenge (p=0·01) and during and after treatment (p=0·002) · ECP decreased after 12 months of treatment (p=0·04) · The treatment was well tolerated. 1 local (oral itching) side-effects in active group
	Lombardi (2001) [31]	Safety	Observational Study	69/-	Perennial or seasonal rhinitis and/or mild asthma	· 17 adverse events corresponding to 7.5% of patients and 0.52 per 1000 doses: 7 episodes of rhinitis, 3 of oral itching, and 1 of abdominal pain. Two cases of urticaria and two of abdominal pain/nause were controlled by a temporary dose-adjustment, and one case of urtìcaria and conjunctivitis required oral antihistamines. · Medical intervention was needed in six patients only during a 3-year period. · No severe systemic side-effect *The events reported as results of Lombardi's study were observed in 198 patients receiving different SLIT treatments (69 patients – Mites ;75 patients – Grasses; 46 – Parietaria; 4 Birch; 1 Olive; 3 Compositae)
	Passalacqua G (2006) [32]	Efficacy and Safety	Randomized, placebo-controlled, double-blind, multicenter	34/34	Mild persistent rhinitis with/without mild intermittent asthma, since at least 2 years	Active vs Placebo: · Fifty-six patients completed the study (28 Active/ 28 Placebo) · A significant difference in the clinical score after 1 year of treatment (P = 0.027) · A significant difference for the symptom *nasal obstruction* after 1 year (P=0.05) and 2 years (P=0.033) · A significant global drug intake at the first year of treatment (P = 0.036) · A significant change in SLIT group was seen for the item *change in health status* (P = 0.05) after the second year of treatment. · No relevant side effect was reported (30 *vs* 43 events) · The need for extra visits was lower in the active group (25% *vs* 43%)
	Cosmi L (2006) [25]	Efficacy	Open, randomized, two arm parallel group: one treated with SLIT, one untreated (UT) and receiving only rescue symptomatic drugs	12 SLIT-treated/ 13 untreated (UT)	Perennial rhinitis and/or rhinitis plus mild asthma	Active vs Control: · Twenty patients (80%) completed the study (11 T and 9 UT). · A significant reduction of symptom medication scores after 12 and 18 months of treatment (P<0.05) · Reduction of Dp-specific IgE after 12 and 18 months (P<0.05 and P<0.005 respectively) of therapy · The serum levels of CXCL10 (an IFN-g-driven chemokine) after 12 and 18, but not after 6 months, of treatment were significantly higher (P<0.05) · IL-10 were significantly increased (P<0.05) in culture supernatants of PBMC from 6 month-treated patients in comparison with those detected at the beginning of therapy
	Giordano T (2006) [33]	Efficacy and safety	Open observational study	27	moderate/ severe rhinitis, with or not moderate asthma, perennial or seasonal	· Improvement of the VAS scores was observed. · Decrease of the drug consumption {p<0.01). · No side effects: Only two mild adverse reactions: somnolence and tiredness *The study observed 39 patients house-dust mite (n. 27), grass pollen (n. 7), olive pollen (n. 3), cat dander (n. 1) and Parietaria pollen (n. 1).

Allergen	Study	Study objective	Study design	No patient	Patology	Results
Lais Betulle-Chemically modified allergen extract of trees pollens (Betula pendula 50%, Alnus incana 50%)	*D'Anneo RW (2010) [34]*	Efficacy and Safety	Prospective, open-label, randomized study included two parallel groups one treated with SLIT, one treated with standard pharmaco -therapy (control group)	15/15	Intermittent or persistent rhinitis or rhino conjunctivitis and/or intermittent, mild-persistent or persistent moderate-severity allergic asthma	SLIT group vs Control: · All patients very well tolerated both the four-day build-up phase and the 12-month maintenance phase · Visual Analogue Scale rises significantly, about 45%, in both groups (p=0.001). · Reduction in the global symptom score SLIT group vs control group, about 52% (p=0.0004). · Smaller rescue drug consumption SLIT group vs control group, about 9%. · The difference between before SLIT (T0) and after 12 months (Tl) was highly significant in skin reactivity (p=0.000003). The control group had a small increase in skin-reactivity (2.6±15.7%) with significance between T0 and Tl (p=0.5226).
	Burastero SE (2009) [35]	Efficacy and Safety	Open observational, parallel grouped: active and placebo	11/11	Seasonal allergic rhino conjunctivitis with or not mild asthma	· Two patients had transient itching in their mouth, spontaneously disappeared. · During the pollen season symptoms/drug usage scores improved of 30% and 40% respectively in actively treated and control patients (p<0.0001); well-days (days without intake of rescue medications and symptoms score less than 2) were in 33% and 23% of patients respectively (p=0.0024).
	L. Bommarito (2009) [36]	Efficacy	Open, randomized, parallel group: three active groups	8 T1+ 8 T2 /5 T3 (Drug Therapy alone)	Allergic rhinoconjunctivitis with/without mild intermittent asthma	· T1 vs T2: significant improvement of both nasal obstruction (p<0.01) and other symptoms (p<0.01). · Significant reduction of antihistamine consumption as well as rescue medication score in T1 vs T3 patients (p<0.05). · T2 vs T3 patients reported less nasal congestion and ocular symptoms in 2008 season (p< 0.01). · No significant AR have been observed.
	Passali D (2010) [37]	Efficacy and Safety	Prospective, open, randomized study, with three parallel groups and control group	4 (Group A) / 3 (Group B) / 3 (Group C) / 3 (control)	Rhinitis and oculo-rhinitis	Treated VS Control · All patients tolerated all the three dosage very well, no patient interrupted · A statistically significant (p < 0.02) reduction of SMSs vs control group · Significant (p < 0.01) decrease in nasal reactivity the three SLI T-treated groups, while the untreated controls remained unchanged · A significant increase in VAS values has been observed in all 3 study groups, in comparison to the controls (p < 0.001). · During up-dosing 4 slight side-effects in 4 patients, 1 somnolence and 1 tiredness, and 2 oral itching. No side-effects were recorded during the maintenance treatment.
	Marogna M (2013) [38]	Efficacy and Safety	Open randomized parallel 4 groups study: Group 1: BUD 400 mcg/day + anti Lt/s Group 2: BUD 800 mcg/day Group 3: BUD 1600 mcg/day Group 4 : BUD 400 mcg/day + SLIT	Group 1 (n=21) / Group 2 (n=21) / Group 3 (n=21) / Group 4 (n=21)	Seasonal mild and persistent asthma and normal lung function associated with AR	· A significantly performance associated with the use of SLIT; only patients of group 4, achieved an appreciable control (mean 24; SEM 0.242). · A significant improvement in allergy symptoms-medications scores (SMS), in patients of group 4 (decrease of 87%) than in all other groups (p < 0.01). · The FEV1 increase and the albuterol intake in group 4 was significantly lower after three years (p < 0.001), · Reduction of nasal eosinophils and nasal corticosteroids in group 4 · Significant difference in the PD20 was detected at baseline between the controls and the 1,000 AU and between the 1,000 and 2,000 AU groups · During the three years of SLIT course, two patients reported one episode of occurred during the maintenance phase and self-resolved without any therapy in less than two hours.

Allergen	Study	Study objective	Study design	No patient	Patology	Results
Lais Grasses-Chemically modified allergen extract of grass pollens (Holcus lanatus 33%, Phleum pratense 33%, Poa pratensis 33%)	*Bordignon V (1994) [39]*	Efficacy	Randomised, placebo-controlled, double-blind parallel study	30/30	Perennial rhino conjunctivitis and/ or asthma at least for 2 years	Active vs Placebo: · A statistically significant reduction of nasal and bronchial symptoms particularly after the second and the third years of treatments ($p < 0.01$). · Significant reduction of drugs consumption ($p < 0.01$)
	Pacor M.L. (1996) [40]	Efficacy	Open non comparative	34	Seasonal rhino conjunctivitis	· After 1 years, reduction of symptoms: sneezing ($p<0.001$), nasal itching ($p<0.001$) and ocular symptoms ($p<0.001$) and improvement at the second year · Significant reduction of antihistamine consumption ($p<0.001$) · Treatment well tolerated and no side effects
	Caffarelli C. (2000) [18]	Efficacy and safety	Randomised, double-blind, placebo-controlled study	24 active / 24 placebo	Seasonal rhinitis and/ or rhino-conjunctivitis and/ or bronchial asthma	Active vs Placebo: · 44 out of 48 patients (91.6%), all 24 in the active treatment group and 20 of 24 given placebo, completed the study: three because they moved away, and one because of a mild side-effect (abdominal pain) · Significant reduction of total symptoms ($P<0.05$) during the pollen season · Treatment well tolerated and compliance was good · EG2/EGl increased significantly only in the placebo group during natural allergen exposure ($P< 0.01$)
	Lombardi C (2001) [41]	Efficacy and safety	Open, controlled study	26 (pharmaco-therapy + SLIT) / 25 (pharmaco-therapy only)	Seasonal rhinoconjunctivitis and/or asthma (mild intermittent or mild persistent)	Active vs Control: · Significant increase ($p=.0.01$) of PD20 at the methacholine · Significant clinical improvement both for rhinitis ($p = 0.001$) and asthma ($p=0.001$) · Reduction of drug intake ($p= 0.001$) · Improvement of rhinitis symptom without modification of drug intake · Treatment well tolerated and no relevant side effects during the 3 years.
	Lombardi C (2001) [31]	Safety	Observational Study	75/-	Perennial or seasonal rhinitis and/or mild asthma	· 17 adverse events corresponding to 7.5% of patients and 0.52 per 1000 doses: 7 episodes of rhinitis, 3 of oral itching, and 1 of abdominal pain. Two cases of urticaria and two of abdominal pain/nause were controlled by a temporary dose-adjustment, and one case of urtìcaria and conjunctivitis required oral antihistamines. · Medical intervention was needed in six patients only during a 3-year period. · No severe systemic side-effect *The events reported as results of Lombardi's study were observed in 198 patients receiving different SLIT treatments (69 patients – Mites ;75 patients – Grasses; 46 – Parietaria; 4 Birch; 1 Olive; 3 Compositae)
	Quercia O (2001) [42]	Efficacy and safety	Prospective, randomized, open controlled trial with three parallel groups.	Group 1 (n=10), Group 2 (n=11) and Group 3 (n=11).	Rhino-conjunctivitis with/without mild intermittent asthma	· Significant VAS improvement in both SLIT groups, after the first and second pollen season, compared to baseline and to Group 3($p<0.05$). · Less symptoms and need for medications resulted during the second season ($p<0.05$). · Lower drug assumption was significantly in both SLIT groups during the second season ($p<0.05$) · Lower global symptoms score in comparison Group 1 and Group 2 vs Group 3 during the second pollen season ($p<0.05$) · Treatment well tolerated, only 2 patients reported local or mild adverse events and one of this has interrupted the study (Group 1 - originally 11).

Allergen	Study	Study objective	Study design	No patient	Patology	Results
Lais Grasses-Chemically modified allergen extract of grass pollens (Holcus lanatus 33%, Phleum pratense 33%, Poa pratensis 33%)	*A.G. Palma Carlos (2006) [43]*	Efficacy and safety	Monocentric randomised, double-blind, placebo controlled	17 Active / 16 Placebo	Seasonal rhinoconjunctivitis with or not intermittentor mild persistent asthmas since at least two years	Active vs Placebo: · 20 patients out of the 33 enrolled (60.6%) completed the study (13 Active/ 7 Placebo) · Statistically significant decrease of symptom scores (conjunctivitis p<0.02, rhinorrea p<0.03 and sneezing p< 0.03) · Statistically significant decrease of nasal reactivity at the second year of treatment (p<0.03) · Lower consumption of inhaled steroids, mean monthly scores (P < 0.02) · Treatment well tolerated; 2 mild local adverse events occurred without interruption of therapy
	Burastero, S.E (2008) [26]	Efficacy	Open, observational pilot study	11	Rhinoconjunctivitis with or not mild asthma for at least 2 years	· Decrease in Allergen-Specific Proliferation to the rPhl p 1 and to the raw grass extract after 2 Months of SLIT (P= .002 and .04) · Increase in Transcription of IL-10 (P < .001) and TGF-β (P = .06), at rPhl p1–Stimulated Lymphocytes · Correlation indexes of pre-treatment and post-treatment changes in IL-10 vs TGF-β expression were 0.17 (P .47) and 0.16 (P .70), respective
	Ariano R (1998) [44]	Efficacy and safety	Randomised, placebo controlled, double-blind parallel study.	15/15	Allergic rhinitis with or without asthma	Active vs Placebo: · Improvement of score symptoms and drug consumption with a statistically significant difference at the end of the treatment (p<0.01) · Comparison of the areas of the skin tests and RAST before and after treatment showed no statistically significant difference in the two groups. · Comparison of nasal or bronchial provocation test before and after treatment with statistically significant difference (p<0.05) No side effect observed: one patient of active group discontinued the treatment owing to digestive troubles (Active Group – 14 out of 15 completed the study)
	Lombardi C (2001) [31]	Safety	Observational Study	46/-	Perennial or seasonal rhinitis and/or mild asthma	· 17 adverse events corresponding to 7.5% of patients and 0.52 per 1000 doses: 7 episodes of rhinitis, 3 of oral itching, and 1 of abdominal pain. Two cases of urticaria and two of abdominal pain/nause were controlled by a temporary dose-adjustment, and one case of urticaria and conjunctivitis required oral antihistamines. · Medical intervention was needed in six patients only during a 3-year period. · No severe systemic side-effect · *The events reported as results of Lombardi's study were observed in 198 patients receiving different SLIT treatments (69 patients – Mites ;75 patients – Grasses; 46 – Parietaria; 4 Birch; 1 Olive; 3 Compositae)
	Arena A (2003) [45]	Efficacy and tolerability	Prospectic Observational Study	24 SLIT / 11 SIT / 9 pharmacological therapy	Rhinitis and/or mild intermittent or persistent asthma or conjunctivitis	· 8 patients interrupted the immunotherapy during the study period: 3 SLIT group and 5 SIT group · The physician's opinion on efficacy, by symptoms and drug consumption reduction, was statistically better in the SLIT group than in the other two groups (p< 0.0001). · The difference between the patient's degree of satisfaction of treatments was statistically significant in favour of SLIT treatments (p< 0.0001). * The events reported as results of a study observed in 110 patients receiving different treatments (Parietaria, Graminacea, Olea, Dermathopaghoides)
	Lombardi C (2004) [46]	Safety	Multicenter observational Study	18	Allergic rhinitis and/or asthma at least 2 years	· 11 mild side effects were reported in 6 (7%*) patients: 6 oral itching, 2 rhinitis, 2 nausea, and 1 generalized itching · Omitted dose was documented in 11 patients. *on a total of 86 patients: 41 received SLIT to mite and 45 to pollens (24 grasses, 18 Parietaria, 3 Ragweed).

Allergen	Study	Study objective	Study design	No patient	Patology	Results
Lais Parietaria-Chemically modified allergen extract of parietaria pollens (Parietaria judaica 50%, Parietaria officinalis 50%)	*Gammeri E (2005) [20]*	Safety and the tolerability	Open sequential Non controlled	34	intermittent/ persistent rhinitis or intermittent/ mild persistent asthma	Only 1 patient out of 105* (0.9 %) had a mild local symptom (gastric pyrosis) that occurred 30 minutes after the last initial dose and spontaneously disappeared as the treatment was continued. *The study observed 105 patients [Dust (n = 56), Parietaria (n = 34) and Timothy-grass (n =15)]
	La Grutta S (2007) [47]	Efficacy	Prospective, open-controlled randomised	33 SLIT / 23 Control *56 pt allergic to House Dust mite with (n-36) or without Parietaria	mild persistent asthma with or not moderate intermittent moderate rhinitis	Active vs Control · All patients completed the study · Greater reduction daily of the mean symptom score (p<0.01) and drug consumption (p<0.001) in the SLIT than in the control group. · MCh PD20 increased only in the SLIT group(p<0.0005) · The reduction of nasal eosinophils was statistically greater (P<0.05) only in the SLIT group.
	D'Anneo RW (2008) [48]	Efficacy and safety	Prospective, randomized, With three parallel Groups receiving either two different dosages of SLIT or the standard chronic	24 (SLIT 1,000 AU/week) / 21 (SLIT 3,000 AU/ week) / 21 (drug therapy)	Seasonal rhinoconjunctivitis and/or asthma (mild intermittent or mild persistent)	· VAS: at the 3rd month: p < 0.05 improvement in group of higher dose vs control; after 6 months, VAS in the SLIT groups is statistically better than control (p < 0.05) · Reduction in rescue medication consumption between 3 and 6 months (p < 0.05) in all 3 groups. · Reduction bronchial reactivity in the SLIT groups (p < 0.001). · Significant increase of MCh PD20 at the end of the study, in both the patients treated with 1,000 AU (p < 0.05) and in those treated with 3,000 AU (p < 0.001) · No adverse events were observed, no patient interrupted the study
	Passali D (2010) [37]	Safety and efficacy	Prospective, open, randomized study, with three parallel groups and control group	4 (Group A) /3 (Group B) / 2 (Group C) / 2 (Control)	Rhinitis and oculo-rhinitis	Treated VS Control · All patients tolerated all the three dosage very well, no patient interrupted · A statistically significant (p < 0.02) reduction of SMSs vs control group · Significant (p < 0.01) decrease in nasal reactivity the three SLI T-treated groups, while the untreated controls remained unchanged · A significant increase in VAS values has been observed in all 3 study groups, in comparison to the controls (p < 0.001). · During up-dosing 4 slight side-effects in 4 patients, 1 somnolence and 1 tiredness, and 2 oral itching. No side-effects were recorded during the maintenance treatment.

Conclusions

The introduction of allergoids was an actual advance for AIT with inhalant allergens, providing a response to the problem of systemic reactions to injective immunotherapy, which rather commonly hindered the performance of the treatment, being rarely able even to result in fatal events. Abundant literature supports the role of allergoids in AIT, including for injective AIT several types, obtained by different chemical treatments of the natural allergens to reduce allergenicity while maintaining the immunogenicity and thus the therapeutic efficacy. Also, a product to be used by the sublingual route is available, which consists of the carbamylated monomeric allergoid, which has good evidence of efficacy and safety. Still, there is room for allergoids characterization, taking into account the allergoids require more sophisticated analytical methods than native extracts (28). In addition, in the current landscape of the regulatory requests governing allergen products, special requirements need to be implemented for control of allergoids (29). We have identified a total of 24 journal articles reporting 313 participants as total number of active patients and 298 participants as total number of placebo/control group (Lais Mites: 64 active/ 61 placebo-control ; Lais Birch 55 active /82 placebo-control; Lais Grass 114 active/ 95 placebo-control; Lais Parietaria 80 active/ 60 placebo-control).

Conflict of interests

C. Cavaliere and S. Masieri declare that they have no conflict of interests, financial or otherwise. C. Incorvaia is a scientific consultant for Stallergenes Italy. FF, CE, GA, MG are employees of Lofarma SPA.

Credit author statement

IC, CC, CE, FF, MG Conceptualization, Resources. CC, IC Writing - Original Draft. FF, GA, MS Writing - Review & Editing.

References

1. Frew AJ. Hundredyearsofallergen immunotherapy. Clin Exp Allergy. 2011; 41(9): 1221-1225.
2. Johansson SG, Bennich H, Wide L. A new class of immunoglobulin in human serum. Immunology. 1968;14(2):265-72.
3. Berings M, Karaaslan C, Altunbulakli C, Gevaert P, Akdis M, Bachert C, et al. Advancesand highlights inallergen immunotherapy: On the way to sustained clinical andimmunologictolerance. J Allergy Clin Immunol. 2017;140(5):1250-67.
4. Committee on Safety in Medicine. CSM update: desensitizing vaccines. BMJ 1986; 293:948.
5. Lockey RF, Benedict LM,Turkeltaub PC,Bukantz SC. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol 1987;79:660-77.
6. Patterson R, Suszko IM, McIntire FC. Polymerized ragweed antigen E. I. Preparation and immunologic studies. J Immunol 1973;110(5):1402-12.
7. Grammer LC, Shaughnessy MA, Patterson R. Modified forms of allergen immunotherapy. J Allergy Clin Immunol. 1985;76(2 Pt 2):397-401
8. Puttonen E, Maasch HJ, Pilström L. Studies of allergen and allergoid preparations from purified timothy (Phleum pratense) pollen extracts. I. Physicochemical characteristics and binding to allergen-specific human IgE. Int Arch Allergy Appl Immunol. 1982;68(1):1-6.
9. Bousquet J, Hejjaoui A, Skassa-Brociek W, Guérin B, Maasch HJ, Dhivert H, et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen extract. J Allergy Clin Immunol. 1987;80(4):591-8.
10. Pastorello EA,Pravettoni V,Incorvaia C,Mambretti M,Franck E,Wahl R,et al. Clinical and immunological effects of immunotherapy with alum-absorbed grassallergoidin grass-pollen-induced hay fever. Allergy.1992;47(4 Pt 1):281-90.
11. Keskin O,Tuncer A,Adalioglu G,Sekerel BE,Saçkesen C,Kalayci O. The effects of grass pollenallergoidimmunotherapy on clinical and immunological parameters in children with allergic rhinitis. Pediatr Allergy Immunol.2006;17(6):396-407.
12. Pfaar O,Hohlfeld JM,Al-Kadah B,Hauswald B,Homey B,Hunzelmann N,et al. Dose-response relationship of a new Timothy grass pollenallergoidin comparison with a 6-grass pollenallergoid. Clin Exp Allergy.2017;47(11):1445-55.
13. Henmar H, Lund G, Lund L, Petersen A, Würtzen PA. Allergenicity, immunogenicity and dose-relationship of three intact allergen vaccines and four allergoid vaccines for subcutaneous grass pollen immunotherapy. Clin Exp Immunol. 2008;153(3):316-23.
14. Lüderitz-Püchel U, Keller-Stanislawski B, Haustein D. Neubewertung des Risikos von:709–18 Test- und Therapieallergenen. Eine Analyse der UAW Meldungen von 1991 bis 2000. Bundesgesundheitsbl Gesundheitsforsch Gesundheitsschutz 2001;44.
15. Rajakulendran M, Tham EH, Soh JY, Van Bever HP. Novel strategies in immunotherapy for allergic diseases. Asia Pac Allergy. 2018;8(2):e14.
16. Galimberti M., Cantone R., Pastore M., Mistrello G. e Falagiani P."Immunotherapy with grass allergoid (Modall). Preliminary results". Italian Journal of Chest Diseases, Suppl. 6 (Nov / Dec. 1986).
17. Passalacqua G, Albano M, Fregonese L, Riccio A, Pronzato C, Mela GS, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. Lancet. 1998 28;351(9103):629-32.
18. Caffarelli C, Sensi LG, Marcucci F, Cavagni G. Preseasonal local allergoid immunotherapy to grass pollen in children: a double-blind, placebo-controlled, randomized trial. Allergy. 2000;55(12):1142-7.
19. Agostinis F, Tellarini L, Canonica GW, Falagiani P, Passalacqua G. Safety of sublingual immunotherapy with a monomeric allergoid in very young children. Allergy. 2005;60(1):133
20. Gammeri E, Arena A, D'Anneo R, La Grutta S. Safety and tolerability of ultra-rush (20 minutes) sublingual immunotherapy in patients with allergic rhinitis and/or asthma. Allergol Immunopathol (Madr). 2005 Jul-Aug;33(4):221-3.
21. Mösges R, Ritter B, Kayoko G, Allekotte S. Carbamylated monomeric allergoids as a therapeutic option for sublingual immunotherapy of dust mite- and grass pollen-induced allergic rhinoconjunctivitis: a systematic review of published trials with a meta-analysis of treatment using Lais® tablets. Acta Dermatovenerol Alp Pannonica Adriat. 2010;19(3):3-10.
22. Scalone G, Compalati E, Bruno ME, Mistrello G. Effect of two doses of carbamylated allergoid extract of dust mite on nasal reactivity. Eur Ann Allergy Clin Immunol. 2013;45(6):193-200.
23. Mösges R, Rohdenburg C, Eichel A, Zadoyan G, Kasche EM, Shah-Hosseini K, et al. Dose-finding study of carbamylated monomeric allergoid tablets in grass-allergic rhinoconjunctivitis patients. Immunotherapy. 2017;9(15):1225-38.
24. Hüser C, Dieterich P, Singh J, Shah-Hosseini K, Allekotte S, Lehmacher W, et al. A 12-week DBPC dose-finding study with sublingual monomeric allergoid tablets in house dust mite-allergic patients. Allergy. 2017;72(1):77-84
25. Cosmi L, Santarlasci V, Angeli R, Liotta F, Maggi L, Frosali F, et al. E. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. Clin Exp Allergy. 2006;36(3):261-72.
26. Burastero SE, Mistrello G, Falagiani P, Paolucci C, Breda D, Roncarolo D, et al. Effect of sublingual immunotherapy with grass monomeric allergoid on allergen-specific T-cell proliferation and interleukin 10 production. Ann Allergy Asthma Immunol. 2008;100(4):343-50.

27. Di Gioacchino M, Perrone A, Petrarca C, Di Claudio F, Mistrello G, Falagiani P, et al. Early cytokine modulation after the rapid induction phase of sublingual immunotherapy with mite monomeric allergoids. Int J Immunopathol Pharmacol. 2008;21(4):969-76.
28. Carnes J, Gallego MT, Moya R, Iraola V. Allergoids for allergy treatment. Recent Pat Inflamm Allergy Drug Discov. 2018;12(2):110-19.
29. Zimmer J, Bonertz A, Vieths S. Quality requirements for allergen extracts and allergoids for allergen immunotherapy. Allergol Immunopathol (Madr). 2017;45 Suppl 1:4
30. Pacor ML, Biasi D, Carletto A, Lunardi C. Effectiveness of oral immunotherapy in bronchial asthma caused by Dermatophagoides pteronyssinus. Recenti Prog Med 1995; 86(12):489-91.
31. Lombardi C, Gargioni S, Melchiorre A, Tiri A, Falagiani P, Canonica GW, et al. Safety of sublingual immunotherapy with monomeric allergoid in adults: multicenter post-marketing surveillance study. Allergy 2001; 56:989-92.
32. Passalacqua G, Pasquali M, Ariano R, Lombardi C, Giardini A, Baiardini I, et al. Randomized double blind controlled study with sublingual carbamylated allergoid immunotherapy in mild rhinitis due to mites. Allergy 2006; 61: 849-54.
33. Giordano T, Quarta C, Bruno ME, Falagiani P, Riva G. Safety, tolerability and efficacy of sublingual allergoid immunotherapy with a 4-day shortened build-up phase. Eur Ann Allergy Clin Immunol 2006; 38:310-2
34. D'Anneo RW, Bruno ME, Falagiani P. Sublingual allergoid immunotherapy: a new 4-day induction phase in patients allergic to house dust mites. Int J Immunopathol Pharmacol 2010; 23:553 -60.
35. Burastero S, Mistrello G, Paolucci C, Breda D, Roncarolo D, Zanotta S et al. Clinical and immunological correlates of pre-co-seasonal sublingual immunotherapy with birch monomeric allergoid in patients with allergic rhinoconjunctivitis. Int J Immunopathol Pharmacol. 2009; 22: 343-52.
36. Bommarito L, Bruno ME, Nebiolo F, Moschella A, Zanierato G, Mistrello G, et al. Efficacy and safety of sublingual immunotherapy with birch monomeric allergoid: a comparison of two different treatment regimens versus pharmacological one. Allergy 2009;64(90) 99-178.
37. Passali D, Mösges R, Passali GC, Passali FM, Ayoko G, Bellussi L. Safety, tolerability and efficacy of sublingual allergoid immunotherapy with three different shortened up-dosing administration schedules. Acta Otorhinolaryngol Ital. 2010;30(3):131-7.
38. Marogna M, Braidi C, Bruno ME, Colombo C, Colombo F, Massolo A, et al. The contribution of sublingual immunotherapy to the achievement of control in birch-related mild persistent asthma: A real-life randomised trial. Allergol Immunopathol (Madr). 2013;41(4):216-24.
39. Bordignon V, Di Berardino L. Efficacy of a new oral immunotherapy for grass. Three years parallel study. Giorn. It. Allergol. Immunol. Clin. 1994; 4:153-59.
40. Pacor ML, Biasi D, Carletto A, Maleknia T, Lunardi C. Oral Immunotherapy in the treatment of rhinoconjunctivitis due to grass pollen. Recenti Prog Med. 1996; 87:4-6.
41. Lombardi C, Gargioni S, Venturi S, Zoccali P, Canonica GW, Passalacqua G. Controlled study of preseasonal immunotherapy with grass pollen extract in tablets: Effect on bronchial hyperreactivity. J Invest Allergol Clin Immunol 2001; 11(1):41-5.
42. Quercia O, Bruno ME, Compalati E, Falagiani P, Mistrello G, Stefanini GF. Efficacy and safety of sublingual immunotherapy with grass monomeric allergoid: comparison between two different treatment regimens. Eur Ann Allergy Clin Immunol. 2011;43(6):176-83.
43. Palma Carlos AG, Santos AS, Branco-Ferreira M, Pregal AL, Palma Carlos ML, et al. Clinical efficacy and safety of preseasonal sublingual immunotherapy with grass pollen carbamylated allergoid in rhinitic patients. A doubleblind placebo-controlled study. Allergol Immunopathol (Madr) 2006; 34:194-198.
44. Ariano R, Panzani RC, Augeri G. Efficacy and safety of oral immunotherapy in respiratory allergy to Parietaria judaica pollen. A double-blind study J Investig Allergol Clin Immunol. 1998;8(3):155-60.
45. Arena A, Barbatano E, Gammeri E, Bruno M, Riva G. Specific immunotherapy of allergic diseases: a three years perspective observational study. Int J Immunopathol Pharmacol 2003; 16:277-82
46. Lombardi C, Gani F, Landi M, Falagiani P, Bruno M, Canonica GW, et al. Quantitative assessment of the adherence to sublingual immunotherapy. J Allergy Clin Immunol 2004; 113:1219-20
47. La Grutta S, Arena A, D'Anneo WR, Gamberi E, Leopardi S, Trimarchi A, et al. Evaluation of the anti-inflammatory and clinical effects of sublingual immunotherapy with carbamylated allergoid in allergic asthma with or without rhinitis. A 12-month perspective randomized, controlled, trial. Eur Ann Allergy Clin Immunol 2007; 39:40-4
48. D'Anneo RW, Arena A, Gammeri E, Bruno ME, Falagiani P, Riva G, et al. Parietaria sublingual allergoid immunotherapy with a co-seasonal treatment schedule. Allergol Immunopathol (Madr). 2008 Mar-Apr;36(2):79-84

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House dust mite allergy and shrimp allergy: a complex interaction

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Key words

Food allergy; peach allergy; lipid transfer protein; SPT; diagnosis.

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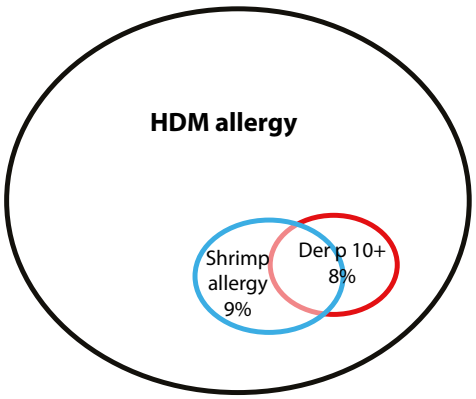
Summary

Background and objective. *Sensitization and allergy to shrimp among Italian house dust mite allergic patients are not well defined and were investigated in a large multicenter study.* **Methods**. *Shrimp sensitization and allergy were assessed in 526 house dust mite (HDM)-allergic patients submitted to the detection of IgE to Der p 10 and 100 atopic controls not sensitized to HDM.* **Results**. *Shrimp allergy occurred in 9% of patients (vs 0% of 100 atopic controls not sensitized to HDM; $p < 0.001$). Shrimp-allergic patients were less frequently hypersensitive to airborne allergens other than HDM than crustacean-tolerant subjects (35% vs 58.8%; $p < 0.005$). Only 51% of tropomyosin-sensitized patients had shrimp allergy, and these showed significantly higher Der p 10 IgE levels than shrimp-tolerant ones (mean 22.2 KU/l vs 6.2 KU/l; $p < 0.05$). Altogether 53% of shrimp-allergic patients did not react against tropomyosin.* **Conclusions**. *Shrimp allergy seems to occur uniquely in association with hypersensitivity to HDM allergens and tropomyosin is the main shrimp allergen but not a major one, at least in Italy. Along with tropomyosin-specific IgE levels, monosensitization to HDM seems to represent a risk factor for the development of shrimp allergy among HDM allergic patients.*

Introduction

House dust mites are one of the main causes of respiratory allergy worldwide, and shrimp represents the second cause of primary food allergy in Italy (1). These two allergies are strictly interconnected as both mites and shrimps are invertebrates and share cross-reacting allergens, the best known being tropomyosin (**table I**). Shrimp allergens identified so far belong to diverse protein families characterized by conserved three-dimensional structures leading to potential IgE cross-reactivity among different members of crustaceans and mollusks (2). It is presently still unclear whether, in patients allergic to both house dust mite and crustaceans, sensitization occurs via the respiratory or the gastrointestinal tract. Prevalence studies of shrimp allergy in house dust mite allergic patients are missing. In the present work we investigated a large population of house dust mite-allergic patients, the vast majority selected within a national multicenter study (3) with the aim to detect the prevalence and features of shrimp allergy.

Figure 1 *- Venn diagram showing the prevalence and serological features of shrimp allergy among 526 HDM-allergic patients.*



Materials and methods

Patients

Five hundred and twenty-six house dust mite-allergic patients (M/F: 261/265; mean age 28.2 years, range 4-79 years) were studied. This population was virtually the same recently investigated to study the clinical significance of Der p 23, a major HDM allergen (3). Methods employed to diagnose HDM allergy included a positive SPT with a commercial extract of either *Dermatophagoides pteronyssinus* (D1) or *Dermatophagoides farinae* (D2), and the measurement of IgE specific for the HDM whole extracts D1, and D2, by ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden). IgE specific for Der p 10, the house dust mite tropomyosin, were measured as well in all study patients. Levels exceeding 0.35 kU/L were considered positive; this cut-off level was chosen with the aim to improve the specificity of in-vitro tests. Further, all patients underwent SPT with a large series of commercial extracts of seasonal (grass, mugwort, ragweed, pellitory, plantain, birch, olive, and cypress) and perennial (Alternaria, cat and dog dander) allergens. Patients were thoroughly interviewed about their tolerance to crustaceans. Those reporting suspect allergic reactions associated with the ingestion of shrimp or other invertebrates (i.e., oral allergy syndrome, contact urticaria, generalized urticaria, asthma, or anaphylaxis) underwent SPT with either commercial extract of shrimp (1:20 w/v; ALK-Abello', Madrid Spain) or fresh shrimp and/or shrimp-specific IgE measurement to confirm sensitization status. Skin tests with fresh material were carried out using the most common seawater shrimp species eaten in Italy, all belonging to the Penaeideae family (*Aristeus antennatus*, *Parapenaeus longirostris*, *Parapeneopsis cornuta* and *Melicertus kerathurum*). Patients scoring positive on SPT and/or on ImmunoCAP were considered as clinically allergic to shrimp.

One hundred randomly selected atopic patients sensitized to different airborne allergens except house dust mites were assessed for crustacean allergy in the same way and were used as controls.

Table I *- House dust mite allergens. Official Shared allergens between house dust mite and shrimp are highlighted.*

Allergen	Biochemical name	MW	Allergen
Dermatophagoides farinae			***Dermatophagoides pteronyssinus***
Der f 1	Cysteine protease	27	Der p 1
Der f 2	NPC2 family	15	Der p 2
Der f 3	Trypsin	29	Der p 3
Der f 4	alpha-amylase	58	Der p 4
			Der p 5
Der f 6	Chymotrypsin	25	Der p 6
Der f 7	Bactericidal permeability-increasing like protein	30	Der p 7
Der f 8	Glutathione S-transferase	32	Der p 8
	Collagenolytic serine protease	29	Der p 9
Der f 10	Tropomyosin	37	Der p 10
Der f 11	Paramyosin	98	Der p 11
Der f 13	Fatty acid binding protein		Der p 13
Der f 14	Apolipophorin	177	Der p 14
Der f 15	Chitinase	98	Der p 15
Der f 16	Gelsolin/villin	53	
Der f 17	Calcium binding protein	53	
Der f 18	Chitin-binding protein	60	Der p 18
Der f 20	Arginine kinase	40	Der p 20
Der f 21		14	Der p 21
Der f 22			
Der f 23	Peritrophin-like protein	19	Der p 23
Der f 24	Ubiquinol-cytochrome c reductase binding protein homologue	13	Der p 24
Der f 25	Triosephosphate isomerase	34	
Der f 26	Myosin alkali light chain	18	
Der f 27	Serpin	48	
Der f 28	Heat Shock Protein	70	
Der f 29	Peptidyl-prolyl cis-trans isomerase (cyclophilin)	16	
Der f 30	Ferritin	16	
Der f 31	Cofilin	15	
Der f 32	Secreted inorganic pyrophosphatase	35	
Der f 33	alpha-tubulin	52	
Der f 34	enamine/imine deaminase	16	
Der f 35		14	
Der f 36		23	Der p 36
	Petrotrophic like protein domain	30	Der p 37

Statistics

Statistical methods as well as ethical issues have been detailed elsewhere (3). Probability levels < 5% were considered statistically significant.

Ethical issues

The clinical part of the study as well as specific IgE measurement were carried out as part of the clinical routine of every participating center. Patients gave an informed consent to the use of their clinical data in an anonymous form. The study was approved by the internal review board of the leading center. In view of the essentially observational nature of the study a formal approval by an external ethical committee was not requested.

Results

The main findings are summarized in **figure 1**. The prevalence of shrimp allergy in the general house dust mite allergic population was 45/526 (9%) vs 0/100 (0%) in the control population ($p< 0.001$). No differences in the prevalence of shrimp allergy between female (7.5%) and male (9.6%) patients was detected. Similarly, patients allergic and not allergic to crustaceans showed the same mean age (30 [16.2] years vs 28.2 [16.2] years, respectively), and no difference in the prevalence of asthma was observed between patients allergic or tolerant to shrimp (40% vs 40%, respectively). In contrast, patients with crustacean allergy were much less frequently hypersensitive to airborne allergens other than house dust mites than tolerant patients (35% vs 58.8%; $p < 0.005$).
The prevalence of hypersensitivity to tropomyosin in the study population was 7.8% (41/526). Of tropomyosin reactors, only 21 (51%) were clinically allergic to crustaceans, whereas 20 (49%) reported good tolerance to shrimp and other invertebrates. Interestingly, those with shrimp allergy showed a significantly higher mean level of IgE to Der p 10 than patients reporting good tolerance to crustaceans (22.2 [SD 28.0] KU/l vs 6.2 [9.6] KU/l; $p < 0.05$). Altogether, Der p 10 reactors were more frequently allergic to crustaceans than patients that did not show IgE specific for Der p 10 (21/41 [51%] vs 24/485 [4.9%]; $p < 0.001$). Nonetheless, notably 24/45 (53%) patients allergic to crustaceans did not react against tropomyosin. Finally, no difference in the prevalence of shrimp allergy was detected between patients monosensitized to Der p 10 (7/14 [50%]) and Der p 10 reactors who were sensitized to other mite allergens also (13/27 [48%]; p: NS).

Discussion

The present study, which was carried out on a large population of patients with clinically defined house dust mite allergy, shows once more to which extent hypersensitivity to house dust mites and to shrimp are strictly linked. In effect, none among the atopic controls reported symptoms suggestive of shrimp allergy whereas the prevalence of shrimp allergy in the study population was nearly 10%. Such prevalence suggests that the cross-reactivity between HDM and other invertebrates involves minor mite allergens. Tropomyosin was the first shrimp allergen to be identified more than 25 years ago (4). Although it has been considered the major shrimp allergen ever since, recent multicenter studies carried out in the Mediterranean area were able to detect tropomyosin hypersensitivity in less than 50% of shrimp allergic patients (5). This observation was fully confirmed by the present study that was carried out on a completely different population, where 53% of shrimp-allergic patients were not tropomyosin reactors. Further, interestingly, among tropomyosin-hypersensitive patients the occurrence of shrimp allergy was strongly related to specific IgE levels, suggesting the clinical relevance of sensitization degree. Nonetheless, the present study confirmed the association between tropomyosin sensitization and shrimp allergy.
A number of shrimp allergens other than tropomyosin have been detected during the last years (2); most of these seem phylogenetically conserved throughout the invertebrates' kingdom and hence able to cross react with homologous house dust mite allergens (5,6). Although in-vitro cross-inhibition experiments were not carried out in the present study it has to be considered that the whole study population was represented by patients with house dust mite-induced respiratory allergy, and no atopic control reported a history of food allergy to shrimps. In one shrimp allergic patients that did not react to recombinant Der p 10 the relevant shrimp allergen, that showed a molecular weight at about 100 kDa on immunoblot analysis was characterized by mass spectrometry (3) as paramyosin, a potentially cross-reacting muscular allergen of invertebrates.
Another interesting finding was the significantly higher prevalence of shrimp allergy among subjects monosensitized to HDM than among those who reacted to different airborne allergens. This observation is in keeping with similar findings in patients with food allergy to lipid transfer protein, that show more severe reactions if they are monosensitized and less severe allergic reactions in case of co-sensitization to airborne allergens (7). These findings might suggest that the dispersion of specific IgE reactivity over a larger number of targets is protective against severe allergic reactions or against food allergy per se.
In conclusion, shrimp allergy seems to occur uniquely in association with hypersensitivity to HDM allergens and, at least in this geographical area, tropomyosin is the main shrimp allergen but not a major one. Along with tropomyosin-specific IgE levels, monosensitization to HDM seems to represent a risk factor for the development of shrimp allergy among HDM allergic patients.

Obituary

This paper is in memory of our colleague Elena Varin.

Author contribution statement

Every author listed participated in the recruitment of patients and in the clinical workup at their own allergy centers. RA conceived and managed the multicenter study and wrote the manuscript. ES and DV revised the manuscript. GC performed the statistical analyses

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Asero R, Antonicelli L, Arena A, Bommarito L, Caruso B, et al. EpidemAAITO: features of food allergy in Italian adults attending allergy clinics: a multi-centre study. ClinExp Allergy 2009;39:547-55.
2. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI Molecular Allergology User's Guide. Pediatr Allergy Immunol 2016;27 Suppl 23:1-250.
3. Celi G, Brusca I, Scala E, Villalta D, Pastorello E, Farioli L, Cortellini G, et al. House dust mite allergy in Italy-Diagnostic and clinical relevance of Der p 23 (and of minor allergens): A real-life, multicenter study. Allergy 2019;74:1787-89. doi: 10.1111/all.13776.
4. Shanti KN, Martin BM, Nagpal S, Metcalfe D DD, Rao PVS. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. J Immunol 1993; 151: 5354-63.
5. Asero R, Mistrello G, Amato S, Ariano R, Colombo G, Conte ME, et al. Shrimp allergy in Italian adults: a multicenter study showing a high prevalence of sensitivity to novel high molecular weight allergens.Int Arch Allergy Immunol 2012; 157:3-10.
6. Giuffrida MG, Villalta D, Mistrello G, Amato S, Asero R. Shrimp allergy beyond Tropomyosin in Italy: clinical relevance of Arginine Kinase, Sarcoplasmic calcium binding protein and Hemocyanin. Eur Ann Allergy ClinImmunol 2014; 46:172-7.
7. Pastorello EA, Farioli L, Pravettoni V, et al. Pru p 3-sensitised Italian peach allergic patients are less likely to develop severe symptoms when also presenting IgE antibodies to Pru p 1 and Pru p 4. Int Arch Allergy Immunol 2011; 156:362–72.

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Clinical assessment of tolerability, immunological and cutaneous reactivity effects of an abbreviated schedule with *Olea europaea* native extract of subcutaneous immunotherapy

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Key words

Olea europaea; subcutaneous immunotherapy; rhinoconjunctivitis; abbreviated schedule; depot extracts.

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Summary

Objectives. *To evaluate the tolerability and efficacy of Olea europaea subcutaneous immunotherapy (SCIT) on patients with rhinoconjunctivitis.* **Methods**. *In this open clinical trial patients were assigned to an abbreviated build-up scheme. The outcomes were: number, percentage, and severity of adverse reactions. Secondary outcomes included: changes in immunoglobulin titers and changes in dose-response skin prick tests.* **Results**. *Only 8 systemic reactions were registered, which represented 7/47 (14.9%) of patients and 8/429 (1.9%) of administered doses. Regarding immunological parameters the significant increases of sIgG and sIgG4 evidenced the changes in the patient immune system. Cutaneous reactivity decreased significantly.* **Conclusions**. *Olea europaea SCIT (Allergovac® depot ROXALL Medicina España S.A.) showed a good safety and tolerability profile. Immunological changes with induction of blocking IgG and decreases in cutaneous reactivity were detected in the patients.*

List of abbreviations

AEs: Adverse events
AIT: Allergen immunotherapy
AR: Allergic rhinitis
ARs: Adverse reactions
ARIA: Allergic Rhinitis and its Impact on Asthma
DBU: Diagnostic biological unit
EAACI: European Academy of Allergy and Clinical Immunology
ELISA: Enzyme-Linked Immune Sorbent Assay
FEV_1: Forced expiratory volume in one second
GLP: Good Laboratory Practice
ICH: International Conference on Harmonisation
ITT: Intention to treat
LR: Local reaction
LTPs: Lipid transfer proteins
MedDRA: Medical Dictionary for Regulatory Activities
OAS: Oral Allergy Syndrome
PP: Per Protocol
RA: Respiratory Allergy
SCIT: Subcutaneous Immunotherapy
SPT: Skin prick test
sIgE: specific immunoglobulin E
sIgG: specific immunoglobulin G
sIgG4: specific immunoglobulin G4
SLIT: Sublingual immunotherapy
SP: Safety population
SPSS: Statistical Package for Social Sciences
SR: Systemic reaction
TSU: Treatment standardized unit

Introduction

Allergic rhinitis (AR) secondary to olive pollinosis is one of the most important causes of respiratory allergy in the Mediterranean area (1,2). In some provinces of southern Spain, *Olea europaea* pollen becomes the main allergen overtaking grass and dust mites allergens in eliciting respiratory allergy symptoms (3).
The production of olive pollen extracts may present differences in allergen composition and potency as a result of the variability in cultivars origin (4-6). Some olive tree species like Frantoio, Gordal or Arbequina are less allergenic than Loaime, Hojiblanca or Picual species, regarding immunoblot detection of Ole e 1 (7). These variations could be higher from one batch to another in the case of Ole e 7 and Ole e 9, as a consequence of the little amount of these minor allergens in the source material pollen (8). In addition, these allergenic differences could be affected by external factors in relation to geographical, climatological and pollution conditions where these trees are cultivated; as soil quality, hours of light received, rainfall values and maturation stage of the plant. Thus, the characterization of the olive pollen raw material by manufacturers is crucial during the supplier selection process, in order to assure the presence of these allergens.
It is well known, that olive pollen extracts show a greatly complex and varied allergogram (9,10). Standard laboratory methods have detected at least 20 protein bands with allergenic activity (11). One of the most studied allergens is Ole e 1, it seems to be involved in pollen hydration or germination processes (12). This protein is considered a major allergen, because almost 70% of allergic patients to *Olea europaea* recognise it (13). Other olive pollen allergens belong to panallergens family, such as profilin (Ole e 2) (14) and calcium binding proteins (Ole e 3 and Ole e 8) (15-17). Ole e 2 is responsible for cross reactivity to vegetable foods and oral allergy syndrome (OAS), and the two latter are related to polysensitization observed with olive pollinosis (9). Ole e 7 belongs to the well-known family of Lipid Transfer Proteins (LTPs) associated with fruit anaphylaxis (9,18,19). In addition, the prevalence of asthma is significantly higher in patients sensitized to Ole e 7 (20). On the contrary, patients sensitized to 1,3-β-gl++ucanase (Ole e 9) have more connection with poor tolerance to allergen immunotherapy and show more severe adverse reactions (11,21).
ROXALL Medicina España S.A. developed a sensitive and specific two-site sandwich ELISA for quantification of Ole e 1 (22). This method is especially useful in manufacturing procedure to guarantee the quality and standardization of allergenic extracts from olive tree pollen, intended for diagnostic and therapeutic clinical use. In addition, the drug substance (*Olea europaea* allergenic extract) used for manufacturing these products has been characterized and the presence of main olive tree pollen allergens has been detected (Ole e 1, Ole e 2, Ole e 3, Ole e 5, Ole e 8, Ole e 9, Ole e 10, and Ole e 11) by Western-blot and mass spectrometry (ROXALL internal files).
On the other hand, two kind of products are available for SCIT, chemically modified or native allergen extracts. In the most of cases, both of them are absorbed into aluminium hydroxide, in order to reduce the number and severity of systemic adverse reactions by binding and slowly releasing allergens (23). Furthermore, this is the most common adjuvant used in allergen immunotherapy (AIT) (24), being able to induce the immune system response although the mechanism is not fully understood (25). According to European Pharmacopeia, the maximum amount of aluminium (Al) content per human dose is restricted to 1,25 mg/mL or lower (26). In spite of the Al concerns regarding safety and tolerability (24,27,28), until now, there are no major safety issues on limited time SCIT course when the overall load of aluminium is carefully monitored (29).
Traditionally, SCIT depot required large series of injections, which included a long up-dosing phase increasing allergen weekly dose until the achievement of maintenance dose after 3-4 months. Abbreviated schedules, using higher concentrations at the beginning, allowed to shorten this process maintaining a good tolerability profile (30-32). Therefore, an open multicentre clinical trial in adult patients with allergic rhinoconjunctivitis (with or without asthma) using standardized native depot *Olea europaea* extract was conducted. The main aim of the current clinical trial was to establish the tolerability and safety levels of an abbreviated treatment schedule in patients with allergic rhinoconjunctivitis sensitized to olive tree pollen. Finally, the effects on immunological and cutaneous reactivity were also evaluated.

Materials and methods

Study design and ethical considerations

This open, multicentre and phase I clinical trial, was conducted at 5 hospitals in Spain. Patients were assigned to a new abbreviated schedule comprising 6 visits for 5 weeks, where the concentration of the olive pollen extract was increased gradually to reach the target maintenance dose, being the whole treatment duration of 17-weeks (**table I**). Tolerability was assessed taking into account the number, percentage and severity of adverse reactions and safety, testing haematological and biochemical parameters. The surrogate efficacy was measured through evaluation of immunological parameters and performing skin prick tests (SPTs).
The study was conducted in accordance with the principles of the Declaration of Helsinki and the ICH guideline on Good Clinical Practice. It was approved by relevant ethics committees and by the Spanish regulatory authorities, (EudraCT 2014-001569-29). Prior to their participation, written informed consent was given by every patient.

Table I *- Treatment schedule.*

Build up phase treatment schedule				
Week	VIAL No	INJECTION VOLUME (mL)	CONCENTRATION (TSU/mL)	DOSE INTERVAL
0		0.2	20	NA
1	**2**	0.5	50	1 week
2	**2**	1	100	1 week
3	**2**	0.2	200	1 week
4	**3**	0.5	500	1 week
5	**3**	1	1000	1 week
Maintenance phase treatment schedule				
Week	VIAL No	INJECTION VOLUME (mL)	CONCENTRATION (TSU/mL)	DOSE INTERVAL
9	**3**	1	1000	4 weeks
13	**3**	1	1000	4 weeks
17	**3**	1	1000	4 weeks

Study population

Patients were included in the study if they followed these inclusion criteria: patients aged 18–60 years with seasonal AR due to *Olea europaea* and clinical history of AR induced by olive tree pollen for at least 2 years prior to the study inclusion. Regarding asthma, only patients with concurrent mild asthma were allowed to participate. A positive SPT against *Olea europaea* at a concentration of 192 µg/mL, (wheal diameter ≥ 3 mm) and specific immunoglobulin E (sIgE) levels ≥ 0.7 kUA/L determined by ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden) were also required. Women of childbearing age should present a negative urine pregnancy test before first vaccine dose administration.

Patients were excluded from study participation if they had received immunotherapy against *Olea europaea* or a cross-reactive allergen in the 5 years prior the study inclusion, or if currently they were receiving immunotherapy for any other allergen. In spite of the good control of asthma, patients with moderate to severe asthma and a forced expiratory volume in 1st second (FEV_1) < 70%, were ineligible. Patients were also excluded if they presented additional clinically relevant sensitization different of *Olea europaea* or 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, beta-blockers, or angiotensin-converting enzyme inhibitors and women who were pregnant or breast-feeding.

Study interventions

A standardized native extract of *Olea europaea* adsorbed onto 0.2% aluminium hydroxide, was used for patients' SCIT treatment, (Allergovac® depot, ROXALL Medicina España S.A., Zamudio, Spain). Injections were administered by trained nurses under supervision of qualified allergologists in Immunotherapy Units.

During the first 5-weeks, patients received increasing doses of *Olea europaea* extract at weekly intervals (±2 days) to reach the target maintenance dose from the maximum concentration (vial 3, 1000 Treatment Standardized Units (TSU)/mL). The concentration of the major allergen Ole e 1 was 11.28 µg/mL. The build-up schedule comprised 6 doses: 3 doses (0,2, 0,5 and 1 mL) from vial 2 (100 TSU/mL), and 3 subsequent administrations (0,2, 0,5 and 1 mL) from vial 3 (**table I**). Dose modifications were allowed in the event of adverse reactions according to the recommendations of Alvarez-Cuesta *et al*. (33).

Outcome measures

Adverse events were collected and recorded for tolerability assessment. As a primary outcome, the incidence of adverse reactions was recorded at Immunotherapy Units during the 30 minutes after each vaccine administration. Likewise, adverse reactions were also collected by checking the patients' diaries designed to register any unpleasant experience outside each participating centres. Adverse reactions were defined as all noxious and unintended responses to any dose of the investigational al-

lergen vaccine administered. These reactions were classified as immediate (within 30 minutes after the vaccine administration) or delayed (> 30 minutes after vaccine administration).
In the same way, adverse reactions were classified as local (LR, reactions taking place at the arm where vaccine was administered), or systemic (SR, generalised symptoms taking place far away from the administration site). According to LRs extension, we consider clinically significant the immediate LR ≥5 cm and the delayed LR ≥10 cm or those implying a dose modification in the next administration (34). Additionally, LRs were described as diffuse inflammation, redness, erythema, local painfulness, pruritus, or reaction in injection site (when two or more local symptoms took place simultaneously). SRs were classified by the investigators according to the European Academy of Allergy and Clinical Immunology EAACI guidelines (33) and the Medical Dictionary for Regulatory Activities (MedDRA).
Skin prick testing was performed using four increasing concentrations of *Olea europaea* extract (100, 1,000, 10,000 and 100,000 DBU/mL, Diagnostic Biologic Units) as well as positive (histamine 10 mg/mL) and negative (saline) controls. It was performed in one day, by duplicating, in opposing rows in the volar surface of the forearm at basal and final visits. The change in cutaneous reactivity (wheal area in mm^2) from baseline to the final visit was measured.
Regarding the immunological effects assessment, serum samples were obtained at baseline and final visits to determinate specific immunoglobulin levels (IgE, IgG and IgG_4) against *Olea europaea* whole extract by ELISA (Enzyme-Linked Immune Sorbent Assay) as previously described (35). Samples were frozen and sent to ROXALL's central laboratory for bioanalysis in accordance with Good Laboratory Practices (GLPs).

Statistical methods

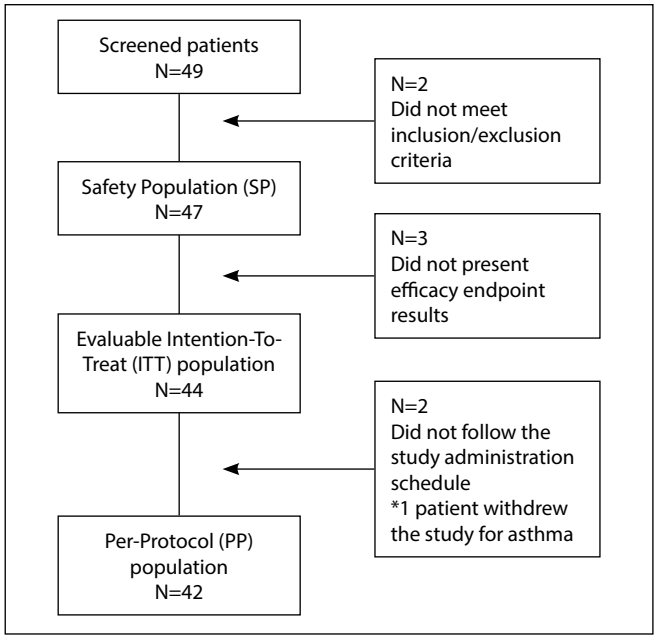
We performed tolerability and safety assessment and descriptive statistical analyses in the safety population (patients who received at least one dose of treatment). Efficacy statistical analyses were applied using the intention-to-treat (ITT) population (patients who met all inclusion/exclusion criteria, received at least one dose of treatment and had available data on efficacy variables) and the per-protocol (PP) population (patients who met previous criterial and moreover achieved their target maintenance dose and completed the study without any major protocol deviation).
For descriptive statistics, we displayed categorical variables by absolute and relative frequencies and continuous variables by the standard deviation and the mean.
Changes in immunoglobulin levels and SPT values from baseline to final visit, were analysed by means of the Wilcoxon non-parametric test for paired samples. A bilateral statistical significance level of 0.05 was applied to all statistical tests. Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) version 17.
Sample size was calculated considering a percentage of adverse events of 71% (34). Establishing a confidence interval of 95% with a precision of ± 13 percentage unit and assuming a 5% of drop outs, the number of patients to provide adequate data on the primary endpoint was 49.

Results

Descriptive data

A total of 49 subjects were enrolled in two out-season periods: from 1/9/15 to 28/2/16 and from 1/9/16 to 28/2/17. Two of them were screening failures, so 47 ones were assigned to receive AIT and were analysed in safety population (SP). Based on a rigorous clinical history and allergy diagnosis tests, sensitization to *Olea europaea* was confirmed, therefore, a vaccine of olive tree pollen extract 100% was indicated. ITT population included 44 patients since 3 patients were excluded due to the absence of data on immunoglobulins or dose-response SPT at final visit. Finally, 42 patients remained in the per protocol population (PP). Major protocol deviations were the reason for the exclusion of 1 patient from this analysis. An additional patient dropped out from the study as a consequence of asthma not related with the treatment by investigator's judgement. Patient´s distribution is shown in **figure 1**. Most patients (70%) showed

Figure 1 *- Study flow chart.*



*Patient's disposition along the study.

sIgE class ≥ 4 against complete *Olea europaea* extract. Subjects´ baseline demographic and clinical characteristics are presented in **table II**.

Tolerability and safety

One hundred and fifty-five adverse events (AEs) were described in the study, being only 37 (23.87%), related to study vaccine administration. In addition, 31 patients (66.0%) reported at least one adverse event (interestingly, one patient reported 17 AEs). The most frequent AE reported by > 5% of patients were, headache (23 events in 8 patients, 17.0%), upper respiratory tract infections (8 events in 7 patients, 14.9%), pharyngitis (11 events in 7 patients, 14.9%), back pain (17 events in 6 patients, 12.8%), cutaneous reaction (11 events in 5 patients, 10.6%), dysmenorrhea (7 events in 3 patients, 6.4%), myalgia (3 events in 3 patients, 6.4%) and pruritus (3 events in 3 patients 6.4%). All AEs were non-serious and most of them (82.6%) were of mild or moderate intensity (mild: symptoms that do not interfere with patients' usual daily activities, moderate: symptoms that interfere in some way with patients' usual daily activities and severe: symptoms that significantly interfere with the subject's usual daily activities). Only 27 (17.4%) were reported as severe, being headache the most frequent severe AE. In the majority of cases (106) were resolved with symptomatic medication.

During the study period, 5 clinically relevant delayed LR in 5 patients (10.6%) were recorded, implying a 1.2% of the administered doses (**table III**). There were no immediate clinically relevant LR. Non clinically relevant LR were present in 5.4% of administered doses.

Regarding systemic reactions, only 8 SRs in 7 patients (14.9%) were recorded; five grade 0 (8.5%) described as: general discomfort, isolated eye itchy, nasal herpes, lonely oral pruritus and oral pruritus plus nausea. With regards to systemic reactions grade I (2.1%), only one described as rhinoconjunctivitis was recorded. Finally, two systemic reactions grade II (4.3%) were documented as generalized urticaria and asthma. There were no systemic reactions grade III or IV. All these SRs occurred during the build-up phase (**table III**). Systemic reactions represented 1.9% of the vaccine administrations.

Most of ARs were of mild intensity and took place in the initiation period. Symptomatic treatment or a change in the next administration dose was the most common action required (**table III**). All patients recovered of the ARs at the end of the study. None of the patients failed to reach the maintenance dose established in the study protocol, in spite of the schedule dose modifications due to 8 adverse reactions.

No clinically relevant changes in blood laboratory parameters were observed following treatment in any patient.

Immunoglobulin levels

For ITT population, mean changes in immunoglobulin levels against *Olea europaea* between baseline and final visit are described (**figure 2**). Statistically significant increases in serum specific IgG and IgG4 titers at final visit were observed compared with basal visit (both $p < 0.001$; Wilcoxon test). Serum specific IgE levels to *Olea europaea* slightly decreased at final visit, achieving statistical significance ($p < 0.001$; Wilcoxon test). As it was expected, these results were maintained in PP population.

Cutaneous reactivity

A dose-response SPT was performed with four ten-fold increasing concentrations (vials 1 to 4). Cutaneous reactivity to *Olea europaea* decreased at final visit compared with baseline values in ITT population. Mean values of wheal area in mm^2 were significantly reduced at final visit compared with baseline in each one of the four tested vials (**figure 3**). Moreover, a statistical significance was achieved with any vial tested ($p < 0.001$; Wilcoxon test from vial 1 to vial 4). These cutaneous results were also reproducible in the PP population.

Table II *- Patients' baseline clinical characteristics.*

Baseline characteristics	**Abbreviated schedule**
Number of patients (SP)[a]	47
Age (years), mean ± (SD)[b]	37.7 ± 11.82
Women, n (%)	28 (59.6)
Rhinitis ARIA classification 47	
Intermittent mild n (%)	0 (0)
Persistent mild n (%)	0 (0)
Intermittent moderate-severe n (%)	2 (4.3)
Persistent moderate-severe n (%)	45 (95.7)
Main concomitant illness	
Asthma n (%)	27 (57.4)
Time from diagnostic (years), mean ± SD	9.8 ± 7.5
IgE *Olea europaea* CAP class n (%)	
2	8 (17.0)
3	6 (12.8)
4	16 (34.0)
5	8(17.0)
6	9 (19.1)

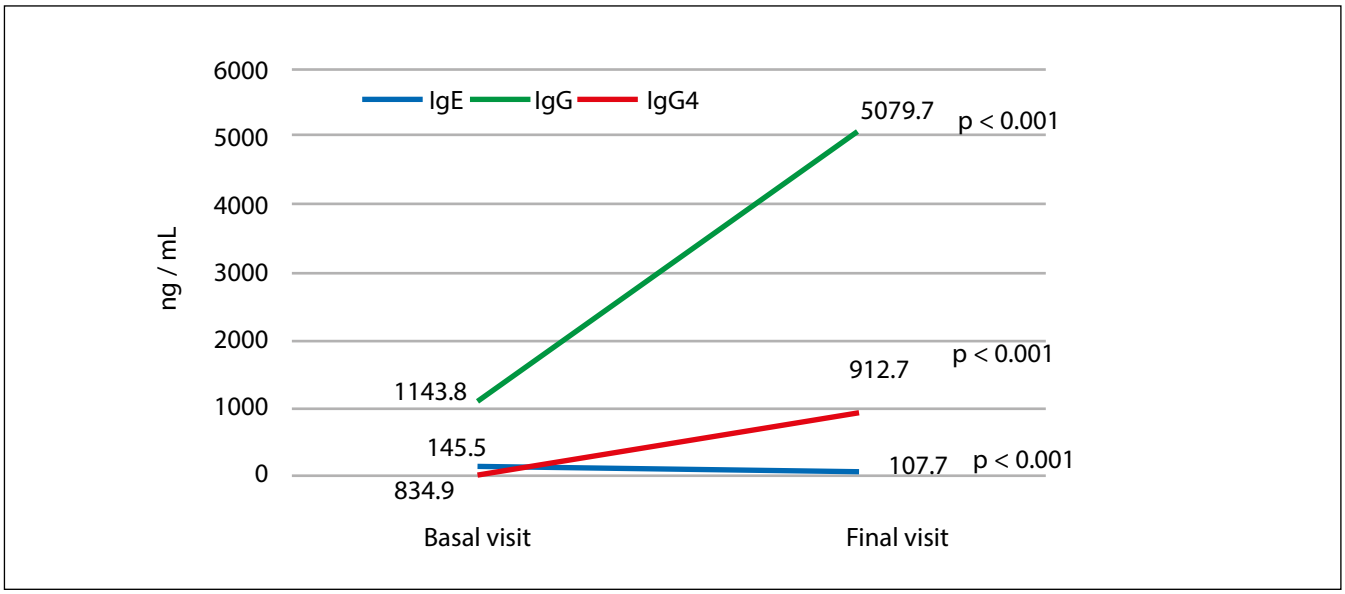
*(SP)[a] safety population, (SD)[b] standard deviation.

Table III *- Summary of adverse drug reactions by administration doses (N=423 doses administered).*

	Initiation Phase n (%)	Maintenance Phase n (%)*	Description	Intensity	Action Taken	Recovery
Clinically relevant immediate LRs	0 (0)	0 (0%)	--	--	--	--
Clinically relevant delayed LRs	4[a] (0.95)	1[b] (0.23)	Skin reaction[a]	mild	dose change	yes
			Erythema + oedema[a]	mild	symptomatic treatment	yes
			Localized oedema[a]	mild	dose change	yes
			Erythema[a]	mild	none	yes
			Injection site reaction[b]	severe	dose change	yes
Systemic reactions						
Grade 0[c]	5 (1.2)	--	General discomfort[c]	mild	none	yes
			Eye pruritus[c]	mild	none	yes
			Nasal herpes[c]	mild	symptomatic treatment	yes
			Mouth pruritus[c]	mild	none	yes
			Pruritus + nausea[c]	moderate	none	yes
Grade I[d]	1 (0.2)	--	Rhinoconjunctivitis[d]	moderate	none	yes
			Asthma[e]	severe	change + treatment	yes
Grade II[e]	2 (0.5)	--	Urticaria[e]	mild	change + treatment	yes

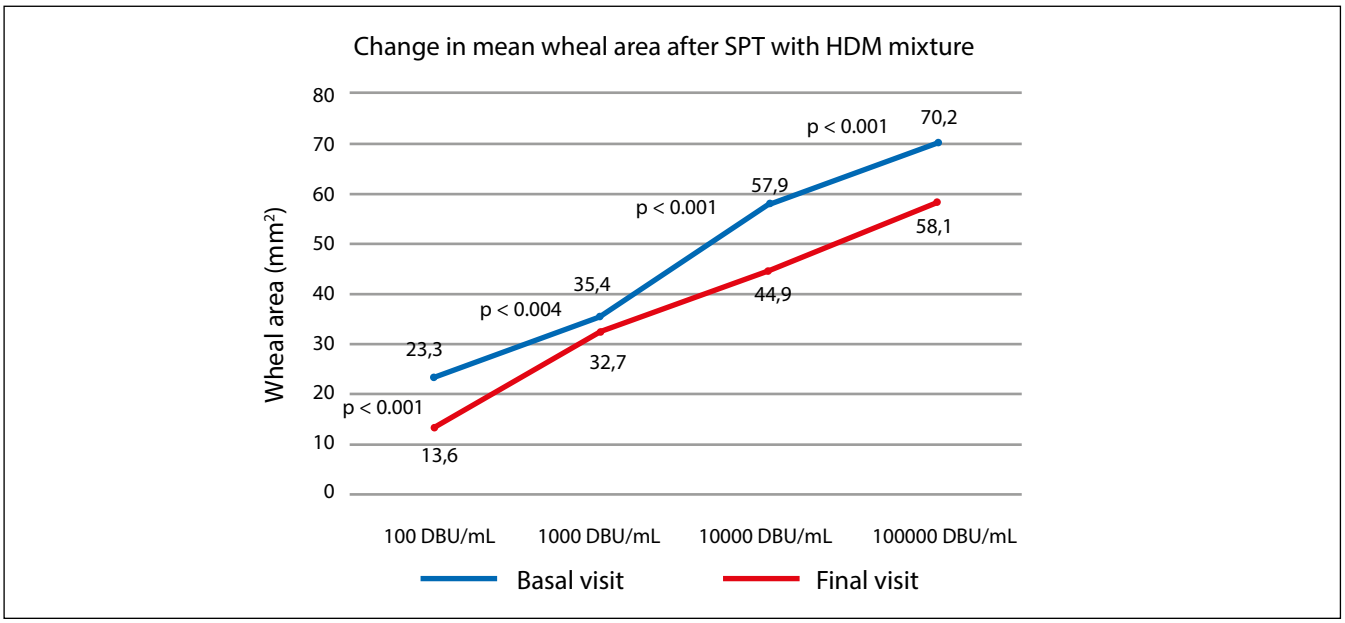
*n (%) number and percentage of adverse reactions, LR (local reaction), [a] Clinically relevant delayed LRs during initiation phase, [b] Clinically relevant delayed LRs during maintenance phase, [c] Systemic reaction grade 0, [d] Systemic reaction grade I and e Systemic reaction grade II

Figure 2 *- Changes in specific Olea europaea pollen extract immunoglobulins.*



Specific *Olea europaea* pollen extract immunoglobulins. Corresponding P-values according to Wilcoxon test are indicated.

Figure 3 *- Change in mean wheal area at final visit versus baseline to Olea europaea.*



Change in mean wheal area after SPT with to *Olea europaea* pollen extract at final visit versus baseline. P-values according to Wilcoxon test are indicated.

Discussion

In Mediterranean countries and especially in provinces of southern Spain (Córdoba and Jaén), rhinoconjunctivitis due to olive tree pollen is one of the most frequent consultations to allergologist (36). It is also a health problem due to the large surface area devoted to this crop in Andalusia (37).

Allergen-specific immunotherapy is the unique etiologic treatment that can alter the course of the respiratory allergy condition, presenting a disease modifying effect and inducing tolerance to the antigen (38). Traditionally, clinicians prescribed allergy immunotherapy following two defined schedules as perennial or pre-seasonal. The first one was generally used in respiratory allergy secondary to perennial allergens, (i.e. mites and moulds) while the pre-seasonal schedule was usually preferred in pollinosis. Both immunotherapy schedules have been shown to be effective in terms of clinical and immunological parameters (39). Currently, perennial schedules are more commonly used in AIT clinical practice with independency of the kind of the allergen responsible. Pre-seasonal and co-seasonal schedules are more frequently used in sublingual immunotherapy (SLIT) (40). In the event of performing a perennial subcutaneous schedule with pollen allergens, it is recommended to carry out the scale up outside the pollen season to diminish the risk of adverse reactions. Not only for the effect of the induction phase, which is related to a significantly higher number of systemic reactions (40), but also for the "priming effect," due to the natural exposure to a high amount of grain pollens. Consequently, the vaccine tolerance can be reduced, because of these two factors (41). On the other hand, classic AIT comprises a build-up phase with increasing doses of allergen extracts administered at short regular intervals until the optimal dose is reached. Afterwards, a maintenance period where the optimal dose is administered approximately at monthly intervals for 3 to 5 years is performed. According to the initial increasing doses schedule, immunotherapy is categorized as rush, cluster, abbreviated and conventional. Conventional schedules implies that maintenance dose is achieved after a long period of time, between 2-3 months (42). The modern tendency is to provide a treatment schedule that allows the attainment of the maintenance dose in the shortest period with the fewest adverse events and the best patient adherence to treatment. For these reasons, the abbreviated schedule can be considered a good AIT option.

A potential risk of fast schedules (rush, accelerated and abbreviated) is an increase in adverse reactions, especially systemic ones (42), although in other published article, an accelerated schedule versus a conventional one with grass SCIT seems to be similar between both regimens (43). The pattern and intensity of adverse reactions in our trial were similar to those reported in other studies, in spite of the difficulties to com-

pare trials due to the differences in the products (44-46). The maintenance dose of 1000 TSU/mL was reached by all study participants. Only 8 systemic reactions in 7 patients (14.9%) and 1.9% of administered doses were recorded and no one was grade III or higher. A randomized unblinded controlled study with SCIT containing a standardized extract of *Olea europaea* reported good clinical results in nasal and bronchial symptoms with a rate of systemic reactions in 8.7% of the patients (44). Another open label clinical trial (45) with 93 patients treated with a short up-dosing SCIT containing *Olea europaea* extract, showed a slightly lower rate of systemic reactions 4.3%. However, non-specific or grade 0 reactions were not taken into consideration for the analysis. Other multicentre randomized clinical trials evaluated the tolerability of two five-step up-dosing schedules for SCIT with grasses. The incidence of systemic adverse reactions was 22.5% for group 1, (weekly injections) and 35.1% in group 2, (3-4 days interval injection), according to Jung K. (31), and 21% and 33% of patients in group 1 and group 2 respectively, according to Pfaar O. (32).

Regarding to the early immunological response produced by the new up-dosing SCIT depot formulation, in five steps, it could be confirmed the statistically significant increment of more than 4-fold for sIgG (x4.4) and more than 6-fold for sIgG4 (x6.3) levels to *Olea europaea*, after 3 months of therapy. Similar results could be observed in other studies, where a rapid increase in sIgG and sIgG4 can be associated with the effect of blocking IgE-binding to allergens and B cells (39,45,46). In spite of the fact that sIgE levels after a short course of AIT, cannot always present the same behavior depending on analytical technique (47), our immunological results, measured using the ELISA technique at the Protein Lab of ROXALL Medicina España S.A. (48), showed a very early decreased which are in line with results published in other bibliography references (48-53).

With respect to cutaneous reactivity to the causative allergen, a statistically significant reduction in immediate skin reactivity to the different concentrations of *Olea europaea* extract was observed, expressed as a decrease in the mean wheal area produced by each concentration tested. This result is in the line of another clinical trial (45) with an extract of *Olea europaea* after a short course of AIT.

Since the main objective of this study was tolerability, the trial was designed without a placebo group. This fact must be taken into account when interpreting the reported surrogate efficacy results because the comparison was made with each patient comparing with himself.

Conclusions

The results of this clinical trial show that the build-up phase and the maintenance phase assayed up to 17 weeks with this abbreviated schedule with native depot *Olea europaea* SCIT, (Allergovac® depot ROXALL Medicina España), have a good tolerability profile, with few systemic and local clinically significant reactions. In addition, the treatment induces a surrogate positive efficacy response. This fact is confirmed by significant immunological and cutaneous reactivity changes in subjects suffering from allergic rhinoconjunctivitis with or without asthma due to sensitization to olive pollen. In spite of these good preliminary results, the reduction of rhinoconjunctivitis symptoms remains to be demonstrated in further clinical trials.

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Conflict of interests

Blanca Saenz-De San Pedro has received research fees from ALK- Abello, Leti, Novartis, Diater, Meda, Shire and Bial. María Pilar Mur has received research fees from ALK- Abello, Leti, Diater, GSK, Chiesi, Astra and Roxall. Lucía Valverde has received collaboration fees from ALK- Abello. María Angela Gonzalo-Garijo has received collaboration fees from ALK-Abelló, Allergy Therapeutics, Bial/Roxall, Diater, Leti, Merck-Allergopharma and Stallergenes. Begoña Madariaga, Juan Andrés Asturias, Leire Begoña, Alberto Martínez and Mª Cruz Gómez are fulltime employees of ROXALL Medicina España S.A. Mercedes Hernández has no conflicts of interest to declare. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

1. D'Amato G, Liccardi G. The increasing trend of seasonal respiratory allergy in urban areas. Allergy 2002;57 (suppl 71):35–6.
2. Nikolaidis C, Katotomichelakis M, Nena E, Makris M, Tsakas M, Michopoulos I, Constantinidis TC and Danielides V. Seasonal variations of allergenic pollen in a Mediterranean region - Alexandroupolis, north-east Greece. Ann Agric Environ Med 2015;22(4):685–9.
3. Domínguez-Ortega J, Quirce S, Delgado J, Dávila I, Martí-Guadaño E and Valero A. Diagnostic and therapeutic approaches in respiratory allergy are different depending on the profile of aeroallergen sensitisation. Allergol Immunopathol (Madr) 2014;42(1):11–8.
4. Alché JD, Castro AJ, Jiménez-lópez JC, Morales S, Zafra A, Hamman-khalifa AM and Rodríguez-García MI. Differential characteristics of olive pollen from different cultivars: biological and clinical Implications. J Investig Allergol Clin Immunol 2007; 17 Suppl1: 69-75.
5. Conde Hernández J, Conde Hernández P, Gónzalez Quevedo Tejerína MT, Conde Alcañiz MA, Conde Alcañiz EM, Crespo Moreira P, Cabanillas Platero M. Antigenic and allergenic differences between 16 different cultivars of Olea europaea. Allergy 2002;57 Suppl 71:60-5.
6. Barber D, Carpizo J, Garcia-Rumbao MC, Polo F, and Juan F. Allergenic variability in Olea pollen. Ann Allergy 1990;64(1):43–6.
7. Castro JA, de Dios Alché J, Cuevas J, Romero PJ, Alché V and Rodríguez-García MI. Pollen from different olive tree cultivars contains varying amounts of the major allergen Ole e 1. Int Arch Allergy Immunol 2003;131(3):164–73.
8. Barber D, Moreno C, Ledesma A, Serrano P, Galán A, Villalba M, Guerra F, Lombardero M et al. Degree of olive pollen exposure and sensitization patterns. Clinical implications. J Investig Allergol Clin Immunol 2007;17(Suppl 1):63–8.
9. Quiralte J, Palacios L, Rodríguez R, Cárdaba B, Arias de Saavedra JM, Villalba M, Florido JF and Lahoz C. Modelling diseases: the allergens of Olea europaea pollen. J Investig Allergol Clin Immunol 2007;17 (Suppl 1):24–30.
10. Rodríguez R, Villalba M, Batanero E, Palomares O, Quiralte J, Salamanca G, Sirvent S, Castro L et al. Olive pollen recombinant allergens: value in diagnosis and immunotherapy. J Investig Allergol Clin Immunol 2007;17(Suppl 1):56–62
11. Villalba M, Rodríguez R and Batanero E. The spectrum of olive pollen allergens. From structures to diagnosis and treatment. Methods 2014;66(1):44–54.
12. Tang W, Ezcurra I, Muschietti J and McCormick S. A cysteine-rich extracellular protein, LAT52, interacts with the extracellular domain of the pollen receptor kinase LePRK2. Plant Cell 2002;14(9):2277-87.
13. Palomares O, Swoboda I, Villalba M, Balic N, Spitzauer S, Rodríguez R, Valenta R. The major allergen of olive pollen Ole e 1 is a diagnostic marker for sensitization to Oleaceae. Int Arch Allergy Immunol 2006;141(2):110-8.14.
14. Ledesma A, Rodríguez R and Villalba M. Olive-pollen profilin. Molecular and immunologic properties. Allergy.1998;53(5):520–6.
15. Quiralte J, Florido F, Arias de Saavedra JM, Gómez A, Sáenz de San Pedro B, González E, and Rodríguez R. Olive allergen-specific IgE responses in patients with *Olea europaea* pollinosis. Allergy 2002;57 (Suppl 71):47–52.
16. Batanero E, Villalba M, Ledesma A, Puente XS and Rodríguez R. Ole e 3, an olive-tree allergen, belongs to a widespread family of pollen proteins. Eur J Biochem 1996;241(3):772–8.
17. Ledesma A, Villalba M and Rodríguez R. Cloning, expression and characterization of a novel four EF-hand Ca2+-binding protein from olive pollen with allergenic activity. FEBS Lett 2000;466(1):192–6.
18. Florido López JF, Quiralte Enriquez J, Arias de Saavedra Alías JM, Sáenz de San Pedro B, Martín Casañez E. An allergen from *Olea europaea* pollen (Ole e 7) is associated with plant-derived food anaphylaxis. Allergy 2002;57(Suppl 71):53–9.
19. Scala E, Abeni D, Pomponi D, Paganelli R, Locanto M, Giani M, Cecchi L and Asero R. Ole e 1, Ole e 7, and Ole e 9: Identifying distinct clinical subsets of olive tree-allergic patients. J Allergy Clin Immunol 2016;137(2):629–31.
20. Alcántara M, Sáenz de San Pedro B, Cañada C, Muñoz MA, Jimeno L, Villalba M and de la Torre F. Steps towards clarifying the clinical relevance of minor olive allergens in areas with extremely high levels of olive pollen. J Investig Allergol Clin Immunol 2017;27(2):138-140.
21. Duffort O, Palomares O, Lombardero M, Villalba M, Barber D, Rodríguez R and Polo F. Variability of Ole e 9 allergen in olive pollen extracts: Relevance of minor allergens in immunotherapy treatments. Int Arch Allergy Immunol 2006;140(2):131–8.
22. Arilla MC, Eraso E, Ibarrola I, Algorta J, Martínez A and Asturias JA. Monoclonal antibody-based method for measuring olive pollen major allergen Ole e 1. Ann Allergy, Asthma Immunol 2002;89(1):83–9.
23. Hogenesch H. Mechanism of immunopotentiation and safety of aluminum adjuvants. Front Immunol 2013;3:406.
24. Kramer MF and Heath MD. Aluminium in allergen-specific subcutaneous immunotherapy - A German perspective. Vaccine 2014;32(33):4140–8.
25. Klimek L, Schmidt-Weber CB, Kramer MF, Skinner MA and Heath MD. Clinical use of adjuvants in allergen-immunotherapy. Expert Rev Clin Immunol 2017;13(6):599–610.
26. EMA, Agency, Medicines E. CHMP Safety Working Party 's response to the PDCO regarding aluminium hydroxide contained in allergen products. EMA J 2010;44.
27. Jensen-Jarolim E. Aluminium in Allergies and Allergen immunotherapy. World Allergy Organ J 2015;8(1): 7.
28. Guimarães LE, Baker B, Perricone C and Shoenfeld Y. Vaccines, adjuvants and autoimmunity. Pharmacol Res. 2015;100:190–209.
29. Gołoś A and Lutyńska A. Aluminium-adjuvanted vaccines-A review of the current state of knowledge. Przegl Epidemiol. 2015;69(4):731–4.
30. Bousquet J, Calvayrac P, Guérin B, Hejjaoui A, Dhivert H, Hewitt B and Michel FB. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. I. In vivo and in vitro parameters after a short course of treatment. J Allergy Clin Immunol 1985;76(5):734–44.
31. Jung K. Safety and tolerability of immunotherapy using various updosing schedules of a new SCIT product with an optimised allergen/aluminium hydroxide ratio. Allergy Eur J Allergy Clin Immunol 2011;66(suppl 95):41–3.
32. Pfaar O, Jung K, Wolf H, Decot E, Kleine-Tebbe J, Klimek L and Wüstenberg E. Immunological effects and tolerability of a new fast updosed immunologically enhanced subcutaneous immunotherapy formulation with optimized allergen/adjuvant ratio. Allergy 2012;67(5):630–7.
33. Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ and Valovirta E. EAACI, Immunotherapy Task Force. Standards for practical allergen-specific immunotherapy. Allergy. 2006;61 (Suppl 82):1–20.

34. Casanovas M, Sastre J, Fernandez-Nieto M, Lluch M, Carnés J and Fernández-Caldas E. Double-blind study of tolerability and antibody production of unmodified and chemically modified allergen vaccines of Phleum pratense. Clin Exp Allergy 2005;35 (10):1377–83.
35. Sola J, Sánchez V, Landeta A, Madariaga B, Martínez A and Álvarez-Cuesta E. A phase I clinical trial with subcutaneous immunotherapy vaccine of Timothy grass pollen extract according to EMA guidelines. Immunotherapy 2015;7(4):343-52.
36. Sociedad Española Alergia e Inmunología Clínica SEAIC (Ed.). Alergologica 2015. Factores epidemiológicos, clínicos y socioeconómicos de las enfermedades alérgicas en España en 2015, 2015th Edition. Draft Grupo de Comunicación Healthcare, Madrid, Spain 2015; 1–352.
37. Vázquez LM, Galán C and Domínguez-Vilches E. Influence of meteorological parameters on Olea pollen concentrations in Córdoba (south-western Spain). Int J Biometeorol 2003;48(2):83-90.
38. Shamji MH and Durham SR. Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. J Allergy Clin Immunol 2017;140(6):1485–98.
39. Gokmen NM, Ersoy R, Gulbahar O, Ardeniz O, Sin A, Unsel M and Kokuludag A. Desensitization effect of preseasonal seven-injection allergoid immunotherapy with olive pollen on basophil activation: the efficacy of olive pollen-specific preseasonal allergoid immunotherapy on basophils. Int Arch Allergy Immunol 2012;159(1):75-82.
40. Calderón MA, Vidal C, Rodríguez Del Río P, Just J, Pfaar O, Tabar AI, Sánchez-Machín I, Bubel P, Borja J, Eberle P, Reiber R, Bouvier M, Lepelliez A, Klimek L, Demoly P and EASSI Doctors' Group. European Survey on Adverse Systemic Reactions in Allergen Immunotherapy (EASSI): a real-life clinical assessment. Allergy 2017;72(3):462-472.
41. Pfaar O, Wolf H, Klimek L, Schnitker J and Wüstenberg E. Immunologic effect and tolerability of intra-seasonal subcutaneous immunotherapy with an 8-day up-dosing schedule to 10,000 standardized quality-units : a double-blind, randomized, placebo-controlled trial. Clin Ther 2012;34(10):2072–81.
42. Serrano P, Justicia JL, Sánchez C, Cimarra M, Fernández-Távora L, Orovitg A, Moreno C, Guerra F, et al. Systemic tolerability of specific subcutaneous immunotherapy with index-of-reactivity – standardized allergen extracts administered using clustered regimens : a retrospective , observational , multicenter study. Ann Allergy Asthma Immunol 2009;102(3):247–52.
43. Pfaar O, van Twuijver E, Hecker H, Boot JD, van Ree R and Klimek L. Accelerated up-dosing of subcutaneous immunotherapy with a registered allergoid grass pollen preparation. Int Arch Allergy Immunol 2013;160(4):420–4.
44. González P, Florido F, Sáenz de San Pedro B, de la Torre F, Rico P and Martín S. Immunotherapy with an extract of Olea europaea quantified in mass units. Evaluation of the safety and efficacy after one year of treatment. J Investig Allergol Clin Immunol 2002;12(4):263-71.
45. Moreno C, Sáenz de San Pedro B, Millán C, Panizo C, Martín S, Florido F. Exploratory study of tolerability and immunological effect of a short up-dosing immunotherapy phase with a standardised allergen extract derived from pollen of *Olea europaea*. Clin Transl Allergy 2015;24(5):1–6.
46. Macchia L, Caiaffa MF, Di Felice G, Pini C, Bariletto G, Strada S and Tursi A. Changes in skin reactivity, specific IgE and IgG levels after one year of immunotherapy in olive pollinosis. Allergy 1991;46(6):410-8.
47. Lawrence MG, Steinke JW, Borish L. Basic science for the clinician: Mechanisms of sublingual and subcutaneous immunotherapy. Ann Allergy Asthma Immunol 2016;117(2):138-42.
48. Sola J, Sánchez V, Landeta A, Madariaga B, Martínez A, Álvarez-Cuesta E. A Phase I clinical trial with subcutaneous immunotherapy vaccine of Timothy grass pollen extract according to EMA guidelines. Immunotherapy 2015;7(4):343-52.
49. Quiralte J, Lara MA, Sánchez GV, Monteserín J, Fernández L, Gómez-Fernández MC, Madariaga B, Arilla C, Asturias JA, Begoña L, Martínez A. Tolerability and surrogate efficacy parameters of a polymerized depot mixture pollen extracts without dilutional effect. Immunotherapy 2019 Aug;11(12):1031-42.
50. Enrique E, de Rojas DH, Alba P, Flores I, Colomer N, Andreu C, Gómez-Fernández MC, Landeta A, Asturias JA, Martínez A, Madariaga-Goirigolzarri B. Tolerability and positive efficacy results after subcutaneous immunotherapy with Parietaria judaica depot extract. Immunotherapy 2018 Oct;10(14):1253-63.
51. Moreno V, Alvariño M, Rodríguez F, Roger A, Peña-Arellano MI, Lleonart R, Pagán JA, Navarro JA, Navarro LA, Vidal C, Ponte-Tellechea A, Gómez-Fernández MC, Madariaga-Goirigolzarri B, Asturias JA, Hernández-Fernandez de Rojas D. Randomized dose-response study of subcutaneous immunotherapy with a Dermatophagoides pteronyssinus extract in patients with respiratory allergy. Immunotherapy 2016;8(3):265-77.
52. Sola J, da Silva Ferreira JA, Dionicio Elera J, Plácido JL, Pereira C, Fonseca J, Panizo C, Inácio LF, Cancelleire N, Zubeldia Ortuño JM, Landeta A, Madariaga B, Martínez A. Timothy grass pollen therapeutic vaccine: optimal dose for subcutaneous immunotherapy. Immunotherapy 2016;8(3):251-63.
53. Hernández Fernández de Rojas D, Antépara Ercoreca I, Ponte Tellechea A, Ibáñez Echevarría E, Jáuregui Presa I, Gamboa Setién P, Asturias JA, Landeta Manzano A, Madariaga Goirigolzarri B. Phase I study of subcutaneous allergen immunotherapy with Dermatophagoides pteronyssinus in patients with allergic rhinoconjunctivitis with or without asthma. Immunotherapy 2015;7(2):89-99.
54. Brozek JL, Bousquet J, Baena-Cagnani CE, Bonini S, Canonica GW, Casale TB, van Wijk RG, Ohta K, Zuberbier T, et al. Global Allergy and Asthma European Network; Grading of Recommendations Assessment, Development and Evaluation Working Group. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision. J Allergy Clin Immunol 2010;126(3):466-76.

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Diagnostic approach to hypersensitivity reactions to iodinated contrast media: a single-center experience on 98 patients

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Contrast media; allergy; hypersensitivity; adverse reaction; premedication.

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Summary
Adverse reactions to iodinated contrast media (ICM) are reported in 1%–3% of diagnostic procedures. They represent a relevant problem involving patients' safety as well as relevant costs for healthcare systems. Premedication with antihistamines and corticosteroids is still widely used, but evidence of its efficacy is lacking and there is a risk for under-estimation of possible severe adverse reactions to ICM in those who undergo premedication.
Data from 98 patients with a previous reaction to ICM that consecutively referred to our unit between 2015 and 2018 were retrospectively analyzed. They underwent an allergologic workup comprehending skin tests and drug provocation tests (DPT) with ICM. The skin test showed a very high negative predictive value (NPV) compared to DPT in patients with a previous immediate adverse reaction, while the NPV in patients with a previous delayed adverse reaction was lower.
After completion of the allergologic workup, 94 patients (95.9%) could tolerate a DPT with the culprit or alternative ICM.
Subsequently, 90 patients were reached by phone to assess if they had been re-exposed to ICM for radiologic procedure. Thirty-nine patients had been re-exposed, without any premedication in 13 cases: 12 of them had tolerated the ICM, while one reacted again despite a negative DPT with the same ICM. Overall, the NPV of this protocol was elevated (92.3%) for patients undergoing DPT and subsequent exposure to the same ICM in a real-life setting.
Collaboration between the prescribing physician, the radiologist and the allergist, and an accurate allergologic workup are essential to ensure maximum safety for the patient.

Introduction

Iodinated contrast media (ICM) are widely used drugs during radiological imaging and angiographic procedures (1). They were first introduced in the 1920s and were gradually replaced by more tolerable compounds that are currently classified as follows: nonionic monomers (iopamidol, iohexol, ioversol, iopentol, iomeprol, iobitridol, and iopromide), nonionic dimers (iodixanol) and ionic dimers (ioxaglate) (**table I**).

Hypersensitivity reactions after contrast media injection are usually divided into immediate (IHR), when occurring within 1 hour, and delayed (DHR), when occurring after more than 1 hour to 7 days (2).
The prevalence of adverse reactions to nonionic ICM is about 1%–3% (3). A consistent part of IHRs is non-IgE-driven and their rate decreased significantly (nowadays 0.7%–3%) after the introduction of nonionic hypo-isosmolar ICM (2). Severe IHRs are usually IgE-mediated and have a frequency of 0.02%–

0.04%, while DHRs could be T-cell mediated and occur in 0.5% to 3% of the administrations (3,4).
According to the European Network for Drug Allergy/European Academy of Allergy and Clinical Immunology (ENDA/EAACI) working group, in a patient with a previous adverse reaction to ICM, an allergological diagnostic workup is required to confirm hypersensitivity and to find a safe alternative ICM (5,6). The drug provocation test (DPT) is still considered the gold standard to assess tolerability to the drug (5,6).
Nevertheless, the value of the allergologic workup is often underrecognized since scientific societies such as the European Society of Urogenital Radiology and American College of Radiology still rely on the use of premedication protocols (even recognizing their questionable efficacy) or the complete avoidance of ICM (7,8).
Recently, the Società Italiana di Allergologia, Asma ed Immunologia Clinica and the Società Italiana Radiologia Medica e Interventistica, in a joint Italian consensus document, confirmed the importance of an allergologic workup that includes a DPT (1).
In this retrospective study, we evaluated the safety and the accuracy of a diagnostic protocol that includes skin tests and DPT for patients with a previous adverse reaction to ICM.

Materials and methods

Study population

We carried out a retrospective study on a population of patients who consecutively referred to our Allergology Unit from 2015 to 2018 for adverse reactions to ICM. Ninety-eight patients were evaluated and included in the study; the characteristics of our study population are shown in **table II**.
All patients signed an informed consent for the diagnostic procedure. All patients were treated according to the Helsinki declaration ethical principles.
The adverse reactions to ICM were classified according to the literature (IHR, <1 hour after ICM administration; DHR >1 hour after ICM administration) (2).
Ring and Messmer severity scale (grades 1–4) was used for classification of IHRs (9), while DHRs were graded as mild (no treatment was required), moderate (the patient responded to appropriate treatment without hospitalization), and severe (the reaction required hospitalization or was life-threatening) (10).
MS Excel (Microsoft Corporation, Redmond, WA, USA) was used for statistical analysis.

Skin tests and in vitro tests

Patients underwent skin tests for ICM according to ENDA criteria with the culprit (when known) and with the ICM commonly used in our geographic area (iohexol, iopromide, iodixanol, iopamidol, ioversol) (5).
Briefly, we performed skin prick tests on the volar surface of the forearm with undiluted ICM; positive (histamine 0.01%) and negative controls (saline solution NaCl 0.9%) and latex prick test (Alk-Abellò, Hørsholm, Denmark) were also included. If the ICM prick tests were negative, intradermal tests (IDT) with a 1:10 dilution were performed.
The result was considered positive in case of a wheal reaction with a mean diameter of ≥3 mm with surrounding erythema 15 minutes after the prick test and 20 minutes after IDT; we also reevaluated the skin reactions 48/72 hours after IDT (2).
Basal tryptase level (ThermoFisher Scientific, Uppsala, Sweden) was assessed in patients who had experienced more severe reactions (grade ≥ 3 IHR and moderate/severe DHR).

Drug provocation tests (DPT)

The ICM for the DPT was chosen according to the results of skin tests and the characteristics of the index reaction. In case of a mild (grade I according to Ring and Messmer in case of a previous DHR), recent (<12 months) reaction with negative skin tests for the culprit (when known), tolerance toward the culprit ICM was proposed. DPT with an alternative ICM was performed in those who did not agree to be challenged with the culprit ICM and in all the other patients not included in the aforementioned situation.

Table I *- Biochemical classification of ICM.*

	Monomers	**Dimers**
Ionic	Replaced by more tolerable compounds	Ioxaglate
Nonionic	Iopamidol, iohexol, ioversol, iopentol, iomeprol, iobitridol and iopromide	Iodixanol

Table II *- Characteristics of our study population.*

Number of patients	98
Sex distribution	53 females (54.1%), 45 males (45.9%)
Median age	65.6 years (range, 23–90 years)
Atopic	34 (34.7%)
Asthma/COPD comorbidity	16 (16.3%)

The dose ICM to be tested was decided according to international literature (total volume 95 mL) (11,12), independently of the subject body weight (6). In case of non-allergologic contraindications to ICM administration (e.g., kidney failure), the patient was excluded from the diagnostic protocol and the case was discussed with the referring physician.
The challenge required a 6-hour in-hospital stay, with supervision of trained medical staff and emergency equipment and an on-call emergency team available.
Briefly, in patients with a previous IHR, the DPT started with a placebo consisting of 50 mL of saline solution, and then the chosen ICM was administered intravenously with an infusion volume of 5 mL, 30 and then 60 mL (cumulative dose, 95 mL), respectively, at 30-minute intervals. An infusion pump was used for this purpose (Infusomat Space Neutrapur; B. Braun, Melsungen, Germany).
In case of DHR, the contrast media was administered in two separate sessions with an interval of 7–14 days in between; 50 mL of saline solution followed by 5 and 30 mL of ICM on the first day, and 30 and 60 mL of ICM on the second session.
Subsequent telephone follow-up was carried out in order to determine whether the patients had been re-exposed to ICM in real-life settings as well as the outcome.

Results

Characteristics of adverse reactions to ICM in our population

The main aspects of the adverse reactions to ICM in our population are shown in **table III**.
Of note, iomeprol was the most commonly reported culprit ICM, at least partially due to its frequent use in our region. In almost

Table III *- Features of the adverse reactions to ICM.*

		Total	Immediate	Delayed
Timing of the index reaction (%)		98	82 (83.7%)	16 (16.3%)
Severity			Grade 1 n = 47 (58.1%) Grade 2 n = 24 (29.6%) Grade 3 n = 10 (12.3%) Grade 4 n = 0	Mild n = 15 (93.7%) Moderate n = 1 (6.3%) Severe) n = 0
On first exposure to ICM (missing information n = 40, 40.8%)		30 (30.6%)	28 (34.1%)	2 (12.5%)
Use of "antiallergic" premedication (missing information n = 16, 16.3%)		26 (26.5%)	18 (22.0%)	8 (50.0%)
Culprit ICM (three patients reported adverse reactions with more than one ICM)	Iomeprol	32 (32.7%)	24 (29.3%)	8 (50.0%)
	Iopamidol	4 (4.1%)	2 (2.4%)	2 (12.5%)
	Iopromide	14 (14.3%)	11 (13.4%)	3 (18.8%)
	Iobitridol	5 (5.1%)	5 (6.1%)	0
	Iodixanol	4 (4.1%)	3 (3.7%)	1 6.3%)
	Unknown	43 (43.9%)	39 (47.6%)	4 (25.0%)
Latency from latest ICM reaction to allergologic workup (missing information n = 2, 2.0%)	Median delay (months)	90.8 (range 1–600)	107.7 (range 1–600)	12.4 (range 1–48)
	Within 12 months	47 (48.0%)	35 (42.7%)	12 (75.0%)
	Within 6 months	31 (31.6%)	23 (28.0%)	8 (50.0%)

one third of the patients, the reactions occurred on the first exposure to ICM and were mainly immediate, but this rate might have been underestimated since information on previous exposure was scarce. Culprit ICM was unknown in almost half of the cases.
We recorded a high rate of grade 1 IHRs. Globally, the use of antiallergic premedication, including steroids and/or antihistamines, without any previous allergologic consultation was common, from 22% in those who had experienced an IHR to 50% in those who had experienced a DHR.
The delay between the adverse reaction and the allergologic evaluation was lengthy, but a gradual reduction of this time interval was noted during the 3 years observation period (an average of 110.7 months in 2015 vs 87.5 months in 2017–18).
Three patients exhibited more than one adverse reaction against ICM, and in these cases the same clinical features (IHR or DHR) relapsed regardless of the use of a different compound.

Skin tests and laboratory results

Skin prick tests to ICM and latex were negative in all our patients. Basal tryptase values were normal in all the tested subjects.
In our population, IDTs for ICM resulted positive in 10 patients (10.2%), the majority of whom were positive to iomeprol (n = 6) (**table IV**); of note, one patient showed a delayed positivity to IDT to all tested ICM. Seven skin positive results correlated to IHRs and the other three to DHRs.
In two of these cases, the culprit ICM was not known. In the case of the patient with multiple IDT positive results, the culprit ICM was iopromide. In all the other cases there was concordance among the result of the IDT and the culprit ICM (skin test positive for iomeron in 6/32 (18.75%) patients that previously reacted to iomeron; skin test with ioversol was positive in the only individual that reacted to ioversol, but ioversol was only tested in this patient).
A complete overview of the results of these 10 patients is shown in **table IV**.
Focusing on the cases evaluated within 1 year since the last reaction (n = 47, 48.0%), the rate of positive skin tests increased to 14.9% (n = 7); however, this difference was not statistically significant compared with the whole population. Even in the cases of grade 3 IHR (with hypotension or worse), the rate of positive skin tests showed an increasing trend (n = 3 on 13 patients, 23.1%) without reaching statistical significance.

ICM provocation test

After the skin tests, all patients underwent a DPT with intravenous ICM. Only four of them received the culprit ICM, and two reacted again, despite negative skin tests (iomeron n = 1, iobitridol n = 1).
Eight patients, on a total of 94, who were challenged with an alternative ICM (8.5%), exhibited an adverse event that did not

Table IV *- Features of patients with positive skin test.*

		Total	IHR	DHR
No. of patients with skin test positive for any ICM		10 (10.2%)	7 (8.5%)	3 (18.8%)
No. of patients with skin test positive for >1 ICM		1 (1.0%)	0	1 (6.25%)
No. of patients with skin test positive for any ICM and ICM reaction despite premedication		4 (4.1%)	2 (2.4%)	2 (12.5%)
No. of patients with skin test positive for any ICM and ICM reaction on first exposure		2 (2.0%)	2 (2.4%)	0
Elicitor	Iomeprol	6*	4*	2
	Iopamidol	0	0	0
	Iopromide	1	1	0
	Iobitridol	1*	1*	0
	Iodixanol	0	0	0
	Ioversol°	1	1	0
	All	1#	0	1#

*In two of these patients (one with skin test positive for iomeprol, one for iobitridol), the culprit ICM was unknown.
#In this case, a delayed positive reaction to all the ICM was observed after IDT.
°A single patient with previous IHR to ioversol underwent skin tests with this ICM, which were positive.

differ from the index reaction regarding the time of onset and the severity.
Hence, we recorded 10 overall adverse events during DPT (two with culprit, eight with alternative ICM) consisting in two immediate erythematous rashes, one immediate and one delayed urticaria, four delayed cutaneous angioedema, one delayed lymphoadenomegaly, and one delayed disphagia. Epinephrine administration was not needed in any of these cases. Results of the DPTs are shown in (**tables V**, **VI**).
Seven patients who had experienced a previous DHR did not tolerate the ICM challenge test (7/16 = 43.8%), despite negative skin tests.

Table V *- Features of the subjects who did not tolerate the first challenge with culprit or alternative ICM.*

Patient no.	Index reaction	Symptoms	ICM	Skin tests	DPT witch culprit	1st challenge	Symptoms	2nd challenge	Symptoms
3	DHR	Dysphagia	Iopromide	Negative	No	Iomeprol	Dysphagia	Iodixanol	No
11	DHR	Urticaria	Iopromide	All positive	No	Iomeprol	Urticaria	STOP	-
17	DHR	Generalized angioedema	Iomeprol	Iomeprol (culprit) positive	No	Iodixanol	Generalized angioedema	Iopromide	No
21	DHR	Angioedema	Iopamiro	Negative	No	Iodixanol	Angioedema	Iopromide*	Angioedema
25	DHR	Angioedema	Iomeprol	Negative	No	Iopromide	Urticaria/ Angioedema	STOP	-
27	DHR	Angioedema	Iomeprol	Negative	No	Iopromide	Angioedema	STOP	-
43	IHR	Cutaneous rash	Iomeprol	Negative	No	Iopromide	Cutaneous rash	Iodixanol	No
54	DHR	Face angioedema	Iomeprol	Iomeprol positive (unknown culprit)	No	Iodixanol	Face angioedema	Iopromide	No
57	IHR	Cutaneous rash	Iobitridol	Negative	Yes	Iobitridol	Cutaneous rash	Iomeprol	No
82	IHR	Urticaria	Iomeprol	Negative	Yes	Iomeprol	Urticaria	Iodixanol	No

STOP = no more DPTs; *= 2nd DPT was not tolerated, no more DPTs were proposed.

Table VI *- Characteristics of the reactions after ICM re-exposure.*

		No. of reactions upon ICM re-exposure	Type of reaction upon ICM re-exposure	Premedication*
Total		4/39 (10.2%)		3/26 (11.5%)
Culprit reaction	IHRs	3/33 (9.1%)	2 IHRs + 1 DHR	2
	DHRs	1/6 (16.7%)	DHR	1

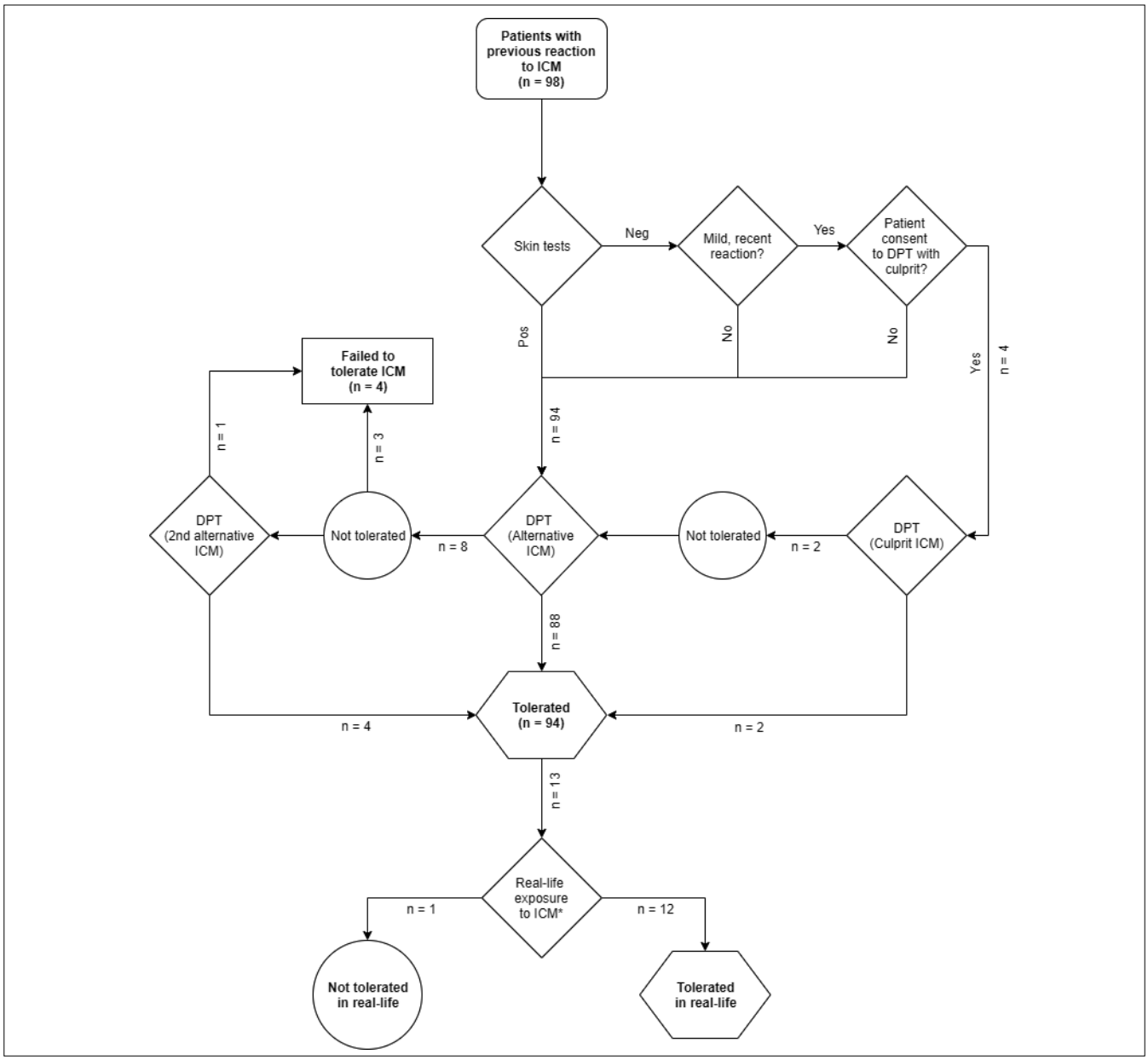
*Premedication was not indicated after our diagnostic workup

After failure of the first DPT, seven patients accepted to undergo a second DPT with a different ICM; the index reaction was a DHR in most cases (**table V**). All of these patients, except one, tolerated the DPT with a second different ICM. Overall, the protocol was completed by 94 patients (95.9%).

Therefore, assuming DPT as the gold standard, in our study population, the negative predictive value (NPV), calculated as no. of true negatives / (no. of true negatives + no. of false negatives), for skin tests was 96.2% in IHRs and 58.8% in DHRs($p < 0.0001$, Fisher's exact test) when administering an ICM different from the culprit.

In the patients who underwent DPT with the culprit ICM, NPV was low (50%) despite negative skin tests. **Figure 1** summarizes the study protocol and outcomes.

Figure 1 *- Study protocol and outcomes.*



* = only not-premedicated patients were considered.

ICM real-life re-exposure and follow-up

All patients were discharged with the indication to use only the tolerated ICM in case of future need of ICM-enhanced radiologic examination, without premedication.
Ninety subjects were reached by phone in the following months and asked standardized questions regarding their re-exposure to ICM as well as the outcome. Thirty-nine of them had undergone ICM re-administration, with anti-histamine/corticosteroid premedication in 26 cases, even if this was advised against after our allergologic evaluation.
Thirteen patients had undergone ICM re-administration without corticosteroid and/or anti-histamine premedication, and only these were considered for the purpose of predictive value calculation. Among these, one experienced an adverse reaction (immediate urticarial rash, 7.7%).
The NPV of our diagnostic protocol was 92.3%, compared with real-life re-exposure.
Considering all the 39 patients that had undergone ICM re-exposure, a total of 4 reactions (10.3%) (**table VII**) were reported, which was a rate higher than that observed in not-premedicated patients (reaction rate in premedicated patients was 3 on 26, 11.5%; p = ns, Fisher's exact test).

Discussion

This retrospective study reports a single-center experience on 98 patients with a previous adverse reaction to ICM who underwent an allergological workup.
The study protocol, adapted from the EAACI/ENDA consensus document (5), was demonstrated to be safe since no severe adverse events nor epinephrine administration occurred during the workup.

Skin tests

Regarding the skin tests, in our population, only a minority (10.2%) of the subjects exhibited a positive skin test. This result agrees with previous data reported by Schrijvers et al. (13.4%) (13) and Sesè et al. (13.5% in IHRs only) (14); other authors reported a higher prevalence of positive skin tests to ICM (29.1% to 64.7%) (12)(15)(16).
It is known that most of the IHRs to ICM are not IgE-mediated, and this is the main reason for the low sensitivity of the skin tests (2). However, the low rate of positive skin tests could also depend on other factors. First of all, the exact ICM involved in the index reaction was unknown in about half of our patients. Although we tested the five most frequently used ICM in the last 5 years, we could have not included the culprit, especially for those who experienced the reaction several years before.
This high rate of missing information regarding the culprit has been reported in other European countries as well, for example in the cohort of Sesè *et al.* (32.4%) (14), and is a reasonable value considered the real-life setting.
Secondly, the severity of the reaction could influence the outcome of skin tests; other authors have described a higher rate of positivity among patients who experienced severe reactions (17) with a reported percentage of positivity of more than a half in case of anaphylaxis and 82% in case of anaphylactic shock (16) (18). Our data confirm these findings since focusing on grade ≥3 IHRs with at least hypotension, the rate of positive skin test showed an increasing trend in respect to patients with a grade <3 reaction (23.1% vs 9.0%, p = ns).
Thirdly, it has been demonstrated in a multicenter trial that skin testing within six months from the latest reaction confers higher sensitivity to the test (15).
Our results highlight the importance of a short time delay between the reaction to ICM and the execution of the allergologic workup, and in particular of skin tests. In this study, the median time delay was elevated (89.0 months (range, 1–600 months)); only 42.8% and 30.6% of the patients, respectively, underwent an allergologic workup within 12 or 6 months from the last reaction. Shortening this delay could have a positive impact on the predictive value of skin tests, as in our population; when performed within 1 year (n = 47) and 6 months (n = 31), the rate of positive skin test increased respectively to 14.9% (7 of 47) and to 12.9% (4 of 31), even if this difference did not reach statistical significance. These findings are similar to those already reported (15)(14), (19).
Even considering DHRs alone, previous studies report a higher rate of positive skin tests in case of DHR (12), (20), and our results confirm this trend.

Table VII *- Summary of negative DPTs (with alternative ICM).*

Total of negative DPT with alternative ICM	Culprit ICM	Alternative ICM used for DPT
88	Known, n = 46 (52.3%)	Iopromide n = 32 (36.4%)
	(Unknown, n = 42, 47.7%)	Iodixanol n = 27 (30.7%)
		Iomeprol n = 25 (28.4%)
		Iobitridol n = 4 (4.5%)

Of note, we reported 30 reactions on first exposure, two of which associated with positive skin tests; they were both IHRs, the culprit emerged and resulted positive in one case, while it was unknown in the other one. Reactions on first exposure to ICM have been already reported before (13); in other series, most of them were DHRs, which occurred more than 1 hour after ICM administration (21). Of interest, in our study, only two subjects out of 30 experienced a DHR on first exposure; they exhibited negative skin tests performed more than 6 months after the adverse event, tolerated the DPT, but one relapsed after re-exposure.
IHR to ICM on first exposure have been also described. The rate of positive skin tests was 43% in subjects with such features in a study by Brockow (4).

ICM provocation test

The choice of ICM for DPTs has been based on results of skin tests, severity and temporal proximity of the index reaction, patient's consent to use the culprit ICM when indicated and potential cross-reactivity between different ICM.
Cross-reactivity between ICM depends on their chemical structure, but is less common in IHRs than in DHRs (6). Recently, Rosado Ingelmo et al. reported an elevated risk of cross reactions between iohexol, iopentol, ioversol, iopentol, and iodixanol, with the most relevant risk between iodixanol and its monomer iohexol, previously described by other authors (6), (12),(22).
There have been several attempts to classify ICM considering their cross-reactivity in skin tests, with little differences between authors. In a recent metanalysis (21), Yoon et al. confirmed the higher cross-reactivity of ICM during skin tests in case of DHRs, but even the higher rate of failure during DPTs in spite of negative skin tests.
Skin tests are currently considered the most reliable tool to choose the alternative ICM to be used for DPT, and a more reliable tool than premedication itself (20). Consequently, DPT has been recognized as essential to establish the diagnosis of ICM allergy, to assess tolerance, and to find a safe alternative ICM (12).
Of interest, the main feature of the patients who did not tolerate the selected ICM was an index DHR (see **table V**). The diagnostic accuracy of skin tests was significantly higher in patients who experienced a previous IHR compared with those who experienced a previous DHR (NVP, 96.2% vs 58.8%, respectively; $p < 0.0001$, Fisher's exact test).
Even if the sensitivity of the skin test with ICM is fairly good, we cannot exclude that a real-life challenge with a bolus administration could result in a more serious—and potentially life-threatening—adverse event in those who fail to tolerate the selected ICM. For this reason, DPT is an essential part of the proposed diagnostic protocol.
We know that DPTs are time- and resource-consuming, and can be performed only in hospital settings, in selected Allergy Units with adequate facilities and trained staff. Nevertheless, even if the NPVs for skin tests with ICM is fairly good, DPTs are still essential for a correct diagnosis. With our protocol, 10.3% of the patients reacted at the DPT after a negative skin test, but no severe adverse reactions were reported. We cannot exclude that some of these patients could have experienced a more severe (or even life-threatening reaction) if a real-life exposure with a bolus of ICM was performed instead of the step-wise administration of the DPT. Moreover, skin tests alone demonstrated a very low NPV in those patients who had experienced a previous DHR to ICM.
In vitro tests, such as the basophil activation test (BAT), could be useful to further improve the accuracy of the protocol. However, BAT is nowadays still a not completely standardized procedure and is not currently available in most laboratories.

ICM real-life re-exposure and follow-up

Overall, thirty-nine subjects were re-exposed to ICM after our allergologic workup, but only in 13 cases a corticosteroid/anti-histamine premedication was not used. In order to avoid bias, these were the only patients considered for the calculation of NPV, which resulted to be very high (92.3%).
Just one of these patients experienced an adverse reaction to ICM in a real-life setting, which was mild.
The fact that a premedication was used in 26 of the 39 patients that had undergone ICM re-exposure, despite this was advised against after our allergologic work-up, confirms that radiologist still rely very much on premedication, despite a low grade of evidence on its efficacy.
Surprisingly, the rate of reaction in premedicated patients was higher than that in not-premedicated patients in our study population, even if the difference was not statistically significant (11.5% vs 7.7%, p = ns). In each of these cases, reaction was mild, which required no epinephrine administration or hospitalization.
As this observation could be attributed to the small size of the population, it once again confirms the scarce utility of antiallergic premedication.
Moreover, it has been demonstrated that premedication protocols are associated with elevated costs (mostly due to the delay of the diagnostic procedure) and adverse effects (mostly due to corticosteroids), which greatly exceed the possible benefits (23, 24). The number needed to treat has been estimated to be 69 to prevent any reaction, 569 to prevent a severe reaction, and 56.900 to prevent a lethal reaction (23).
Despite the elevated NPV of our study protocol, four patients (on a total of 39) who had tolerated the chosen ICM reacted to the same compound in the real-life setting. The total dose of administered ICM was not significantly different between

the DPT and the radiologic exam. Possibly, a difference in the means of its administration should be taken into account; for the radiologic examination, the ICM is administered all at once, while in our study protocol it was administered in a three-step protocol that took about 120 minutes to be completed. One can speculate that a slower administration, as in our study protocol, could reduce the incidence of mild reactions due to direct histamine-releasing effects, while it should not modify the risk of immune-mediated adverse reactions.

Conclusions

We have reported here the results of the application of a protocol to diagnose ICM allergy and find a safe alternative in subjects with previous adverse reactions to ICMs.
This protocol is based on skin test and DPT, which is considered the gold standard for the diagnosis of ICM allergy but is potentially dangerous for the risk of severe adverse events. Our protocol demonstrated to be safe as no serious adverse event or epinephrine administration was reported in any of our 98 patients. It also demonstrated to be accurate as 92.3% of our patients subsequently tolerated ICM administration in a real-life setting without any antiallergic premedication.
On the other hand, some critical issues arouse that could limit the efficacy of the protocol; the late presentation of the patient to the allergist after an adverse reaction to ICM and the missing information about the culprit ICM represent important reasons of diagnostic failure.
Therefore, we believe that this protocol could be proposed to be used for the management of patients with previous reactions to ICM, where a BAT is not available.
The choice of premedication does not represent a valid alternative the allergologic workup, as an increasing body of evidence demonstrates discouraging data regarding premedication; a high number needed to treat is needed to prevent severe-lethal reactions and an unfavorable cost/harm ratio, since unnecessary premedication increases adverse events (glycometabolic failure and infections, to name a few), hospital stay and costs (23).
Hence, the use of premedication with antihistamines and steroids before the administration of an ICM should be evaluated on the single case, when the allergologic workup is not possible (e.g., radiologic examination is urgent) or contraindicated (e.g., renal failure).
A strong interplay between the prescribing physician, the radiologist, and the allergist is a key factor to ensure maximum safety for the patient.

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Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Romanini L, et al. Documento di Consenso SIRM-SIAAIC Gestione dei Pazienti a Rischio di Reazione Avversa a Mezzo di Contrasto, 2018. Available: https://www.sirm.org/documenti/documento-di-consenso-sirm-siaaic-per-la-gestione-dei-pazienti-a-rischio-di-reazione-avversa-da-mezzo-di-.
2. Brockow K. Immediate and Delayed Cutaneous Reactions to Radiocontrast Media. Chemical immunology and allergy 2012; 97: 180–90.
3. Brockow K, Sánchez-Borges M. Hypersensitivity to Contrast Media and Dyes. Immunol. Allergy Clin. North Am. 2014; 34(3):547–64.
4. Brockow K. Immediate and Delayed Reactions to Radiocontrast Media: Is There an Allergic Mechanism?. Immunology and Allergy Clinics of North America 2009; 29(3): 453-468. Elsevier Ltd.
5. Brockow K, et al. Skin test concentrations for systemically administered drugs. An ENDA/EAACI Drug Allergy Interest Group position paper. Allergy Eur. J. Allergy Clin. Immunol. 2013; 68:702-12.
6. Rosado Ingelmo A, et al. Clinical Practice Guidelines for Diagnosis and Management of Hypersensitivity Reactions to Contrast Media. J. Investig. Allergol. Clin. Immunol 2016. 26(3): 144–55.
7. Jakobsen JÅ, et al. Safety of ultrasound contrast agents, Eur. Radiol 2005. 15: 941–945.
8. ACR Manual On Contrast Media 2018. Available: https://www.acr.org/-/media/ACR/Files/Clinical-Resources/Contrast_Media.pdf. (Accessed: 20-Aug-2019).
9. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet (London, England) 1977; 1(8009): 466–9.
10. Brockow K, et al. Management of hypersensitivity reactions to iodinated contrast media. Allergy 2005. 60(2): 150–8.
11. Salas M, et al. Diagnosis of immediate hypersensitivity reactions to radiocontrast media. Allergy Eur J.Allergy Clin Immunol 2013; 68(9): 1203–1206.
12. Torres MJ et al. Diagnostic evaluation of patients with nonimmediate cutaneous hypersensitivity reactions to iodinated contrast media. Allergy 2012; (67)7:929–35.
13. Schrijvers R, Breynaert C, Ahmedali Y, Bourrain JL, Demoly P, Chiriac AM. Skin Testing for Suspected Iodinated Contrast Media Hypersensitivity. J. Allergy Clin. Immunol. Pract 2018; 6(4): 1246–54.

14. Sesé L, et al. Immediate hypersensitivity to iodinated contrast media: Diagnostic accuracy of skin tests and intravenous provocation test with low dose. Clin. Exp. Allergy 2016; 46(3):472–478.
15. Brockow K, et al. Skin testing in patients with hypersensitivity reactions to iodinated contrast media - a European multicenter study. Allergy 2009; 64(2): 234–41.
16. Kim MH et al. Anaphylaxis to Iodinated Contrast Media: Clinical Characteristics Related with Development of Anaphylactic Shock. PLoS One 2014; 9(6):100-54.
17. Joint Task Force on Practice Parameters, American Academy of Allergy, Asthma and Immunology, American College of Allergy, Asthma and Immunology, and Joint Council of Allergy, Asthma and Immunology, Drug allergy: an updated practice parameter. Ann Allergy Asthma Immunol 2010; 105(4): 259–73.
18. Kim SH, et al. Outcomes of premedication for non-ionic radio-contrast media hypersensitivity reactions in Korea, Eur J Radiol 2011; 80(2): 363–7.
19. Caimmi S, et al. Clinical value of negative skin tests to iodinated contrast media. Clin Exp Allergy 2010; 40(5): 805–10.
20. Yoon SH, et al. Skin tests in patients with hypersensitivity reaction to iodinated contrast media: a meta-analysis. Allergy 2015; 70(6): 625–37.
21. Bircher AJ, Brockow K, Grosber M, Hofmeier KS. Late elicitation of maculopapular exanthemas to iodinated contrast media after first exposure. Ann. Allergy. Asthma Immunol 2013; 111(6): 576–7.
22. Hasdenteufel F, et al. Delayed hypersensitivity reactions caused by iodixanol: An assessment of cross-reactivity in 22 patients. J Allergy Clin Immunol 2011; 128(6): 1356–57.
23. Davenport MS, Cohan RH. The Evidence for and Against Corticosteroid Prophylaxis in At-Risk Patients. Radiol Clin North Am 2017; 55(2): 413–21.
24. Tramèr MR, von Elm E, Loubeyre P, Hauser C. Pharmacological prevention of serious anaphylactic reactions due to iodinated contrast media: systematic review. BMJ 2006; 333 (7570): 675.

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Unmet needs and relationship between general practitioners (GPs) and allergists living in Campania region (southern Italy)

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Allergists, allergy; allergic rhinitis; allergic sensitization; bronchial asthma; Campania region; general practitioner; hypersensitivity.

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To the Editor,

It is widely recognized that the prevalence of allergic diseases is increasing in all industrialized countries and that it determines increasing problems in managing such high number of patients. In Italy, the necessity of optimizing economic resources as well as the lack of specialist' turnover have the consequence that general practitioners (GPs) are called to manage individuals suffering from less severe / life-threatening allergic conditions and, consequently, to select those cases requiring specialized consultation. Several studies have investigated competences and role of GPs in managing respiratory (1-7), cutaneous (8), food/drug-related (9,10) allergic symptoms. Based on this background, the aim of our study was to assess, by a questionnaire, how GPs living in Campania region approach patients suffering from different allergic diseases in "real life", their knowledge about some debated topics in order to point out pitfalls and unmet needs in their relationship with allergists.

A board of experts belonging to Italian Association of Hospital and Territorial Allergists and Immunologists (AAIITO – Campania region) developed a questionnaire made of 10 multiple choice questions covering some aspects in management strategies of common allergy conditions adopted by GPs working in Campania region (13.595 Km^2, 5.833.332 inhabitants at 30 November 2014).

Between 10th of January 2018 and 28th of February 2018, a self-administered anonymous questionnaire was e-mailed to a sample of GPs randomly selected from the National Registers of Physicians and working in Campania region. E-mails containing questionnaire were sent and collected by twenty allergists belonging to AAIITO-Campania according to the five regional provinces. Compared to the total number of contacted GPs, 31% (n. 730) of these were available to effectively participate in the survey and to complete the questionnaire (**figure 1**).

Figure 1 shows the percentages of each answers (including the case of no-answer) in response to the 10 multiple choice questions.

Question 1. GPs do not shy away from the responsibility of managing patients suffering from allergic diseases independently (43%). Otherwise, the patient is referred (37%) to the allergist or (19%) to other specialists (e.g. otorhinolaryngologist, pulmonologist, dermatologist, gastroenterologist).

Question 2. Only 2, 50% of the GPs sends the patient to allergist both diagnostics (23%) and therapeutic (26%) purposes.

Question 3. About half of GPs (52%) manage personally asthmatic patients whereas, the remaining percentage send them to pulmonologist (34%) and to allergist (14%).

Question 4. High percentages (64%) of GPs manage directly patients suffering from suspected allergic rhinitis, and only a minor percentage of rhinitics were sent to otorhinolaryngologist (14%) and to allergist (19%).

Question 5. About 59% of GPs consider that allergen immunotherapy (AIT) should be administered only in selected allergic patients. It is noteworthy the about 21% of GP consider AIT not based on scientific evidence and 19% suitable for "all" allergic patients (19%).

Question 6. Only 23% of GPs consider allergy consultation in response to the question. It is noteworthy that 19% of GP consider suitable tests for "food intolerance", not approved by the scientific community.

Question 7. It is very appreciable that 45% of GPs assumes responsibility for using an alternative drug in patients with suspected allergic drug reaction. However, the request of an allergy consultation is correctly performed by other GPs.

Question 8. Allergists are the preferred specialists (50%) in response to question 8, followed by dermatologists (31%). Thirteen percent of GPs prefer to wait for the result of medical treatment before electing the reference specialist.

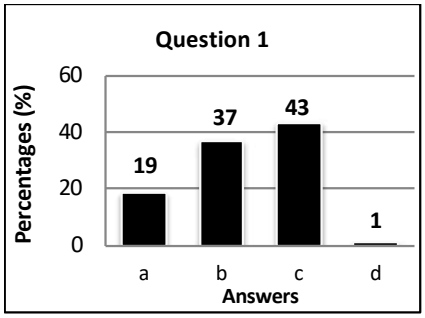
Question 9. A high percentage of GPs (79%) do not perform diagnostic tests for allergic disorders.

Question 10. Too long waiting lists are considered the most important reason of difficulties in sending patients to allergists (53%) followed by the lack of nearby territorial structures (20%) and the spending limits imposed by the National Health System (19%).

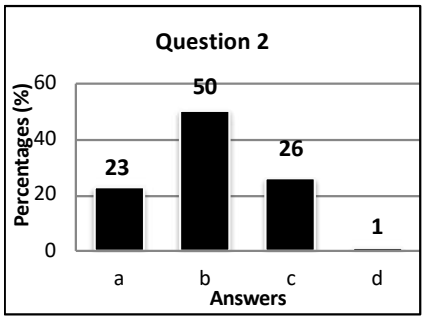
The overall evaluation of the answers of 730 GPs working in Campania region shows that a remarkable percentage of them manages personally patients suffering from suspected allergic diseases and particularly those with bronchial asthma and allergic rhinitis. Of course, we had no possibility to establish if GPs-suggested diagnostic approach and related therapies, could be considered corrected or not. This topic should be object of a future research. An allergy consultation is usually requested for diagnostic / therapeutic purposes or in the case of severe / life-threatening conditions such as drug / sting venom allergy or anaphylaxis. GPs have found some difficulties in the management of dyspeptic / gastrointestinal disorders of suspected allergic aetiology, some of them advice food intolerance tests generally not considered a scientifically validated diagnostic measures. Although the most of GPs consider AIT suitable for

a well-defined allergic patient, others show poor knowledge of mechanisms and potential role of this therapy in allergic respiratory disorders. In the case of drug allergy, the advice of an alternative drug is frequent and this is an important aspect because, sometimes, patient's condition needs an immediate decision. In other cases, the confirmation of the diagnosis of drug allergy and/or the testing of an alternative drug are correctly associated to the request of an allergy consultation. However, about 26% of GPs prefer to avoid any prescription of drugs without allergist's suggestion. Only a minority of GPs usually manage chronic urticaria probably because the well-known difficult diagnostic and therapeutic approach, in this case allergist is the preferred

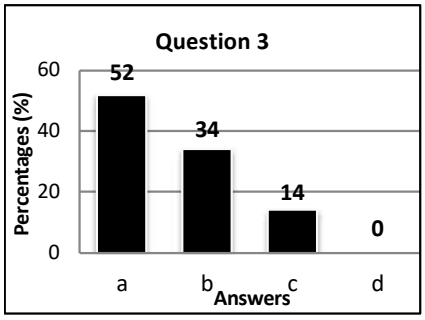
Figure 1 *- Percentages of each answers (including the case of no-answer) in response to the 10 multiple choice questions.*



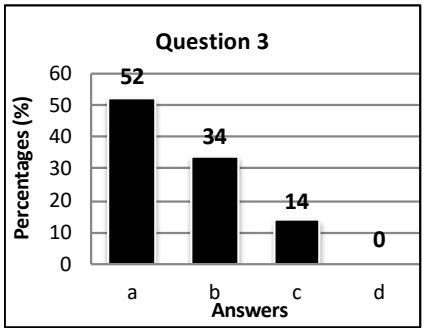
1.In patients with a suspected allergic disease I prefer:
a. Send the patient to an organ specialist (otorhinolaryngologist, pulmonologist, dermatologist, gastroenterologist)
b. Send the patient to allergologist
c. No answer
d. Manage the patient personally



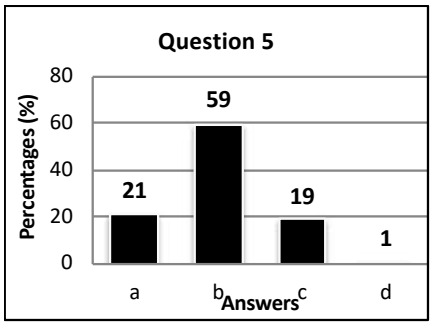
2. When do you solicit an allergy advice?
a. Only for diagnostics
b. Only for diagnostics and therapeutic purposes
c. Only for management of exclusive problems (e.g. drug / hymenoptera venom allergy)
d. No answer



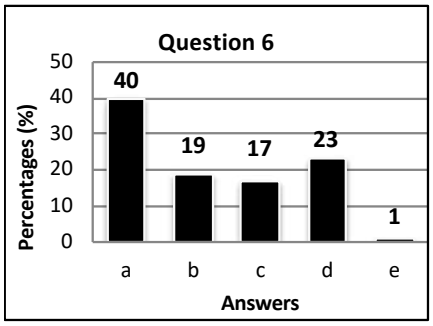
3. In the case of a patient with suspected bronchial asthma, in most cases, I prefer:
a. Personally manage the patient
b. Send the patient to the pulmonologist
c. Send the patient to the allergist
d. No answer



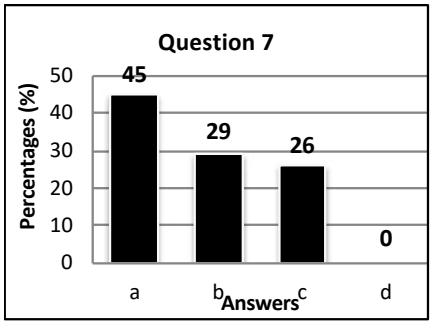
4. In the case of a patient with suspected allergic rhinitis, in most cases, I prefer:
a. Personally manage the patient
b. Send the patient to the otorhinolaryngologist
c. Send the patient to the allergist
d. No answer



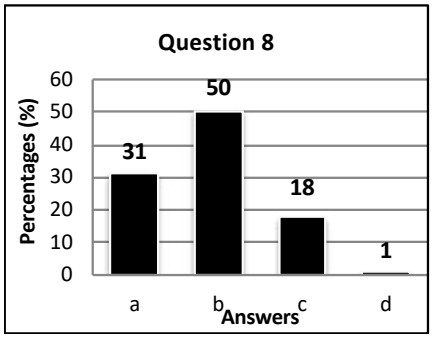
5. How to consider specific allergen immunotherapy (AIT)
a. I do not consider it a scientifically validated therapy
b. Efficacy and safety is to be reserved only for a narrow minority of patients
c. It is recommended for the majority of allergic patients
d. No answer



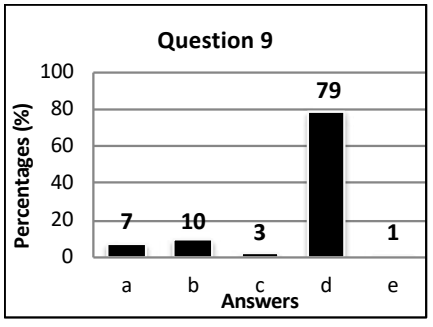
6. In the presence of a patient with dyspeptic / gastrointestinal disorders (abdominal pain, meteorism, irregular alve), for which you think it is advisable to investigate any hypersensitivity to specific foods, what advice?
a. Investigations for Celiac disease or Lactase deficiency
b. Tests for food intolerances (e.g. food-specific IgG, Cytotest, other tests for intolerances)
c. Food allergy tests (prick test or specific IgE)
d. Allergic specialist advice
e. No answer



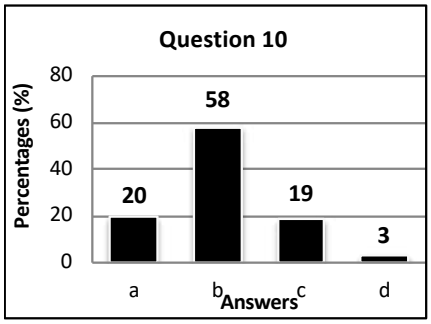
7. Your patient has had a suspected allergic reaction to an antibiotic: what advice?
a. An alternative drug
b. An alternative drug but I send the patient to the allergist anyway
c. I send the patient to the allergist
d. No answer



8. In the presence of a patient with chronic urticaria (ie, persisting for more than 6 weeks), in most cases (in addition to prescribing symptomatic therapy):
a. I send the patient to the dermatologist
b. I send the patient to the allergist
c. I await the successful outcome of the treatment, as probably the urticaria will pass spontaneously
d. No answer



9. Do you test for allergies or intolerance?
a. Yes: skin prick tests for inhalant allergens
b. Yes: skin prick tests for inhalant and food allergens
c. Yes: tests for food intolerances
d. No
e. No answer



10. What difficulties do you find in guiding the allergic patient to the specialist?

a. Lack of nearby territorial structures
b. Excessively long waiting lists
c. Spending limits imposed by the National Health System
d. No answer

specialist. Very few GPs, probably those with special interest on allergic diseases, perform diagnostic tests for respiratory or food allergy. An important unmet need of GPs on allergy topics is the difficult communication with allergists because the paucity of these specialists in Campania region as well as for bureaucratic reasons (waiting lists too long). Finally, it is important to outline that we cannot compare our results with those of other authors because no study has used the same questions.

In conclusion, the results of our questionnaire administered to GPs of Campania region suggest a comfortable willigness of these GPs to manage personally some categories of allergic patients particularly those suffering from respiratory symptoms. Further efforts should be done to correct some pitfalls in managing other allergic conditions such as those skin or food-related and therapies (AIT). A better knowledge of the allergic diseases and a stronger collaborative alliance between allergists and GPs are desirable for a good management of allergic disorders in Campania primary care.

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Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Heffler E, Crimi C, Mancuso S, Campisi R, Puggioni F, Brussino L, Crimi N. Misdiagnosis of asthma and COPD and underuse of spirometry in primary care unselected patients. Respir Med. 2018;142:48-52.
2. Wang Y, Cho SH, Lin HC, Ghoshal AG, Bin Abdul Muttalif AR, Thanaviratananich S, Tunceli K, Urdaneta E, Zhang D, Faruqi R. Practice Patterns for Chronic Respiratory Diseases in the Asia-Pacific Region: A Cross-Sectional Observational Study. Int Arch Allergy Immunol.2018; 177:69-79.
3. Grover HL, Higgins BG. GPs have key role in improving outcomes in acute asthma. Practitioner. 2016; 260:15-9.
4. Bosnic-Anticevich S, Kritikos V, Carter V, Yan KY, Armour C, Ryan D, Price D. Lack of asthma and rhinitis control in general practitioner-managed patients prescribed fixed-dose combination therapy in Australia. J Asthma. 2018; 55:684-94.
5. Todkill D, Loveridge P, Elliot AJ, Morbey R, Lusignan S, Edeghere O, Smith G. Socioeconomic and geographical variation in general practitioner consultations for allergic rhinitis in England, 2003-2014: an observational study. BMJ Open. 2017;7:e017038.
6. Morrow S, Daines L, Wiener-Ogilvie S, Steed L, McKee L, Caress AL, Taylor SJC, Pinnock H. Exploring the perspectives of clinical professionals and support staff on implementing supported self-management for asthma in UK general practice: an IMP2ART qualitative study. NPJ Prim Care Respir Med. 2017;27:45.
7. Magnoni MS, Latorre M, Bettoncelli G, Sanchez-Herrero MG, Lopez A, Calvo E, Rizzi A, Caminati M, Senna G, Paggiaro PL. Asthma control in primary care: the results of an observational cross-sectional study in Italy and Spain. World Allergy Organ J. 2017;10:13.
8. Caminati M, M. S. Magnoni MS, Rizzi A, Braido F, Foresi A, Bettoncelli G, Infantino A, D'Andria C, Antonicelli L, Paggiaro PL, Falcone F, Senna G. Asthma management among different specialists: results from a national Italian survey. Eur Ann Allergy Clin Immunol 2014;46:74-82
9. Vena GA, Cassano N, Pegoraro V, Cataldo N, Heiman F, Cricelli I, Colombo D, Zagni E, Cricelli C, Lapi F. Medication patterns in chronic spontaneous urticaria: results from a nationwide investigation in the primary care setting in Italy. G Ital Dermatol Venereol. 2018;153:39-42.
10. Ciprandi G, Comite P, Ferrero F, Montaruli R, Mussap M. Prescriptive appropriateness using inhalant and food allergen panels: a comparison between General Practitioners' and Allergists' prescription in Genoa (Italy). Eur Ann Allergy Clin Immunol. 2017;49: 80-3.
11. Kasternow B, Karim MY. Introduction to drug allergy, and whom to refer for specialist assessment? Clin Med (Lond). 2016;16:588-92.

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Successful desensitization procedure to lenalidomide in a patient with delayed hypersensitivity confirmed with a positive LTT

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Key words

Drug allergy; desensitization; lenalidomide; multiple myeloma; lymphocyte transformation test.

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To the Editor,

Lenalidomide is an immunomodulatory oral synthetic-derivative of thalidomide which is indicated in association with dexamethasone in refractory multiple myeloma (MM) and when it relapses. Lenalidomide acts inducing apoptosis of tumour cells and changes in micro-environmental conditions of tumour stroma and angiogenesis and stimulating the host immune response through the activation of cytotoxic T-lymphocytes and Natural Killer-cells (1,2).

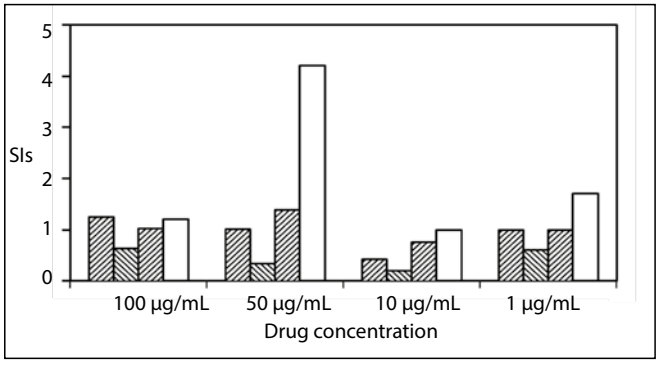
Adverse drug reactions (ADRs) to lenalidomide range from 6% to 43%, mostly morbilliform, urticarial and maculopapular exanthema, occurring within the first month of treatment (3). Some cases of severe cutaneous ADRs have also been reported such as Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS), erythema multiforme, Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). According to a meta-analysis conducted on ten trials, the overall incidence of all-grade and high-grade rash was 27.2% and 3.6%, respectively (4). In most cases, the relationship between the drug and rash development was suggestive leading to the removal of the drug, but they could not be ascertained with an objective test.

We present the case of a 77-year-old man with MM (lambda-light-chain disease, stage Durie Salmon IIIB, ISS3) diagnosed 2 years before, with renal impairment and bone lesions. His personal background included a colostomy for diverticulitis, inguinal herniorraphy and a transurethral resection for prostatic hypertrophy. He presented a previous allergic reaction to colistin,

but he had no history of either food or latex allergy, rhinitis or asthma. A third-line treatment with lenalidomide (10 mg/24h on days 1 to 21 of a 28-day cycle)-dexamethasone was initiated in February 2017 upon evidence of disease progression. In December 2017, after 5 days on the 10th cycle, he experienced an acute pruritic exanthema, developing a generalized morbilliform eruption on the trunk and folds with residual flaking skin. No mucosa involvement was observed. Neither pustules, vesicles or blisters were present. No eosinophilia, enlarged lymph nodes, elevated creatinine or hepatitis signs were detected. With the suspicion of a toxicodermic reaction, the discontinuation of lenalidomide was decided and the patient was treated with oral prednisolone for two weeks.

In an attempt to clarify the underlying mechanism of this reaction, a lymphocyte transformation test (LTT) with lenalidomide was performed. This method is performed by incubating fresh peripheral-blood mononuclear cells from patient previously separated over a density gradient (Histopaque-1077, Sigma-Aldrich) for 6 days at 10^6 cells/mL, at different concentration of the suspected drug. In this case, the test was performed in triplicates with lenalidomide at 0.1 µg/mL-100µg/mL. Drug was provided by the Hospital Pharmacy. Phytohemagglutinin (5 µg/mL) was used as positive control. Proliferation was determined by the addition of (^{3}H) thymidine (0.5 µCi/well) for the final 18 hours of the incubation period. The result is expressed as stimulation index (SI), which is the relationship between proliferation of lymphocytes in the presence or absence of the drug (basal proliferation). A positive result is suggestive of sensitization to the drug although a negative result does not exclude sensitization (5). The positive control result was 181 counts per minute (cpm) and that of basal proliferation was 58 cpm. A positive response, defined as an SI of over 2 in at least one of the doses tested, was obtained with lenalidomide. LTT with lenalidomide in 3 different healthy controls showed no proliferative responses (**figure 1**).

Figure 1 *- Lymphocyte transformation test results for lenalidomide. The test is considered positive when the stimulation index (SI) is greater than 2. Stripped bars represent SIs of three healthy controls. Open bars show SIs of the patient.*



A progression of the disease was verified in May 2018 and the haematologist decided to reintroduce the drug as the treatment of choice. Different strategies have been suggested for hypersensitivity dermatologic reactions induced by lenalidomide, including drug discontinuation or antihistamine and corticosteroid premedication. There are some few published reports of rapid inpatient desensitization in patients with acute urticarial rash (6) and an outpatient 6-week desensitization protocol for a target dose of 10 mg, in 5 patients with cutaneous delayed reaction (7). Considering the presence of an immunological mechanism causing the reaction and assessing all possible treatment options, we decided to perform a desensitization procedure. After assessing the safety of drug handling for small doses, a first attempt was initiated with a dose escalation procedure, rising daily the dose from 1 mg, which was planned to last 5 days (1, 2, 2.5, 5, 10 mg). Under specialist supervision in our outpatient clinic, the heart rate, blood pressure, pulse oximetry and peak-flow rate were monitored. This first attempt was interrupted at the third day of treatment, four hours later to the drug intake, the patient reported the presence of an intense armpits and scalp itching in absence of skin lesions, which persisted 48 hours after the removal of the drug. Cutaneous symptoms were accompanied by a single and self-limited episode of diarrhoea. Since the first attempted desensitization protocol failed, we designed a new one with dose escalation every 3 days based on previous recommendations in delayed reactions to allopurinol (8). We also restarted the procedure from a lower initial dose, adding concomitant bilastine 20 mg/24 h. Table I shows the adjusted 14-day protocol that was carried out from the initial dose of 0.1 mg of lenalidomide up to 10 mg/24h according to the dose prescribed by the hematologist. Escalating doses were tolerated, achieving the dose of 10 mg, since he continued to receive this daily dose of 10 mg for the next two months, without appearance of new episodes of itching, diarrhea, or skin involvement.

We report the case of a patient who developed a delayed erythematous morbilliform skin eruption in course of taking lenalidomide. For the first time, the implication of this drug was established by a positive LTT. Although LTT has not been completely standardized yet for many drugs, it should be considered a useful *in vitro* diagnostic tool, especially in non-immediate reactions. LTT reflects the reactivation and proliferation of memory cells that are present in the peripheral blood of allergic patients and it is not necessarily associated with more severe clinical symptoms and a dose-response pattern (9). For some drugs, LTT could offer a better diagnostic value than patch and intradermal tests to identify allergic subjects (10, 11). For drugs such as beta-lactams, LTT can reach a 92.8% of specificity, obtaining positive results even 10 or more years after the occur-

rence of the reaction, without further exposure to the drug (12). Moreover, LTT is safe for patients, which is absolutely relevant for severe reactions. In addition, we could propose an effective and safe alternative with a 14-day desensitization procedure, although it needs to be further validated in more patients.

In summary, this is the first reported case of a patient with hypersensitivity to lenalidomide, demonstrated by a positive LTT, in whom a short successful outpatient oral desensitization procedure was performed.

References

1. Rajkumar SV. Multiple myeloma: 2016 update on diagnosis, risk-stratification, and management. Am J Hematol 2016; 91:719-34.
2. Anderson KC. Lenalidomide and thalidomide: Mechanisms of action-similarities and differences. Semin Hematol 200; 42:S3-S8.
3. Patrizi A, Venturi M, Dika M, Maibach H, Tacchetti P, Brandi G. Cutaneous adverse reactions linked to targeted anticancer therapies bortezomib and lenalidomide for multiple myeloma: new drugs, old side effects. Cutan Ocul Toxicol 2014;33:1-6
4. Nardone B, Wu S, Garden BC, West DP, Reich LM, Lacouture ME. Risk of rash associated with lenalidomide in cancer patients: a systematic review of the literature and meta-analysis**.** Clin Lymphoma Myeloma Leuk 2013;13:424-9.
5. Pichler WJ, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. Allergy 2004: 59: 809–20.
6. Seki JT, Banglawala S, Lentz EM, Reece DE. Desensitization to lenalidomide in a patient with relapsed multiple myeloma. Clin Lymphoma Myeloma Leuk 2013; 13:162-5
7. Lee MJ, Wickner P, Fanning L, Schlossman R, Richardson P, Laubach J, et al. Lenalidomide desensitization for delayed hypersensitivity reactions in 5 patients with multiple myeloma. Br J Haematol. 2014; 167:127-31
8. Fam AG, Dunne SM, Iazzetta J, Paton TW. Efficacy and safety of desensitization to allopurinol following cutaneous reactions. Arthritis Rheum 2001; 44:231-8.
9. Lochmatter P and Pichler WJ. In vitro tests in drug hypersensitivity diagnosis. Immunol Allergy Clin North Am 2009; 29: 537-54.
10. Gonzalez-Cavero L, Dominguez-Ortega J, Gonzalez-Muñoz M, Mayor-Ibarguren A, Tomás M, Fiandor A, et al. delayed allergic reaction to terbinafine with a positive lymphocyte transformation test. J Investig Allergol Clin Immunol 2017; 27: 136-7.
11. Monge-Ortega OP, Cabañas R, Fiandor A, Domínguez-Ortega J, González-Muñoz M, Quirce S, et al. Overlap between DRESS syndrome and exanthema induced by sulfadiazine in a patient treated with sulfamethoxazole: utility of the lymphocyte transformation test for identification of the culprit drug. J Investig Allergol Clin Immunol 2018; 28:132-4.
12. Luque I, Leyva L, Torres MJ, Rosal M, Mayorga C, Segura JM, Blanca M, Juárez C. In vitro T-cell responses to beta-lactam drugs in immediate and nonimmediate allergic reactions. Allergy 2001; 56:611-8.

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