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5/2020

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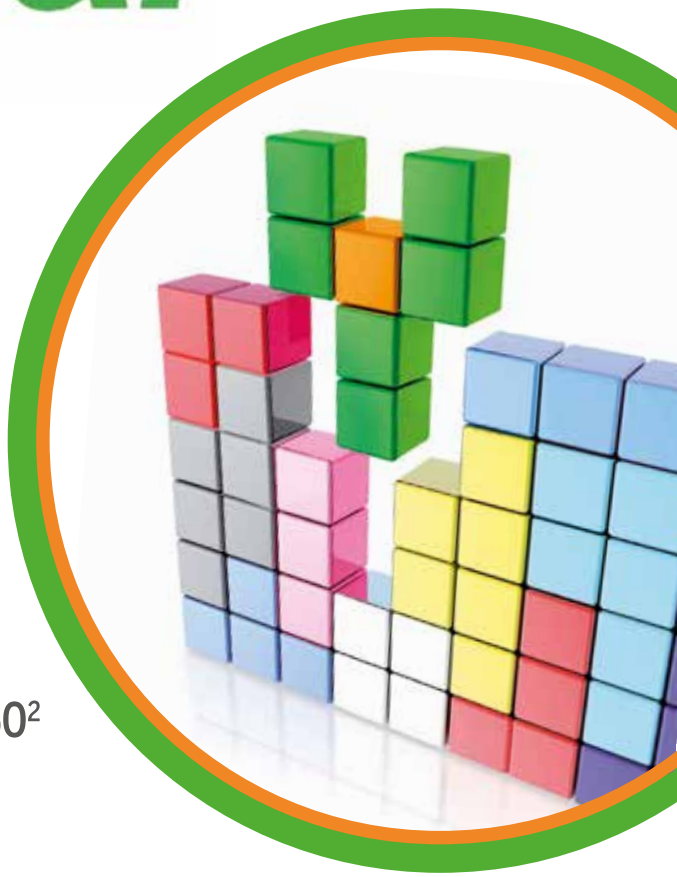
BILASTINA

Ayrinal

È un H1-antistaminico



- Di seconda generazione[1]
- Non sedativo[2]
- Efficace nel ridurre i sintomi della rinocongiuntivite allergica[2]
- Efficace nel ridurre i sintomi dell'orticaria[2]
- Che non interferisce col citocromo P450[2]
- Che non potenzia gli effetti sedativi del lorazepam* sul SNC[2]
- Alla dose di 20 mg non riduce la performance psicomotoria con l'assunzione di alcool[2]

Classe C
€ 10,80
Posologia:[2]
una compressa da 20 mg al dì un'ora prima o due ore dopo l'assunzione di cibo o di succhi di frutta

Prima di prescrivere e per informazioni complete sul medicinale (indicazioni terapeutiche, posologia e modo di somministrazione, controindicazioni, avvertenze speciali e opportune precauzioni di impiego, effetti indesiderati, ecc.), consultare il Riassunto delle Caratteristiche del Prodotto di Ayrinal 20 mg compresse incluso.

* 3 mg/die per 8 giorni

Medicinale soggetto a prescrizione medica.

1. Demonte A., et al. *Bilastine safety in drivers who need antihistamines: new evidence from high-speed simulator driving test on allergic patients.* European Review for Medical and Pharmacological Sciences 2018; 22: 820-828. **2. Riassunto delle caratteristiche di prodotto di AYRINAL 20 mg compresse.**

malesci

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

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Key words

Allergen immunotherapy; safety; efficacy; allergoids; monomeric allergoids.

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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.

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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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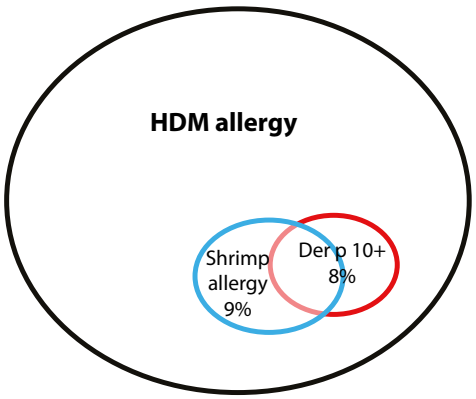
Summary

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.

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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

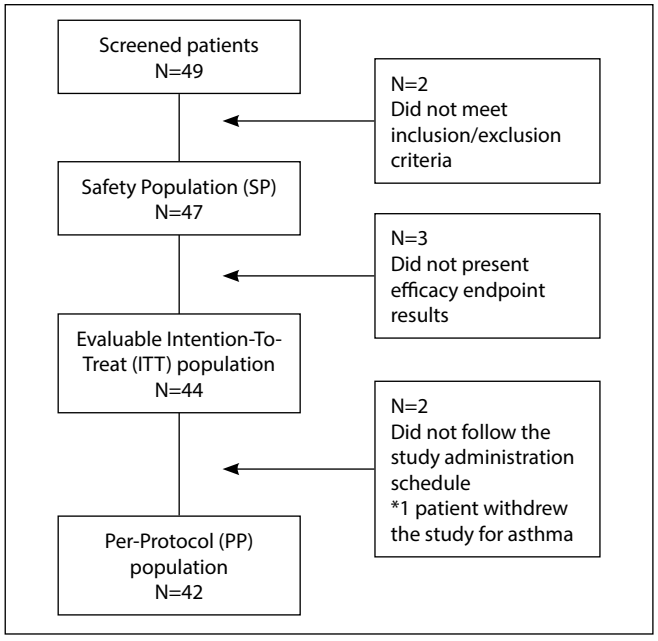
We performed tolerability and safety assessment and descriptive statistical analyses in the safety population (patients who received at least one dose of treatment). Efficacy statistical analyses were applied using the intention-to-treat (ITT) population (patients who met all inclusion/exclusion criteria, 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)

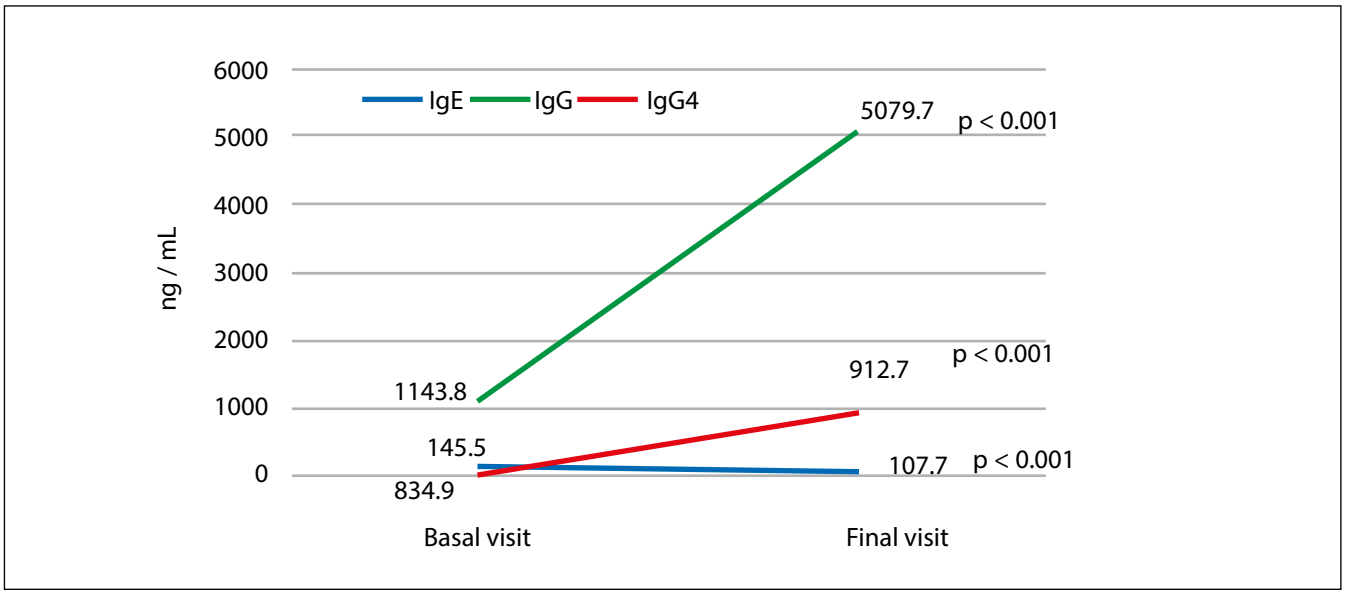
*(SP)[a] safety population, (SD)[b] standard deviation.

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

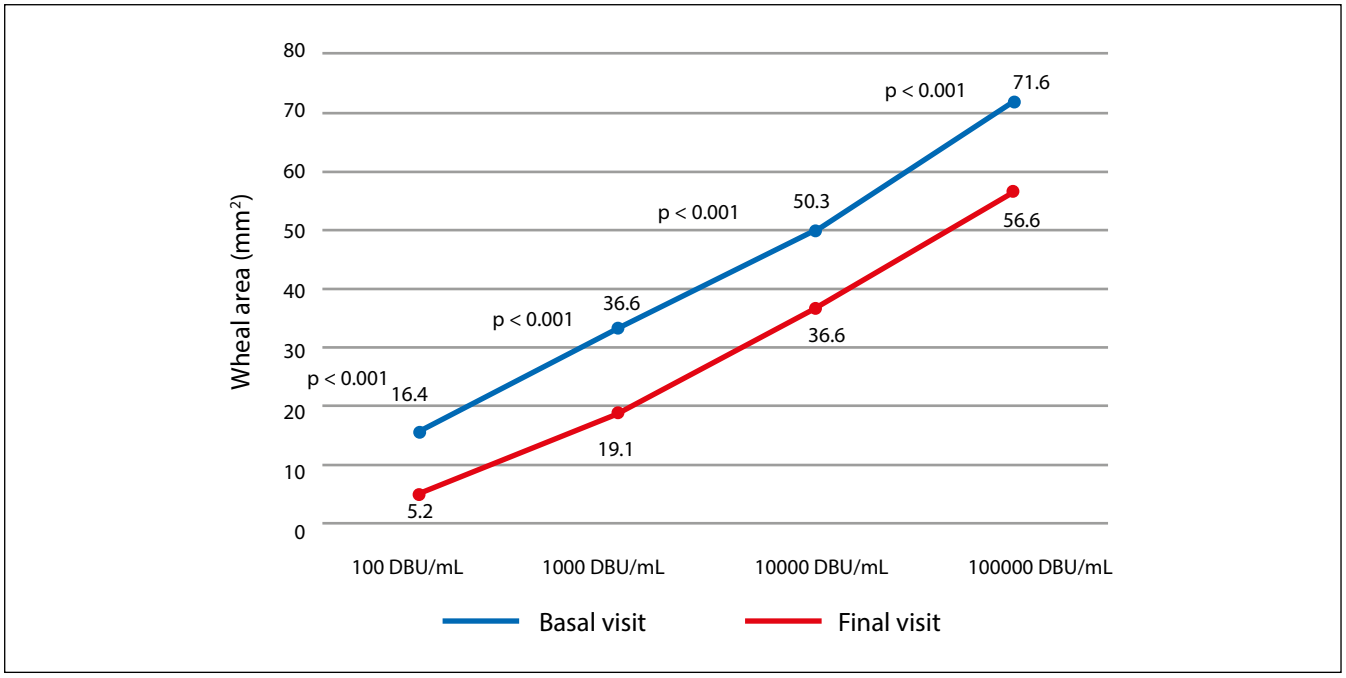
*n (%) number and percentage of adverse reactions, LR (local reaction), [a] Clinically relevant delayed LRs during initiation phase, [b] Clinically relevant delayed LRs during maintenance phase, [c] Systemic reaction grade 0, [d] Systemic reaction grade I and e Systemic reaction grade II

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.*



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.

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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

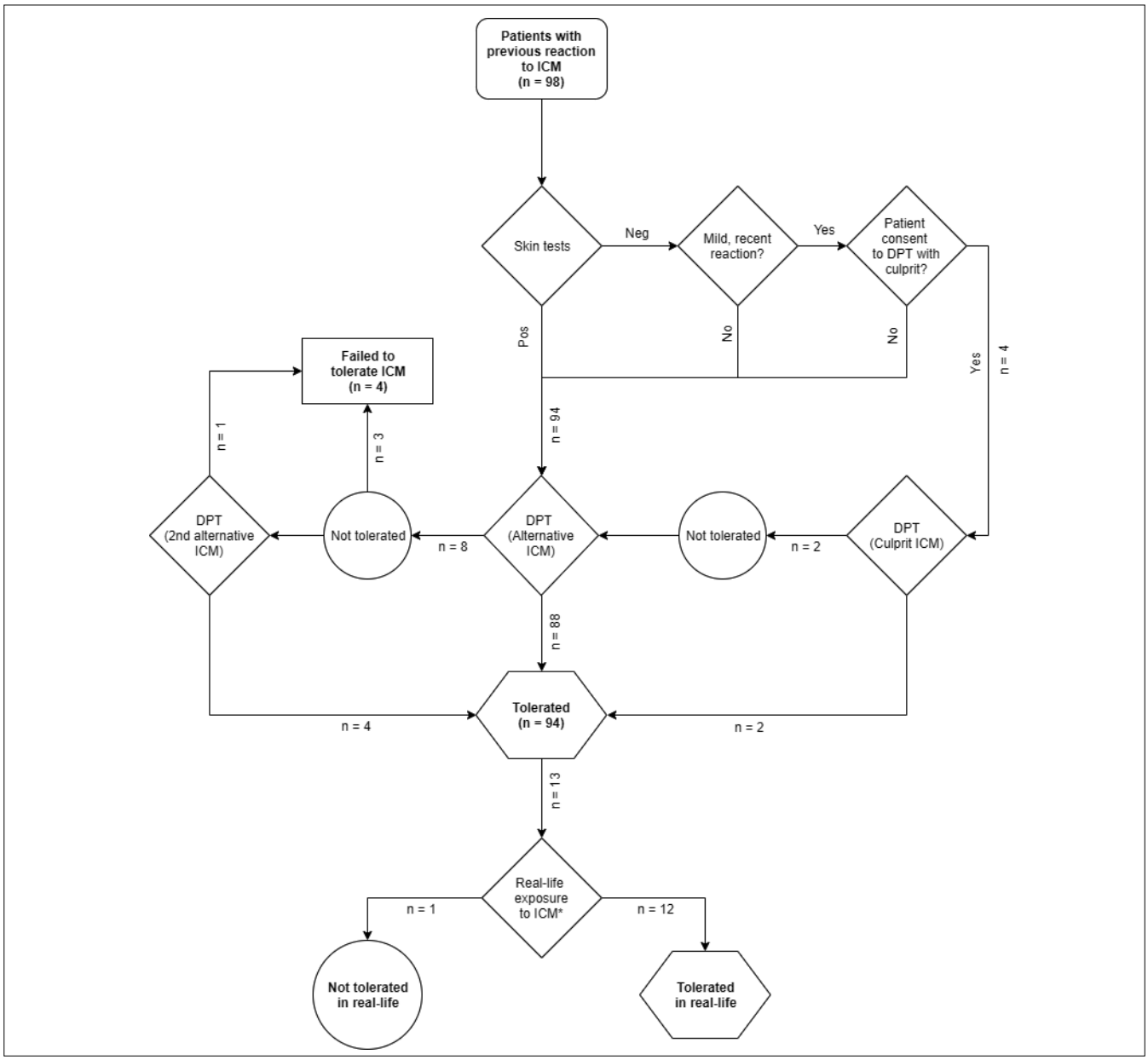
*Premedication was not indicated after our diagnostic workup

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.

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

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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.

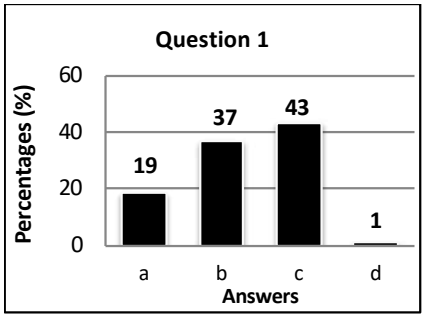
Question 9. A high percentage of GPs (79%) do not perform diagnostic tests for allergic disorders.

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%).

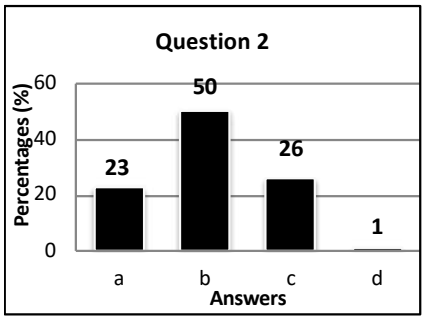
The overall evaluation of the answers of 730 GPs working in Campania region shows that a remarkable percentage of them manages personally patients suffering from suspected allergic diseases and particularly those with bronchial asthma and allergic rhinitis. Of course, we had no possibility to establish 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

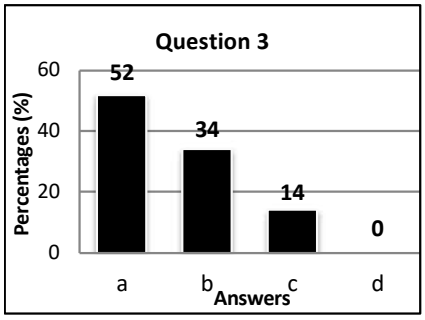
Figure 1 *- Percentages of each answers (including the case of no-answer) in response to the 10 multiple choice questions.*



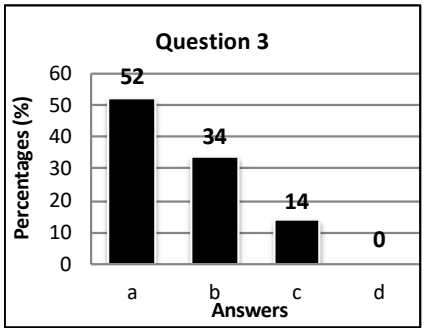
1.In patients with a suspected allergic disease I prefer:
a. Send the patient to an organ specialist (otorhinolaryngologist, pulmonologist, dermatologist, gastroenterologist)
b. Send the patient to allergologist
c. No answer
d. Manage the patient personally



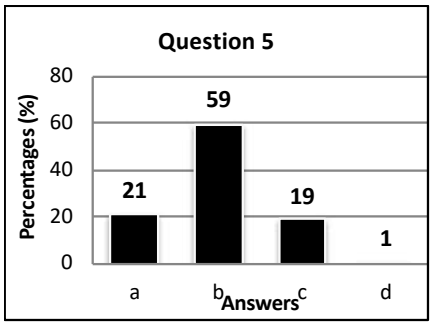
2. When do you solicit an allergy advice?
a. Only for diagnostics
b. Only for diagnostics and therapeutic purposes
c. Only for management of exclusive problems (e.g. drug / hymenoptera venom allergy)
d. No answer



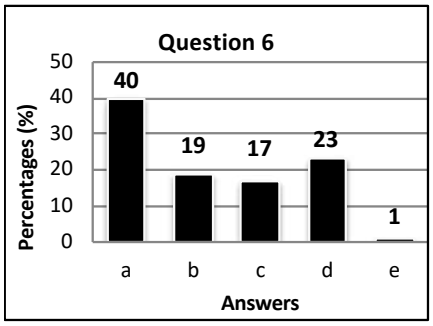
3. In the case of a patient with suspected bronchial asthma, in most cases, I prefer:
a. Personally manage the patient
b. Send the patient to the pulmonologist
c. Send the patient to the allergist
d. No answer



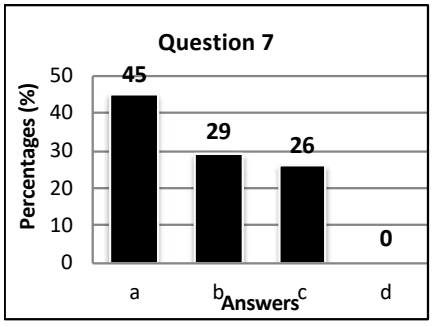
4. In the case of a patient with suspected allergic rhinitis, in most cases, I prefer:
a. Personally manage the patient
b. Send the patient to the otorhinolaryngologist
c. Send the patient to the allergist
d. No answer



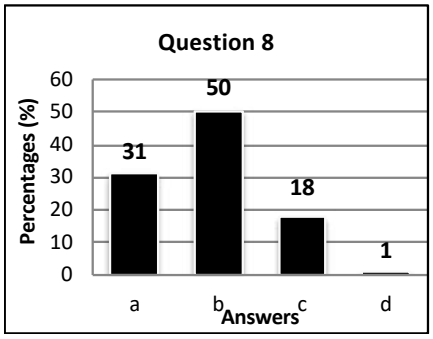
5. How to consider specific allergen immunotherapy (AIT)
a. I do not consider it a scientifically validated therapy
b. Efficacy and safety is to be reserved only for a narrow minority of patients
c. It is recommended for the majority of allergic patients
d. No answer



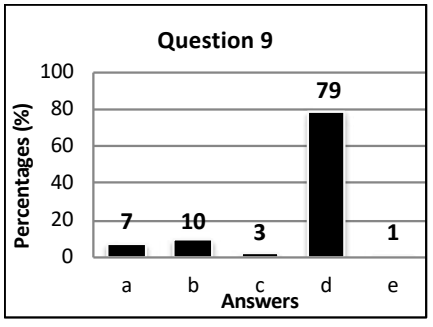
6. In the presence of a patient with dyspeptic / gastrointestinal disorders (abdominal pain, meteorism, irregular alve), for which you think it is advisable to investigate any hypersensitivity to specific foods, what advice?
a. Investigations for Celiac disease or Lactase deficiency
b. Tests for food 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



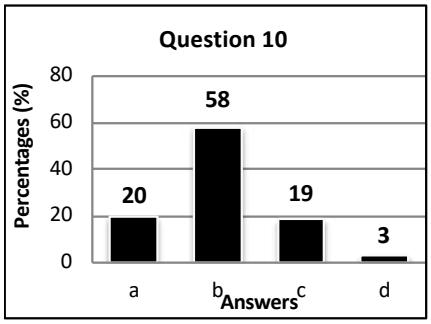
7. Your patient has had a suspected allergic reaction to an antibiotic: what advice?
a. An alternative drug
b. An alternative drug but I send the patient to the allergist anyway
c. I send the patient to the allergist
d. No answer



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.

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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).

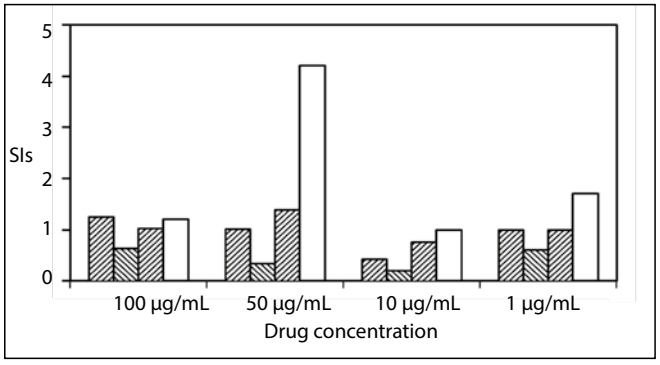
Adverse drug reactions (ADRs) to lenalidomide range from 6% to 43%, mostly morbilliform, urticarial and maculopapular exanthema, occurring within the first month of treatment (3). Some cases of severe cutaneous ADRs have also been reported such as Drug Reaction with Eosinophilia and Systemic Symptoms (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.

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AYRINAL BILASTINA

RIASSUNTO DELLE CARATTERISTICHE DEL PRODOTTO

1. DENOMINAZIONE DEL MEDICINALE. AYRINAL 20 mg compresse. **2. COMPOSIZIONE QUALITATIVA E QUANTITATIVA.** Ogni compressa contiene 20 mg di bilastina. Per l'elenco completo degli eccipienti, vedere paragrafo 6.1. **3. FORMA FARMACEUTICA.** Compressa. Compresse bianche, ovali, biconvesse con linea di incisione (lunghezza 10 mm, larghezza 5 mm). La linea di incisione sulla compressa serve solo per agevolarne la rottura al fine di ingerire la compressa più facilmente e non per dividerla in dosi uguali. **4. INFORMAZIONI CLINICHE. 4.1. Indicazioni terapeutiche.** Trattamento sintomatico della rinocongiuntivite allergica (stagionale e perenne) e dell'orticaria. AYRINAL è indicato negli adulti e negli adolescenti (12 anni di età ed oltre). **4.2. Posologia e modo di somministrazione. Posologia.** *Adulti e adolescenti* (12 anni di età ed oltre). 20 mg di bilastina (1 compressa) una volta al giorno per alleviare i sintomi della rinocongiuntivite allergica (SAR e PAR) e dell'orticaria. La compressa deve essere assunta un'ora prima o due ore dopo l'assunzione di cibo o succhi di frutta (vedere paragrafo 4.5). Durata del trattamento: Per la rinocongiuntivite allergica il trattamento deve essere limitato al periodo di esposizione agli allergeni. Per la rinite allergica stagionale il trattamento può essere interrotto dopo la scomparsa dei sintomi e ripreso alla loro ricomparsa. Nella rinite allergica perenne può essere proposto ai pazienti un trattamento continuato durante il periodo di esposizione agli allergeni. Nell'orticaria la durata del trattamento dipende dal tipo, dalla durata e dal decorso dei disturbi. *Popolazioni speciali:* Anziani: Non sono necessari aggiustamenti del dosaggio nei pazienti anziani (vedere paragrafi 5.1 e 5.2). Compromissione renale: Studi condotti negli adulti in speciali gruppi di rischio (pazienti con compromissione renale) indicano che non è necessario un aggiustamento della dose negli adulti (vedere paragrafo 5.2). Compromissione epatica: Non esiste esperienza clinica in pazienti adulti con compromissione epatica. Tuttavia, dato che la bilastina non viene metabolizzata e viene eliminata immodificata nell'urina e nelle feci, non si prevede che la compromissione epatica aumenti l'esposizione sistemica oltre il margine di sicurezza nei pazienti adulti. Pertanto, non è necessario alcun aggiustamento del dosaggio nei pazienti adulti con compromissione epatica (vedere paragrafo 5.2). *Popolazione pediatrica:* - Bambini dai 6 agli 11 anni di età con un peso corporeo di almeno 20 kg. Bilastina 10 mg compresse orodispersibili e bilastina 2,5 mg/ml soluzione orale sono appropriate per la somministrazione a questa popolazione. - Bambini sotto i 6 anni di età e sotto i 20 kg. I dati attualmente disponibili sono descritti nei paragrafi 4.4, 4.8, 5.1 e 5.2, ma non possono essere effettuate raccomandazioni relativamente alla posologia. Pertanto la bilastina non deve essere usata in questa fascia di età. La sicurezza e l'efficacia di bilastina nei bambini con compromissione renale ed epatica non sono state stabilite. Modo di somministrazione: Uso orale. La compressa deve essere deglutita con acqua. Si raccomanda di assumere la dose giornaliera in un'unica somministrazione. **4.3. Controindicazioni.** Ipersensibilità al principio attivo o ad uno qualsiasi degli eccipienti elencati al paragrafo 6.1. **4.4. Avvertenze speciali e precauzioni d'impiego. Popolazione pediatrica:** L'efficacia e la sicurezza della bilastina nei bambini al di sotto dei 2 anni di età non sono state stabilite ed esiste una limitata esperienza clinica nei bambini tra i 2 e i 5 anni di età, pertanto la bilastina non deve essere usata in queste fasce di età. Nei pazienti con compromissione renale da moderata a grave la co-somministrazione della bilastina con inibitori della P-glicoproteina, quali ad esempio chetoconazolo, eritromicina, ciclosporina, ritonavir o diltiazem, può aumentare i livelli plasmatici della bilastina e pertanto aumentare il rischio di effetti avversi. Pertanto, la co-somministrazione della bilastina ed inibitori della P-glicoproteina deve essere evitata in pazienti con compromissione renale da moderata a grave. **4.5. Interazioni con altri medicinali ed altre forme di interazione.** Studi di interazione sono stati condotti solo negli adulti e sono riepilogati di seguito. Interazione con il cibo: il cibo riduce significativamente la biodisponibilità orale della bilastina del 30%. Interazione con il succo di pompelmo: l'assunzione concomitante della bilastina 20 mg con il succo di pompelmo diminuisce la biodisponibilità della bilastina del 30%. Questo effetto può verificarsi anche con altri succhi di frutta. Il grado di diminuzione della biodisponibilità può variare a seconda dei diversi produttori e dei frutti. Il meccanismo di questa interazione è l'inibizione dell'OATP1A2, un trasportatore di uptake per il quale la bilastina è un substrato (vedere paragrafo 5.2). I medicinali che sono substrati o inibitori dell'OATP1A2, come ritonavir o rifampicina, possono analogamente avere il potenziale di diminuire la concentrazione plasmatica della bilastina. Interazione con chetoconazolo o eritromicina: l'assunzione concomitante di bilastina 20 mg una volta al giorno e chetoconazolo 400 mg una volta al giorno o eritromicina 500 mg tre volte al giorno ha aumentato l'AUC della bilastina di 2 volte e la C_{max} di 2-3 volte. Questi cambiamenti possono essere spiegati dall'interazione con le proteine di trasporto intestinale, in quanto la bilastina è un substrato per P-gp e non viene metabolizzata (vedere paragrafo 5.2). Questi cambiamenti non sembrano avere effetti sul profilo di sicurezza della bilastina e chetoconazolo o eritromicina, rispettivamente. Analogamente altri medicinali che sono substrati o inibitori di P-gp, come la ciclosporina, possono potenzialmente aumentare la concentrazione plasmatica della bilastina. Interazione con diltiazem: l'assunzione concomitante della bilastina 20 mg una volta al giorno e diltiazem 60 mg una volta al giorno ha aumentato la C_{max} della bilastina del 50%. Questo effetto può essere spiegato dall'interazione con le proteine di trasporto intestinale (vedere paragrafo 5.2) e non sembra avere effetti sul profilo di sicurezza della bilastina. Interazione con alcool: la performance psicomotoria dopo l'assunzione concomitante di alcool e della bilastina 20 mg una volta al giorno è stata simile a quella osservata dopo l'assunzione di alcool e placebo. Interazione con lorazepam: l'assunzione concomitante della bilastina 20 mg una volta al giorno e lorazepam 3 mg una volta al giorno per 8 giorni non ha potenziato gli effetti sedativi sul SNC del lorazepam. *Popolazione pediatrica:* Sono stati effettuati studi di interazione solo negli adulti. Poiché non c'è esperienza clinica riguardo l'interazione della bilastina con altri medicinali, cibo o succhi di frutta nei bambini, i risultati ottenuti negli studi di interazione nella popolazione adulta devono essere presi in considerazione quando la bilastina viene prescritta ai bambini. Non esistono dati clinici nei bambini per dimostrare se cambiamenti nell'AUC o C_{max} dovuti ad interazioni influenzano il profilo di sicurezza della bilastina. **4.6. Fertilità, gravidanza e allattamento.** Gravidanza: i dati relativi all'uso della bilastina in donne in gravidanza non esistono o sono in numero limitato. Studi condotti sugli animali non indicano la presenza di effetti negativi diretti o indiretti riguardanti la tossicità riproduttiva, il parto o lo sviluppo postnatale (vedere paragrafo 5.3). A scopo precauzionale, è preferibile evitare l'uso di AYRINAL durante la gravidanza. Allattamento: L'escrezione della bilastina nel latte non è stata studiata nell'uomo. I dati farmacocinetici disponibili sugli animali hanno evidenziato escrezione della bilastina nel latte (vedere paragrafo 5.3). La decisione in merito alla continuazione o all'interruzione dell'allattamento o ad interrompere/astenersi dalla terapia con AYRINAL deve tenere in considerazione il beneficio dell'allattamento per il bambino e il beneficio della terapia con la bilastina per la madre. Fertilità: non esistono dati clinici oppure sono in numero limitato. Uno studio condotto nei ratti non ha indicato alcun effetto negativo sulla fertilità (vedere paragrafo 5.3). **4.7. Effetti sulla capacità di guidare veicoli e sull'uso di macchinari.** Uno studio condotto negli adulti per stabilire gli effetti della bilastina sulla capacità di guidare ha dimostrato che il trattamento con dosi di 20 mg non influenza la capacità di guida. Tuttavia, dato che la risposta individuale al medicinale può essere differente, i pazienti devono essere avvertiti di non guidare o usare macchinari fino a quando non avranno stabilito la propria risposta alla bilastina. **4.8. Effetti indesiderati. Sintesi del profilo di sicurezza in pazienti adulti e adolescenti:** L'incidenza di eventi avversi in pazienti adulti e adolescenti affetti da rinocongiuntivite allergica o da orticaria idiopatica cronica trattati con 20 mg di bilastina nei trial clinici è stato paragonabile all'incidenza in pazienti trattati con placebo (12,7% rispetto a 12,8%). Durante lo sviluppo clinico, sono stati condotti studi di fase II e III che hanno incluso 2525 pazienti adulti ed adolescenti trattati con diversi dosaggi di bilastina, di cui 1697 sono stati trattati con bilastina 20 mg. In questi studi 1362 pazienti hanno ricevuto placebo. Le reazioni avverse più comunemente segnalate dai pazienti che hanno ricevuto 20 mg di bilastina per l'indicazione rinocongiuntivite allergica o orticaria idiopatica cronica sono state mal di testa, sonnolenza, capogiri e affaticamento. Questi eventi avversi si sono verificati con una frequenza paragonabile nei pazienti trattati con placebo. ***Tabella riassuntiva delle reazioni avverse in pazienti adulti e adolescenti:*** Nella tabella che segue sono riportate le reazioni avverse possibilmente correlate alla bilastina e segnalate in oltre lo 0,1% dei pazienti trattati con 20 mg di bilastina nel corso dello sviluppo clinico (N = 1697). Le frequenze sono assegnate come segue: Molto comune (≥1/10); Comune (da ≥1/100 a <1/10); Non comune (da ≥1/1.000 a <1/100); Raro (da ≥1/10.000 a <1/1.000); Molto raro (<1/10.000); Non nota (la frequenza non può essere definita sulla base dei dati disponibili). Le reazioni rare, molto rare e con frequenza non nota non sono state incluse nella tabella.

Classificazione per Sistemi ed Organi		Bilastina 20 mg N=1697	Bilastina Tutte le dosi N=2525	Placebo N=1362
Frequenza	**Reazione avversa**			
Infezioni e infestazioni				
Non comune	***Herpes orale***	2 (0,12%)	2 (0,08%)	0 (0.0%)
Disturbi del metabolismo e della nutrizione				
Non comune	***Aumento dell'appetito***	10 (0,59%)	11 (0,44%)	7 (0.51%)
Disturbi psichiatrici				
Non comune	***Ansia***	6 (0,35%)	8 (0,32%)	0 (0.0%)
	Insonnia	2 (0,12%)	4 (0,16%)	0 (0.0%)
Disturbi del sistema nervoso				
Comune	***Sonnolenza***	52 (3.06%)	82 (3.25%)	39 (2.86%)
	Cefalea	68 (4.01%)	90 (3.56%)	46 (3.38%)
Non comune	***Capogiri***	14 (0.83%)	23 (0.91%)	8 (0.59%)
Disturbi dell'orecchio e del labirinto				
Non comune	***Tinnito***	2 (0,12%)	2 (0,08%)	0 (0.0%)
	Vertigini	3 (0,18%)	3 (0,12%)	0 (0.0%)
Patologie cardiache				

Classificazione per Sistemi ed Organi		Bilastina 20 mg N=1697	Bilastina Tutte le dosi N=2525	Placebo N=1362
Frequenza	**Reazione avversa**			
Non comune	***Blocco di branca destra***	4 (0,24%)	5 (0,20%)	3 (0.22%)
	Aritmia sinusale	5 (0,30%)	5 (0,20%)	1 (0.07%)
	Prolungamento del tratto QT all'elettrocardiogramma	9 (0,53%)	10 (0,40%)	5 (0.37%)
	Altre alterazioni all'ECG	7 (0,41%)	11 (0,44%)	2 (0.15%)
Patologie respiratorie, toraciche e mediastiniche				
Non Comune	***Dispnea***	2 (0,12%)	2 (0,08%)	0 (0.0%)
	Fastidio nasale	2 (0,12%)	2 (0,08%)	0 (0.0%)
	Secchezza del naso	3 (0,18%)	6 (0,24%)	4 (0.29%)
Disturbi gastrointestinali				
Non comuni	***Dolore all'addome superiore***	11 (0,65%)	14 (0,55%)	6 (0.44%)
	Dolore addominale	5 (0,30%)	5 (0,20%)	4 (0.29%)
	Nausea	7 (0,41%)	10 (0,40%)	14 (1.03%)
	Fastidio gastrico	3 (0,18%)	4 (0,16%)	0 (0.0%)
	Diarrea	4 (0,24%)	6 (0,24%)	3 (0.22%)
	Bocca secca	2 (0,12%)	6 (0,24%)	5 (0.37%)
	Dispepsia	2 (0,12%)	4 (0,16%)	4 (0.29%)
	Gastrite	4 (0,24%)	4 (0,16%)	0 (0.0%)
Disturbi della cute e del tessuto sottocutaneo				
Non comune	***Prurito***	2 (0,12%)	4 (0,16%)	2 (0.15%)
Disturbi generali e condizioni relative alla sede di somministrazione				
Non comune	***Affaticamento***	14 (0,83%)	19 (0,75%)	18 (1.32%)
	Sete	3 (0,18%)	4 (0,16%)	1 (0.07%)
	Miglioramento della condizione pre-esistente	2 (0,12%)	2 (0,08%)	1 (0.07%)
	Piressia	2 (0,12%)	3 (0,12%)	1 (0.07%)
	Astenia	3 (0,18%)	4 (0,16%)	5 (0.37%)
Esami diagnostici				
Non comune	***Aumento della gamma-glutamiltransferasi***	7 (0,41%)	8 (0,32%)	2 (0.15%)
	Aumento dell'alanina amino transferasi	5 (0,30%)	5 (0,20%)	3 (0.22%)
	Aumento dell'aspartato aminotransferasi	3 (0,18%)	3 (0,12%)	3 (0.22%)
	Aumento della creatinina nel sangue	2 (0,12%)	2 (0,08%)	0 (0.0%)
	Aumento dei trigliceridi nel sangue	2 (0,12%)	2 (0,08%)	3 (0.22%)
	Aumento del peso corporeo	8 (0,47%)	12 (0,48%)	2 (0.15%)

Frequenza non nota (non può essere definita sulla base dei dati disponibili): palpitazioni, tachicardia, reazioni di ipersensibilità (quali anafilassi, angioedema, dispnea, eruzione cutanea, edema localizzato/gonfiore locale ed eritema) e vomito sono state osservate nel periodo post-marketing. Descrizione di alcune reazioni avverse in pazienti adulti e adolescenti. Sono state osservate sonnolenza, cefalea, capogiri e affaticamento sia nei pazienti trattati con bilastina 20 mg che con il placebo. Le frequenze segnalate sono state 3,06% rispetto a 2,86% per sonnolenza; 4,01% rispetto a 3,38% per cefalea; 0,83% rispetto a 0,59% per capogiri e 0,83% rispetto a 1,32% per affaticamento. Le informazioni raccolte nel corso della vigilanza post-marketing hanno confermato il profilo di sicurezza osservato durante lo sviluppo clinico. *Sintesi del profilo di sicurezza nella popolazione pediatrica*. Durante lo sviluppo clinico, la frequenza, la tipologia e la severità delle reazioni avverse negli adolescenti (di età compresa tra 12 e 17 anni), sono state le stesse osservate negli adulti. Le informazioni raccolte in questa popolazione (adolescenti) durante la vigilanza post-marketing hanno confermato i risultati degli studi clinici. In uno studio clinico controllato a 12 settimane, la percentuale dei bambini (2-11 anni) che hanno riscontrato eventi avversi (EA) dopo il trattamento con bilastina 10 mg per la rinocongiuntivite allergica o per l'orticaria idiopatica cronica, era paragonabile con la percentuale del gruppo che riceveva il placebo (68,5% rispetto a 67,5%). Gli EA collegati al medicinale riportati più comunemente da 291 bambini (2-11 anni) che ricevevano bilastina 10 mg (formulazione in compressa orodispersibile) durante gli studi clinici (*260 bambini esposti nello studio di sicurezza clinica, 31 bambini esposti nello studio farmacocinetico), erano cefalea, congiuntivite allergica, rinite e dolore addominale. Gli eventi avversi correlati al medicinale si sono verificati con una frequenza comparabile nei 249 pazienti che ricevevano il placebo. Tabella riassuntiva delle reazioni avverse nella popolazione pediatrica: Nella tabella che segue sono riportate le reazioni avverse possibilmente correlate alla bilastina segnalate in oltre lo 0,1% dei bambini (2-11 anni) trattati con bilastina nel corso dello sviluppo clinico. Le frequenze sono assegnate come segue: Molto comune (≥1/10); Comune (da ≥1/100 a <1/10); Non comune (da ≥1/1.000 a <1/100); Raro (da ≥1/10.000 a <1/1.000); Molto raro (<1/10.000); Non nota (non può essere stabilita sulla base dei dati disponibili). Le reazioni rare, molto rare e con frequenza non nota non sono state incluse nella tabella.

Classificazione per Sistemi e Organi		Bilastina 10 mg (n=291)[#]	Placebo (n=249)
Frequenza	**Reazione avversa**		
Infezioni e infestazioni			
Comune	Rinite	3 (1,0 %)	3 (1,2 %)
Patologie del sistema nervoso			
Comune	Cefalea	6 (2,1 %)	3 (1,2 %)
Non comune	Capogiri	1 (0,3 %)	0 (0,0 %)
	Perdita di conoscenza	1 (0,3 %)	0 (0,0 %)
Patologie dell'occhio			
Comune	Congiuntivite allergica	4 (1,4 %)	5 (2,0 %)
Non comune	Irritazione degli occhi	1 (0,3 %)	0 (0,0 %)
Patologie gastrointestinali			
Comune	Dolore addominale/addominale superiore	3 (1,0 %)	3 (1,2 %)
Non comune	Diarrea	2 (0,7 %)	0 (0,0 %)
	Nausea	1 (0,3 %)	0 (0,0 %)
	Gonfiore delle labbra	1 (0,3 %)	0 (0,0 %)
Patologie della cute e del tessuto sottocutaneo			
Non comune	Eczema	1 (0,3 %)	0 (0,0 %)
	Orticaria	2 (0,7 %)	2 (0,8 %)
Patologie sistemiche e condizioni relative alla sede di somministrazione			
Non comune	Affaticamento	2 (0,7 %)	0 (0,0 %)

[#]260 bambini esposti nello studio di sicurezza clinica, 31 bambini esposti nello studio farmacocinetico.

Descrizione di alcune reazioni avverse nella popolazione pediatrica: Cefalea, dolore addominale, congiuntivite allergica e rinite sono state osservate nei bambini trattati con bilastina 10 mg che con il placebo. La frequenza segnalata era 2,1% rispetto a 1,2% per cefalea; 1,0% rispetto a 1,2% per dolore addominale; 1,4% rispetto a 2,0% per congiuntivite allergica e 1,0% rispetto a 1,2% per rinite. Segnalazione delle reazioni avverse sospette: La segnalazione delle reazioni avverse sospette che si verificano dopo l'autorizzazione del medicinale è importante, in quanto permette un monitoraggio continuo del rapporto beneficio/rischio del medicinale. Agli operatori sanitari è richiesto di segnalare qualsiasi reazione avversa sospetta tramite il sistema nazionale di segnalazione all'indirizzo https://www.aifa.gov.it/content/segnalazioni-reazioni-avverse. **4.9. Sovradosaggio.** Le informazioni inerenti il sovradosaggio acuto di bilastina derivano dalle esperienze raccolte in trial clinici condotti durante lo sviluppo e la vigilanza post-marketing. Nel corso degli studi clinici, dopo la somministrazione di bilastina a dosi superiori di 10 o 11 volte la dose terapeutica (220 mg come dose singola o 200 mg/die per 7 giorni) a 26 volontari sani adulti, la frequenza degli eventi avversi occorsi durante il trattamento è stata di due volte superiore rispetto al placebo. Le reazioni avverse segnalate con maggior frequenza sono state capogiri, cefalea e nausea. Non sono stati segnalati eventi avversi gravi e nessun prolungamento significativo nell'intervallo QTc. Le informazioni raccolte nel corso della vigilanza post-marketing sono in linea con quanto riportato negli studi clinici. Una valutazione critica dell'effetto di dosi multiple di bilastina (100 mg x 4 giorni) sulla ripolarizzazione ventricolare mediante un "approfondito

studio incrociato sul QT/QTc" che ha coinvolto 30 volontari sani adulti, non ha evidenziato un prolungamento significativo del QTc. Non ci sono dati per il sovradosaggio nei bambini. In caso di sovradosaggio si raccomanda un trattamento sintomatico e di supporto. Non esiste alcun antidoto noto alla bilastina. **5. PROPRIETÀ FARMACOLOGICHE. 5.1. Proprietà farmacodinamiche.** Categoria farmacoterapeutica: antistaminici per uso sistemico, altri antistaminici per uso sistemico Codice ATC R06AX29. Meccanismo d'azione: La bilastina è un antagonista istaminergico non sedativo, ad azione prolungata con selettiva affinità antagonista per il recettore H_1 periferico e nessuna affinità per i recettori muscarinici. La bilastina ha inibito reazioni cutanee eritemato-pomfoidi indotte dall'istamina per 24 ore in seguito a somministrazioni di dosi singole. Efficacia clinica e sicurezza: Nei trial clinici eseguiti in pazienti adulti ed adolescenti con rinocongiuntivite allergica (stagionale e perenne), la bilastina 20 mg, somministrata una volta al giorno per 14-28 giorni, è stata efficace nell'alleviare i sintomi quali starnuti, fastidio nasale, prurito nasale, congestione nasale, prurito agli occhi, lacrimazione e rossore oculare. La bilastina ha mantenuto efficacemente sotto controllo i sintomi per 24 ore. In due trial clinici condotti in pazienti con orticaria idiopatica cronica, la bilastina 20 mg, somministrata una volta al giorno per 28 giorni è stata efficace nell'alleviare l'intensità del prurito ed il numero e le dimensioni dei pomfi, oltre ai disturbi provocati dall'orticaria. Nei pazienti sono migliorate le condizioni del sonno e la qualità della vita. Nei trial clinici condotti con la bilastina non è stato osservato un prolungamento clinicamente rilevante dell'intervallo QTc o alcun altro effetto cardiovascolare, anche a dosi di 200 mg al giorno (10 volte la dose clinica) per 7 giorni in 9 soggetti, oppure anche quando co-somministrata con inibitori P-gp, quali chetoconazolo (24 soggetti) ed eritromicina (24 soggetti). Inoltre è stato eseguito uno studio approfondito sul QT su 30 volontari. Nei trial clinici controllati alla dose raccomandata di 20 mg una volta al giorno, il profilo di sicurezza per il SNC della bilastina è stato simile al placebo e l'incidenza della sonnolenza non è stata statisticamente diversa dal placebo. La bilastina a dosi fino a 40 mg ogni giorno non ha influenzato la performance psicomotoria nei trial clinici e non ha influenzato la capacità di guida in un test di guida standard. Nei pazienti anziani (≥ 65 anni) inclusi in studi di fase II e III non sono state evidenziate differenze nell'efficacia o nella sicurezza rispetto ai pazienti più giovani. Uno studio post-autorizzativo su 146 pazienti anziani, non ha mostrato differenze sul profilo di sicurezza rispetto alla popolazione adulta. Popolazione pediatrica: Gli adolescenti (di età compresa tra 12 e 17 anni) sono stati inclusi nello sviluppo clinico. Nel corso degli studi clinici la bilastina è stata somministrata a 128 adolescenti (81 in studi in doppio cieco sulla rinocongiuntivite allergica). Un ulteriore gruppo di 116 adolescenti è stato randomizzato per la somministrazione di comparatori attivi o placebo. Non è stata osservata alcuna differenza in efficacia e sicurezza tra adulti e adolescenti. Secondo le linee guida, la comprovata efficacia negli adulti e negli adolescenti può essere estrapolata per i bambini, avendo dimostrato che l'esposizione sistemica a 10 mg di bilastina nei bambini dai 6 agli 11 anni di età, con un peso corporeo di almeno 20 kg, è equivalente all'esposizione a 20 mg di bilastina negli adulti (vedere paragrafo 5.2). L'estrapolazione dai dati raccolti negli adulti e negli adolescenti viene ritenuta appropriata per questo medicinale in quanto la patofisiologia della rinocongiuntivite allergica e dell'orticaria è la medesima per tutte le fasce d'età. In uno studio clinico controllato della durata di 12 settimane con bambini tra i 2 e gli 11 anni di età (totale 509 bambini, 260 trattati con bilastina 10 mg: 58 tra 2 anni e < 6 anni, 105 tra 6 anni e < 9 anni e 97 tra 9 anni e < 12 anni e 249 trattati con placebo: 58 tra 2 anni e < 6 anni, 95 tra 6 anni e < 9 anni e 96 tra 9 anni e < 12 anni), alla dose pediatrica raccomandata di 10 mg di bilastina una volta al giorno, il profilo di sicurezza della bilastina (n=260) era simile al placebo (n=249), con reazioni avverse al farmaco osservate nel 5,8% e 8,0% dei pazienti trattati rispettivamente con bilastina 10 mg e con il placebo. Durante questo studio, entrambi i gruppi di trattamento, bilastina 10 mg e placebo, hanno mostrato una lieve diminuzione nei punteggi di sonnolenza e sedazione nel Questionario Pediatrico del Sonno, con nessuna differenza statisticamente significativa. In questi bambini dai 2 agli 11 anni di età, non sono state osservate differenze significative nel QTc in seguito alla somministrazione giornaliera di 10 mg di bilastina comparata con il placebo. I questionari sulla Qualità della Vita specifici per i bambini con rinocongiuntivite allergica o orticaria cronica hanno mostrato un aumento generale nei punteggi oltre le 12 settimane con nessuna differenza statisticamente significativa tra i due gruppi di trattamento (bilastina e placebo). La popolazione totale dei 509 bambini comprendeva: 479 soggetti con rinocongiuntivite allergica e 30 soggetti con diagnosi di orticaria cronica. 260 bambini sono stati trattati con la bilastina, 252 (96,9%) per la rinocongiuntivite allergica e 8 (3,1%) per l'orticaria cronica. In analogia, 249 bambini sono stati trattati con placebo, 227 (91,2%) per la rinocongiuntivite allergica e 22 (8,8%) per l'orticaria cronica. L'agenzia Europea dei Medicinali ha dispensato dall'obbligo di presentare i risultati degli studi con la bilastina per tutti i sottoinsiemi della popolazione pediatrica al di sotto dei 2 anni di età (vedere paragrafo 4.2 per informazioni sull'uso pediatrico). **5.2. Proprietà farmacocinetiche.** Assorbimento: La bilastina viene rapidamente assorbita dopo la somministrazione orale raggiungendo la massima concentrazione nel plasma in circa 1,3 ore. Non si è osservato fenomeno di accumulo. La biodisponibilità media della bilastina dopo somministrazione orale è del 61%. Distribuzione: Studi *in vitro* e *in vivo* hanno mostrato che la bilastina è un substrato del Pgp (vedere paragrafo 4.5 "Interazione con chetoconazolo, eritromicina e diltiazem") e OATP (vedere paragrafo 4.5 "Interazione con succo di pompelmo"). La bilastina non risulta essere un substrato del trasportatore BCRP o dei trasportatori renali OCT2, OAT1 e OAT3. In base agli studi *in vitro,* non si prevede che la bilastina inibisca i seguenti trasportatori nella circolazione sistemica: P-gp, MRP2, BCRP, BSEP, OATP1B1, OATP1B3, OATP2B1, OAT1, OAT3, OCT1, OCT2 e NTCP, poiché solo una modesta inibizione è stata rilevata per P-gp, OATP2B1 e OCT1, con una IC_{50} stimata ≥ a 300 µM, molto più elevata rispetto alla C_{MAX} plasmatica clinica calcolata e per ciò queste interazioni non saranno clinicamente rilevanti. Tuttavia, sulla base di questi risultati, l'azione inibitoria della bilastina sui trasportatori presenti nella mucosa intestinale, per esempio P-gp, non può essere esclusa. Alle dosi terapeutiche la bilastina è legata per l'84-90% alle proteine del plasma. Biotrasformazione: La bilastina non ha indotto o inibito l'attività degli isoenzimi CYP450 negli studi *in vitro*. Eliminazione: In uno studio di bilanciamento di massa condotto su volontari sani adulti, dopo la somministrazione di una singola dose di 20 mg di ^{14}C-bilastina, quasi il 95% della dose somministrata è stata recuperata nelle urine (28,3%) e nelle feci (66,5%) come bilastina immodificata, confermando quindi che la bilastina non è significativamente metabolizzata nell'uomo. L'emivita media di eliminazione calcolata in volontari sani è stata di 14,5 h. Linearità: La bilastina presenta una farmacocinetica lineare nell'intervallo di dosi studiato (da 5 a 220 mg), con bassa variabilità interindividuale. Compromissione renale: In uno studio in soggetti con compromissione renale, la media (DS) dell'$AUC_{0-\infty}$ è aumentata da 737,4 (±260,8) ngxh/ml nei soggetti senza compromissione (GFR: > 80 ml/min/1,73 m^2) a: 967,4 (±140,2) ngxh/ml nei soggetti con compromissione lieve (GFR: 50-80 ml/min/1,73 m^2), 1384,2 (±263,23) ngxh/ml nei soggetti con compromissione moderata (GFR: 30 - <50 ml/min/1,73 m^2), e 1708,5 (±699,0) ngxh/ml nei soggetti con compromissione grave (GFR: < 30 ml/min/1,73 m^2). L'emivita media (DS) della bilastina era 9,3 h (± 2,8) nei soggetti senza compromissione, 15,1 h (± 7,7) nei soggetti con compromissione lieve, 10,5 h (± 2.3) nei soggetti con compromissione moderata e 18,4 h (± 11,4) nei soggetti con compromissione grave. L'escrezione urinaria della bilastina era essenzialmente completa dopo 48-72 h in tutti i soggetti. Questi cambiamenti farmacocinetici non si prevede presentino un'influenza clinicamente rilevante sulla sicurezza della bilastina, dato che i livelli di bilastina nel plasma nei pazienti con compromissione renale rientrano ancora nell'intervallo di sicurezza della bilastina. Compromissione epatica: Non esistono dati sulla farmacocinetica per i soggetti con compromissione epatica. La bilastina non viene metabolizzata negli umani. Dato che i risultati dello studio sulla compromissione renale indicano che l'eliminazione renale è il maggior contribuente dell'eliminazione, si prevede che l'escrezione biliare sia coinvolta solo marginalmente nell'eliminazione di bilastina. Non si prevede che le alterazioni nella funzione epatica abbiano un'influenza clinicamente rilevante sulla farmacocinetica di bilastina. Anziani: Sono disponibili solo un quantitativo limitato di dati di studi farmacocinetici in soggetti oltre i 65 anni di età. Non sono state osservate differenze statisticamente significative nella farmacocinetica della bilastina negli anziani oltre i 65 anni di età rispetto alla popolazione di adulti di età compresa tra 18 e 35 anni. Popolazione pediatrica: Non sono disponibili dati di farmacocinetica negli adolescenti (di età compresa tra 12 e 17 anni) in quanto, per questo prodotto, l'estrapolazione dei dati nell'adulto sono ritenuti appropriati. I dati farmacocinetici nei bambini sono stati ottenuti da uno studio di farmacocinetica di fase II che comprendeva 31 bambini dai 4 agli 11 anni di età, con rinocongiuntivite allergica o orticaria cronica, trattati con una compressa orodispersibile di bilastina 10 mg somministrata una volta al giorno. L'analisi farmacocinetica dei dati delle concentrazioni plasmatiche ha mostrato che l'esposizione sistemica di una dose pediatrica di bilastina 10 mg una volta al giorno risulta equivalente a quella osservata dopo una dose di 20 mg negli adulti e negli adolescenti, essendo il valore medio di AUC pari a 1014 ng x h/ml per i bambini dai 6 agli 11 anni. Questi risultati sono stati ampiamente al di sotto della soglia di sicurezza basandosi sui dati di una dose da 80 mg una volta al giorno negli adulti, in conformità con il profilo di sicurezza del farmaco. I risultati confermano che la scelta di bilastina 10 mg per via orale una volta al giorno, è la dose terapeutica appropriata per la popolazione pediatrica nella fascia di età dai 6 agli 11 anni con un peso corporeo di almeno 20 kg. **5.3. Dati preclinici di sicurezza.** I dati non-clinici sulla bilastina non evidenziano rischi particolari per l'uomo sulla base di studi convenzionali di sicurezza farmacologica, tossicità a dosi ripetute, genotossicità e potenziale cancerogeno. Negli studi di tossicità riproduttiva gli effetti della bilastina sul feto (perdita pre-e post-impianto nei ratti ed ossificazione incompleta delle ossa craniali, dello sterno e degli arti nei conigli) sono stati osservati solo a dosi tossiche per la madre. I livelli di esposizione al NOAEL (No Observed Adverse Effect Level) sono sufficientemente in eccesso (> 30 volte) rispetto all'esposizione umana alla dose terapeutica raccomandata. In uno studio sull'allattamento, è stata riscontrata bilastina nel latte dei ratti in allattamento cui era stata somministrata una singola dose orale (20 mg/kg). Le concentrazioni di bilastina presenti nel latte equivalgono a circa la metà di quelle presenti nel plasma materno. La rilevanza di questi risultati nell'uomo non è nota. In uno studio di fertilità nei ratti, la bilastina somministrata per via orale fino a 1000 mg/kg/die non ha indotto alcun effetto sugli organi riproduttivi maschili e femminili. Gli indici di accoppiamento, fertilità e gravidanza non sono stati influenzati. Come evidenziato in uno studio di distribuzione nei ratti mediante determinazione delle concentrazioni di farmaco tramite autoradiografia, la bilastina non si accumula nel SNC. **6. INFORMAZIONI FARMACEUTICHE. 6.1. Elenco degli eccipienti.** Cellulosa microcristallina, Sodio Amido glicolato (tipo A) (derivato dalle patate), Silice colloidale anidra, Magnesio stearato. **6.2. Incompatibilità.** Non pertinente. **6.3. Periodo di validità.** 5 anni. **6.4. Precauzioni particolari per la conservazione.** Questo medicinale non richiede alcuna condizione particolare di conservazione. **6.5. Natura e contenuto del contenitore.** Il medicinale è confezionato in un blister, che consiste di due parti: laminato, composto da poliamide orientata (lato esterno del laminato), alluminio e PVC (lato interno del laminato), Foglio in alluminio. Il foglio in alluminio è termosaldato con una lacca termosaldante (copolimero PVC-PVAC e resine di butilmetacrilato) al laminato dopo la formatura e il riempimento con le compresse. Ciascun blister contiene 10 compresse. I blister sono confezionati in astucci di cartone. Confezioni da 10, 20, 30, 40 o 50 compresse. È possibile che non tutte le confezioni siano commercializzate. **6.6. Precauzioni particolari per lo smaltimento e la manipolazione.** Il medicinale non utilizzato ed i rifiuti derivati da tale medicinale devono essere smaltiti in conformità alla normativa locale vigente. **7. TITOLARE DELL'AUTORIZZAZIONE ALL'IMMISSIONE IN COMMERCIO.** Menarini International Operations Luxembourg S.A. 1, Avenue de la Gare, L-1611 – Lussemburgo. *Concessionario per la vendita:* Malesci Istituto Farmacobiologico S.p.A. Via Lungo l'Ema, 7 – Loc. Ponte a Ema, Bagno a Ripoli - Firenze. **8. NUMERO(I) DELL'AUTORIZZAZIONE PER L'IMMISSIONE IN COMMERCIO.** AYRINAL 20 mg compresse: 10 compresse – A.I.C. 040854010, 20 compresse – A.I.C. 040854022, 30 compresse – A.I.C. 040854034, 40 compresse – A.I.C. 040854046, 50 compresse – A.I.C. 040854059. **9. DATA DELLA PRIMA AUTORIZZAZIONE / RINNOVO DELL'AUTORIZZAZIONE.** Data di prima autorizzazione: 3 Aprile 2012. Data del rinnovo più recente: 8 settembre 2015. **10. DATA DI REVISIONE DEL TESTO. Febbraio 2020.**

CONFEZIONI	PREZZO AL PUBBLICO	CLASSE	NOTA
20 mg 20 cpr	10,80	C	-

Medicinale soggetto a prescrizione medica

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