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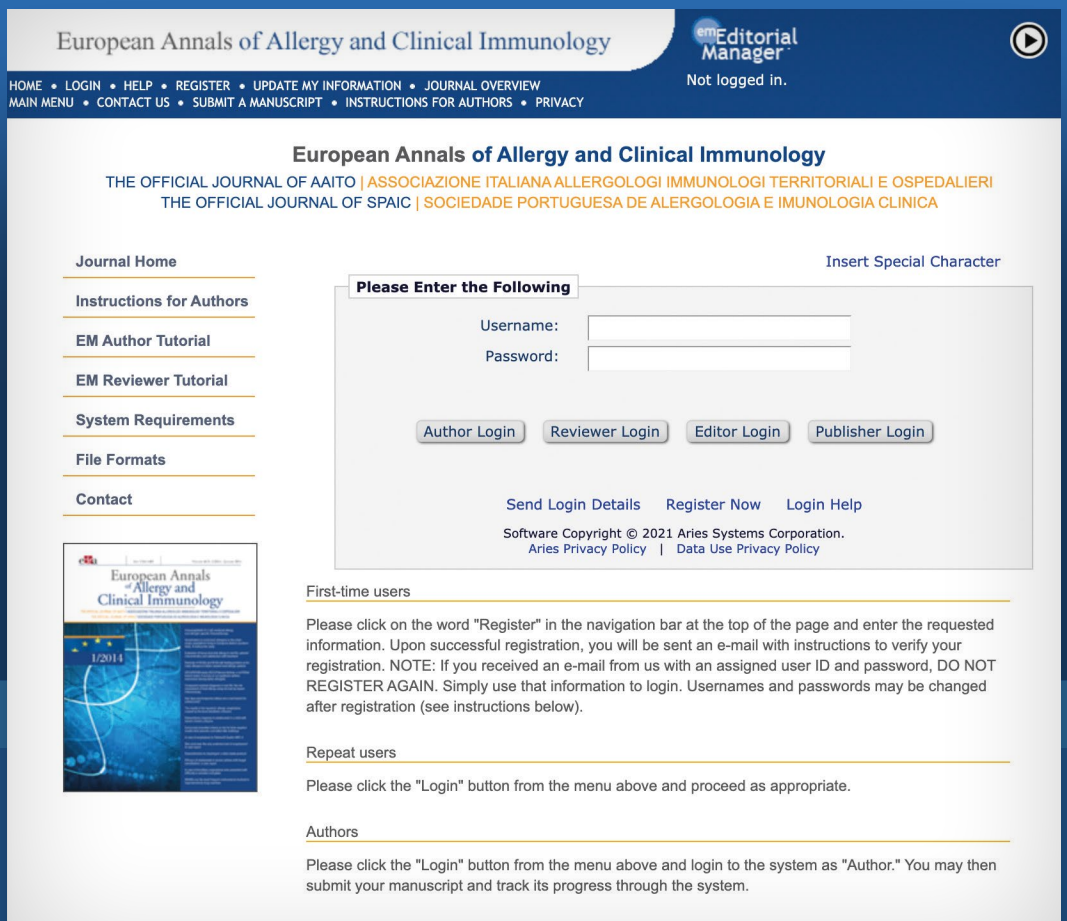
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edra REVIEW | Eur Ann Allergy Clin Immunol | Vol 57, N.2, 51-67, 2025

Giuseppe Parrinello[1], Desré Ethel Fontana[2], Danilo Villalta[1]

An overview of hidden food allergens: need for change to the priority food allergen lists?

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Key words

Hidden allergens; food allergy; food labelling; priority food allergen lists; molecular allergy.

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Impact statement

The adoption of plant-based diets and new sustainable foods is contributing to the onset of new emerging food allergens. Diagnostic implementations and priority food allergen lists revision will be needed.

Summary

Many food allergens not actually included in the European priority list of allergenic foods have the potential to cause severe allergic reactions and could escape correct identification and behave as "hidden allergens". Moreover, the adoption in recent years of novel diets based on plant products and new sustainable foods or the use of specific food additives have contributed to the onset of new emerging allergens of public health importance. The knowledge of hidden allergens is important both for physicians and for patients to improve the prevention, diagnosis and treatment of food allergies, in order to decrease eventual improper diagnosis of idiopathic anaphylaxis. In this review, the characteristics of the most frequent hidden allergens and their diagnostic tools are described. A detailed history with a careful review of the ingredient lists, an understanding of possible cross-reactions or contaminations with other foods, together with an allergological evaluation consisting of "in vivo" or "in vitro" tests and, where necessary, an oral food challenge, are recommended for the successful identification of the culprit allergen.

In future, it will be very important to implement these diagnostic tools, especially in the field of molecular allergology, and reporting allergens on labels should become mandatory.

Introduction

Dietary changes of the last years, based on the adoption of plant products and new sustainable foods with low environmental and economic impact, have contributed to the onset of new emerging allergens of public health importance (1, 2). According to the current European labeling regulations, these "new allergens" are not always labeled in food products and so they can act as "hidden allergens". A hidden allergen is defined as a substance that is unrecognized or not declared on the product label. The omission of these allergens is not always intentional, and it can be due to different reasons such as misleading labels, allergenic foods that can contaminate other safe foods, carelessness, use of uncommon terms in the food list or ingredient switching (3). The first priority list of allergenic foods for the European Union was established by the European Parliament and the Council through Directive No 1169/2011 and it currently includes fourteen allergens: cereals containing glutens, milk, crustaceans, eggs, fish, peanuts, soybeans, tree nuts (*i.e.,* almonds, hazelnuts, cashews, pecan nuts, Brazil nuts, pistachio nuts, macadamia nuts and Queensland nuts), celery, mustard, sesame seeds, sulphur dioxide and sulphites (at concentrations of more than 10 mg/kg or 10 mg/liter expressed as SO_2), lupin and molluscs. The criteria used to include these foods on the priority list are based on the following parameters: the evidence for an IgE mechanism, the adverse reactions caused by IgE-mediated reactions, the potency, the severity and

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the prevalence in the general population through double-blind, placebo-controlled food challenge or other published reports in accordance with the International Life Sciences Institute-Europe (4). However, the foods included on this and on priority lists from other countries don't always fully comply with all the expected scientific criteria, probably due to a variable weighting of the importance of the aforementioned factors (*i.e.,* prevalence, severity and potency) in terms of impact on public health (5). Food allergens not included in the actual labeling regulations lists can act as "hidden allergens" and they can cause severe allergic reactions or anaphylaxis. Awareness thereof is important for both physicians and patients in order to improve the prevention, diagnosis and treatment of food allergies, leading to a decrease of eventual improper diagnosis of idiopathic anaphylaxis. Aim of this review is to describe the main hidden allergens, to summarize their characteristics, the foods and substances where they usually are found, their more common reported allergic reactions and the diagnostic tools currently available for their identification, focusing on the implementation of diagnostic strategies and the need for their future mandatory labeling on foods products. We also include a section related to complementary and alternative medicine, as the increasing use of herbal medicines or treatments in Western countries has contributed to the rise of sensitization to these substances, which in some cases can act as hidden food allergens.

Hidden allergens have been divided based on their sources in the following sections: vegetable allergens (*i.e.,* herbs and spices, legumes and seeds), additives (*i.e.,* thickeners, food dyes and prebiotics), food substitutes (*i.e.,* wheat and meat substitutes) and complementary and alternative medicine (**table I**).

Methods

In this overview, a literature search was carried out in the PubMed/Medline database using the PubMed search engine as of December 2023. The research included a combined set of keywords: "Hidden allergens", "Hidden food anaphylaxis", "Food labelling", "Food allergen priority", "Spices allergy", "Legumes allergy", "Seeds allergy", "Pine nut allergy", "Additives allergy", "Buckwheat allergy", "Oat allergy", "Millet allergy", "Quinoa allergy" and "Psyllium allergy". The search was mainly limited to articles published over the last 24 years (2000-2023). No language restrictions were adopted. The overview was performed based on the relevant literature, including case reports, case series, clinical trials, retrospective and prospective studies, retrospective series reviews, scoping and narrative reviews. From a result of more of 57,000 entries, 158 relevant articles were selected based on titles, abstracts and relevance to the topic. To obtain additional information sources, the lists of references of relevant articles were examined and a further selection of their content was assessed. Duplicated results, articles not accessible or with titles or abstract not adherent to the topic, were excluded. A priority to plants-derived "hidden allergens" or additives used as ingredients in food products and to healthy food alternatives was given. In this review, other sources of "hidden allergens" like insects, cereal mites, parasites (*e.g., Anisakis simplex*) or contaminations with known declared allergens present in non-food items, were not taken into consideration.

Vegetable allergens

Herbs and spices

The definition of these terms varies in different references. In the culinary definition "spices" include aromatic seasonings obtained from the bark, buds, fruit, root, and seeds of various trees and plants, while "herbs" usually come from the leafy part of the plant (6). They belong to different botanical families: Apiaceae, Lamiaceae, Lauraceae, Leguminosae, Liliaceae, Myristicaceae, Myrtaceae, Piperaceae, Solanaceae, Zingiberaceae, *etc*. Spice allergy is more frequent in adults, due to the greater exposure in this class compared to children, with a frequency ranging from 2 to 6.4% of adults with food allergy (7, 8). According to data from French CICBAA database based on 589 cases of food allergy, the greatest sensitizations are to Apiaceae and Liliaceae families (8). Herbs and spices are commonly used in various food or commercial preparations such as soups, meat dishes, sauces, gravy, ready meals, seasoning, curries, barbeque, pizza, *etc*.

Excluding celery and mustard, which are subject to food labelling requirements in the European Union, other spices are commonly involved in allergic reactions. Among the Apiaceae botanic family coriander, fennel, parsley and cumin are the most involved in allergic reactions. Coriander is an ingredient present in curry, in Italian or Thai seasoning and in Masala powder. It is also used as a "natural" flavoring in beer even if is not always reported in alcoholic drinks and in one case it was reported to have caused anaphylaxis (9). Fennel allergy was described in the context of a pollen food allergy syndrome with mugwort involving a homologue to Api g 5 (profilin) recognized in fennel (10) or of a non-specific lipid transfer protein (nsLTP) syndrome (11), while forms of contact urticaria were described with parsley (12). Cumin was also directly involved in rare cases of anaphylaxis (13) and it can be mistaken for other allergenic foods. Moreover, undeclared contaminants such as peanuts, almonds, Brazil nuts, cashews, hazelnuts, and pistachio were found in cumin used in the manufacture of many food products in the United States (14).

Among the Liliaceae family, garlic and onion were described to be involved in clinical manifestations such as contact dermatitis, urticaria, occupational asthma, rhinitis and also anaphylaxis (15, 16). In addition to profilin and nsLTP, associations between mugwort pollen and food allergies to vegetables, herbs and spices from the Apiaceae (*e.g.,* celery, carrot, parsley, fennel, cumin, coriander, aniseed) and other botanic families (*i.e.,* Solanaceae and Liliaceae) seem to involve another family protein called defensins: ancient

Table I *- Class of food allergens and their associated botanic families, hidden allergens, sources and clinical manifestations.*

Class of allergens	Botanic family	Hidden allergen	Foods and substances containing the hidden allergen	Clinical manifestations
Herbs/spices	*Apiaceae*	Coriander	Curries, seasoning, Masala powder, beers and drinks	Anaphylaxis (9)
	//	Fennel	Curries, sausages, pizza, soups, pasta dishes	Oral allergy syndrome (10) Systemic reactions (11)
	//	Parsley	Seasoning, pasta dishes	Contact urticarial (12)
	//	Cumin	Curries, Masala powder, seasoning	Rare anaphylaxis (13)
	Liliaceae	Garlic and onion	Seasoning, ketchup, Masala powder, pizza, soups, pasta dishes	Contact dermatitis, urticaria, occupational asthma, rhinitis and anaphylaxis (15,16)
	Iridaceae	Saffron	Risotto alla Milanese, pasta dishes	Systemic reactions (18) Respiratory symptoms (rhinitis, conjunctivitis) (19)
	Piperaceae	Black, green and white pepper	Seasoning, curries, lemon pepper, pizza, sauces, soups, barbeque	Systemic reactions (19)
	Solanaceae	Paprika, Cayenne, Chili pepper	Curries, seasoning, pasta dishes, soups, barbeque	Rhinoconjunctivitis, Anaphylaxis (20-23)
	Anacardiaceae	Pink peppercorns	Meat dishes, pasta dishes, seasoning	Cross-reactivity in subject with cashew-nut allergy (24)
	//	Sumac	Mediterranean, Middle East and African dishes	Systemic reaction in subject with cashew nut allergy (25)
Legumes	*Fabaceae*	Pea, lentil, chickpea, bean, *etc.*	Prepackaged food, gluten-free pasta, vegan and meat substitutes (peas, lentils, chickpeas) Yoghurts, pizza, milkshakes, vegetarian burgers (peas)	From mild reactions to anaphylaxis (26)
	//	Fenugreek	Curry powder, spice mixes (Greek, Indian, Turkish, Iranian and Egyptian dishes)	Anaphylaxis, cross-reactivity in subjects with peanuts allergy (30)
Seeds	*Cucurbitaceae*	Pumpkin seeds	Appetizers, condiments in bread or salads, snacks	Anaphylaxis (31-33)
	Compositae	Sunflower seeds	Appetizers, oil, margarine or bread products	Rhinitis, asthma, oral allergy syndrome, urticaria, angioedema, contact dermatitis (38,39) and anaphylaxis (40,41)
	Pinaceae	Pine nuts	Both in raw or roasted form and as ingredients in breads, cakes, cookies, sauces, pesto sauce, candies, vegetable and meat dishes	Oral allergy syndrome, urticaria, angioedema and anaphylaxis (42)
	Linaceae	Flaxseeds	Bakery products	Anaphylaxis (49-53)
	Lamiaceae	Chia seeds	Bread and bread products	Anaphylaxis (56) and dermatitis (57)
	Cannabaceae	Hemp seeds	Energy bars, yoghurts, bakery products	Anaphylaxis, asthma, angioedema (60)
	Papaveraceae	Poppy seeds	Ingredients in cakes, bread and for garnish	Mild oral symptoms, contact urticaria, anaphylaxis (61-65)

→

Class of allergens	Botanic family	Hidden allergen	Foods and substances containing the hidden allergen	Clinical manifestations
	Chenopodiaceae	Quinoa seeds	Wheat substitutes, salads, pasta dishes, burgers, meatballs	Anaphylaxis (66,67), occupational asthma (68)
Additives				
Thickeners		Pectin (*i.e.*, E440)	Jellies, jams, candies, smoothies, desserts, fruit juices, milk drinks, medicines	Occupational asthma, rhinitis, dermatitis, anaphylaxis (72-78)
		Gelatin (*i.e.*, E441)	Candies, desserts, drinks, vaccines, medications	Urticaria, anaphylaxis (82-84)
	Fabaceae	Guar gum, carob gum, arabic gum, tragacanth gum	Bakery products, thickened milk products, condiments, canned soups, medicines	Occupational rhinitis/asthma, urticarial, angioedema, anaphylaxis (88,89)
Food dyes		Carmine (*i.e.*, E120)	Sweets, ice lollies, soft drinks, syrups, colored food (*e.g.*, cakes, biscuits), cosmetics, medicines, tissues	Asthma, dermatitis and anaphylaxis (90,91)
	Bixaceae	Annatto (*i.e.*, E160b)	Dairy and bakery products, vegetable oils, drinks	Urticaria, anaphylaxis (93,94)
Prebiotics		Inulin	Naturally in artichokes, chicory, salsify, processed food (*e.g.*, ice creams, butters, margarine, candies, yoghurts, cereals), medical tests (*i.e.*, intravenous test of renal function)	Anaphylaxis (95,96)
Food substitutes				
Wheat substitutes	*Poligonaceae*	Buckwheat	Gluten-free products, noodles, bread, gallettes, pancakes, porridge, kasha, burgers, pizza, polenta taragna, pizzoccheri, buckwheat pillows, soaps	Occupational rhinitis/asthma, urticaria, angioedema and anaphylaxis (99-101)
	Poaceae	Oat	Gluten-free products, biscuits, cereals, soaps, cosmetics	Rhinitis, asthma, contact dermatitis, anaphylaxis (103,104)
	//	Millet	Gluten-free products, cereals	Asthma, systemic reactions, anaphylaxis (105-109)
	Amaranthaceae	Amaranth	Gluten-free products, soups, sauces	Anaphylaxis (112)
Meat substitutes		Mycoprotein	Vegetarian or vegan ready meals	Pruritus, urticaria, respiratory symptoms, anaphylaxis (113)
Complementary and alternative medicine	*Plantaginaceae*	Psyllium (Ispaghula)	Cereals, ice creams, desserts, laxatives	Occupational rhinitis, asthma and anaphylaxis (117)

peptides present in plants and in other live creatures, including humans, with antifungal and anti-bacterial properties highly stable to thermal and acidic treatment. Sensitization to Art v 1, the major allergen from mugwort pollen belonging to defensin-like protein family, was proven to be linked to allergic reactions to celery, horse chestnut seeds, mango and sunflower seeds due to cross-reactivity with homologous defensins discovered in these plant foods. Up to now, few food defensins have been characterized and to determine if they could have a role in the mugwort pollen-related food syndromes, further allergenic defensins in herbs and spices should be identified (17).

Saffron is a spice derived from the flowers of *Crocus sativus* and may induce allergic reactions. Among the molecular allergens identified there are an Ole e 1-like protein (Cro s 1), a profilin (Cro s 2) and nsLTP (Cro s 3). The latter two molecules were shown to be involved in respiratory and severe systemic reactions, respectively (18, 19). Allergies to paprika, Cayenne, Chili pepper and peppercorns are rare. Paprika, Cayenne and Chili pepper are of the *Capsicum* genus which belongs to the Solanaceae family and allergy symptoms such as rhinoconjuncitivitis or rare cases of anaphylaxis to these spices have been described (20-22). Ten allergens have been reported in bell pepper (*Capsicum*

annuum) allergy, but only three of these are actually certified by World Health Organization/International Union of Immunological Societies (WHO/IUIS): an osmotin, member of the thaumatin-like protein family (Cap a 1), a profilin (Cap a 2) and a gibberellin-regulated protein (Cap a 7). This last molecule was found to be involved in a case of anaphylaxis after ingestion of chili pepper in a 16-year-old Japanese girl with known allergy to Japanese cedar pollen (23). Black, green and white pepper are instead all derived from *Piper nigrum* plant which belongs to Piperaceae family; while pink peppercorns are dried berries derived from *Schinus molle* trees belonging to Anacardiaceae family. In literature, a cross-reactivity among Anacardiaceae species in subjects sensitized to cashew nuts with an involvement of seed storage proteins (albumin and legumin type) was described (24). At the same time, allergic reactions to sumac, another spice of the Anacardiaceae family, were reported in subjects known for cashew nut allergy (25).

Key points: spice allergy is uncommon, even if probably under-diagnosed due to the different botanical families involved. Clinical manifestation ranges from respiratory, dermatologic and gastrointestinal symptoms to anaphylaxis. The main molecular allergens involved are pathogenesis-related class 10 proteins (PR-10), profilins, nsLTP, giberellin-regulated proteins (GRPs), seed storage proteins and defensins with the last four protein families generally associated to more severe and systemic reactions. Several spice allergens are degraded by digestion and the sensitization to these allergens mostly occurs via inhalation or through cross-reacting pollen.

Legumes

Legumes include different edibles species (peanuts, soybeans, lupins, lentils, peas, beans *etc*.) all belonging to the Fabaceae botanic family. At present, only peanut, soybean and lupin are reported on the European priority allergy list of allergenic foods, failing to include other legumes that are so-called "non-priority legumes" and can act as hidden allergens. Over the last years, there has been a notable increase of non-priority legumes allergies, probably due to the increase in the consumption of these food in Western countries. They are generally used in vegan meat substitutes or in gluten-free pasta and in other food products such as yoghurt, pizza, milkshake and chicken burgers. Allergenic symptoms are similar for all legumes and can involve the cutaneous, cardiovascular, respiratory, and gastrointestinal systems causing oral allergy syndrome, angioedema, urticaria, rhinitis, asthma or anaphylaxis and even death in extreme and rare cases. Inhalation of vapor, powder, or flour from some legumes could cause respiratory symptoms such as rhinitis, asthma, and hypersensitivity pneumonitis (26). In a recent scoping review including 47 articles about non-priority legumes food allergy, the greatest part of the articles (38.3%) were published in the last five years (2015-2020) demonstrating the growing interest in this type of problem. Of the 47 articles included, 21.3% focused exclusively on the adult population and 38.3% on children. All the articles primarily focused on reporting prevalence that ranged from 0.5% to 39.6% depending on the studies. Lentil was the most commonly analysed non-priority legume (46.8%), followed closely by pea (40.4%); while none of these articles focused on the labelling of these allergens, highlighting how more studies are needed in this area (27).

Co-sensitization among legumes is frequently seen and varies from 36.7% to 100% according to a recent study conducted in adults with a legume allergy in which 16 individual proteins from 10 legumes (peanut, soybean, green pea, chickpea, blue and white lupine, black and green lentil, fava and white bean) were compared and it seems largely due to the 7S/11S globulin fractions or individual 7S and 11S globulins. However, this is not always clinically relevant, because in peanut and soybean-allergic patients, co-allergies for other legumes were uncommon (≤ 16.7%). On the other hand, in green pea, lupine, lentil, and bean-allergic patients, co-allergy for peanut (64.7%-77.8%) or soybean (50%-64.7%) was frequently seen (28). The situation in the pediatric population is not dissimilar: in a study based on 195 peanut-allergic children, Mueller *et al*. found that 64% of them were sensitized, but only 17% had an allergy to one other legume. Most allergies were found to lentil (21%), lupin (19%) and pea (15.4%) with severe reactions affecting 50% of examined subjects (29). Among legumes it could be important to consider fenugreek (*Trigonella foenum-graecum*), often used in the culinary field as a constituent of spice mixes (*i.e.,* curry powder), which is not always correctly labelled in food products. Peanut-allergic patients can react to fenugreek due to the demonstrated cross-reactivity between homologous fenugreek and peanut allergens (30). Except for peanut, soybean and lupin, the following molecular allergens of some "non-priority legumes" are actually registered in the WHO/IUIS allergen database: Len c 1 (vicilin), Len c 2 (seed biotynilated protein) and Len c 3 (nsLTP) from lentil (*Lens culinaris);* Pis s 1 (vicilin), Pis s 2 (convicilin) and Pis s 3 (nsLTP) from green pea (*Pisum sativum*); Cic a 1 (late embryogenesis protein 4) from chickpea; Pha v 3 (nsLTP) from green bean (*Phaseolus vulgaris*); Vig r 1 (pathogenesis-related protein, PR-10), Vig r 2 (vicilin), Vig r 3 (cupin), Vig r 4 (seed albumin), Vig r 5 (fragment of Vig r 2) and Vig r 6 (cytokinin-specific binding protein, CSBP) from green gram (*Vigna radiata*).

Key points: non-priority legumes allergy is a growing problem in recent years and the necessity of their labeling in food allergen lists is becoming indispensable. Clinical manifestations range from mild reactions to anaphylaxis involving different organ systems. Sensitization to seed storage proteins (especially 7S/11S globulins and partially 2S globulins) seems to be involved in most allergic reactions to non-priority legumes, even if other molecular allergens are under investigation.

Seeds

Under this term foods of various species belonging to different botanical families are included. In the European Union, only sesame and mustard seed are actually reported as allergens on food labels. However, other seeds such as pumpkin, sunflower, flaxseed, pine nuts, Chia, hemp, quinoa and poppy are increasingly present in our diets and contribute to the rise in their hypersensitivity in the general population. Prevalence data on seed hypersensitivity are scarce except for sesame or mustard seeds and are mainly based on case reports or case series. Here are summarized the characteristics of the other eight edible seeds, the more common allergic reactions caused by these seeds and their actually identified molecular allergens.

Pumpkin seeds

Pumpkin seeds belongs to the Cucurbitaceae family. It is usually consumed toasted as an appetizer, added to bread or to salads as a condiment or in various snacks. Until now, few cases of pumpkin seed allergy were reported in literature, but it was demonstrated in different situations that subjects with a pumpkin seed allergy usually tolerate the pumpkin flesh, whose sensitivity would instead seem to be associated with sensitization to peach (31-35). From a molecular point of view, two seed storage proteins were identified: a 2S albumin (Cuc ma 5) and an 11S globulin (Cuc ma 4). The prior is considered the first marker of sensitization and was linked to more severe reactions; while the latter is also considered a marker of symptom severity and cross-reactivity with other vegetable sources (melon seed, mustard seed and cashew nut) (36). Moreover, in pumpkin seeds, a homologue of birch profilin (Cuc ma 2) was identified and seems to be involved in pollen-food syndromes (37).

Sunflower seeds

Sunflower seed allergies are rare even if severe allergic reactions to these seeds are described, including anaphylaxis. They belong to the Compositae family and are consumed in the form of seeds, oil, margarine or bread products. Allergy to sunflower seeds included rhinitis, asthma, angioedema, acute urticaria, contact dermatitis, oral allergy syndrome and rarely anaphylaxis (38, 39). The main allergens identified in sunflower seeds include a defensin-like protein (Hel a 1), a profilin (Hel a 2) and a nonspecific lipid transfer protein (Hel a 3), which are involved in cross-reactions to homologous mugwort pollens proteins: Art v 1, Art v 4 and Art v 3, respectively. Hel a 2 was also found to react with profilins of ragweed, olive tree and *Mercurialis perennis* (38). Moreover, 2S albumins proteins (Hel a 15, Hel a 16 and Hel a 17) and oleosins have been identified as allergens responsible for severe reactions, even if patients with sunflower seeds allergy not always had reactions to other nuts or sunflower oil (40, 41).

Pine nuts

Pine nuts belongs to the Pinaceae family, evergreen trees belonging to the old conifers division of Gymnosperms. They have been consumed for over two thousand years in the Mediterranean region. They are frequently used in raw or roasted form and as ingredients in breads, cakes, cookies, sauces, pesto sauce, candies and vegetable or meat dishes. Until now, approximately fifty cases of pine nut allergy have been reported in literature, of which mostly anaphylactic reactions (42). Generally, it is an independent allergy with a high monosensitization rate and poor cross-reactivity with other tree nuts derived of Angiosperms (*e.g.,* peanuts, nuts, walnuts, cashew and pistachio). A 2S albumin (Pin p 1) is considered the major allergen of pine nut (*Pinus pinea*) together with a 7S globulin (Pin p-vicilin), even if other several proteins of different molecular weights (17 kDa; 30, 44, and 50 kDa; and <14 kDa) have been detected (43-46). In an Italian multicentric study including 12 patients with a history of pine nut allergy, an IgE reactivity against allergens present in the lipophilic fraction of the pine nut extract was shown in a patient with negative skin and serological tests, suggesting a potential allergenic role of oleosins in the development of pine nut allergy (47).

Flaxseeds

Flaxseeds are derived from *Linum usitatissimum*, an annual plant of Central Asia and of Arab origin belonging to the Linaceae family, and they were originally used in the textile industry for its fibers. The oil obtained from the seeds is also exploited for the production of printer inks, paints, healing agents, moisturizing creams and laxatives. Moreover, flaxseeds and derivatives are also used as animal feed (birds, dogs, cats, horse *etc*.). Recently, they are included as food in our diets for their nutritional benefits, anti-inflammatory, antioxidant, and cardioprotective properties (48). For this reason, sensitization to flaxseeds in the general population has caused a noticeable increase of different allergic reactions, including anaphylaxis, to be published in literature (49-52). Recently it was shown that seed storage proteins such as a 2S albumin (Lin u 1) and an 11S globulin were involved in severe allergic reactions to flaxseeds (53,54).

Chia seeds

Chia seeds are a "superfood" introduced in our diets for their functional and nutritional proprieties. They are of Mexican origin and Chia (*Salvia hispanica L.*) is an annual plant belonging to the Lamiaceae family originally cultivated in South America by pre-Columbian populations for thousands of years. Since 2005, Chia seeds were introduced to the European Union as a novel ingredient in bread and bread products (55). Rare cases of anaphylactic reactions and dermatitis after ingestion of Chia seeds were described (56, 57). Albunni *et al.* (58) demonstrated that legumins and vicilins contained in Chia seed possess similar IgG or IgE binding epitopes as those in sesame proteins or other nuts (*i.e.,* hazelnuts) and legumes (*i.e.,* peanuts) and that this could translate into possible cross-reactivity reactions to these seeds in patients with other food allergies.

Hemp seeds

Hemp seeds are a variety of *Cannabis sativa* plant species, belonging to Cannabaceae, that is grown for the industrial uses of its derived products. The seeds are highly nutritious foods and they are used in various food products including energy bars, yoghurts and bakery products. Also in this case, the widespread availability and use of hemp seeds in the food industry contribute to the rise of hemp seed allergy (59). Allergic reactions have been reported after their ingestion in patients without previous direct exposure to cannabis. In these cases, a possible sensitization from an indirect exposure or a cross-reactivity with homologous pollen proteins in patients with tree or weed pollinosis is presumed (60). Sensitizations to nsLTP, PR-10 and profilin homologues (Can s 3, Can s 5 and Can s 2, respectively) appear to be involved in most allergic reactions to hemp seeds (43). Other molecular allergens registered in the official allergen database WHO/IUIS are an oxygen evolving Enhancer Protein 2 (Can s 4) and a thaumatin-like protein (Can s 7).

Poppy seeds

Poppy seeds are derived from *Papaver somniferum* and belong to Papaveraceae family. They are generally used as ingredients in cakes, breads and for garnish and are rarely considered a cause of food allergy (61). Various types of allergic reactions to these seeds were described: from mild oral symptoms and contact urticaria to anaphylaxis (61-65). Furthermore, a cross-reactivity with hazelnut, buckwheat and sesame was shown, in part explained by a sensitization to homologous seed storage proteins: Pap s 1 (7S vicilin), Pap s 2 (11S globulin) and a 2S albumin (Pap s 2S) present in poppy seeds (65). In the WHO/IUIS allergen database another protein of 10 kDa (Pap s 3) corresponding to a small hydrophilic seed protein belonging to the family of the late embryogenesis abundant protein 5 (LEA-5) was recently registered.

Quinoa seeds

Quinoa seeds are derived from *Chenopodium quinoa*, an herbaceous annual plant belonging to Chenopodiaceae family. A flour is obtained from quinoa seeds which is especially used as wheat substitute for celiac or gluten sensitive patients since it does not contain gluten. Allergy reactions to quinoa are rare and few case reports of anaphylaxis after ingestion or occupational allergy after exposure to quinoa flour are reported in literature (66-68). In a recent study conducted in rats, Ballegaard AR *et al.* showed that quinoa proteins (especially 11S globulin) were found to have an inherent medium to high immunogenicity and sensitizing capacity. Regarding the cross-reactivity with peanut and tree nuts (*i.e.,* hazelnut, walnut, cashew nut, Brazil nut) the highest percentage of identification was found for 11S globulin, profilin and oleosin. Further studies are necessary to demonstrate if this medium-high level of allergenicity of quinoa seeds is also present in humans (69).

Key points: hypersensitivity reactions to seeds range from respiratory (*i.e.,* rhinitis, asthma) and dermatologic (*i.e.,* urticaria, angioedema, dermatitis) symptoms to oral allergy syndrome and anaphylaxis. The main molecular allergens involved in severe allergic reactions are seed storage proteins (cupins, vicilins or legumins), nsLTP and oleosins, while PR-10 proteins and profilins are mainly involved in mild or oral allergy symptoms. Sensitization could occur by ingestion, contact or through pollen exposure with a cross-reaction among homologous pollen proteins. The impact of seed allergy will increase in the coming years due to their large availability and consumption in the food industry and so their entry in food allergen lists should be considered.

Additives

Additives include different compounds which are added to products to perform specific functions (*i.e.,* coloring, sweetening, thickening or preserving foods). In the European Union they are identified by letter E followed by a specific number. Product labeling must specify additive proprieties by referring to its name and E number (70). Additives are potentially involved in different kind of allergic or immunological reactions (IgE-mediated, non-IgE-mediated, and mixed IgE/non-IgE-mediated reactions) with different clinical manifestations mainly involving cutaneous, respiratory and gastrointestinal systems. However, the prevalence of these adverse reactions remain difficult to estimate due to symptom subjectivity and the lack of reliable markers of reactivity (71). Among additives, in the European Union only sulphites contained in foods above a certain concentration (≥ 10 mg/kg or 10 mg/liter expressed as SO_2) are subjected to obligatory labeling. It has been reported that other additives for which an IgE-mediated mechanism has been demonstrated that have not been subjected to mandatory labeling could potentially act as hidden allergens. The characteristics and the allergic reactions to these additives are listed below and they were divided into three categories: thickeners, food dyes and prebiotics.

Thickeners

Pectin (i.e., E440)

Pectin is a structural heteropolysaccharide present in most primary cell walls of vegetable tissues. It is used for its emulsifying and thickening proprieties in various foods (*i.e.,* candies, jellies, jams, smoothies, fruit juices, milk drinks) but also in medications (*i.e.,* barium suspensions). Case reports of reactions to pectin present in foods or medications have been reported for many years in literature and some of these were also associated with occupational asthma or they occurred in patients with known cashew allergy (72-78). The mechanism underlying the cross-reactivity between cashew/pistachio and pectin has not been determined, but it is purported to be due to a novel carbohydrate allergen or a cross-reaction between proteins in tree nuts and apples or citrus

fruits (79-81). For this reason, pectin should always be considered in patients with cashew or pistachio allergy reporting allergic reactions to an unknown trigger.

Gelatin (i.e., E441)

Gelatin can be derived from mammalian meats or fish. It can be found as a stabilizing agent in different foods such as candies, desserts and drinks as well as in vaccines (*i.e.,* influenza, MMR, *Varicella* spp., *Varicella zoster,* rabies, typhoid, yellow fever) or in medications (*i.e.,* hemostatic agents, capsules, suppositories, erythropoietin, plasma volume expanders and colloids). Allergic reactions including anaphylaxis to desserts containing mammalian or fish derived gelatin are reported in literature, even without previous reactions to vaccines (82-84). Fish gelatin is a hydrolyzed collagen type I derived from fish skin and bones, while commercial bovine gelatin consists of denatured type I collagen derived from mammalian tendon, cartilage or skin and it has been demonstrated that they do not cross-react with each other (85). Moreover, a sensitization to galactose-α-1,3-galactose (alpha-gal) might risk the development of allergic reactions to mammalian gelatin-containing foods (86,87).

Guar gum (i.e., E412)

Guar gum is a polysaccharide extracted from guar beans (*Cyamopsis tetragonoloba*), members of the Fabaceae family, used for its thickening and stabilizing proprieties in various foods such as baked foods, thickened milk products, condiments and canned soups. Guar gum has been shown to cause occupational rhinitis and/or asthma and in one instance a case of anaphylaxis has also been described (88,89). Potential allergic reactions to other gums including carob, tragacanth and acacia gum were also reported (88).

Food dyes

Food dyes are used to enhance or change the color of many food and beverage products. Allergic reactions to natural dyes such as carmine, annatto and saffron are described.

Carmine (i.e., E120)

Carmine is a red dye extracted from dried female cochineal insects (*Dactylopius coccus*). Over 30 cases of IgE-mediated hypersensitivity reactions to carmine red were reported and most were anaphylactic reactions, as well as cases of delayed anaphylaxis (90, 91). Cutaneous exposure through cosmetics seems important to sensitization to this dye (92).

Annatto (i.e., E160b)

Annatto is a yellowish orange pigment derived from the seeds of *Bixa Orellana* and is used as an ingredient in dairy and bakery products, vegetable oils and drinks. In literature, few cases of anaphylactic reactions to this dye have been described (93, 94).

Saffron (i.e., E164)

Saffron is a yellow food coloring extracted from the dried stigma of flowers of *Crocus sativus* that may induce allergic reactions. See the section above "Herbs and spices".

Prebiotics

Inulin

Inulin is a non-digestible carbohydrate present in many natural foods such as artichokes, chicory and salsify. Because of its health benefits, it is recently used as a prebiotic added to various processed foods (*e.g.,* ice creams, butters, margarine, candies, yoghurts, cereals, *etc.*). Rare cases of anaphylaxis to inulin were reported and in one of them specific IgE antibodies directed to a specific inulin protein were shown (95, 96).

Key points: allergic reactions to food additives is not frequent, although it's overrated and often reported by patients. However, the recognition of IgE mediated allergies is important to avoid severe and life-threatening reactions. Little is known about the molecular allergens involved and needs to be investigated.

Wheat substitutes

Celiac disease affects about 1% of the general population and gluten-free diet is the primary form of treatment of this disease (97). However, during the last decade, an increment of individuals self-reporting wheat sensitivity who decide to exclude gluten from their diet was observed, despite not having a diagnosis of celiac disease. Recent observational studies from across the world suggest that about 10% (range 4.3-14.9%) of the population is self-reporting wheat sensitivity and in Italy it stands at about 12.2% (98). The progressive market promotion of gluten-free diets has notably influenced the increase in the avoidance of wheat and other cereals containing gluten (*i.e.,* barley, rye) with greater use of wheat substitutes such as oat, buckwheat, millet, quinoa and amaranth.

Buckwheat

Buckwheat is considered a pseudocereal, because unlike other cereals it does not belong to the Poaceae family but to the Poligonaceae family instead, even if its proprieties and its food use are similar to other cereals. Two species of cultivated buckwheat are known: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). Buckwheat is used in different types of food such as noodles, bread, pancakes, porridge and kasha, pre-boiled and dried whole buckwheat grain. In Italy, it is also used for famous traditional dishes as polenta taragna (a hot porridge from the north of Italy) and pizzoccheri (a type of pasta from Valtellina, a small region near Como's lake in Northern Italy). Allergic reactions to buckwheat are described after the ingestion of buckwheat food products, in the work environment or when sleeping on pillows containing

buckwheat husks. Clinical manifestations range from occupational rhinitis or asthma, dermatitis, urticaria and angioedema to anaphylaxis (99). Buckwheat allergy is more common in Asian countries (*e.g.,* China, Korea and Japan) and for this reason buckwheat is included in their priority allergenic food lists. However, in Europe it could be considered a potential cause of hidden anaphylaxis (99-101). Nowadays, Fag e 1 (13S globulin), Fag e 2 (2S albumin), Fag e 3 (alpha-hairpinin), Fag e 4 (hevein-like antimicrobial peptide), Fag e 5 (vicilin-like protein) are the molecular allergens identified of *Fagopyrum esculentum* and Fag t 1 (legume-type protein), Fag t 2 (2S albumin) and Fag t 3 (seed storage protein of cupin superfamily) are those of *Fagopyrum tataricum.* Sensitization to Fag e 2 (a highly stable 2S albumin) is often related with severe reactions including anaphylaxis and is thus considered an important allergen in buckwheat anaphylaxis. In addition, an oleosin (Fag t 6) was recently implicated in allergic reactions to tartary buckwheat (*Fagopyrum tataricum*) (43,102). However, in the official allergen database WHO/IUIS, only Fag e 2, Fag e 4, Fag e 5 of *Fagopyrum esculentum* and Fag t 2 and Fag t 6 of *Fagopyrum tartaricum* are actually registered.

Oat

Oat (*Avena sativa*) is a cereal from the Poaceae family generally well tolerated by the majority of patients with Celiac disease. Oat is occasionally involved in forms of occupational allergies (*i.e.,* rhinitis or Baker's asthma) or contact dermatitis in children with atopic dermatitis who regularly used oat-based creams (103). Moreover, rare cases of anaphylaxis after ingestion were also reported (104). To date no major molecular allergens have actually been identified.

Millet

Millet is another cereal belonging to the Poaceae family, which is usually consumed in Western countries as a "healthy" alternative cereal or as a gluten-free substitute for wheat. Millet allergy is rare and has been mainly recognized in industrialized countries (*i.e.,* USA, Japan and Central Europe) where reactions especially included severe anaphylaxis (105-109). Sensitization to this cereal seems to occur mostly with inhalation through millet containing birdseed. Millet allergens have not yet been identified in detail; however, a potential cross-reactivity with homologues allergens present in other cereals (*i.e.,* rice, wheat and corn) has been reported (110). Recently a case of wheat-induced anaphylaxis in a bird-keeper caused by an early sensitization to millet was also described (111). No specific molecular allergens have been identified to date.

Amaranth

Amaranth seeds are also used as a wheat substitute and they have also been reported to cause anaphylaxis (112).

Quinoa

See the section above "Seeds".

Meat substitutes

Over the last 20 years, meat substitutes (*i.e.,* Quorn) composed of mycoproteins derived by a fungus called *Fusarium venenatum* have been marketed. They are usually part of composite dishes within which they may not always be obvious as allergens because they could take on the flavor of herbs or spices or could be confused for other types of meat. In a web survey conducted in USA, Jacobson *et al.* reported 312 allergic reactions to mycoproteins (12,5% anaphylaxis) of which 72,4% occurred at the first individual exposure (113). This last data suggests a possible cross-reactivity with other foods or allergens. In this regard, a possible link between sensitization to mold and allergic reactions to mycoproteins was found (114, 115).

Complementary and alternative medicine

Complementary and alternative medicine include a huge range of therapies, practices and healthcare systems mainly based on the use of herbal medicines or products. In Western countries, during the last decades its prevalence has noticeably increased. Allergic reactions and anaphylaxis after the use of herbal treatments that include Phleum pretense (6.5%), Andrographis paniculata (5%), Echinacea purpura (3.8%), Ginkgo biloba (3.6%) and bee products have been described in literature (116).

Psyllium (ispaghula)

Psyllium, or ispaghula, is a natural hydrophilic mucilloid of the genus *Plantago.* It was traditionally used as a laxative and recently for its lipid-lowering property. Psyllium was known to be associated with a form of occupational allergy well described in health care workers and pharmaceutical plant employees with clinical manifestations ranging from rhinitis and asthma to anaphylaxis (117). Psyllium is also used as a natural thickener in many foods (*e.g.,* cereals, ice creams, desserts) and in sensitized subjects it can act as an hidden allergen.

Key points: the growing use of food substitutes or complementary medicine in Western countries has led to a rise of hypersensitivity reactions to these allergens. Clinical manifestations range from respiratory (*i.e.,* rhinitis, asthma) and dermatologic (*i.e.,* urticaria, dermatitis) symptoms to anaphylaxis. Therefore, the occupational exposure of these substances seems to play an important role for their sensitization.

Also, for these allergens, with the exception of buckwheat, little is known about the molecular allergens involved in the allergic reactions.

Diagnosis

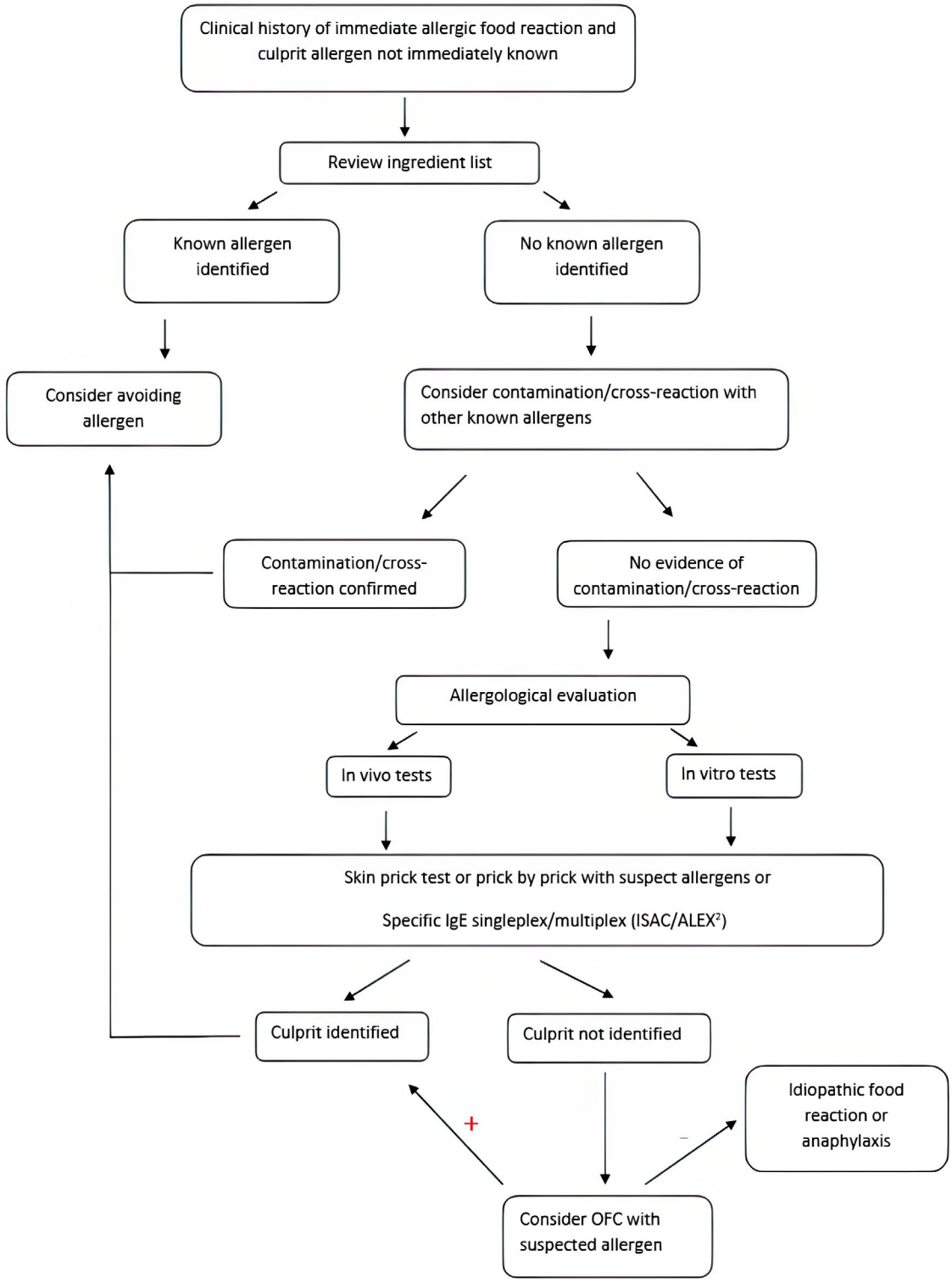
The identification of the culprit allergen is not always simple because most of these allergens are not actually subjected to man-

datory labelling in food products and many diagnostic tools are not available in common practice. However, a careful history and review of ingredient lists with the understanding of possible cross-reactions/contaminations with other foods is recommended in successfully identifying the culprit allergen. Prick by prick and/or commercial skin prick test together with the research of currently available specific commercial IgE (both in singleplex or multiplex methods) could be used to detect IgE sensitization to these potential allergens even if the oral food challenge (OFC) with the culprit agent remain the gold standard for the diagnosis (**figure 1**). Concerning the laboratory diagnostics, various specific IgE extracts for herbs and spices (*e.g.,* coriander, fennel, parsley, cumin, black and green pepper, paprika, garlic, onion, *etc*.) both ImmunoCAP and multiplex methods (ImmunoCAP ISAC or ALEX² microarrays) are available, although no specific molecular allergens are actually included. The diagnostic tools currently available for the investigation of legume allergies are mainly based on the priority legumes (*i.e.,* peanuts and soybeans) and little or nothing on the non-priority ones (*e.g.,* peas, lentils, chickpeas, beans, *etc*.) and again only specific IgE extracts without molecular allergens. Similarly, few seeds extracts are actually available (*e.g.,* pumpkin seeds, sunflower seeds, quinoa seeds, flaxseed, poppy seeds, hemp seeds and pine nuts) and from a molecular point of view, only the lipid transfer protein of hemp seeds (Can s 3) and the 2S albumin of poppy seed (Pap s 2S) (both only in the ALEX² microarray) can be currently assessed. Fewer diagnostic extracts are available for additives (*i.e.,* guar gum, carob gum, arabic gum, bovine gelatin and carmine red), wheat substitutes (*i.e.,* buckwheat, oat and millet) and other hidden allergens (*i.e.,* psyillum or ispaghula). Regarding buckwheat allergy, only the 2S albumin of *Fagopyrum esculentum* (Fag e 2) can be currently assessed but only with multiplex methods (ImmunoCAP ISAC or ALEX²) (**table II**).

Discussion

The progressive change in eating habits, together with the development of the food industry, the adoption of new technologies and globalization phenomena, have contributed to a great variability and availability of all sorts of foods. In this contest, a growing minority of people may become allergic to other lesser-known allergenic substances which are not always adequately reported in the current food allergen lists. A large variety of food reactions caused by hidden allergens has been reported in literature and, in various cases, severe systemic reactions up to anaphylaxis have been described. Sensitization to new vegetable allergens (*i.e.,* herbs and spices, legumes, seeds) and food substitutes are constantly increasing in the general population. In the European Anaphylaxis Registry during the period of 2007 to 2020 in the subgroup of "other tree nuts", pine nuts were the elicitors in over 19% of cases after almond and pistachio, while in the group of seeds, sunflower and pumpkin seeds were involved in 18% and 12% of cases, respectively (118). In children and adolescents, data on anaphylaxis taken from the European registry during the period of 2007 to 2015 have shown that among the subgroup of vegetables, celery was the main elicitor in 42% of cases, but other vegetables (*i.e.,* carrot, lettuce, tomato and cabbage) were the elicitors in over 58% of cases. In the subgroup of cereals, with the exclusion of wheat which was the leading elicitor in the 62% of cases, other cereals (*i.e.,* buckwheat, barley, sweet corn, rye, rice and spelt) were involved in over 38% of cases and in the legumes subgroup, pea and other legumes (*i.e.,* bean, chickpea, lupine and lentil) were the elicitors in over 12% of cases. The main elicitors remain peanuts and soy (88% of cases). Among the tree nuts subgroup, pine nuts were involved in over 5% of anaphylaxis and in the spices subgroup, sesame remained the main elicitor even if other spices (*i.e.,* curry, poppy, pepper, mustard, sunflower and pumpkin seed) were involved in above 44% of anaphylaxis (119). These data show how the ever-increasing impact of these allergens in terms of public health should not be underestimated. More difficult to estimate is the real prevalence of additives adverse reactions because symptoms are prone to subjectivity and markers of reactivity are not available (71). In addition, only in a few case reports or case series an IgE mediated mechanism was shown, so that it is difficult to estimate their real prevalence and the impact on public health. However, their reporting on product labels could avoid potentially serious and unwanted adverse reactions. Up to now, little is known about some hidden allergens from a molecular point of view, and this is true especially for various spices, some seeds (*e.g.,* Chia seeds, flaxseeds and quinoa seeds), additives (*e.g.,* pectin, gelatin, inulin and food dyes) and wheat substitutes (*e.g.,* oat, millet and amaranth) and this knowledge gap should be filled in the future. At the same time, for those allergens where a molecular profile is more defined such as some legumes, other seeds (*e.g.,* pumpkin, sunflower, hemp, poppy seeds and pine nuts) and buckwheat, there currently is a lack of diagnostic tools capable of identifying their molecular allergens and diagnoses are based on skin prick test or prick by prick with extracts or using commercial specific IgE for whole extracts. Future researches in this regard will be necessary to implement diagnostic possibilities at our disposal. We acknowledge the limitations of this review compared to a scoping or a systematic review, since we have included multiple study designs (*e.g.,* case reports, case series, retrospective and prospective studies, retrospective series and narrative reviews) with a lack of assessment of the quality and evidence of the included studies. Moreover, our review include a literature search that was limited to only one electronic database (*i.e.,* PubMed/Medline) and it is possible that we have not considered all available data or documentations actually present in literature. Future studies based on a larger amount of data with a more stringent and systematic use of the inclusion or exclusion criteria of the published

Figure 1 *- Diagnostic algorithm approach to evaluate allergic food reactions caused by a hidden allergen.*



OFC: oral food challenge.

Table II *- In vivo and in vitro diagnostic tools available for the diagnosis of hidden allergens except the European declared allergens celery, mustard, peanut, soybean, lupin and sesame.*

Class of allergens	*In vivo* tests	*In vitro* tests		
	Skin prick tests/prick by prick	Specific IgE singleplex (ImmunoCAP)	Specific IgE multiplex microarrays	
			ISAC	ALEX²
Herbs and spices	SPT and PBP for single herb or spice	Anise, basil, bay leaf, black pepper, cumin, chili pepper, clove, coriander, dill, fennel, garlic, ginger, green pepper, lovage, mace, marjoram, mint, onion, oregano, paprika, parsley, tarragon, thyme, vanilla, *etc*. No commercial specific IgE for saffron, pink peppercorns and sumac	//	Anise, cumin, garlic, onion, oregano, paprika
Legumes	SPT and PBP for single legume	Pea, green and white bean, chickpea, fenugreek, lentil, lupin seed, carob	//	Chickpea, white bean, lentil, pea
Seeds	PBP for specific seed	Pumpkin seed, sunflower seed, flaxseed, hemp seed, quinoa seed, poppy seed, pine nut	//	Pumpkin seed, sunflower seed, poppy seed (both extract and Pap 2S albumin), hemp seed (both extract and Can s 3), quinoa
Additives				
Thickeners	PBP with pectin and gelatin containing foods, guar gum, carob gum, arabic gum and tragacanth gum	Bovine gelatin, guar, carob and arabic gum No commercial specific IgE for pectin, fish gelatin and tragacanth gum	//	//
Food dyes	PBP with fresh carmine and annatto	Carmine red No commercial specific IgE for annatto	//	//
Prebiotics	PBP with inulin containing foods	No commercial IgE available	//	//
Food substitutes				
Wheat substitutes	PBP or SPT with buckwheat, oat, millet and amaranth	Buckwheat (*Fagopyrum esculentum),* oat and millet No commercial specific IgE for amaranth	Buckwheat (Fag e 2)	Buckwheat (both extract and Fag e 2), oat and millet
Meat substitutes	PBP with Quorn	No commercial specific IgE available	//	//
Complementary and alternative medicine	PBP with fresh psyllium	Ispaghula	//	//

SPT: skin prick test; PBP: prick by prick.

studies could provide broader and more detailed information on this complex topic.

Conclusions

Hidden allergens are potential causes of misdiagnosed allergic reactions or idiopathic anaphylaxis. During the last decade, the growing trend of wheat or meat exclusion with the adoption of plant-based diets or new sustainable foods is contributing to the exposure to new emerging allergens. Food allergies are on the rise, with an increasing prevalence worldwide and the main allergens involved in anaphylactic reactions in adults are predominantly of plant origin, mainly legumes and nuts (120). In this review, we tried to summarize the clinical implications and the molecular characteristics of several potential hidden allergens: our findings showed how there is a need to expand our knowledge, especially on vegetables allergens (*i.e.,* herbs and spices, legumes and seeds), additives and food substitutes, both from a molecular point of view and from potential cross-reactivity. Moreover, it will be important to take into consideration other potential allergens used in the context of complementary and alternative medicine. The European Union declaration is currently mandatory for a few plant derived allergens: cereals containing glutens, peanuts, soybeans, tree nuts, celery, mustard, sesame seeds and lupin. At present, no declarations for other cereals (*e.g.,* buckwheat, oat, millet, quinoa, rice), legumes (*e.g.,* peas, lentils, chickpeas, beans), vegetables (*e.g.,* garlic, onions, fennel, parsley, cumin, carrot, tomatoes) and several seeds (*e.g.,* pumpkin, sunflower, flaxseed, poppy, hemp seeds), potentially causing severe allergic reactions, are yet proposed. Curiously, among additives none for the allergens for which an IgE-mediated mechanism has been demonstrated (*e.g.,* carmine red, guar gum, gelatin, pectin) are subject to mandatory declarations and only sulphites, for which the hypersensitivity mechanism still remains unclear and debated, are reported. Therefore, the need to cover more allergens groups re-evaluating the inclusion of same debated allergens, would suggest a revision of the current list of priority food allergens in order to avoid the onset of unpredictable and life-threatening allergic reactions.

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Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Clinical efficacy and safety evaluation of *Dermatophagoides farinae* drops in the treatment of allergic rhinitis with epistaxis

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Key words

Allergic rhinitis; epistaxis; house dust mite; sublingual immunotherapy; efficacy.

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Impact statement

The symptoms of rhinitis and epistaxis were both improved after SLIT treatment, indicating the remarkable efficacy and safety for AR patients with epistaxis.

Summary

Background*. Epistaxis is frequently observed in allergic rhinitis (AR) patients. However, few studies focus on the outcome of epistaxis with treatment of AR patients. This study aimed to retrospectively analyze the efficacy and safety of AR patients with epistaxis treated with sublingual immunotherapy (SLIT).* ***Methods****. A total of 74 patients aged 4-60 years with house dust mite (HDM)-induced AR accompanied by epistaxis and who completed 1 year of SLIT treatment with standard Dermatophagoides farinae (D. farinae) drops were enrolled in this study. The symptom scores, total medication scores (TMS), combined symptom and medication score (CSMS), visual analog scales (VAS), and bleeding score (BS) were assessed, as well as the nasal endoscopic examinations were performed to observe nasal signs.* ***Results.*** *The levels of symptom scores, TMS, CSMS, VAS, and BS at 0.5 year and 1 year of SLIT treatment were significantly lower than those at the baseline (all $p < 0.01$). Also, statistical differences were seen in CSMS ($p < 0.05$) and VAS ($p < 0.01$) between 0.5 year and 1 year. As expected, BS was positively correlated with CSMS ($r = 0.617$, 95%CI 0.517-0.699) and VAS ($r = 0.777$, 95%CI 0.719-0.822) at all three time points.* ***Conclusions****. SLIT with D. farinae drops was effective and safe for AR patients with epistaxis, resulting in improving the symptoms of rhinitis while relieveing the symptoms of epistaxis.*

Introduction

Allergic rhinitis (AR) has evolved as the most common allergic disease during the past few decades, which carries a significant disease burden both at individual and societal levels (1-3). In China, a huge economic burden of 51.28 billion EUR per year was constituted by ARs (4). Not only that, the life quality of AR patients was also seriously affected. In outpatient practice, epistaxis is a common concomitant symptom and frequently observed in AR patients (5-9). Potentially, the pathological changes including edema of the nasal mucosa, nasal vasodilatation, angiogenesis, and increased vascular permeability associated with AR lead to increased fragility of the nasal mucosa, which is prone to blood vessel damage and epistaxis (5). At the same time, repeated picking/rubbing and nose blowing behavior related to itching/rhinorrhea caused by AR could also damage the nasal mucosa and

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frequently lead to epistaxis (8, 9). In general, epistaxis makes AR patients bear greater psychological and physiological burden. Until now, few studies have focused on epistaxis and AR (7-9). A preceding study reported on the outcome of epistaxis with treatment of underlying AR. The result had shown that there was a clinically significant improvement in the frequency and severity of epistaxis after symptomatic drug treatment of AR, indicating that the valid treatment of AR could be beneficial to the prevention and treatment of epistaxis (9). Compared with drug therapy, AIT was regarded as an etiotropic therapy with remarkable clinical efficacy and safety, especially the emerging immunotherapy method of sublingual immunotherapy (SLIT) (10-13). In clinical practice, SLIT could improve the nasal signs including nasal mucosa edema and telangiectasia, the nasal symptoms, and the use of anti-allergic drugs in AR patients (14-16). These improvements could further lead to a healthier nasal mucosa, reduced picking/rubbing and nose blowing behavior, and less risk of epistaxis for higher doses of intranasal corticosteroids used to treat AR (17). As far as we know, only a few studies were reported on the outcome of SLIT for the treatment of AR with epistaxis (18). Therefore, this retrospective study aimed to further investigate the clinical efficacy and safety of SLIT treatment with *D. farinae* drops in AR patients accompany with epistaxis.

Materials and methods

Ethical approval and consent to participate

This study protocol was approved by the Medical Ethics Committee of the Affiliated Jiang Ning Hospital of Nanjing Medical University (No.2023-03-055-K01) and the patients (or their guardians) signed informed consent forms.

Study subjects

This was a retrospective study conducted in the Affiliated Jiangning Hospital of Nanjing Medical University from April 2020 to May 2022. The complete data of 74 patients aged 4-60 years with house dust mite (HDM)-induced intermittent or persistent AR with epistaxis were included in the study. All patients met the treatment criteria of SLIT and had received one whole year treatment with standard *Dermatophagoides farinae* (*D. farinae*) drops. The treatment criteria of SLIT included: 1) patients have been diagnosed with moderate-to-severe AR according to Allergic Rhinitis and Its Impact on Asthma and combined with epistaxis; 2) patients have a clinical history of mite allergy and sensitization to *D. farinae* with/without *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) as assessed by a positive skin prick test (SPT) with grade ≥ 2 (Zhejiang Wolwo Bio-Pharmaceutical Co., Ltd., Zhejiang, China); 3) patients without immunodeficiency, non-stable or severe systemic diseases such as poorly controlled cardiovascular diseases, immune diseases, or malignant tumors, receiving β-blockers or angiotensin-converting enzyme inhibitors, serious psychological barriers or failed to understand the risks and limitations of treatment; pregnancy or lactation, or planning pregnancy within 1 year.

Sublingual immunotherapy

Patients were treated with standardized *D. farinae* drops (Zhejiang Wolwo Bio-Pharmaceutical Co., Ltd., Zhejiang, China) labeled from No. 1 to No. 5. The total protein concentration of No. 1-5 was 1, 10, 100, 333, and 1,000 μg/mL, respectively. In the up-dosing phase of SLIT, patients were administrated with increasing doses starting from No. 1 to No. 3 during the first 3 weeks and 50, 100, 150, 200, 300, 400, and 500 μL were given day after day in a week, respectively. Then, the patients aged < 14 years were instructed to have 150 μL of No. 4 per day from the fourth week to the end of the treatment. While patients aged ≥ 14 years were instructed to have 150 μL of No. 4 per day during the fourth and fifth weeks, and then take 100 μL of No. 5 per day from the sixth week until the end of the treatment. Drops were instructed to be kept under the tongue for 1-3 minutes before being swallowed. The first SLIT administration was given in doctors' office and the patient should be observed for at least 30 minutes.

Clinical assessments

During the treatment, patients (or their guardians) were required to record symptoms and medicine use through the electronic diary. The symptom scores, total medication scores (TMS), combined symptom and medication score (CSMS), visual analog scales (VAS), and bleeding score (BS) were assessed at baseline, 0.5 year, and 1 year after SLIT. The total nasal symptom score (TNSS) was defined as the sum of 4 nasal symptom scores, including sneezing (number/day, ≤ 2 = 0, 3 to 5 = 1, 6 to 10 = 2, ≥ 11 = 3), rhinorrhea (times/day, none = 0, 1 to 4 = 1, 5 to 9 = 2, ≥ 10 = 3), nasal itching (no symptom = 0, intermittent itching = 1, tolerable itching = 2, intolerable itching = 3), and nasal obstruction (no symptom = 0, congestion without mouth breathing = 1, severe congestion with occasional mouth breathing = 2, severe congestion with mouth breathing almost all day = 3). TMS were assessed according to the daily dosage recommended by the drug instructions for controlling AR symptoms (none = 0, oral antihistamines or anti leukotrienes = 1, topical glucocorticoid = 2, oral glucocorticoid = 3). Then, CSMS was calculated ultimately according to the formula: CSMS = TNSS/4 + TMS, which is used to reflect the severity of symptoms and medication intake simultaneously (15, 16, 19-22). VAS ranges from 0 to 10 and assesses the severity of patients' symptoms by themselves. 0 expressed "no symptoms" and 10 indicated "maximum symptoms". BS were scored from 0 to 4 (none or bleeding less than 5 times/year = 0; mild, bleeding more than 5 times/year or each bleeding time less than 10 minutes = 1; moderate, bleeding more than 10-20 times/year, and each bleeding time more than 20 minutes = 2; relatively

severe, require bandage, cauterize, or administer antifibrinolytic drugs = 3; severe, require blood transfusion, decompression, or emergency hospitalization = 4) (23). The score in baseline is the average situation of the previous week reviewed by the patient. The scores at 0.5 year and 1 year were the average daily scores of 1 week before and 1 week after each follow-up time point.

Considering that AR patients were often accompanied by a series of changes in nasal signs, such as pale edema of the nasal mucosa, runny nose, swelling of the inferior turbinate, *etc.*, the nasal signs of patients at 3 time points were also collected through nasal endoscopy in this study.

Adverse events

The occurrence rate, duration, and severity of adverse events (AEs) were recorded during the whole study to assess safety. All AEs were addressed under the instruction of the physicians.

Patient management

Initial clinical education and follow-up education were carried out for all patients. The patient education includes: 1) the nature causes and hazards of AR with epistaxis; 2) the characteristics of SLIT and its relationship with anti-allergic drugs; 3) the methods, courses of treatment, costs, efficacy, and safety of SLIT; and 4) advices on how to avoid allergen and deal with AEs. The patient files were established to record the symptoms, medication use, and AEs during the whole treatment. Telephone follow-ups were provided to solve problems during the treatment process.

Statistical analysis

Statistical analysis was performed with SPSS software 21.0. The intergroup comparisons of clinical characteristics were performed by the Mann-Whitney U test or Wilcoxon signed rank test. The 2-tailed level of statistical significance was set at $p = 0.05$ and the effect size was calculated using Cliff's Delta. The Spearman bivariate analysis was performed to determine potential differences and correlation coefficients between CSMS, VAS, and BS. The 95% confidence interval of the correlation coefficient was estimated by the bootstrap method.

Results

The evaluation of four nasal symptoms

The four typical symptom scores of AR were drawn in **figure 1**. Compared with the baseline (sneezing: $\bar{x}$ = 1.46, 95%CI 1.27-1.64; rhinorrhea: $\bar{x}$ = 1.42, 95%CI 1.23-1.61; nasal obstruction: $\bar{x}$ = 1.59, 95%CI 1.39-1.80; nasal itching: $\bar{x}$ =1.58, 95%CI 1.36-1.80), the individual nasal symptom score of 0.5 year (sneezing: = 0.82, 95%CI 0.68-0.97, effect size = 0.42; rhinorrhea: $\bar{x}$ =0.77, 95%CI 0.61-0.93, effect size = 0.42; nasal obstruction:$\bar{x}$ = 0.74, 95%CI 0.57-0.92, effect size = 0.51; nasal itching: $\bar{x}$ = 0.73, 95%CI 0.54-0.92, effect size = 0.49; all $p < 0.01$) and 1 year (sneezing: $\bar{x}$ = 0.46, 95%CI 0.32-0.60, effect size = 0.65; rhinorrhea: $\bar{x}$ = 0.42, 95%CI 0.27-0.57, effect size = 0.63 ; nasal obstruction: $\bar{x}$ = 0.41, 95%CI 0.26-0.55, effect size = 0.69; nasal itching: $\bar{x}$ = 0.42, 95%CI 0.27-0.57, effect size = 0.66; all $p < 0.01$) after SLIT treatment both significantly declined. Meanwhile, the score of sneezing (effect size = 0.31, $p < 0.01$), rhinorrhea (effect size = 0.30, $p < 0.01$), and nasal obstruction (effect size = 0.26, $p < 0.05$) exhibited significant differences between 0.5 and 1 year. As for nasal itching, the score at 1 year showed a lower level compared with 0.5 year, but there was no significant difference ($p > 0.05$).

The evaluation of TMS, CSMS, VAS, and BS

Except for the improvement of symptoms, the levels of TMS, CSMS, VAS, and BS were also evaluated in detail. Compared with the baseline (TMS: $\bar{x}$ = 1.84, 95%CI 1.75-1.92; CSMS: $\bar{x}$ = 3.35, 95%CI 3.16-3.54; VAS: $\bar{x}$ = 7.49, 95%CI 7.14-7.83; BS: $\bar{x}$ =1.81, 95%CI 1.63-1.99), the level of these outcomes at 0.5 year (TMS: $\bar{x}$ = 0.01, 95%CI -0.01-0.04, effect size = 1.00; CSMS: $\bar{x}$ = 0.78, 95%CI 0.66-0.90, effect size = 0.98; VAS: $\bar{x}$ = 3.04, 95%CI 2.58-3.50, effect size = 0.92; BS: $\bar{x}$ =0.42, 95%CI 0.27-0.57, effect size = 0.80; all $p < 0.01$) and 1 year (TMS: $\bar{x}$ = 0.08, 95%CI 0.02-0.14, effect size = 0.99; CSMS: $\bar{x}$ = 0.51, 95%CI 0.35-0.66, effect size = 0.98; VAS: $\bar{x}$ = 1.86, 95%CI 1.43-2.30, effect size = 0.96; BS: $\bar{x}$ =0.19, 95%CI 0.10-0.28, effect size = 0.92; all $p < 0.01$) after SLIT treatment were significantly declined (**figure 2**). Not only that, but there were also statistical differences in CSMS (effect size = 0.37, $p < 0.05$) and VAS (effect size = 0.36, $p < 0.01$) between 0.5 year and 1 year. However, no statistical difference was seen in TMS and BS scores between the two time points ($p > 0.05$).

Nasal endoscopy finding

The nasal endoscopic examinations were performed at baseline, 0.5 year, and 1 year. And the representative endoscopy findings of 2 cases of AR patients were shown in **figure 3**. For both patients, A and B, the nasal signs were significantly improved after SLIT treatment compared with the baseline, with the improvements including color change of the turbinate mucosa (from pale to light red), reduction of nasal secretion and blood, and disappearance of nasal mucosal edema.

Correlation analysis

In this study, the correlation of BS with CSMS and VAS was calculated and analyzed. A positive correlation of BS was observed with CSMS (r = 0.617, 95%CI 0.517-0.699, **figure 4A**) as well as VAS (r = 0.777, 95%CI 0.719-0.822, **figure 4B**).

Adverse events

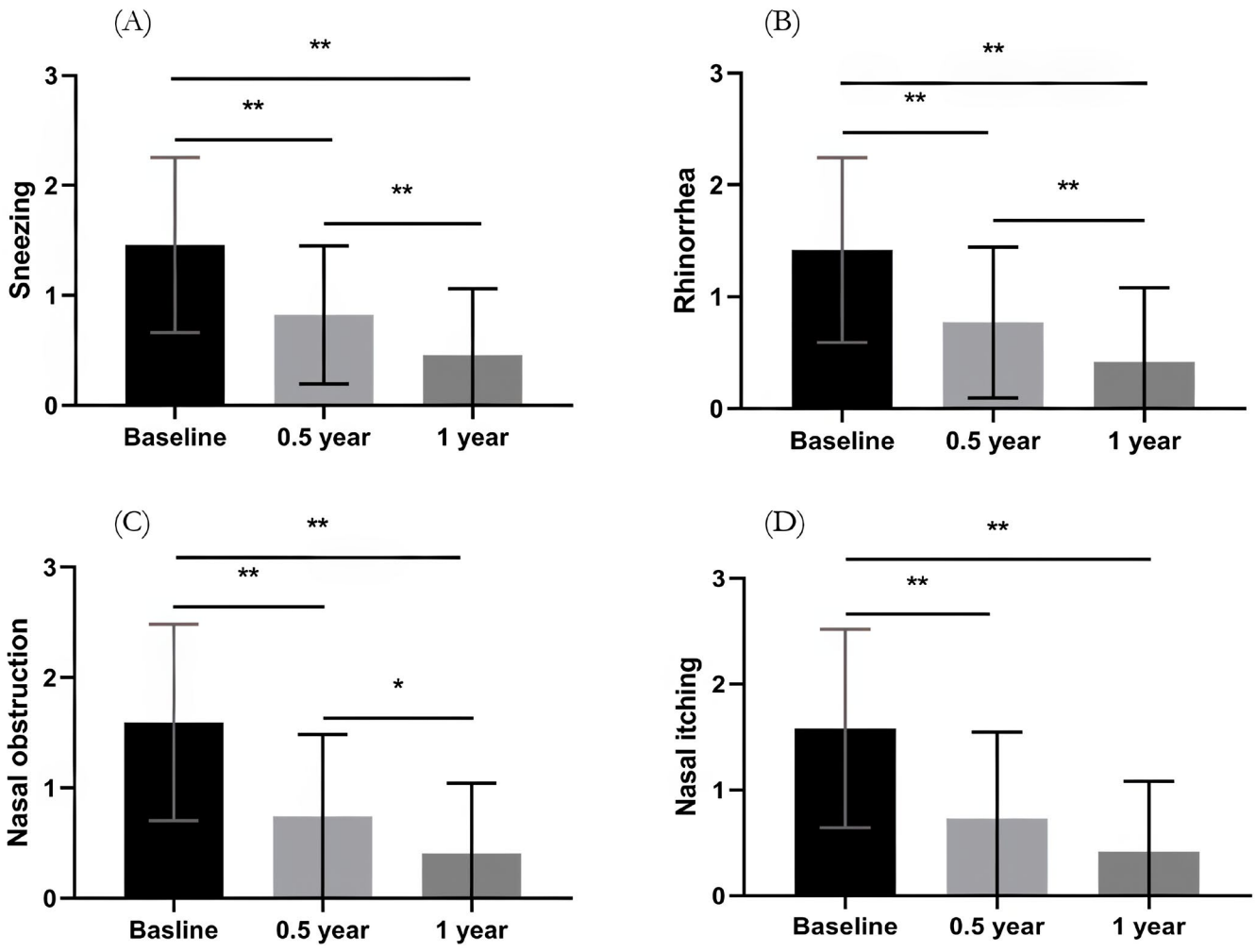
All of the AEs, based on five levels (0-4 scale) according to the grading system proposed by World Allergy Organization (WAO) immunotherapy committee (24), were promptly addressed under

the instruction of the physician. No severe systemic AEs, anaphylaxis, acute attack of asthma, or use of adrenaline were reported. Seven patients reported 7 local AEs, including 3 skin local rash, 1 hypoglossal edema, and 3 symptom aggravation. All AEs in this study were grade 1 and relieved within a week without medication.

Discussion and conclusions

AR is a highly prevalent chronic disease that limits the self-image and psychosocial interaction of patients (2). Until now, the clinical effect of SLIT on HDM-induced AR had been proven by plenty of clinical trials (15, 16, 25-27). A previous study showed that the early effect of SLIT was generally observed in 3 to 6 months (28). Similarly, the individual nasal symptom scores, TMS, CSMS, and VAS signally declined after 0.5 year of SLIT in this study. Besides, these clinical scores continued decreasing with prolonged treatment when compared with 0.5 year. Our results further substantiated the previous findings that the longer duration of SLIT treatment contributes to the better efficacy

Figure 1 *- Analysis of four individual AR typical symptoms* ***(A)*** *sneezing,* ***(B)*** *rhinorrhea,* ***(C)*** *nasal obstruction, and* ***(D)*** *nasal itching at the baseline, 0.5 year, and 1 year of SLIT treatment.*



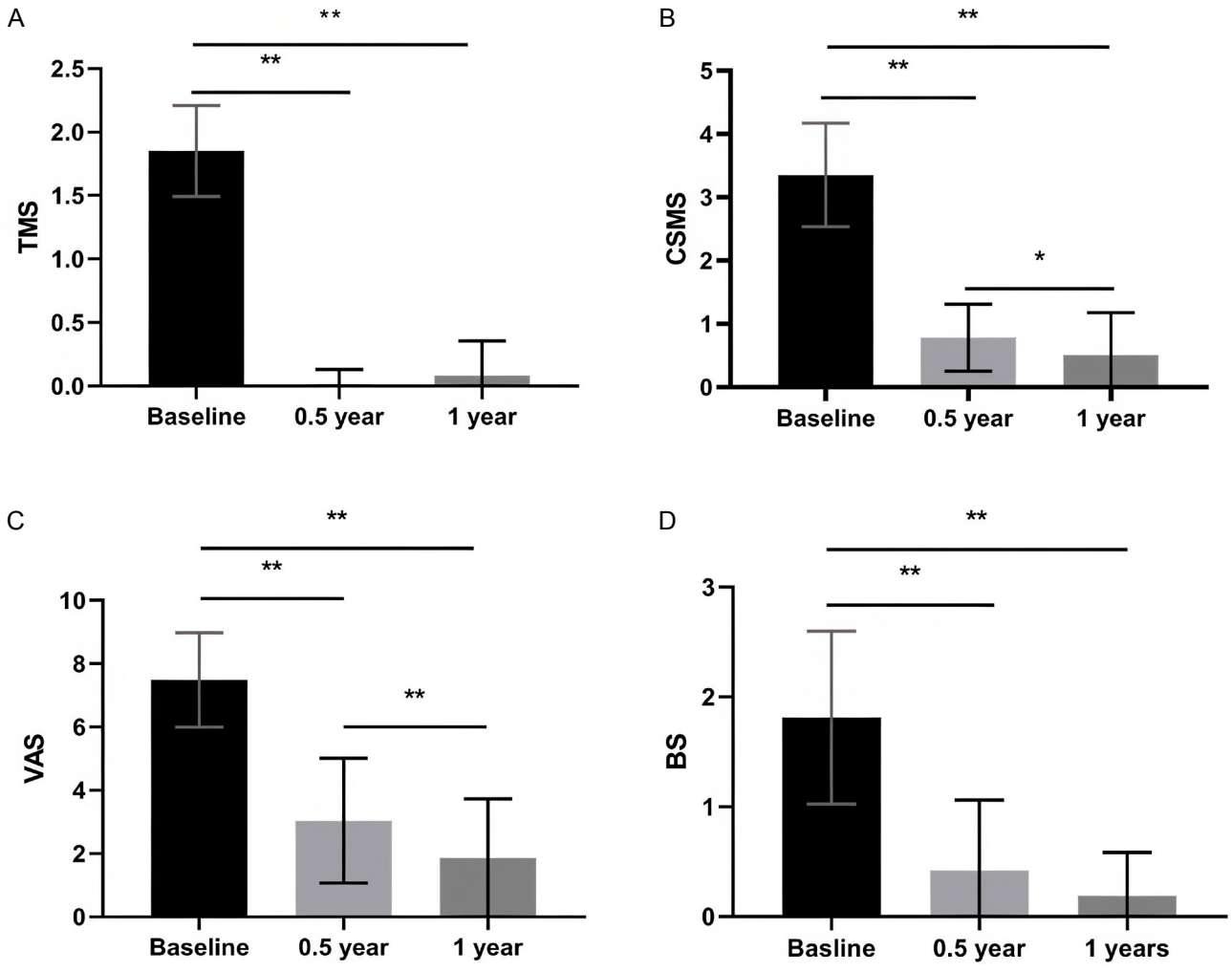
*p < 0.05; **p < 0.01, significant difference between different time points; AR: allergic rhinitis.

(29, 30). Also, our findings manifested that SLIT performed a significant efficacy on AR patients.

In outpatient practice, epistaxis is frequently observed in AR patients (7-9, 31). A few literatures confirmed the improvement of nasal symptoms and nasal bleeding after the treatment of AIT in AR patients co-existing with epistaxis (18, 32, 33). Shao *et al.* (18) focused on the efficacy of SLIT with *D. farinae* drops on pediatric patients by evaluating the score of epistaxis symptoms. In our study, the same criteria was used to assess the improvements of epistaxis. Our results exhibited significantly declined in BS scores after 0.5 year and 1 year SLIT treatment, which is consistent with the conclusions of the previous published study (18). Our study also first analyzed that BS was positively correlated with CSMS and VAS.

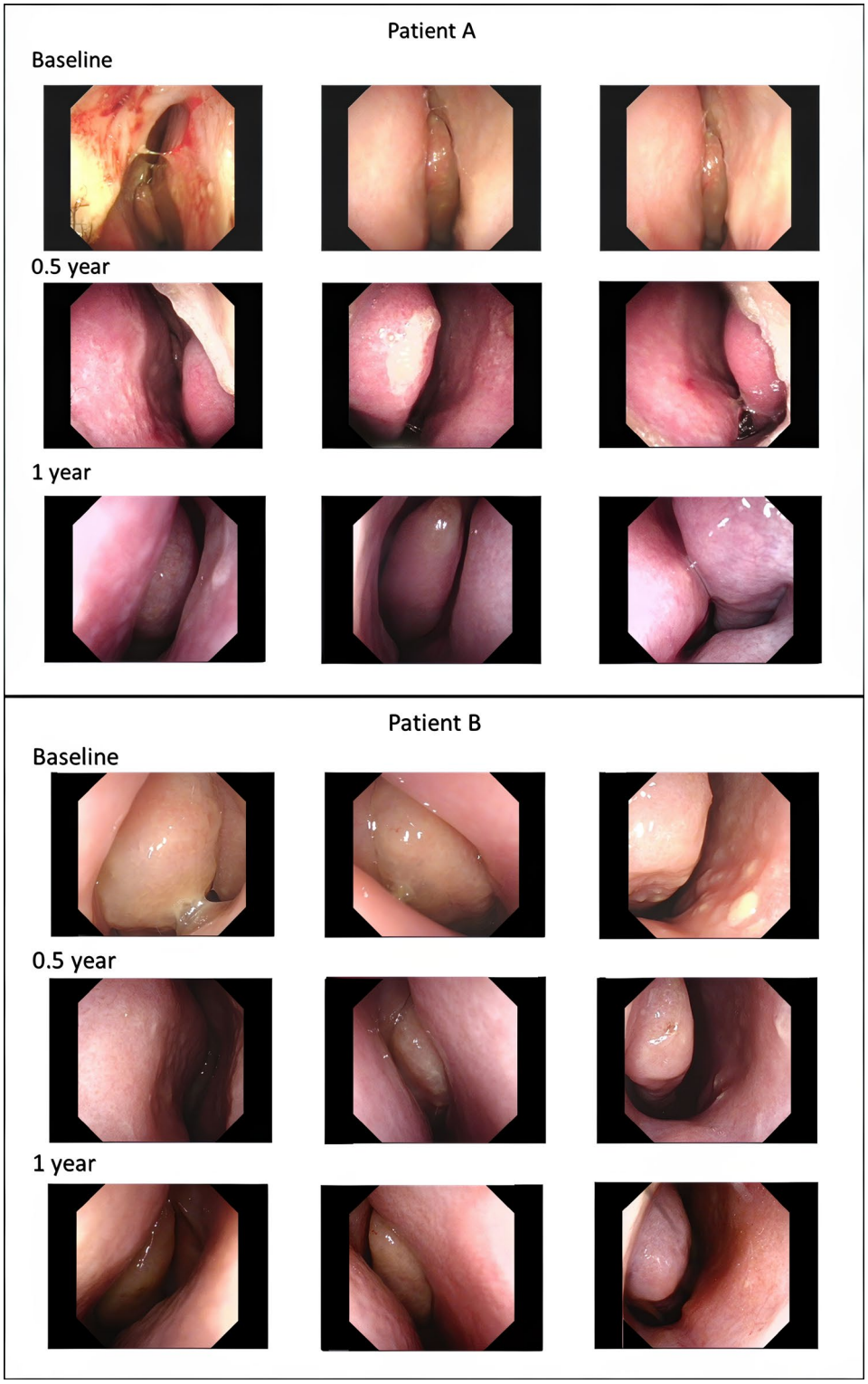
In addition, the changes of patients' nasal signs were observed using nasal endoscopy. After treatment, the nasal mucosa returned to

Figure 2 - *The comparison at the baseline, 0.5 year, and 1 year of SLIT treatment of* ***(A)*** *TMS;* ***(B)*** *CSMS;* ***(C)*** *VAS;* ***(D)*** *BS.*



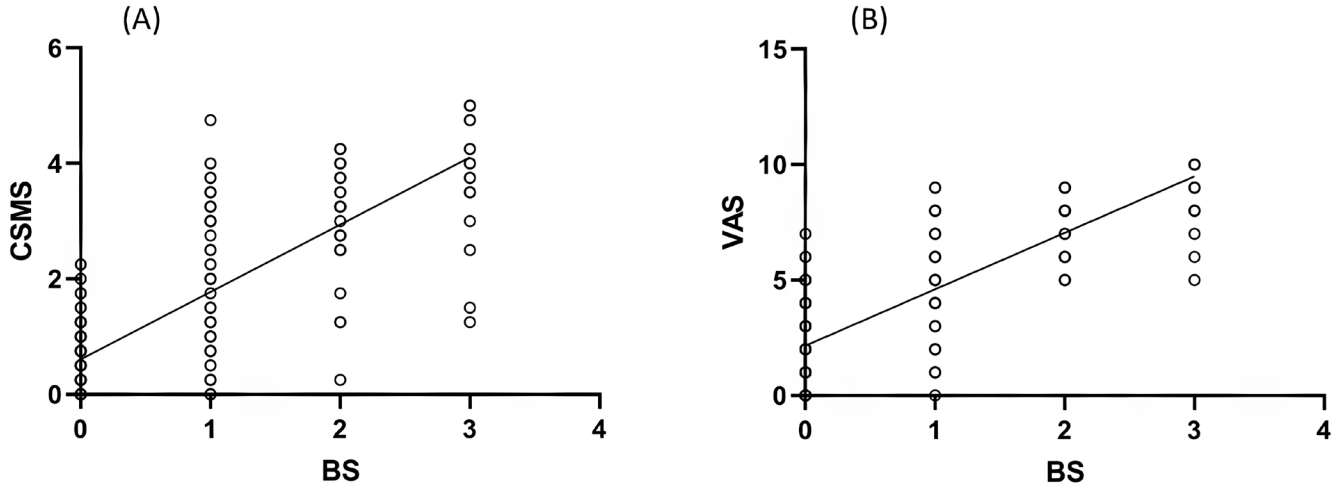
**p < 0.01, *p < 0.05: significant difference between different time points; TMS: total medication score; CSMS: combined symptom and medication score; VAS: visual analogue scale; BS: bleeding score.

Figure 3 *- Representative endoscopy findings in 2 cases of AR patients at baseline, 0.5 year, and 1 year after treatment.*



AR: allergic rhinitis.

Figure 4 *- Correlation between different clinical outcomes:* ***(A)*** *CSMS and BS;* ***(B)*** *VAS and BS (95% CI).*



CSMS: combined symptom and medication score; BS: bleeding score; VAS: visual analogue scale; CI: confidence interval.

a healthy state with nasal secretions, hyperaemia, and inflammation alleviated. Overall, the alleviated or even disappeared symptoms of AR and epistaxis might be probably due to the improvement of nasal signs.

The safety of SLIT has been demonstrated in multiple articles of numerous clinical trials (34-37). The incidence of AEs in Chinese AR patients undergoing SLIT ranged from 8.4% to 27.7% according to the summarized results of several preceding reports (2, 35-37). In our study, the AE rate of SLIT in AR patients with epistaxis was 9.5%, suggesting that the concomitant disease such as epistaxis did not increase the occurrence of AEs. Meanwhile, the main AEs were local AEs such as transient oral swelling and skin local rash. These results identified that SLIT was generally safe and could be well tolerated in AR patients with epistaxis.

Some limitations of this study should be acknowledged. First, the number of patients was small. Second, the impact of HDM level in the environment which might be variable in different seasons wasn't considered. Thirdly, the course of treatment was short, the long-term efficacy of SLIT for AR with epistaxis was not investigated. In the future, studies with larger sample sizes, larger geographical areas, and longer treatment duration would be researched to further provide more evidence on the short-term and long-term efficacy of SLIT in AR patients with epistaxis.

In conclusion, our study preliminary confirmed the efficacy and safety of SLIT on HDM-induced AR patients accompanied by epistaxis. There was clinical improvement of epistaxis after SLIT treatment of AR with *D. farinae* drops in this study.

Fundings

None.

Contributions

JP, CQH, YJT: conceptualization, writing – original draft. ZRD: methodology, writing – review & editing. LC: methodology, data curation. HFY formal analysis.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Hypersensitivity to non-steroidal anti-inflammatory drugs on a pediatric Portuguese cohort

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Key words

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Impact statement

Non-steroidal anti-inflammatory drugs are the second cause of drug hypersensitivity suspicions in children. As most of those suspicions are not confirmed after a diagnostic workup it is very important to perform a correct investigation in order to avoid unnecessary restrictions.

Summary

Background. *Non-steroidal anti-inflammatory drugs (NSAID)/analgesics (paracetamol) are among the most common causes of drug hypersensitivity reactions in children, with a reported prevalence of around 0.3% in the pediatric population.*

Paracetamol and ibuprofen are the most commonly reported culprits in the pediatric population.

Our objective was to describe the allergy workup to NSAID/paracetamol of a pediatric population monitored in an allergy outpatient clinic. ***Methods.*** *Retrospective observational study by consulting the medical records of patients evaluated in a pediatric outpatient clinic with history of NSAID/paracetamol, between January 2016 to August 2022.* ***Results.*** *A total of 43 patients have been evaluated for NSAID/paracetamol suspected allergy: 53.5% females, mean age of 9.8±5.1 years, 47.7% atopic. The drugs reported as culprits were ibuprofen (75.6%), paracetamol (17.8%), metamizole (4.4%) and naproxen (2.2%) and clinical manifestations were mainly urticaria/angioedema and maculopapular exanthema.*

Skin tests were performed in 7 patients: paracetamol (n = 5) and metamizole (n = 2), which were all negative. Fourty-six drug provocation tests (DPT) were performed: 28 with the culprit drug and 18 with an alternative one; only 2 were positive (ibuprofen - culprit NSAID group): one immediate periorbital angioedema and one delayed lip edema with oropharyngeal tightness.

Conclusions. *The investigation of allergy to NSAID/paracetamol in children remains a challenge. In our population, ibuprofen was the most common NSAID reported. There were only 2 (4.3%) mild reactions on DPT. We could allow the use of the culprit NSAID/analgesic in 11 patients and an alternative one in 9 patients.*

This study highlights the importance of DPT in children for a correct diagnosis of NSAID hypersensitivity and selection of an alternative drug.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAID) and beta-lactam antibiotics are among the most common causes of drug hypersensitivity reactions. The reported prevalence of NSAID/paracetamol hypersensitivity is about 6% in the general population and 0.3% in the pediatric population. Paracetamol and ibuprofen are the most commonly reported culprit agents in the pediatric population, as they are the most prescribed drugs in this age group (1-8).

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Atopy and atopic diseases (*e.g.*, rhinitis, eczema and asthma) are reported to be the most important risk factors for drug hypersensitivity reactions, both in adults and children (2, 4, 6, 8).
In the pediatric population, NSAID/paracetamol are mainly prescribed as antipyretics or anti-inflammatory agents during viral infections. Often in children, viral diseases are accompanied by a maculopapular rash, which can mimic an allergic reaction. For this reason, it is very important to clarify the drug allergy label in children, in order to avoid unnecessary restrictions (1-4, 7-15).
In the pediatric population, the clinical presentations of NSAID/paracetamol hypersensitivity reactions are diverse and may range from maculopapular exanthema or nonimmediate urticaria, to life-threatening reactions, as anaphylaxis or severe cutaneous adverse reactions (SCAR) (2-4, 8, 9, 16-18).
The diagnostic workup includes a detailed clinical history with identification of the culprit drug, reaction time, clinical manifestations, treatment needed, other drugs taken, presence of comorbidities. The gold standard for the diagnosis of NSAID/ hypersensitivity is the drug provocation test (DPT). When an immediate immune reaction is suspected, skin tests (prick and intradermal) may be indicated, only validated for metamizole and paracetamol, but can be painful and poorly tolerated by children. In mild, nonimmediate reactions, it has been proposed to perform the DPT without prior skin tests (1, 2, 8, 9, 14, 15, 19, 20).
With this study, we aimed to: 1) describe the characteristics and clinical manifestations of NSAID/paracetamol hypersensitivity in a tertiary pediatric allergology outpatient clinic; 2) identify the main NSAID/analgesic reported as culprits; 3) describe the diagnostic workup performed in order to confirm/exclude the NSAID/ paracetamol allergy label.

Materials and methods

Population and study design

Retrospective observational study including all children and adolescents (0-18 years old) with a suggestive history of NSAID/paracetamol hypersensitivity reaction that completed an allergy workup in the Pediatric Outpatient Clinic.
Data refer to a period of 7 years, from January 2016 to August 2022, and were collected from the records in the patients' clinical files.

Clinical characterization and allergy investigation

In addition to the demographic characteristics, the clinical evaluation included a complete clinical history with identification of the culprit drug (according to parents' reports), a detailed characterization of the reactions according to time (immediate − less than 1 hour to 6 hours after the last intake; delayed − more than 6 hours after the last intake) (2, 7), clinical manifestations (maculopapular exanthema, urticaria/angioedema, gastrointestinal symptoms, and severe reactions such as anaphylaxis and SCAR) and the presence of atopy, defined by the presence of other allergic diseases such as rhinitis, asthma and/or atopic dermatitis, confirmed with positive skin prick tests and/or specific IgE for aeroallergens. No isolated respiratory symptoms were identified (3, 5).

Skin Prick and Intradermal Tests

Skin prick and intradermal tests (if negative prick test) were performed when there was a suspicion of an immediate immune reaction to paracetamol or metamizole, with the formulations and concentrations according to the EAACI/ENDA (European Academy of Allergy and Clinical Immunology/European Network on Drug Allergy) group (2, 19). Sodium chloride 0.9% was used as a negative control for both prick and intradermal tests and histamine 10 mg/mL as a positive control for prick tests. The results were recorded after 20 minutes and considered positive if the largest diameter of the papule was equal to or greater than 3 mm for skin prick tests and at least 3 mm wider than the initial papule with surrounding erythema for intradermal tests (2, 14, 21, 22).

Drug Provocation Tests

In order to confirm or exclude the diagnosis of NSAID/paracetamol hypersensitivity, an open DPT was performed. In patients with an initial severe reaction (like anaphylaxis or SCAR) or with a strong suggestive history, a DPT with an alternative NSAID was performed. The DPT was performed in our Pediatric Outpatient Clinic, by oral route with three doses (1/10, 1/3 and 2/3 of the therapeutic dose) every 30 minutes, with the total cumulative dose calculated as individual therapeutic dose (adjusted to weight and age), either for immediate or delayed reactions, according to EAACI/ENDA group recommendations (2, 4, 5, 8, 19). After the last dose administration, children remained under surveillance for 2 hours and delayed reactions surveillance was also carried out. Those patients with delayed reactions extended the administration at home according to the time of the initial reaction.
The DPT was considered positive when objective signs occurred: exanthema, urticaria, angioedema, rhinitis, bronchospasm/wheezing, cough, vomiting/diarrhea (3, 5). In this case, the DPT was stopped, and the reaction was immediately treated accordingly. If subjective symptoms occurred, the supervising physician decided either to repeat the last step, divide the next step into two doses, or proceed as planned. If the patient could complete the DPT without further objective signs or symptoms, the DPT was considered negative (2, 7, 15).

Statistical analysis

Statistical analysis was performed with GraphPad Prism software version 8.00 (Graphpad Software Inc., San Diego, USA).
Descriptive analysis included the frequency of positive results (in percentage) for qualitative variables compared with the Fisher's test. For quantitative variables, the average ± standard deviation with 95% confidence intervals was described. Normality was ver-

ified by the Shapiro-Wilk and Kolmogorov-Smirnov tests. For the comparison between two unpaired groups, Mann-Whitney tests were used, or unpaired, depending on the situation. Values of $p < 0.05$ were considered significant.

Ethical issues

The clinical part of the study as well as *in vivo* tests were carried out as part of the clinical routine evaluation.

All caregivers and patients (if aged 16 years or older) signed an informed consent form before carrying out the investigation (either skin tests and/or drug provocation tests), which describes the possible use of anonymized data for studies purposes.

The study followed the recommendations of the Ethics Committee and of the World Medical Association (Declaration of Helsinki revised in 2013).

Results

A total of 43 patients were included (53.5% females, mean age of 9.8 ± 5.1 years old; mean age at the reaction of 7.1 ± 5.1 years old) that were referred to our Outpatient Clinic for a suspected NSAID/paracetamol hypersensitivity during the defined period. There was an average delay of 3 ± 3.8 years between the reaction and the referral. The clinical and demographic characterization of the population is described in **table I**.

Table I *- Clinical and demographic characterization of the first reactions.*

Variables	
Total number of patients	n = 43
Age, years old	9.8 ± 5.1 [3-18]
Gender, female / male	23 (53.5) / 20 (46.5)
Age in the reaction, years old	7.1 ± 5.1 [1-16]
Atopy	21 (47.7)
Underlying infection	12 (27.3)
Suspected drug	
Ibuprofen	32
Paracetamol	8
Metamizole	2
Naproxen	1
Immediate / delayed reactions	28 (65.1) / 15 (34.9)
Clinical manifestations	
Urticaria/Angioedema	27
Maculopapular exanthema	6
Anaphylaxis	5
Gastrointestinal symptoms	4
Stevens-Johnson syndrome	1

Data presented as n (%), mean ± SD; SD: standard deviation.

Atopy was present in about half of the patients (47.7%), not associated with the severity of the first reaction.

Twelve patients had a presumptive diagnosis of concomitant infection [viral tonsillitis (n = 6), acute sinusitis (n = 4), epididymitis (n = 1) and fever of unknown origin (n = 1)], of which 4 were concomitantly taking other drugs at the time of the initial reaction, namely antibiotics (amoxicillin, cefotaxime, fluconazole, gentamicin, clotrimazole) and analgesics (tramadol).

According to parents' reports, the drugs suspected of causing the reactions were: ibuprofen (n = 32; 74.4%), paracetamol (n = 8; 18.6%), metamizole (n = 2; 4.7%) and naproxen (n = 1; 2.3%). Seven patients had more than one episode of drug hypersensitivity, either with the same or different NSAID.

Regarding the clinical manifestations of the reactions, 28 (65.1%) were immediate reactions and 15 (34.9%) were delayed reactions (**table I**).

The clinical manifestations of the reactions are detailed in **table I**. Four of the five patients with anaphylaxis were adolescents (> 12 years of age at reaction), being ibuprofen the most frequent NSAID identified as the culprit. Severe reactions (like anaphylaxis and Stevens-Johnson syndrome) were reported with only ibuprofen or paracetamol.

In 7 patients with suspected immediate reaction (anaphylaxis or urticaria), skin prick and intradermal tests were performed before DPT. Of these 7 patients, 5 were tested with paracetamol and 2 with metamizole, and skin tests were all negative. Six patients performed DPT after the skin tests, which were all negative. One patient did not undergo DPT by choice.

The other patients were directly submitted to DPT.

We performed 46 DPT in 43 patients, in patients with both immediate and delayed reactions, only excluding the ones with SCAR: 28 (60.9%) with the culprit NSAID/analgesic and 18 (39.1%) with an alternative one (**table II**).

In the culprit NSAID group, the following DPT were performed: ibuprofen (n = 18); paracetamol (n = 8), metamizole (n

Table II *- Characterization of drug provocation tests performed.*

Drug provocation test (DPT)	Drug	Reaction
Culprit drug (n = 28; 60.9%)	• 18 ibuprofen • 8 paracetamol • 1 metamizole • 1 naproxen	• 1 immediate periorbital angioedema • 1 delayed lip edema with oropharyngeal tightness
Alternative drug (n = 18; 39.1%)	• 10 nimesulide • 5 paracetamol • 2 etoricoxib • 1 celecoxib	No reactions were recorded

= 1) and naproxen (n = 1). Two (7.1%) DPT were positive (both with ibuprofen), with the same clinical manifestations as in the first reaction: 1 immediate periorbital angioedema and 1 delayed lip edema with oropharyngeal tightness. All reactions resolved with oral antihistamine and no severe reactions were recorded.
Confirmation of reactions with ibuprofen by DPT occurred in those patients who did not have a concomitant diagnosis of infection in the initial reaction.
In the alternative NSAID group, the following DPT were performed: nimesulide (n = 10); paracetamol (n = 5); etoricoxib (n = 2) and celecoxib (n = 1). No reactions were recorded on these DPT.

Discussion and conclusions

NSAID/paracetamol are among the most common causes of drug hypersensitivity reactions in children. According to the literature, the reported prevalence of NSAID/paracetamol hypersensitivity is lower in children than in adults and varies depending if it has been proven by DPT or based on clinical history (2, 3, 7, 8, 16, 17).
The percentage of atopy differs in different studies, in a range between 30 to 60% of patients (1, 2, 6, 9, 10, 17, 20), although one study refers up to 93% of patients (4); in our study, we had about half of the population (47.7%) with atopy, which is in accordance with the literature.
The literature describes maculopapular exanthema and nonimmediate urticaria as the most frequent manifestations of NSAID hypersensitivity in children. Although in our study the most common symptom was urticaria, there was a higher proportion of nonimmediate urticaria, which is in accordance with what is described in the literature and may be explained by differences in drug habits between countries (2, 3, 8, 10).
Concomitant infections, fever syndromes or the use of other drugs may play a role in the pathophysiology of hypersensitivity drug reactions, besides infections can mimic a real drug reaction (1, 2, 14, 23, 24). In our study, a suspected infection was present in approximately 28% of the population, so a high percentage of suspicious could have resulted from manifestations of an underlying disease. In our population, positive DPT (4.3%) only occurred in patients with suspected reaction to NSAID/paracetamol, without concomitant infection. A study with a larger sample will be needed to validate these results.
In our population, the most frequent reactions were urticaria (with and without angioedema) and maculopapular exanthemas. Similar data is described in previous studies, demonstrating that cutaneous reactions, such as maculopapular rash and non-immediate urticaria are the most common manifestations of hypersensitivity to NSAID/paracetamol in children. NSAID/paracetamol are among the most frequent causes of drug induced anaphylaxis, which is consistent to what we found in our cohort where, although the most frequent manifestations are mucocutaneous, anaphylaxis is present in about 11% of the population (2-4, 6, 8).
In our study, the drugs most frequently involved in the hypersensitivity reactions were ibuprofen (75.6%) and paracetamol (17.8%). Compared with the literature, the frequency and type of NSAID involved varies, but it is unanimous that ibuprofen together with paracetamol are the main elicitors (2, 3, 7, 14-16), with paracetamol being the most common in children younger than 6 years old, as occurred in our study (3, 14).
We also identified as culprits metamizole in two patients (4.4%) and naproxen in one patient (2.2%), drugs more often prescribed in older children, together with aspirin, nimesulide and COX-2 inhibitors (16).
It is important to state that the differences observed regarding the culprit NSAID between studies reflect variations in prescription patterns and demographic differences of the studied populations.
Skin tests in children are poorly validated (extrapolated from adults), logistically demanding and can be painful. The most standardized NSAID skin tests are for metamizole, although some medications can be tested with non-irritating concentrations, as in the case of paracetamol (4, 6). Nowadays, the standard use of skin testing for the diagnosis of NSAID hypersensitivity is not recommended (1). In the literature, skin testing has been used for the diagnosis of immediate reactions to metamizole and paracetamol in children (1). In our study, we performed skin tests with metamizole and paracetamol in children with immediate reactions.
Some authors propose as possible approach to perform an initial DPT with aspirin and, if negative, perform another one with the culprit drug. If the DPT with aspirin is positive, patients are directly defined as cross-intolerant and an alternative NSAID should be found (7, 11, 16, 17). However, it is important to state that, according to the prescription profile of our country, it is not usual to prescribe aspirin to children under 18 years old. For this reason, we generally do not perform DPT with aspirin. Although most COX-II inhibitors are not indicated for fever or for children under 12 years of age, their safety has been proven in this age group, mainly with nimesulide, meloxicam and etoricoxib, which were the NSAID tested in our population (4, 8, 11, 17). We only performed DPT with the culprit drug when the reactions were non-severe and only got two (4.3%) positive DPT (ibuprofen), with the same clinical manifestations as in the first reaction: 1 immediate periorbital angioedema and 1 delayed lip edema with oropharyngeal tightness, demonstrating its safety and feasibility.
To conclude, drug hypersensitivity reactions in children are an important topic of debate. Antibiotics and NSAID are the most common suspected drugs. It is during childhood that most people take NSAID for the first time, with paracetamol and ibuprofen being the most used.
In our study, the most common NSAID reported as culprit was the Cox-1 inhibitor ibuprofen, which is similar to what is described

in the literature. There were 2 (4.3%) reactions on DPT, being mostly urticaria/angioedema, also according to other studies. Therefore, we could allow the use of the culprit NSAID in 11 patients and an alternative one in 9 patients.

This study highlights the importance of DPT in children for a correct diagnosis of NSAID/paracetamol hypersensitivity and selection of an alternative drug.

There are still few studies on hypersensitivity to NSAID/paracetamol in children, so the allergy workup keeps representing a challenge, being crucial to decide the clinical approach for each patient and try to stablish the culprit drug or alternative ones, so they do not need to perform unnecessary evictions.

For this reason, we believe that the presented data increases our knowledge about NSAID/paracetamol hypersensitivity in pediatric populations and provides information about the clinical characteristic of such patients, being the biggest case series from a single center in our country.

Fundings

None.

Contributions

MIS, JC, CL, JV, RG, EP, AMN, AL: conceptualization. MIS, JC, CL, JV, RG: project administration. MIS: formal analysis; MIS, JC, AL: writing – original draft, writing- review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Clavulanic acid sensitization seems more involved in cutaneous than systemic reactions in amoxicillin-clavulanate drug reactions

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Key words

Clavulanic acid; amoxicillin; drug allergy; delayed reaction; systemic reaction.

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Impact statement

A significant percentage of mucocutaneous reactions after AX-CLA administration are due to CLA sensitization. In these cases, to not completely exclude AX, in vivo tests with clavulanate are useful.

Summary

Background. *Beta-lactams (BLs) allergy is considered a major health issue, as BLs are the most frequently involved in drug allergic reactions. Amoxicillin (AX) is the main sensitizer among all BLs. AX is commercialized alone or combined with clavulanic acid (CLA) in order to increase the antibiotic spectrum. The growing prescriptions of AX-CLA formulations contributed to increase the role of CLA as an allergy inducer. At present, little is known about the clinical characteristics of hypersensitivity reactions to clavulanate. The aim of this study was to assess the difference in the prevalence of cutaneous vs systemic reactions in patients with a documented history of allergic reactions to amoxicillin-clavulanate and tested positive for clavulanate or penicillin/amoxicillin.* ***Methods.*** *Between January 2017 and March 2023, out of 88 outpatients with suspected BLs allergy we selected 59 patients with a reaction to AX-CLA. Hypersensitivity reactions were classified according to onset time as immediate or delayed and according to clinical presentation as mucocutaneous or systemic reactions (anaphylaxis). All patients underwent recommended test protocols for diagnosing BLs hypersensitivity to identify the culprit drug. Sensitization was assessed through serologic and skin tests.* ***Results.*** *Patients whit immediate and delayed mucocutaneous reactions to AX-CLA are more sensitized to CLA 12/41 (29%) than AX or BLs determinants 9/41 (22%); on the opposite patients with immediate systemic reactions are more sensitized to AX or BLs determinants 13/18 (72%) than CLA 2/18 (11%), $p < 0.00$. There was no difference in immediate vs delayed reaction regarding CLA or AX and BLs determinants sensitization.* ***Conclusions.*** *Our study suggests that patients who presented only muco-cutaneous reactions were more often sensitized to CLA rather than AX.*

Introduction

Beta-lactams (BLs) are amongst the most commonly prescribed antibiotics in the community (1, 2) and the first choice for treating the majority of bacterial infections (3).
BLs allergy is considered a major health issue, as BLs are the most frequently involved in drug allergic reactions.
BLs hypersensitivity can be "immediate" or ''delayed" (4, 5). Immediate allergic reactions, ranging from cutaneous to systemic, usually appear within 1 hour, but may occur up to 6 hours after the last administered dose, and are mostly mediated by specific IgE antibodies (6).
Delayed reactions may occur at any time starting from 1 hour after drug administration, commonly after many days of treatment, and are often associated with a T-cell-dependent type of allergic mechanism (7, 8). Maculopapular exanthemas (MPE) and urticaria are the most common clinical features of delayed

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reactions; less common presentations include fixed drug eruption and severe cutaneous adverse reactions (SCARs) (9-11).
Amoxicillin (AX) is the most frequently involved drug in sensitization among all BLs (6, 12). AX is commercialized alone or combined to clavulanic acid (CLA) in order to increase the antibiotic spectrum, as CLA inhibits bacterial beta-lactamases that nullify the effect of AX in resistant bacteria (13). Recent studies have shown that in younger people, AX-CLA is by far the most important drug triggering allergic reactions, accounting for up to 80% of BLs allergy cases (14). In the last years, the growing prescriptions of AX-CLA formulations contributed to increase the role of CLA as an allergy inducer (15, 16).
In the realm of literature, only a small number of allergic reactions associated with CLA have been documented. These reactions are predominantly attributed to type I immediate hypersensitivity, and to a lesser degree, delayed hypersensitivity (17-20). One of the significant challenges in this context is the constrained accessibility of skin testing for CLA and the absence of validated tests to measure serum-specific IgE (sIgE) levels in response to this drug (13).
At present, little is known about the clinical characteristics of hypersensitivity reactions to clavulanate.
The aim of this study was to assess the difference in the prevalence of cutaneous *vs* systemic reactions in patients who had a documented history of allergic reactions to amoxicillin-clavulanate and tested positive for clavulanate or penicillin/amoxicillin.

Materials and methods

We identified patients (n = 88) who were visited at our outpatient allergy department for an allergic reaction, immediate or delayed, after BLs intake (AX-CLA, amoxicilline, oxacilline) between January 2017 and March 2023, focusing on those who reported an adverse reaction to AX-CLA (n = 59).
Symptoms were collected from patients' medical records or clinical history. Hypersensitivity reactions were classified into four categories based on timing and clinical presentation: respectively immediate (within 1 hour up to 6 hours) *vs* delayed reactions (from 1 hour after the initial drug administration) and mucocutaneous *vs* systemic reactions.
Mucocutaneous reactions included urticaria, angioedema, generalized erythema, maculopapular exanthema and mucosal involvement. Systemic reactions included blood pressure drop related symptoms (*e.g.*, dizziness, fainting, need to lie down), wheezing, dyspnea, laryngeal edema, bronchospasm, dysphonia, dysphagia and all typical features of anaphylaxis.
All patients underwent serum specific IgE assay (ImmunoCAP®, Thermo-Fisher) for BLs (penicilloyl G, penicilloyl V, amoxicillin, ampicillin and cefaclor). In case of specific IgE positivity (cut off > 0.10 kUA/L), patients had their diagnostic process interrupted and they were challenged for alternative drugs instead.
Negative patients underwent skin prick test (SPT) and intradermal test (IDT).
Written informed consent was obtained from the patients to perform *in vivo* cutaneous tests and oral provocation tests.
Patients underwent SPT/IDT followed by OPT (5), using the following validated reagents provided by DIATER Laboratories (DAP; Madrid, Spain): benzylpenicilloyl-polylysine (PPL), minor determinant mixture (MDM), amoxicillin (20 mg/mL) and CLA (20 mg/mL). The maximum concentrations used were as follows: PPL 5 × 10-5 M, MDM 2 × 10-2 M, AX 20 mg/mL, and CLA 20 mg/mL.
The procedure was stopped when SPT or IDT at 15 minutes reading was positive. Patients were monitored for 2 hours after the last IDT.
Skin Tests (ST) were also evaluated at 48 h and 7 days to document delayed reactions. When negative, oral provocation test (OPT) was performed, according to a standardized BLs protocol (5). Patients with a positive clavulanate IDT, underwent OPT with amoxicillin 1,000 mg in a 3-days administration.
Data were tabulated using Excel 2020. Data are presented as frequencies of occurrence.
Comparisons between groups were performed with Fisher's exact test for categorical variables. All tests of hypotheses were considered significant when two-sided probability values were $p < 0.05$.

Results

We examined a total of 59 adults (41 females, 18 males) reporting a hypersensitivity reaction (HR) temporally associated with AX-CLA. Patients' age ranged from 21 to 92 years (mean age: 54.22 years). 41 subjects (69%) reported cutaneous symptoms (13 immediate, 28 delayed) and 18 (31%) systemic symptoms (18 immediate, none delayed) (**table I**).

Mucocutaneous reactions

13/41 patients (32%) presented an immediate reaction, 28/41 (68%) presented a delayed reaction.
7 patients suffering from mucocutaneous reactions (17%) had a positive immunoCAP for BLs; 6 of them did not continue in ST procedure because of the high IgE levels and the possible risk of reaction. Only one patient with very low IgE levels continued in ST procedure (penicillin G and V specific IgE levels 0.29 and 0.60 kUA/L respectively, for a total IgE level 6428 kUA/L).
ST resulted as follow: 1) 12 patients showed IDT positivity to clavulanate (8 with an immediate reaction, 4 with a delayed positivity after 72 h); 2) 1 patient showed an immediate IDT positivity to PPL (ID 1:100), 2 showed a delayed IDT positivity to undiluted amoxicillin (20 mg/mL); 3) 20 patients were negative to skin tests and 6 patients did not perform ST because they all displayed an immunoCAP positivity to BLs.

Table I *- Types of reactions to AX-CLA and related sensitizations.*

Reaction	Symptoms	Positivity for PPL, MDM or AX (IgE assay and/or IDT)	IDT positive for CLA at immediate reading	IDT positive for CLA at delayed reading	Negative IgE assays for BBL and negative STs	Total
Immediate	Muco-cutaneous	6	3	0	4	13
	Systemic	13	1	1	3	18
Delayed	Muco-cutaneous	3	5	4	16	28
	Systemic	0	0	0	0	0
Total		**22**	**9**	**5**	**23**	**59**

Amongst patients experiencing a mucocutaneous delayed reaction (n = 28), 5 patients showed an immediate IDT positivity to clavulanate at 20 mg/mL, 4 showed a delayed IDT positivity for clavulanate at 20 mg/mL after 72 h, 1 showed a delayed IDT positivity to amoxicillin 20 mg/mL after 72 h, 16 showed negative ST, and 2 were not submitted to STs.

Systemic reactions

All patients (n = 18) presenting systemic symptoms had immediate reactions. 11 patients (61%) with systemic reactions displayed a positive immunoCAP for BLs. Only 2 patients with low specific IgE levels to BLs underwent ST because the reaction was reported in infancy.

ST performed in 9 patients resulted as follow: 1) 2 showed IDT positivity to clavulanate, of which 1 showed an immediate IDT positivity while the other one a delayed positivity after 72 h; 2) 4 showed an immediate IDT positivity to BLs (3 presented an IDT positivity to amoxicillin 20 mg/mL, 1 presented an IDT positivity to PPL and MDM 1:1), no one showed a delayed IDT positivity to BLs; 3) 3 had negative ST.

No patient resulted positive to both PPL/MDM or amoxicillin and clavulanate.

All the patients that showed an isolated clavulanate IDT positivity, both immediate and delayed, tolerated amoxicillin oral provocation challenge (**table II**).

No adverse events were recorded while performing ST and OPT.

Overall, patients who experienced mucocutaneous reactions after taking AX-CLA are more sensitized to clavulanate, while those who experienced systemic reactions are mainly sensitized to amoxicillin or BLs determinants. The result is statistically significant both considering the ST positivity alone ($p = 0.04$) or ST positivity and/or BLs ImmunoCAP positivity ($p < 0.00$).

No difference was found in the demographic data between patients with and without positive ST reaction to clavulanate.

There was no statistically significant difference in immediate *vs* delayed reaction regarding clavulanate or BLs test positivity.

Discussion and conclusions

Up to now, few studies focused on the different clinical characteristics of hypersensitivity reactions after AX-CLA administration. Our study suggests that patients who presented only muco-cutaneous reactions were more often sensitized to CLA rather than AX: AX-CLA hypersensitivity reactions probably differ according to the immunologic response either to clavulanate or to amoxicillin and BLs determinants. These data are in contrast with a recent Spanish study, reporting a high frequency (nearby 30%) of immediate systemic reactions in subjects sensitized to CLA (17). Since clavulanate seems to be a major culprit, in case of both immediate or delayed mucocutaneous reactions, our study suggests to test both CLA and BLs, in order not to exclude a BL as possible therapeutic strategy.

Our study does not demonstrate a statistical higher frequency of delayed rather than immediate reactions to AX-CLA in patients with delayed positive skin tests to clavulanate, probably due to a limited study population. Looking at the trend of this study anyway, a further implementation in diagnostic test to CLA will reach statistical significancy.

This study has some limitations: firstly, the little number of patients, partially due to COVID pandemic period; secondly, the lack of validated tests quantifying specific IgE to CLA. At the moment the only *in vitro* test to diagnose a CLA immunologic reaction is Basophil Activation Test (BAT), but this technology is limited to few laboratories, and not well standardized (21).

Table II *- Clinical characterics of patients with CLA skin test positivity.*

Sex	Age (years)	Timing of reaction	Reaction	Skin test	OPT
Male	30	30 minutes after AX-CLA (first dose)	Generalized urticaria	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	70	6th day of AX-CLA therapy	Generalized eritematous rash, pruritus	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	23	7th day of AX-CLA therapy	Maculo-papular rash	Delayed IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	20	6th day of AX-CLA therapy	Generalized urticaria	Delayed IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	23	24 hours after AX-CLA (first dose)	Maculo-papular rash, pruritus	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	56	8th day of AX-CLA therapy	Generalized urticaria	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	45	30 minutes after AX-CLA (first dose)	Urticaria, dyspnea, larynx edema	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Male	35	24 hours after AX-CLA (first dose)	Urticaria, dyspnea, nausea and vomiting	Delayed IDT positivity to CLA both 5 mg/mL and 20 mg/mL	Amoxicillin tolerated
Male	37	24 hours after AX-CLA (first dose)	Eritematous rash	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	34	4th day of AX-CLA therapy	Generalized urticaria	Delayed IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	62	5th day of AX-CLA therapy	Facial angioedema	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Male	63	2th day of AX-CLA therapy	Maculo-papular rash, pruritus	Delayed IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	82	60 minutes after AX-CLA (first dose)	Generalized urticaria	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated
Female	59	60 minutes after AX-CLA (first dose)	Generalized pruritus, edema of extremities	Immediate IDT positivity to CLA 20 mg/mL	Amoxicillin tolerated

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Contributions

FR, VP: conceptualization. CC, AS: writing – original draft, formal analysis. AF, VL: methodology. All authors: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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LETTER TO THE EDITOR
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sIgE/sIgG4 profile in platinum desensitization: is there immunological tolerance?

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Key words

Desensitization; hypersensitivity reaction; immunological tolerance; platinum drugs.

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To the Editor,

hypersensitivity reactions (HSR) to platinum drugs have significantly increased worldwide (1, 2). Drug desensitization (DD) is safe and effective, enabling a temporary state of tolerance to the implicated drug (3). It remains to be understood if, similarly to allergen immunotherapy (4, 5), successive DD could be accompanied by an immunological shift, allowing progressive simplification of DD protocols in the long-term. We aimed to assess for the first-time platinum specific IgE/IgG4 (sIgE/sIgG4) profile along multiple and consecutive DD.

Prospective cohort study including oncologic patients > 18 years-old with HSR to platinum drugs initiating DD in our Allergy & Clinical Immunology Unit from January 2021 to June 2022. HSR diagnosis was considered in the presence of suggestive HSR symptoms and, when possible, confirmed with skin test (ST) with the culprit drug 2-4 weeks after the reaction. Patients were enrolled in the DD program when there were no therapeutic alternatives. ST with platinum drugs and the 12-step DD protocol were performed in our Unit (6). Control group included oncologic patients treated at the Oncology Unit receiving at least 7 infusions of platinum drugs with tolerance. The study was approved by the hospital's ethics committee (532/19) and all patients signed an informed consent.

Demographic and clinical data were obtained in the first interview and registered anonymously. Brown's grading system (7) was used to classify HSR's severity.

A blood sample (~5 ml) was collected before platinum infusion, in the first DD and then every two DD for the patients included, and before the 8th and 12th treatments for controls. Samples

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were analyzed in the Research Institute for Medicines. sIgE and sIgG4 were determined for platinum drugs in all patients using a Bovine Serum Albumin (BSA) standard binding method. The platinum salts were conjugated to human serum albumin by mixing an excess of the drugs in phosphate buffer at pH 7.4 and then by incubating for 24 hours followed by a second conjugation procedure using the same conditions. After conjugation, the excess drug was separated by dialysis, and the drug conjugates were immobilized by PureProteome Albumin Magnetic Beads. The PureProteome Albumin Magnetic Beads are conjugated to an antibody specific for human serum albumin. These magnetic beads provide a rapid, scalable, and reproducible means to bind > 98% of albumin from serum and plasma samples, facilitating the detection and analysis of proteins of interest. A cut-off of 0.10 kUA/L was used for negative *in vitro* testing.

A total of 7 patients fulfilled criteria to enroll the study, although 3 were excluded due to discontinuation of platinum therapy. Control group was represented by 3 patients. Of the 4 patients with HSR, 3 were women, median age 68.5 years [45-78 years]. Two patients had positive intradermal ST in the concentrations of 0.5 mg/ml and 0.05mg/ml, both with HSR grade II, the other 2 did not undergo skin testing due to urgent need of platinum desensitization (**table I**). There were no breakthrough HSR during desensitizations.

Regarding sIgE/sIgG4 profile (**figure 1** and **table IS**), a progressive reduction in sIgE was observed for all patients, with an initial median of 2.86 kU/L [1.31-3.29 kU/L] decreasing to 0.12 kU/L [0.11-0.18 kU/L] in the last DD (**figure 1A**). In parallel, an increase in sIgG4 was found, with an initial median of 0.38 logAU/mL [0.18-0.81 logAU/mL] rising to 2.43 logAU/mL [1.85-3.14 logAU/mL] at the last DD (**figure 1B**).

The present study reports for the first time a trend in favor of an immunological shift along multiple and consecutive platinum DD, resembling the tolerance mechanisms induced by allergen immunotherapy.

Data on long-term tolerance in subsequent desensitization protocols is sparce. Tüzer *et al.* (8) noticed a decrease in the frequency and severity of reactions with repeated DD protocols, demonstrating a possible role of IL-10 in the temporary tolerance induced by DD (8), with an increase during DD procedures and a decrease between treatments.

It remains unknown if the cytokine profile along multiple DD, similarly to the sIgE/sIgG4 profile hereby demonstrated, favors a long-lasting immunological tolerance. In fact, sIgE could be of interest, not only for the diagnosis, as previously demonstrated by our group (9), but also for risk stratification. As for sIgG4 antibodies, their precise role is controversial. They are still considered to demonstrate a response to immunotherapy, although levels of allergen-specific IgG do not predict or correlate with a clinical response to immunotherapy (5).

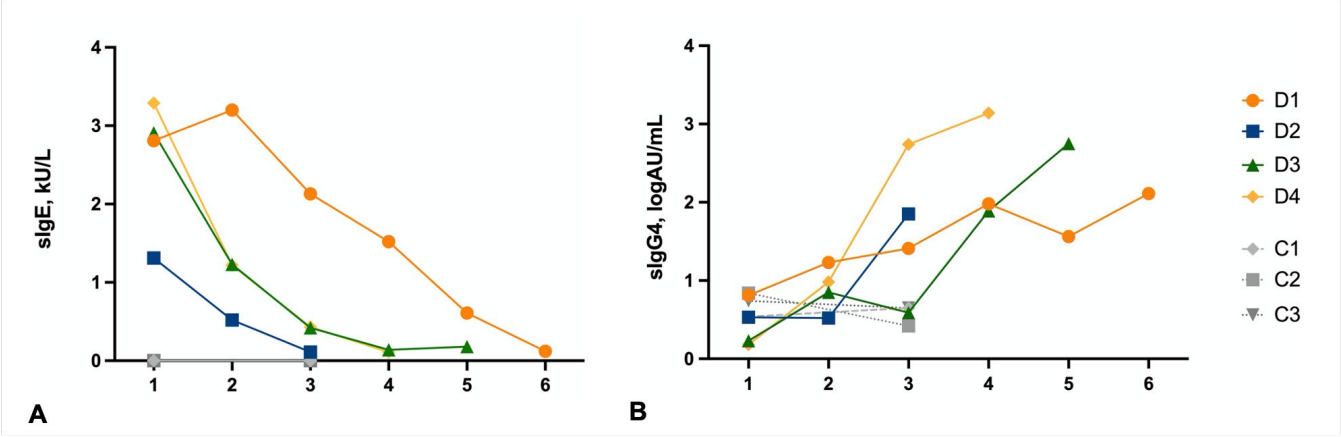
Despite the study's main limitations, namely the short sample and the absence of breakthrough HSR during DD not allowing to document different immunological DD profiles, this first report has an important adding value in fulfilling the gap knowledge on the immunological profile along DD treatments.

Table I *- Characterization of the study population.*

Patient	Gender	Age	Atopy	Drug allergy	Neoplasia	Drug	Previous infusions	Hypersensitivity reaction	Grade of severity	Total IgE (kU/L)	Skin tests
1	F	45	Yes	No	Colorectal	Oxaliplatin	15	Flushing, generalized pruritus, cough, dyspnea, abdominal pain, sudoresis, hypotension	III	296	-
2	F	61	No	No	Ovarian	Carboplatin	9	Flushing, palmoplantar pruritus	I	57.8	-
3	F	76	No	No	Colorectal	Oxaliplatin	10	Generalized pruritus, chest tightness, general malaise	II	40.9	Positive (ID 0.5 mg/mL)
4	M	78	No	No	Colorectal	Oxaliplatin	14	Nausea, general malaise, heat feeling, paresthesia and palmar pruritus.	II	72.6	Positive (ID 0.05 mg/mL)

F: Female; M: Male; ID: Intradermal skin tests.

Figure 1 *- Specific IgE (**A**) and IgG4 (**B**) along consecutive desensitizations of the study population.*



C: control; D: patient; sIgG4: Specific IgG4; sIgE: Specific IgE.

Fundings

None.

Contributions

RB, JC: study design. RB, JC: data collection. JG, CA, AGS: data analysis. RB: writing – original draft. JG, LC, EP, JC: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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LETTER TO THE EDITOR

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Monica Colque-Bayona[1], Javier Dominguez-Ortega[1,2], Margarita Tomás-Pérez[1,2]

Cat and dog specific immunotherapy impact on quality of life and self-reported satisfaction in a real-world setting

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Key words

Specific immunotherapy; quality of life; cat; dog; allergy.

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To the Editor,

cat and dog respiratory allergy represents a growing health problem and its negative repercussions in quality of life (QoL) is an important aspect to be considered (1). In addition to avoidance measures and symptomatic medication, allergen-specific immunotherapy (AIT) is an accepted treatment option and is the only that targets the underlying pathophysiology and thus shows disease modifying effects (2). Previous studies of cat and/or dog AIT focused on safety (3-5) and efficacy (4-8), but the evidence of its impact on QoL after cat or dog AIT is limited (5, 9).

In this prospective, single arm, single center, longitudinal pilot study, we assessed the impact on QoL of cat and dog AIT in real-world conditions. Patients included were allergic to cat and/or dog and had indication of AIT according to standard clinical practice. Subcutaneous immunotherapy (SCIT) with native cat or dog extracts was prescribed. Immunotherapy doses and schedules were performed according to manufactures' recommendations. The following parameters were analyzed at baseline and after one year: clinical characteristics, medication, asthma exacerbations (AE), total Immunoglobulin E (tIgE), specific IgE (sIgE) to the whole cat and dog extract and to allergen components (Fel d1, Fel d2, Can f1, Can f2, Can f3 and Can f5) by CAP System (Thermo Fisher Scientific; Waltham, Massachusetts, USA), IgG4, spirometry, rhinitis classification according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines (10), Asthma Control Test (ACT) and, to assess patients QoL, the validated versions in Spanish for the Allergic Rhinitis Quality of Life Questionnaire (ESPRINT-15) (11) and the Asthma Quality of Life Questionnaire (AQLQ) (12) were used. In addition, patients assessed their nasal symptoms (congestion, rhinorrhea, sneezing) and ocular symptoms (itching, tearing, conjunctival hyperemia and

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eye swelling) using a numerical rating scale (NRS) from 0 to 10, with 0 points being no symptom, and 10 points being the most severe symptom. Finally, patients completed a visual analogue scale (VAS) of satisfaction with the AIT. Statistical analysis was performed using Wilcoxon and McNemar tests. The study was approved by the ethics committee of Hospital Universitario La Paz (HULP-PI 3443) and the participants signed an informed consent document.

Thirteen patients were included (7 women and 6 men) with a mean age of 32 years old. Of these, 46.1% were allergic to cat only, 30.7% to dog only and 23% to both cat and dog. We summarized baseline characteristics in **table I**.

Table I *- Baseline characteristics.*

Variables	Patients (n = 13)	Missing data (%)
Sex (n, %)		0
Women	7 (53.8)	0
Men	6 (46.2)	0
Age (mean years ± SD)	32.31 ± 9.0	0
Exposure to cat or dog (n, %)		0
Permanent	7 (53.8)	0
Sporadic	6 (46.2)	0
Allergic comorbidities (n, %)		0
Urticaria	3 (23.1)	0
Atopic dermatitis	3 (23.1)	0
Food allergies	1 (7.7)	0
Drug allergies	0	0
Sensitization to aeroallergens (n, %)		
Cat	13 (100)	0
Dog	8 (61.5)	0
Grasses	9 (69.2)	0
Reed grass	6 (46.2)	0
Olive tree	5 (38.5)	0
Shade plantain	3 (23.1)	0
Mite	6 (46.2)	0
Alternaria	1 (7.7)	0
Clinical allergy (n, %)		0
Cat only	6 (46.1)	0
RC	2 (15.3)	0
RC and asthma	4 (30.7)	0
Dog only	4 (30.7)	0
RC	0	0
RC and asthma	4 (30.7)	0
Cat and dog allergy	3 (23.0)	0
RC only	1 (7.7)	0
RC and asthma	2 (15.3)	0

SD: standard deviation; RC: rhinoconjunctivitis.

After one year of AIT, the severity of rhinitis decreased significantly according to ARIA classification: 77% reported moderate/severe rhinitis at baseline and 100% had mild rhinitis after one year. Concordantly, there is a significant improvement in the NRS for ocular and nasal symptoms (p = 0.001 and p = 0.002, respectively). Furthermore, there is a statistically significant improvement in mean ACT score one year after AIT (p = 0.011) and 50% of patients had an increase greater than the minimal clinical important difference (MCID): ACT ≥ 3 (13).

Regarding to QoL, AIT contributed significantly to the improvement of rhinitis according to the ESPRINT-15 questionnaire (p = 0.003); as for QoL in asthma there were no significant differences (p = 0.139). Based on MCID for questionnaires responses, 69.2% of the patients had an increase > 0.9 (MCID) in ESPRINT-15 (11) and 70% had an increase of > 0.5 (MCID) in AQLQ (12) after one year of AIT. Otherwise, it was recorded that 88.3% patients had an increase of MCDI in both ESPRINT-15 and AQLQ.

As to medication use, patients were asked yes or no whether they needed medication in relation to direct contact with cat or dogs at baseline and after one year of AIT. There was a marked decrease in the use of antihistamines (from 92.3% to 61.5%) and in the inhaled short-acting ß2-agonists (SABAs) (from 46.2% to 15.4%). No changes were observed in the use of the treatment step for asthma, 3 or 4 according to the Global Initiative for Asthma Management and Prevention (GINA) guidelines (14), being treated with a combination of low doses of inhaled corticosteroids (ICS) and long-acting beta agonists (LABA), remaining on the same therapeutic step. Regarding AE, it was observed that 30% of patients had at least one AE the year before AIT, requeiring rescue medication with SABAs and oral corticosteroids, and after one year of AIT only 15.3% reported AE.

During the first year of AIT, a good overall adherence and tolerance was observed; only 30.8% of the patients had local adverse reactions that were controlled with antihistamines and no systemic reaction were recorded. Moreover, patient satisfaction with AIT was 7.8 in the VAS. No significant differences were observed in other parameters such as IgE, sIgE, IgG4 and spirometry values. Results of the outcomes analyzed after one year of AIT are shown in **table II**.

Based on our results, patients receiving AIT improve their RC symptoms, and therefore, a significant improvement on QoL according to ESPRINT-15 was demonstrated. For asthma, AIT was effective since we found a decrease in use of SABAs that corresponded with significant increase in ACT score. However, although a 70% of patients improved in QoL, no significant changes were found in AQLQ, probably due to the small size of the included cohort. Previous studies in the literature described similar positive findings in terms of quality of life after cat or dog AIT (5, 9). Inside the allergological study, unfortunately, we could not asessed the SPT after one-year, althougth in previous studies (8, 9, 15) AIT was succeeded in reducing the surface of the wheal, demonstrating the efficacy of AIT.

Although this is a pilot study and the interpretation of the results should considerate its limitations as the small sample, the absence of a defined control group, the lack of randomization, the different extracts used and the possible variability in exposure to allergens over the study, we were able to demonstrate the positive impact of dog and cat AIT on the RC QoL, a safety profile in a real-world setting along with a high satisfaction rate. Even so, we believe that further studies with larger samples should be conducted to reinforce our findings.

Table II *- Result of the outcomes analyzed after one year of AIT.*

Variable	Baseline	After one year	P-value	Missing data
Rhinitis severity (ARIA) (n, %)				
Mild	3 (22.9)	13 (100)	NA	0
Intermittent	2 (15.3)	11 (84.6)	NA	0
Persistent	1 (7.6)	2 (15.3)	NA	0
Moderate/severe	8 (77)	0	NA	0
Intermittent	4 (30.8)	0	NA	0
Persistent	6 (46.2)	0	NA	0
ACT (mean ± SD)	17.1 ± 5.1	22.1 ± 2.02	0.011	0
NRS (mean ± SD)				
Ocular	19.5 ± 10.2	4.8 ± 5.7	0.001	0
Nasal	20.8 ± 8.4	9.5 ± 7.6	0.002	0

→

Variable	Baseline	After one year	P-value	Missing data
Allergologic study (mean ± SD)				
Skin Prick Test (mm²)				
Cat	7.5 ± 2.5	NA	NA	0
Dog	4.7 ± 3.2	NA	NA	0
Total IgE (kU/L)	547.7 ± 562.9	697.3 ± 739.5	0.13	0
Total Cat IgE (kU/L)	56.1 ± 43.8	55.1 ± 40.2	0.91	0
Fel d 1 (kU/L)	51.9 ± 37.7	45.9 ± 37.50	0.88	0
Fel d 2 (kU/L)	2.6 ± 4.3	2.3 ± 3.9	1.0	2 (15.38)
Total Dog IgE (kU/L)	75.5 ± 104.3	99.6 ± 150.5	0.46	0
Can f 1 (kU/L)	20.7 ± 39.1	29.7 ± 47.1	0.28	0
Can f 2 (kU/L)	19.0 ± 9.5	21.0 ± 33.7	0.14	0
Can f 3 (kU/L)	11.9 ± 8.4	12.1 ±18.9	0.65	1 (7.69)
Can f 5 (kU/L)	13.4 ± 26.6	4.6 ±2.9	0.68	0
IgG4 (mg/dL)	66.7 ± 25.1	71.0 ± 31.0	0.81	1 (7.69)
Spirometry				
FEV_1 (L)	3.6 ± 1.0	2.6 ± 0.6	0.71	3 (23.0)
FVC (L)	4.5 ± 0.9	3.6 ± 0.7	0.71	3 (23.0)
FEV_1/FVC (%)	88.6 ± 9.1	89.2 ± 10.2	0.68	3 (23.0)
Treatment characteristics				
Antihistamines	12 (92.3)	8 (61.5)	0.125	0
Nasal corticosteroids	1 (7.7)	1 (7.7)	-	0
Oral corticosteroids	0	0	-	0
Inhaled corticosteroids (ICS)	0	0	-	0
SABA	6 (46.2)	2 (15.4)	0.125	0
ICS with LABA	6 (46.2)	5 (38.4)	1.0	0
QoL questionnaires				
ESPRINT-15 (mean ± SD)	2.56 ± 1.56	1.07 ± 0.88	0.003	0
AQLQ (mean ± SD)	5.16 ± 1.7	6.06 ± 0.98	0.139	0

SD: standard deviation; NA: non-analyzed; RC: rhinoconjunctivitis; ARIA: Allergic Rhinitis and its Impact on Asthma; ACT: Asthma Control Test: NRS: numerical rating scale: IgE: Immunoglobulin E; IgG4: Immunoglobulin G4;FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; ICS: inhaled corticosteroids; SABA: short-acting ß2-agonists;LABA: long-acting beta agonists; QoL: quality of life; ESPRINT: Allergic Rhinitis Quality of Life Questionnaire; AQLQ: Asthma Quality of Life Questionnaire.

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Contributions

MCB: conceptualization, data collection, interpretation, writing - original draft, writing - review & editing. JDO: clinical management, data interpretation, writing - original draft, writing review & editing. MTP: clinical management, data interpretation, writing - original draft, writing - review & editing.

Conflict of interests

JDO: has received speaker's honoraria from ALK and Letipharma. Other authors declare no conflict of interests.

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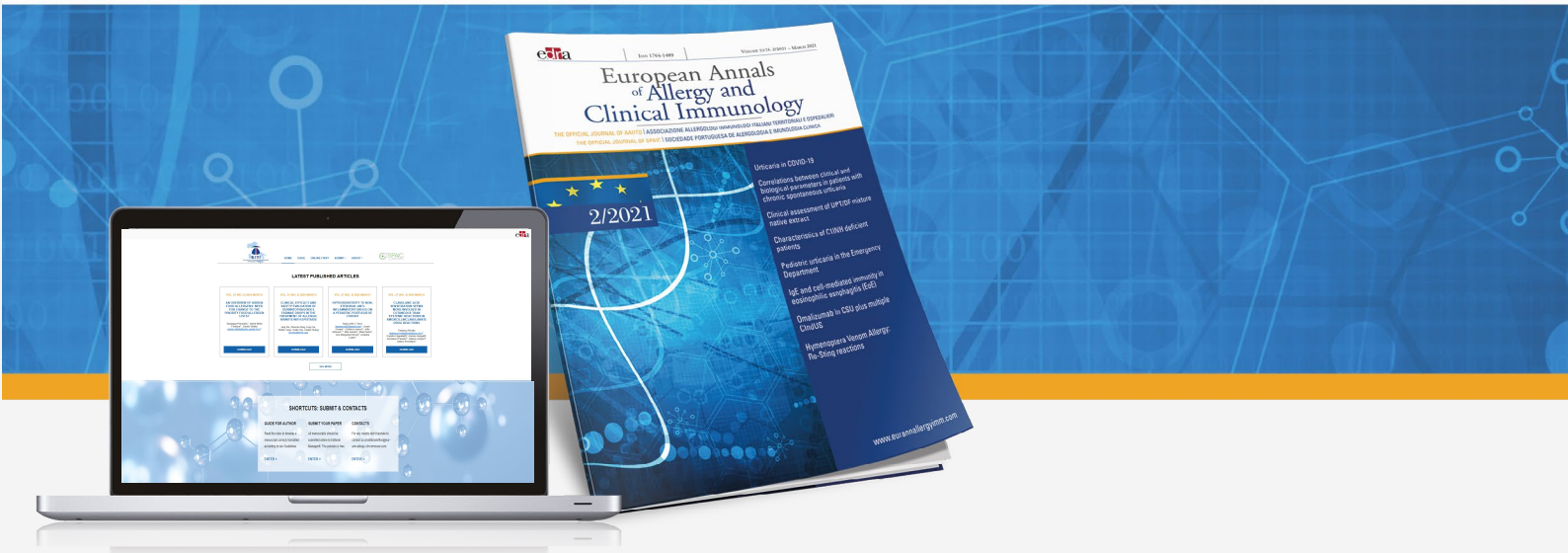
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