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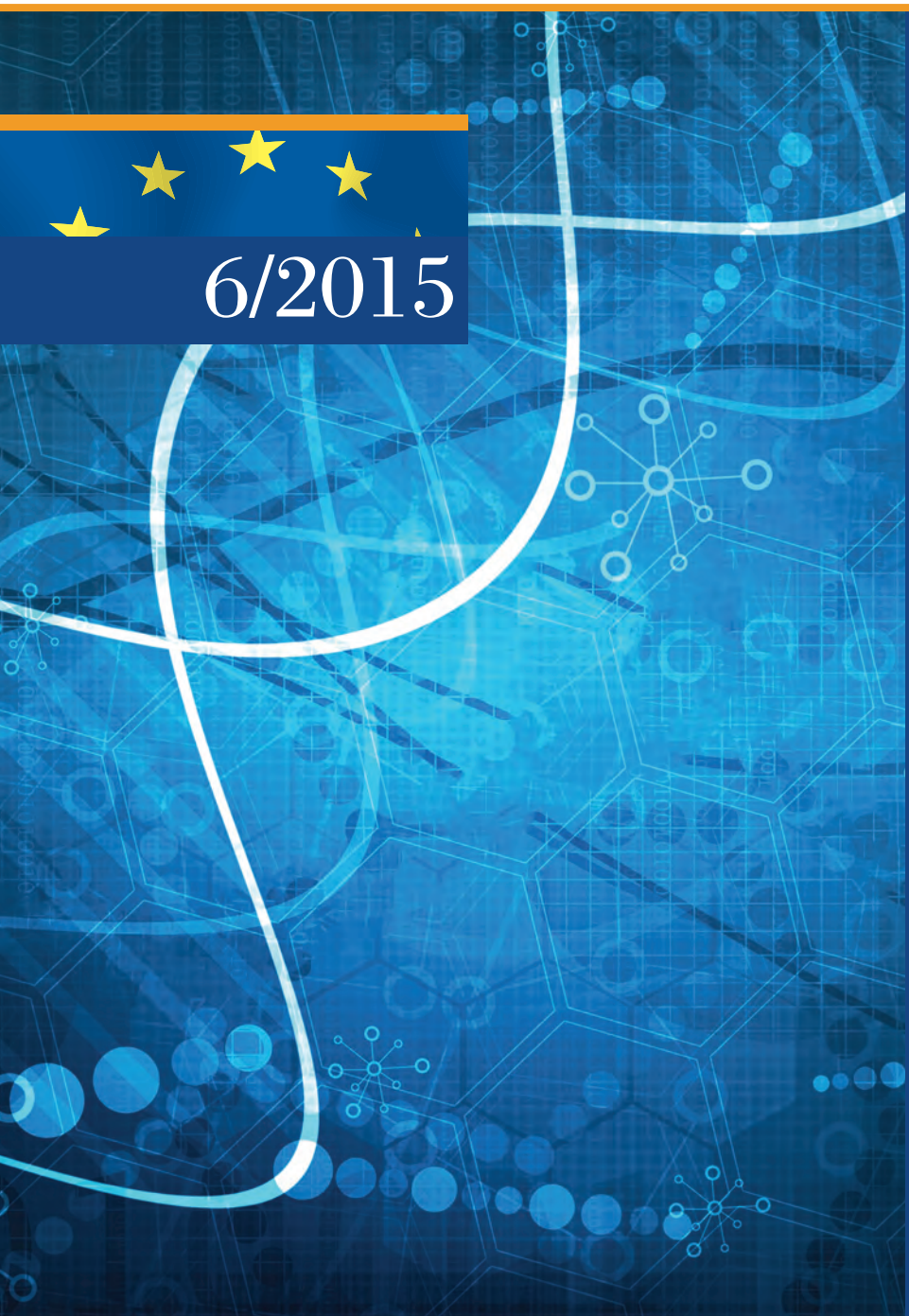

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Editors in Chief
Riccardo Asero
Mário Morais-Almeida

Publishing Director
Nicola Miglino

Publishing Editor
Chiara Scelsi
c.scelsi@lswr.it
Tel. 02 88184.257

Production Manager
Walter Castiglione
w.castiglione@lswr.it
Tel. 02 88184.222

Sales & Marketing
Ludovico Baldessin
l.baldessin@lswr.it
Tel. 02 88184.354

Traffic
Donatella Tardini
d.tardini@lswr.it
Tel. 02 88184.292

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A.K.G. Hassan[1], Y.P. Venkatesh [1]

An overview of fruit allergy and the causative allergens

[1]Department of Biochemistry and Nutrition, CSIR - Central Food Technological Research Institute (CSIR-CFTRI), Mysore 570020, Karnataka, India

Key words

Allergens; cross-reactivity; fruit allergy; oral allergy syndrome; tropical fruits.

Corresponding author
Yeldur P. Venkatesh
Department of Biochemistry & Nutrition
CSIR-CFTRI, KRS Road, Mysore 570020
Karnataka, India
Phone: +91 821 251 4876
Fax: +91 821 251 7233
E-mail: venkatyp@yahoo.com

Summary

Plant allergens, being one of the most widespread allergenic substances, are hard to avoid. Hence, their identification and characterization are of prime importance for the diagnosis and treatment of food allergy. The reported allergies to fruits mainly evoke oral allergy syndrome caused by the presence of cross-reactive IgE to certain pollens and thus, allergy to fruits has also been linked to particular pollens. Many fruit allergies are being studied for their causative allergens, and are being characterized. Some tropical or exotic fruits are responsible for region-specific allergies for which only limited information is available, and generally lack allergen characterization. From a survey of the literature on fruit allergy, it is clear that some common fruits (apple, peach, musk melon, kiwi fruit, cherry, grape, strawberry, banana, custard apple, mango and pomegranate) and their allergens appear to be at the center of current research on food allergy. The present review focuses on common fruits reported as allergenic and their identified allergens; a brief description of allergens from six rare/tropical fruits is also covered.

Abbreviations: LTP, lipid transfer protein; NRL, natural rubber latex; nsLTP, non-specific LTP; OAS, oral allergy syndrome; PR-, pathogenesis-related; STP, skin prick test; TLP, thaumatin-like protein.

Introduction

Food allergy constitutes adverse immune response against food proteins that generally are harmless. From objectively confirmed results, ~5-8% children and 2-3% adults suffer from food allergy. Allergy to vegetables have been described for celery, asparagus, avocado, bell pepper, cabbage, carrot, fennel, lettuce, potato, pumpkin, turnip and zucchini (1-3). An enquiry into the fruits causing allergy leads one to a listing of 12-15 fruits to be commonly associated. Most of these are available worldwide in vegetable and fruit markets; however, a few rare fruits, especially tropical fruits and berries also can be observed to cause allergy in susceptible individuals.

The reported fruit allergic reactions are frequently observed to be associated with oral allergy syndrome (OAS) conjoined with pollen-fruit-vegetable syndrome, triggered upon consumption of raw vegetables or fresh fruits. This is most commonly attributed to cross-reacting, homologous proteins found in plant foods and pollens. Since conserved proteins and distinct epitopes of proteins are found throughout the plant kingdom, expression of homologous proteins in plant foods is not surprising (4-6). Regional variations have been observed in OAS. In a study of 274 adults in England who were allergic to at least one pollen (birch, grass, and/or mugwort), 34% were sensitive to apple, 25% to potato, 23% each to carrot and celery, 22% to peach, and 16% to melon (7). In contrast, OAS is most commonly due to hazelnut, kiwi and apple in Denmark (8). Pollen-allergic adults in Sweden most often reported symptoms with hazelnut, apple, tomato, carrot, and peanut (9). In Spain, peach is the most common fruit which causes allergy (10).

Pollen-food syndromes have been observed to be associated with specific plants. One of them is birch-fruit-vegetable syndrome. Foods belonging to the family Rosaceae, which include apple, pear, peach and almond, most commonly cause symptoms in birch-allergic patients. Another is celery-birch-mugwort-spice syndrome; celery has been found to have cross-reactivity with both birch and mugwort pollens. In areas where birch trees are prevalent, celery allergy is due to Bet v 1 homologs. However, celery allergy does exist in birch-free areas; in these cases, mugwort pollen allergens may be the primary sensitizer (4). Bet v 1 and profilins have also been identified in various spices (11), including anise (Pim a 1 and 2), coriander (Cor s 1 and 2), cumin (Cum c 1 and 2), fennel (Foe v 1 and 2), and parsley (Pet c 1 and 2). Cross-reactivity between mugwort and mustard has also been demonstrated, and accordingly, celery-birch-mugwort-spice syndrome has been used to describe these cross-reactivities (12). Celery root (which is mainly consumed in Switzerland) has been associated with systemic symptoms in the "mugwort-celery-spice syndrome" (13), whereas celery stick is more often associated with OAS in birch pollen-allergic subjects (14).

Melon-induced OAS in ragweed-allergic subjects has been observed to be associated with profilin sensitization (15). Such cross-reaction syndromes have also been observed for mugwort-peach association, plantain-melon association, pellitory-pistachio association, goosefoot-fruit association, and Russian thistle-saffron association (16).

Latex-fruit syndrome was first reported by M'Raihi et al. (17), wherein an allergic reaction to banana was observed in a latex-allergic patient. Soon thereafter, cross-reactivity between latex and various fruits was demonstrated, and generally, this is termed latex-fruit syndrome (18). Some studies have reported that up to 88% of latex-allergic adults have evidence of specific IgE to plant-derived foods (19,20). Several homologous proteins are found to be present in allergenic plant foods as well as in latex which include Hev b 2 (β-1,3-glucanase), Hev b 11 (class I chitinase) and Hev b 8 (profilin) (4,21,22). Hev b 6 (prohevein) is the latex allergen that has received most attention as a possible cause of the latex-fruit allergy syndrome.

Different fruit processing conditions may induce alteration of immune-reactive epitopes on allergenic proteins. Processing was shown to destroy existing epitopes on a protein and generate new ones (formation of neoallergens) as a result of conformational changes (23). Upon surveying the fruit allergy reports, 10 to 12 common fruits and their allergens can be observed to be at the center of the current allergy research on fruits, which include apple, peach, kiwi, musk melon, grape, cherry, strawberry, banana, mango and pomegranate (listed in **table 1**). The present review focuses on such fruits reported as allergenic and the allergens identified from them; in addition, a brief account of some important rare fruits causing allergy is also covered.

Table 1 *- Common fruits and their identified allergens.*

Fruit	**Allergens [kDa - allergen name (nomenclature)]**
Apple (*Malus domestica*)	23.0 - TLP (Mal d 2) 17.5 - Bet v 1 homolog (Mal d 1) 14.0 - Profilin (Mal d 4) 9.0 - LTP (Mal d 3)
Peach (*Prunus persica*)	23.0 - TLP (Pru p 2) 17.5 - Bet v 1 homolog (Pru p 1) 14.0 - Profilin (Pru p 4) 9.0 - LTP (Pru p 3)
Musk melon (*Cucumis melo*)	67.0 - serine protease (Cuc m 1.01) 54.0 - serine protease (Cuc m 1.02) 36.0 - serine protease (Cuc m 1.03) 16.0 - PR-1 protein (Cuc m 3) 14.0 - Profilin (Cuc m 2)
Gold kiwi, green kiwi (*Actinidia chinensis*, *A. deliciosa*)	43.0 - Chitinase (Act d 3) 30.0 - Actinidin (Act d 1) 28.0 - Kiwellin (Act d 5) 23.0 - TLP (Act d 2) 11.0 - Cystatin (Act d 4)
Sweet cherry (*Prunus avium*)	23.0 - TLP (Pru av 2) 17.5 - Bet v 1 homolog (Pru av 1) 14.0 - Profilin (Pru av 4) 9.0 - LTP (Pru av 3)
Grape (*Vitis vinifera*)	30.0 - Chitinase, hevein-like (Vit v 5) 23.0 - TLP (Vit v TLP) 17.5 - Bet v 1 homolog (Vit v 8) 14.0 - Profilin (Vit v 4) 9.0 - LTP (Vit v 1)
Strawberry (*Fragaria ananassa*)	35.0 - Bet v 6 homolog (isoflavone reductase) 17.5 - Bet v 1 homolog (Fra a 1) 14.0 - Profilin (Fra a 4) 9.0 - LTP (Fra a 3)
Banana (*Musa acuminata*)	33.0 - β-1,3-glucanase (Mus a 5) 31.0 - Class I chitinase (Mus a 2) 21.0 - TLP (Mus a 4)
Custard apple (*Annona cherimola*)	45.0 - Class I chitinase (Ann c Chitinase)
Mango (*Anacardium occidentale*)	14.0 - Bet v 1-like (Man i 14kD) 30-45 Class I chitinase (Man i Chitinase)
Pomegranate (*Punica granatum*)	28.0 - PR-4 protein (Barwin family) 17.0 - PR-4 protein 16.0 - PR-4 protein 9.0 - LTP (Pun g 1)

Common fruits causing allergy and their allergens

Apple (Malus domestica**):** Allergy to apple, a fruit of the Rosaceae family, is usually presented with mild oropharyngeal symptoms. Mal d 1, homologous to the major birch pollen allergen Bet v 1 was the first apple allergen to be characterized (24). Later, other allergens were identified which include the thaumatin-like protein (TLP; Mal d 2), the non-specific lipid transfer protein (nsLTP; Mal d 3) and the profilin (Mal d 4).

In Northern and Central Europe, the occurrence of allergy to apple is frequently related to birch pollinosis, and sensitization is due to cross-reactivity between Bet v 1 and Mal d 1, whereas in Southern Europe this fruit allergy is observed together with allergy to peach caused by the allergens Pru p 3 and Mal d 3. Symptoms related to Mal d 1 are generally mild and local, representative of a chemically-labile protein. Mal d 3, on the other hand, is a highly stable protein due to the presence of four disulfide bonds. Mal d 3 and its homologs in other fruits and vegetables have been repeatedly detected as the main elicitors for true food allergy. Systemic manifestations mainly occur in the Mediterranean area and are observed to be based on cross-reactivity between apple LTP and peach LTP, with the latter considered as the primary sensitizer. LTP allergenicity is not reduced by high-temperature processing (25,26). Little is known about the way of sensitization to Mal d 2 (TLP). However, due to the presence of 8 conserved disulfide bridges, TLPs are expected to be resistant to pH or heat. Mal d 4 is a minor allergen and seems to be pollinosis-related; Bet v 2, the birch pollen profilin, sensitizes approximately 20% of the pollen-allergic patients. Profilins seem to be highly cross-reactive allergens with other fruits and vegetables of the Rosaceae, Vitaceae and Solanaceae families as well as with several pollens.

Peach (Prunus persica**):** The two fruits from Rosaceae most frequently involved in allergy cases are apple and peach. Different clinical phenotypes of peach allergy are observed across Europe in relation to different allergen sensitization patterns to the peach allergens. In a series of studies on Rosaceae fruit allergy in the Mediterranean area, peach has been shown as the first triggering food to subsequently associate with other Rosaceae fruits such as apple, due to cross-reactivity of their LTPs (27). In areas rich in birch trees of Central and Northern Europe, peach allergy is linked to birch pollinosis and apple allergy. These patients present mild oropharyngeal symptoms upon peach ingestion. As in the case of apple, 4 homologous allergens have been identified in peach so far: Pru p 1 (a Bet v 1 homolog), Pru p 2 (a TLP), Pru p 3 (a profilin) and Pru p 4 (a LTP) (28, 29).

Musk melon (Cucumis melo**):** As a member of the Cucurbitaceae family which includes several warm season vegetables (squash, cucumber and pumpkin) and fruits (watermelon), musk melon has been reported as a frequent cause of fruit allergy, both in some areas from the U.S. and the European Union. Primary melon allergy is extremely rare, and most cases of melon allergy occur in pollen-allergic subjects. Profilin (Cuc m 2) has been identified as a major allergen from this fruit (30). Other allergens described from this fruit are cucumisin (Cuc m 1, a subtilisin-like protease), and Cuc m 3, a 16 kDa pathogenesis-related (PR) protein belonging to the PR-1 family (31); no plant allergen homologous to Cuc m 3 has been detected till date. The Cuc m 1 serine protease is present in the melon extract in several molecular forms which arise during the process of maturation and subsequently as degradation fragments and, similar to Hev b 6, they have been named Cuc m 1.01 (67 kDa), Cuc m 1.02 (54 kDa) and Cuc m 1.03 (36 kDa); cucumisin and its several N-terminal fragments are major allergens of melon. The ubiquitous distribution of this protein family (cucumisin-like proteases) in many plant species, its high structural similarity and the inhibition data suggest its potential role as a panallergen in plant foods (31).

Kiwi (Actinidia **spp.):** A popular fruit, very rich in vitamin C, is available in two varieties: one with green flesh and the other with yellow flesh. Allergy to green kiwifruit (*A. deliciosa*) was the first to be documented in the early 1980s, and has been reported increasingly in recent years. Moreover, a closely related species, gold kiwi fruit (*A. chinensis*) became available in the international market in 1999 and shares IgE cross-reactivity and the presence of common allergens with green kiwifruit (32). Although allergy to kiwifruit is commonly associated with mild and local symptoms (mainly OAS) and with hypersensitivity to pollens, severe anaphylactic reactions also occur frequently. Further, kiwi allergy also has to be considered in relationship with the latex-fruit syndrome, together with sensitization to avocado, chestnut and banana, which are the main plant foods linked to latex allergy (32).

Among the several putative kiwifruit allergens detected, only two of them have been studied sufficiently in different groups of kiwi-allergic patients till now. Act d 1 (originally Act c 1) corresponds to the 30 kDa thiol-protease actinidin, which is well established as a major kiwi allergen (33). Act d 2 is a 24 kDa TLP, whose sensitization prevalence is still controversial. Besides, N-terminal amino acid sequences of putative relevant allergens, namely an 11 kDa cystatin (Act d 4), a 28 kDa kiwellin (Act d 5), 43 and 45 kDa chitinases (Act d 3) have been reported. Class I chitinases with an N-terminal hevein-like domain and latex hevein have been identified as the major cross-reactive components involved in this latex-fruit syndrome (34). Identification of major allergens in kiwifruit has so far resulted in conflicting and confusing results both in terms of number and relevance of allergens. In fact, different studies reported different dominant allergens, probably due to differences in both experimental procedures and study population used (32).

Cherry (Prunus avium**):** Allergy to cherry fruit is often reported in the context of allergy to other fruits of the Rosaceae family

and pollinosis to trees because of cross-reactive allergens. Allergic reactions to cherry are reported by 19-29% of birch pollen-allergic patients (35). Pru av 2, identified as a TLP from sweet cherry, was recognized by the majority of cherry-allergic patients. Pollen-related cherry allergy is caused by the presence of cross-reactive IgE epitopes on homologous proteins. Four allergens from sweet cherry have been identified so far. Pru av 1 and Pru av 4 are homologous to the birch pollen allergens Bet v 1 and Bet v 2, respectively, and are in part responsible for the cross-reactivity between birch pollen and cherry. Pru av 3 is a nsLTP sharing high amino acid sequence identities with nsLTPs from other Rosaceae fruits. Pru av 2 was first identified in 1996 as the most abundant soluble protein (29 kDa) in ripe cherries accumulating during the ripening process. Later, TLP (23 kDa) from cherry was described as a potential major allergen and named as Pru a 2, which was revised as Pru av 2 (36).

Grape (Vitis vinifera**):** As one of the oldest cultivated plants all over the world, it grows in a temperate climate, especially around the Mediterranean, and its fruit, the grape, is consumed either directly or as processed products (juice, jam and wine). Western Europe is the world's biggest producer of grapes; France, Italy and Spain are the major producers of wine that is consumed throughout the world. Allergic reactions to wine are commonly believed to be caused mainly by sulfites (37). Giannoccaro et al. (38) reported a patient allergic to grape and cherry. Pastorello et al. (39) characterized the major allergens of grape as endochitinase 4A (~30 kDa) and a LTP that was homologous to and cross-reactive with peach LTP; however, a 24 kDa TLP was found to be a minor allergen. In another study, severe allergic reactions to grapes have been described as part of a LTP-associated clinical syndrome (40).

Endochitinase 4A is very likely the allergen in *vino novello* (young wine) and *vino Fragolino*. Researchers have observed several patients with severe allergic reactions after eating grapes and, in some of them, also after drinking two particular kinds of red wine, namely *vino Fragolino* and *vino novello.* Some technical differences in the process of making non-aged wine might explain why the patients were allergic only to *vino novello* or *vino Fragolino*. Polymerization of polyphenols causes the tiny residual proteinaceous material in red wines to coalesce, so that it can be filtered off once the wine has aged, thus theoretically explaining why the patients tolerated older wine. Grape chitinases account for 50% of the soluble proteins in grapes, persisting through the vinification process. Another protein persisting in wine throughout vinification is the 24 kDa TLP, which has been found as another important allergen in grapes. The identification of a 9 kDa LTP as a major grape allergen seems very interesting because it could explain why grape allergy is often associated with allergic reactions to fruits, such as peach and cherry (39).

Strawberry (Fragaria ananassa**):** Nicknamed an 'accessory' fruit due to its seeds on the outside, strawberry is not only eaten fresh, but also used as a common ingredient in many food products such as jam, yogurt, ice cream, and breakfast cereals; strawberry is an important ingredient in the food industry. The strawberry Fra a 1 allergen is a homolog of the major birch pollen allergen Bet v 1. Mass spectrometric analysis indicated the presence of strawberry homologs to the Bet v 1 allergen in both the 20 and the 18 kDa protein bands. They are synthesized by red ripe strawberry fruits while white strawberry fruits of a mutant genotype, which is known to be tolerated by individuals affected by strawberry allergy, are devoid of them (41-43). The presence of a strawberry homolog to the 35 kDa Bet v 6 allergen, an isoflavone reductase, was also suggested to be a strawberry allergen. A 9 kDa LTP (Fra a 3) with 74% homology to apple LTP (Mal d 3) has been detected, which could be a possible strawberry allergen (44).

Banana (Musa acuminata**):** Allergy to banana is relatively frequent; the relevance of banana as a source of food allergy was confirmed in two patients by double-blind food challenge. It has been observed that 20-50% of patients allergic to natural rubber latex (NRL) have experienced symptoms after eating banana. Even though evidence for cross-reacting allergens in NRL and banana has also been reported (45), most cases of banana allergy are associated with profilin sensitization; banana-latex association is, by far, less frequent. Three major allergens from banana have been identified (46-48): Mus a 2 (31 kDa class I chitinase), Mus a 4 (21 kDa TLP), and Mus a 5 (33 kDa β-1,3-glucanase).

Custard apple (Annona cherimola**):** Allergic cases reported for custard apple (also known as cherimoya) have, for most of the cases, been in cross-reaction to latex. Cross-reactivity with latex allergy was found for 40-45 kDa proteins; the 45 kDa protein was identified as chitinase. It has also been reported that the N-terminal hevein-like domain of the chitinase is responsible for cross-reactivity with latex (49). The first case of allergy to custard apple was reported in 1997 wherein a 20-25 kDa band was detected. A 14 kDa acyl carrier protein was also reported as an allergen but not confirmed. Several reports of allergy to custard apple have appeared in the literature (50-52). The 20-25 kDa protein identified as the allergen by IgE-immunoblotting is likely to be a TLP.

Mango (Anacardium occidentale**):** This delicious fruit belongs to the Anacardiaceae family (*Sumac* species), which also includes cashews and pistachios. Rubin and Shapiro (53) were the first to report an anaphylactic reaction following the ingestion of mango. Renner et al. (54) identified 2 major allergens with a molecular mass of 27 kDa in two patients, in addition to a 15 kDa allergen in one patient and a 32 kDa allergen in another. Mango profilin has been shown to cross-react with birch pollen profilin Bet v 2 (55).

Pomegranate (Punica granatum**):** This fruit is commonly consumed in raw and processed forms such as juice, wines, flavors, and extracts, but has rarely been reported to cause immediate hypersensitivity after ingestion. Allergy to pomegranate was first reported by Igea et al. (56) wherein an IgE-mediated allergy could not be demonstrated. An extremely rare case of anaphylaxis to mannitol present in pomegranate (0.25 g per 100 g edible portion) was described by Hegde et al. (57); the presence of mannitol-specific IgE was further demonstrated in the serum of the allergic subject (58). Cross-reactivity has been demonstrated for LTPs present in different fruits including pomegranate (59). By 2-D electrophoresis, Bolla et al. (60) have separated different nsLTP isoforms possessing different IgE-binding properties, which might reflect peculiar allergenic potencies; the contribution of Pru p 3 to prime sensitization is not central as in other plant nsLTPs. Currently, it appears that nsLTP is the major pomegranate allergen (60).

Important allergens causing fruit allergy

Birch pollen-associated allergy in relation to fruits is a well-known clinical phenomenon especially in northern Europe. Following a primary sensitization to birch pollen allergen, a subsequent IgE cross-reaction with homologous proteins in the consumed fruit occurs. Bet v 1, the major birch pollen allergen, shares common epitopes with major food allergens in a large number of different fruits and berries, e.g., cherry (Pru av 1), apple (Mal d 1), pear (Pyr c 1) and peach (Pru p 1). Patients suffering from type I hypersensitivity caused by birch pollen frequently demonstrate allergy to many fruits.

Plant nsLTPs are a widely distributed superfamily of related proteins (PR-14 defense proteins). They are divided into two subfamilies according to their molecular masses: the 9 kDa nsLTP1 and the 7 kDa nsLTP2; several nsLTPs with allergenic activity have been identified in fruits and pollens. The most frequently implicated foods belong to the Rosaceae fruits, but nsLTPs with allergenic activity have also been detected in tree nuts, peanut, lupine, maize, mustard, fennel, and several other fruits and vegetables.

The family of TLPs (PR-5 defense proteins) plays an important role in the plant's defense against pathogens. Several members of the TLP family have been identified as major allergens in Cupressaceae pollens such as Jun a 3, Cup a 3, and Cry j 3 as well as in plant foods such as cherry, apple, kiwi, banana, grape, sapodilla and bell pepper. Recombinant TLPs have been characterized as important allergens of bell pepper, several fruits (kiwi, apple, cherry and grape) as well as of cypress, mountain cedar and Japanese cedar pollens. Despite the vast experimental data, the clinical relevance of TLP is still debated because hypersensitivity to this allergen is exceedingly rare in an isolated form (23).

The latex-fruit syndrome is the result of cross-reactivity between NRL proteins and fruit proteins. Class 1 chitinases (Hev b 6, hevein-like proteins), β-1,3-glucanases (Hev b 2), and other cross-reactive proteins have been implicated. The commonly reported cross-reactive foods include banana, avocado, kiwi and chestnut. The group of defense-related plant proteins, class 1 chitinases, cross-react with the pan allergen hevein. Cross-reactivity with these proteins is noted for banana, avocado, kiwi, chestnut, papaya, tomato, cherimoya, passion fruit, mango and wheat. Prohevein (Hev b 6) behaves as a major allergen, since it reacts with IgE in most sera of subjects with latex allergy (4).

Plant allergens, being one of the most widespread allergenic substances, are hard to avoid. Therefore, their identification and characterization aid in the diagnosis and treatment of allergic diseases. Although serum IgE level is low in the general population, those with hereditary risk of atopy produce excessive levels of IgE, and in conjunction with the relatively high occurrence of the conserved proteins and epitopes in plant-derived foods, completes the disease triangle resulting in sensitization and allergic reactions. In most cases, protocols for the diagnosis of food allergy make use of whole food extracts. However, depending on the experimental procedure used and on the food characteristics (e.g., the ripening stage of a fruit), whole food extracts may be variable in both the number and amount of the allergenic components; this heterogeneity may be at least one of the causes of some conflicting and confusing results reported in the field of allergy. Moreover, results obtained by using whole food extracts do not provide information about individual sensitivity towards single allergenic components of the investigated food, which should be particularly useful in planning and monitoring desensitizing immunotherapy. Availability of purified and characterized allergens would help solving these clinical problems, and also allow controlled and reproducible production of hypoallergenic derivatives (61-63).

Allergy caused by fruits with lower incidence and rare/ tropical fruits

Apart from the common fruits available in the vegetable and fruit market, many allergic reactions have been reported for some common fruits as well as for tropical and region-specific fruits. Since only limited number of cases is encountered, the causative allergens have not been identified in many cases as characterization of the allergens is lacking. Despite the low incidence of allergy to these fruits, isolation and characterization of some allergens have been carried out. Allergy to some common fruits with a lower incidence of allergy including rare/tropical fruits is summarized in **table 2**. These include orange (64-66), mulberry (67, 68), lychee (69, 70), raspberry (71, 72), pineapple (73, 74) and sapodilla (75, 76). Since many tropical and exotic fruits are exported to other countries, increased consumption of these rare fruits is likely to cause a moderate increase in the incidence of these fruit allergy in future.

Table 2 *- Allergens from fruits with low incidence of allergy and some rare/tropical fruits.*

Fruit	Remarks	Reference
Orange (*Citrus sinensis*)	Germin-like glycoprotein (Cit s 1; 23.7 kDa), profilin (Cit s 2), LTP (Cit s 3)	64-66
Mulberry (*Morus alba, M. nigra*)	Bet v 1-related allergens (Mor a 1), nsLTP1 (Mor n 3), profilin (Mor a 4)	67, 68
Lychee (*Litchi chinensis*)	Profilin (Lit c 1), 35 kDa isoflavone reductase (Lit c IFR), 28 kDa triose-phosphate isomerase (Lit c TPI)	69, 70
Raspberry (*Rubus idaeus*)	Rub i 1 (Mal d 1 homolog), Rub i 3 (Mal d 3 homolog), 30 kDa protein (class III chitinase), cyclophilin (Bet v 7 homolog)	71, 72
Pineapple (*Ananas comosus*)	Systemic reactions; profilin (Ana c 1), bromelain (Ana c 2)	73, 74
Sapodilla (*Manilkara zapota*)	Mainly oral allergy syndrome; acidic TLP (Man za TLP 1), basic TLP (Man za TLP 2)	75, 76

Conclusions

It can be observed that most fruits available in the market elicit allergic reactions in susceptible individuals. The prevalence of fruit allergy appears to result from the increased imports and exports of fruits sensitizing susceptible individuals. It can also be observed that similar allergens are present in most of the fruits, and show structural similarity with homologous allergens from pollens and other vegetables/fruits. Although observations on the similarities and differences in allergenic structures may lead to many speculations regarding fruit allergens, substantial experimental data is required to establish allergen properties and uniqueness. Since the protein content of fruits is very low, a detailed study on the fruit proteins is lacking in many situations. The lack of patient data on rare fruit allergy makes it difficult to characterize new allergens form these fruits. Nonetheless, proteomic studies of all fruits should be performed to locate the many isoforms and differential expressions of fruit allergens which should pave the way for preparation of fruit extracts or recombinant allergens for fruit allergy diagnosis and immunotherapy.

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P. D. Pigatto

Conctact dermatitis: some important topics

University of Milan, Department of Biomedical, Surgical and Odontoiatric Sciences and Ospedale Galeazzi

Key words

Contact dermatitis; patch test; allergy irritancy

Summary

Allergic contact dermatitis (ACD) is a type IV delayed hypersensitivity reaction. The gold standard for diagnosis is patch testing. The prevalence of positive patch tests in referred patients with suspected ACD ranges from 27 to 95.6 %. The relationship between ACD and atopic dermatitis (AD) is complicated with conflicting reports of prevalence in the literature; however, in a patient with dermatitis not responding to traditional therapies, or with new areas of involvement, ACD should be considered as part of the work-up.

Corresponding author
Paolo Daniele Pigatto
Department of Biomedical, Surgical and Odontoiatric Sciences
University of Milan and Ospedale Galeazzi
Via Galeazzi 4, 20161 Milano, Italy
E-mail: paolo.pigatto@unimi.it

Contact dermatitis is a category of diseases whose common denominator is an external inciting factor, in contrast to the endogenous dermatoses, e.g., atopic dermatitis and psoriasis. Included in this group are: irritant contact dermatitis (ICD); contact urticaria (CU); protein contact dermatitis (PCD); and allergic contact dermatitis (ACD) (1). The most common form of all the contact dermatoses is ICD. It does not require prior sensitization, but rather is caused by direct damage to keratinocytes by an irritating substance (e.g., an alkaline or acidic chemical). This leads to a localized release of proinflammatory cytokines and the subsequent development of an eczematous dermatitis (2). Importantly, besides avoidance of the causative agent (which in acute cases can usually be identified by the subject), therapy targeted at barrier repair is paramount and can include the use of ceramides, pseudoceramides, and filaggrin degradation products (3). While beyond the scope of this article, it is important to mention the Type I immediate hypersensitivity reactions seen in the skin, as they have important clinical consequences. CU specifies the appearance of pruritic wheals, as the unique symptom after contact with the triggering substance (4). In this type of reaction, the subject will experience degranulation of mast cells in the dermis as well as a perivascular leukocyte infiltrate which triggers the release of histamine and other inflammatory mediators, that, in turn, cause local vasodilation, itch, and swelling in the skin (i.e. wheal and flare formation) (2). There are two subcategories of CU, namely non-immunologic contact urticaria (NICU) and immunologic contact urticaria (ICU). NICU involves the release of vasogenic mediators without the involvement of immunologic processes; it is typically less severe than ICU and occurs 45-60 min after contact (4,5). ICU requires a prior sensitization phase and occurs 15-20 min after contact, and in contrast to NICU, ICU can spread beyond the localized contact point (5). Additionally, this category includes PCD, which is thought to be caused

by a combination of type I and type IV reactions (4). Clinically, rather than the urticarial response, the skin lesions are characterized by chronic or recurrent eczematous dermatitis upon exposure to specific proteins (e.g., as meat, fish, vegetable, and latex) (4,6). Across the board, the first line treatment in this category of diseases is avoidance of the eliciting trigger. In addition, treatments that inhibit the release and effect of mast cell mediators and possibly other inflammatory mediators can ameliorate or suppress symptoms. Specifically, anti-histamines can be considered for urticaria and topical corticosteroids and/or calcineurin inhibitors can be used for dermatitis (4). ACD is a type IV (delayed) hypersensitivity reaction, a complex type of reaction, which requires a prior sensitization, and elicitation. The sensitization phase is characterized by an exogenous allergen entering the epidermis through an impaired skin barrier. These allergens then bind with self-proteins to create complete antigens that are taken up and expressed by dendritic cells on the cell surface of major histocompatibility complexes (MHC) (7-10). The elicitation phase occurs upon repeated exposure to the allergen at which time a clinical dermatitic response occurs. The repeated exposure can occur trans-epidermally or systemically through ingestion, inhalation, or intravenous entry (11). As opposed to ICD, which clinically consists of well-demarcated, erythematous, and sometimes follicular papules and plaques localized to the area of contact, ACD usually expands beyond the contact area. In addition, there can be transfer of the allergen from one body area to another or activation of dermatitis at distant sites via 'recall reactions', which are flares at sites of prior allergen exposure (1). In contrast to ACD, a pearl in the diagnosis of ICD is that the dermatitis will spare 'protected' areas. For example, in diaper dermatitis, the folds are spared, as the skin-skin contact prevents urease and fecal enzymes from touching and breaking down the skin in these areas, further underscoring the role of barrier integrity, maintenance, and repair in the treatment of ICD.

Clinical Relevance of Contact Sensitization

The gold standard for the diagnosis of ACD is patch testing; however, not all positive patch test (PPT) reactions are clinically relevant to a patient's dermatitis. A PPT reaction that is not found to be clinically relevant is termed 'contact allergy' rather than 'allergic contact dermatitis' (12). The prevalence range of PPT reactions with suspected ACD is 27 - 95.6% (13-17), while the relevancy of these PPT is much less frequent.

Patch Testing

The first indication for patch testing is uncontrollable or worsening chronic dermatitis of greater than 2 months duration. The second is a failure to improve following standard treatment protocols (18(26). Given the surface area for patch testing, once there is a high index of suspicion for ACD, a detailed exposure history guides the testing for relevant allergens. This is performed either by selecting potential allergens based on history of exposures or by screening with standardized series of allergens and potentially the patient's own personal care products.

Standard patch testing series have been suggested by both the Contact Dermatitis Group (19) and by centers in the US (18). Notably, a 24-h application period can be efficacious in patients with atopic dermatitis as it can reduce the irritation reactions that may be seen in these subgroups (20,21). In addition to standard comprehensive patch testing, the commercially available Thin-Layer Rapid Use Epicutaneous Patch Test (T.R.U.E.™, Smart Practice; Phoenix) has received FDA-indication for use in adults. The T.R.U.E.™ Test consists of three panels of allergens/mixes and one negative control as uniform dried gel coatings on polyester sheeting. Hypoallergenic adhesive surgical tape secures these patches to the skin. Per the prescribing instructions, it is recommended that the patches be applied for 48 h with reads at 72 and 96 h (22). Since that time, the TRUE test was expanded to include 35 allergens and the negative control. Of note, PREA-2 is currently under way to determine the safety and efficacy of these additional 7. Patch test readings are based on recommendations from the International Contact Dermatitis Research Group (ICDG) (23). A doubtful reaction by definition consists of faint macular erythema. A weak positive (1+) reaction is non-vesicular with erythema, mild infiltration, and potentially discrete papules. A strong positive reaction (2+) is vesicular with erythema, moderate infiltration and papules. Finally, an extreme positive reaction (3+) denotes a coalescing papular-vesicular plaque with deep erythema and significant infiltration, which may become bullous or ulcerative and often expands beyond the margin of the patch well. Notably, irritant reactions may present as pustules or patchy follicular erythema with no infiltration and are not indicative of a true allergy (23). The irritant reactions often appear within the first 48 h of patch testing and improve by 96 h, as opposed to contact allergy reactions, which typically worsen between 48 and 96 h.

If possible, patients should refrain from taking oral corticosteroids during the patch test. In adults, a dose of 20 mg in a 75-kg male is known to significantly suppress patch test reactions (24). In addition, topical corticosteroids should not be applied to the testing area for the 3-7 days prior to patch testing, as this can result in false negative reactions (25). Flare up reactions of the patient's dermatitis may be elicited during patch testing. For this reason, all prior dermatitis sites (excluding the test site) should continue to be treated with topical corticosteroids or immune modulators throughout the duration of the patch test (26). Patients can take oral antihistamines for symptomatic management of the pruritus, and this will not alter the results

of the patch testing. Although systemic immunosuppression is not optimal during patch testing, some patients' dermatitis is so wide-spread that these agents may be warranted, and the minimal suppressive dose may need to be determined to suppress the dermatitis and yet still allow for the patch test to function (25).

Discussion and Conclusions

ACD is a common condition in the general population which has been previously under-recognized, as it is often difficult to distinguish clinically from other eczematous skin eruptions such as AD and chronic irritant reactions (10). The negative impact of ACD extends to include a decrease in quality of life secondary to pruritus, loss of sleep, and feelings of inferiority among peer groups, in addition to a significant economic burden. Rates of contact sensitization are higher than historic literature had predicted, which may be secondary to an increase in allergen exposure associated with new trends (e.g., body piercings, the use of cosmetic products, and participation in sports and hobbies) (17,20), or improved recognition of ACD with patch testing being more frequently performed in the population. In the studies reviewed, the rate of PPT reactions ranged from 27 to 95.6% while the relevancy of the PPT ranged from 30.5 to 92.6%. These data may not be applicable to the population at large, given that these studies were done at major referral centers on selected patients.

The goal of patch testing is to optimize true PPT and reduce false PPT

This is most effectively done with comprehensive patch testing; however, comprehensive patch testing can be time/labor intensive and requires the practitioner to have access to a wide range of allergens. Since the advent of the T.R.U.E.™ test, the number of practitioners providing patch testing in their clinics has greatly increased due to the increased convenience afforded by this commercially available tool (27). That being said, not all relevant allergens are included in the T.R.U.E.™ test, such as the relevant allergens CAPB and dialkyl-thioureas.

When planning for patch testing, it is important for both the clinician and the patient to have realistic expectations. When a relevant allergen is identified, an avoidance regimen is prescribed. In patients with extensive chronic dermatitis, 8-12 weeks of avoidance may be needed before a true assessment of clinical improvement can be made. Even with the most compliant patients, avoidance regimens may be difficult to follow, especially with ubiquitous allergens with a multitude of potential exposure sources or when the product manufacturing ingredients (e.g., shin guards) are not available. Products that are marketed as 'natural' can also cause ACD. It is well known that some 'unscented' products contain a masking fragrance, thus are not 'fragrance-free'. 'Fragrance-free' products can contain essential oils that can also lead to contact sensitization (27).

Resources such as the ACDS Contact Allergen Management Program (CAMP), available at http://www.contactderm.org, and the Contact Allergen Replacement Database (CARD), available at https://card.preventice.com, can be helpful in providing a list of products that patients are allowed to use, in addition to giving them allergen information sheets. It is also important to note that 'allergen avoidance' may require adaptive measures to prevent contact of the allergen with the patients' skin. For example, if a patient is allergic to a component of a shin guard, the shin guard can be lined with canvas as an adjunct to the patient wearing a protective sock underneath, to prevent direct skin contact. In addition, patients can be given instructions on the repeat open application test (ROAT) or 'use test' for testing new products prior to full body application. This test consists of applying a product, twice a day for 1 week, to a designated area on the upper inner arm while monitoring for an eczematous skin reaction.

One population in particular can especially benefit from patch testing: the AD patients. Although the exact prevalence of ACD in patients with AD remains unclear, it is known that ACD can be misdiagnosed as AD and/or the concurrent presence or development of ACD can lead to AD flares. As a result, in those patients with moderate-severe dermatitis, correct use of patch testing can allow for cessation of systemic immunosuppressant therapies, a decrease in the need for topical corticosteroid therapy, and ultimately a drastic improvement in their quality of life.

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M. Verburg, J.M. Oldhoff, R.J.B. Klemans, A. Lahey-de Boer, M.S. de Bruin-Weller, H. Röckmann, C. Sanders, C.A.F.M. Bruijnzeel-Koomen, S.G.M.A. Pasmans, A.C. Knulst

Rush immunotherapy for wasp venom allergy seems safe and effective in patients with mastocytosis

Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, The Netherlands

Key words

Immunotherapy; mastocytosis; tryptase; urticaria pigmentosa; yellow jacket venom allergy; wasp venom allergy.

Corresponding author
R. Klemans
Department of Dermatology/Allergology
University Medical Center Utrecht
PO Box 85500
3508 GA Utrecht
The Netherlands
Phone: +31 88 7553 235
E-mail: r.j.b.klemans-3@umcutrecht.nl

Summary

Background. *Patients with mastocytosis and wasp venom allergy (WA) may benefit from venom immunotherapy (VIT). However, fatal insect sting reactions have been described in mastocytosis patients despite previous immunotherapy. We investigated the safety and efficacy of (rush) VIT in patients with mastocytosis and WA.* ***Objective.*** *To investigate the safety and efficacy of (rush) VIT in patients with mastocytosis and WA.* ***Methods.*** *We describe nine patients with cutaneous mastocytosis and WA who received VIT. Cutaneous mastocytosis was confirmed by histopathology and systemic mastocytosis was diagnosed according to World Health Organization criteria. VIT was given according to a rush protocol. Given the difference in safety and efficacy of VIT in patients with WA and honeybee venom allergy, we reviewed the literature for VIT with the focus on WA patients with mastocytosis and addressed the difference between patients with cutaneous versus systemic mastocytosis.* ***Results.*** *Nine patients had WA and mastocytosis, of whom six had cutaneous mastocytosis, two combined cutaneous and systemic mastocytosis and one systemic mastocytosis. All patients received rush IT with wasp venom. Most patients had only mild local side effects, with no systemic side effects during the course of VIT. One patient had a systemic reaction upon injection on one occasion, during the updosing phase, with dyspnoea and hypotension, but responded well to treatment. Immunotherapy was continued after temporary dose adjustment without problems. Two patients with a previous anaphylactic reaction were re-stung, without any systemic effects.* ***Conclusions.*** *VIT is safe in cutaneous mastocytosis patients with WA, while caution has to be made in case of systemic mastocytosis. VIT was effective in the patients who were re-stung.*

Introduction

Mastocytosis is a disease characterized by the proliferation of mast cells in skin and/or bone marrow and/or other tissues. The symptoms are the consequence of the release of histamine and other mediators from mast cells and can vary from itching and flushing to anaphylactic shock. Clinical presentations can be cutaneous (urticaria pigmentosa, diffuse cutaneous mastocytosis, mastocytoma) but systemic disease with or without skin involvement may also occur (indolent/aggressive systemic mastocytosis, mast cell leukemia) (1). Mastocytosis patients have an increased risk of a severe allergic reaction following hymenoptera stings compared to patients without mastocytosis. An immunoglobulin E (IgE)-mediated mechanism has been postulated, and although specific IgE could not be detected in some mastocytosis patients with a reaction to hymenoptera venom (2), IgE was detected in all but one patient when using the basophil activation test in the diagnostic work-up, making an IgE-mediated mechanism likely in most if not all patients (3).

Immunotherapy is a well-accepted treatment for patients with wasp (yellow jacket) venom allergy (WA) without mastocytosis. It prevents a systemic reaction in 90-98% of cases (4).

Table 1 *- Safety and efficacy according to protocol followed during up-dosing phase.*

Author	C/R	SSE	Re-stung	SS Re-stung
Bonnadonna[9]	15 C	2	13	2
Gonzalez de Olano[11]	10 C	1	5	1
Total	25 C	3	18	3
Gonzalez de Olano[11]	5R	2	4	1
Total	5R	2	4	1
Our data	9R	1	2	0
Oude Elberink[7]	2 nd	1	2	0
Müller[6]	1 nd	0	0	-
Fricker[10]	4 nd	0	3	1

C/R: conventional/rush immunotherapy; nd: not determined; SSE: systemic side effects; SS: systemic symptoms.

Table 2 *- Safety and efficacy depending of type of mastocytosis.*

Author	Number of patients	SSE IT	Re-stung	SS Re-stung
Systemic mastocytosis				
Bonnadonna[9]	15	2	13	2
Gonzalez de Olano[11]	15	3	9	2
Oude Elberink[7]	2	1	2	2
Fricker[10]	1	0	1	0
TOTAL	33	6	25	6
Our data	3	1	1	0
Cutaneous mastocytosis				
Fricker[10]	3	0	2	0
Müller[6]	1	0	0	-
TOTAL	4	0	2	0
Our data	6	0	1	0

SSE: systemic side effects; IT: immunotherapy; SS: systemic symptoms.

H1 antihistamines were reported to reduce local and systemic reactions related to immunotherapy with hymenoptera venom (5). VIT in hymenoptera venom allergy (HVA) patients with (cutaneous) mastocytosis was first described in 1983 in one patient with yellow jacket anaphylaxis. This patient had no systemic side effects during the course of VIT (6). In 1997 two fatal reactions were described in mastocytosis patients with yellow jacket anaphylaxis, both from The Netherlands, after a field sting 1 and 9 years respectively after stopping VIT (duration 2.5 and 5 years) and despite emergency treatment (7). In the first patient VIT was stopped because of systemic side effects. These fatalities raised questions regarding the safety and efficacy of VIT in mastocytosis patients. To date studies are limited to case reports and small observational studies, reflecting the fact that HVA and mastocytosis occur infrequently in combination. Two studies reported a high frequency of systemic side effects during VIT and limited efficacy: 86 to 100% systemic reactions following a re-sting (7,8). Other studies reported encouraging results with regard to safety and efficacy (4,9-11). VIT in WA is safer and more effective than in honeybee allergy (HA) (12). Whether this is similar in patients with co-existing mastocytosis is unknown. However, in a multicenter trial by Ruëff et al. there was a significant association between side effects during VIT and elevated baseline serum tryptase concentration (BTC, a marker for SM) in patients with WA but not with HA (13). So there may be a difference in the efficacy and safety of VIT in HA and WA patients with and without mastocytosis. Most studies, however, do not distinguish between HA and WA in patients with mastocytosis and hymenoptera venom allergy. Moreover studies on patients with urticaria pigmentosa-type mastocytosis are rare.

We report on nine mastocytosis patients (six with urticaria pigmentosa-type mastocytosis) with WA to add to the limited and sometimes conflicting experience with this type of therapy in this rare disease. Moreover we summarize the most important patient characteristics in the studies published to date (**table 1 and 2**).

Methods

Patient characteristics

Between 1990 and 2009, nine patients with yellow jacket venom allergy and mastocytosis were treated with immunotherapy. Inclusion criteria were: 1) severe WA grade IV according to Müller, 2) cutaneous and/or systemic mastocytosis. WA was confirmed by positive intracutaneous (IC) tests and/or venom-specific IgE. Cutaneous mastocytosis was confirmed by skin biopsy and systemic mastocytosis was confirmed according to WHO criteria (1). IC tests, baseline serum tryptase concentration (BTC) and venom-specific IgE were assessed before and

during VIT. IC tests were performed with ten-fold increasing doses of yellow jacket venom ranging from 0.0001 to 0.1 microgram/ml. Testing was conducted on the volar surface of either forearm, with yellow jacket dilutions in conjunction with a normal saline solution as the negative control and histamine hydrochloride as the positive control. Intracutaneous test results were measured with calipers and were considered positive if the intracutaneous skin test with yellow jacket venom (or bee venom, as a control) resulted in a wheal diameter of 5 mm or more and was at least 3 mm larger than the negative control. Venom-specific IgE antibodies in the serum were measured by CAP-FEIA, Phadia, The Netherlands. A value of > 0.35 kU/L was considered positive. Serum BTC levels were measured and a value above 13.5 ng/ml was considered as elevated (2). Patients with systemic symptoms and/or a BTC > 20 ng/ml were referred to the hematologist to consider a bone marrow biopsy. The study was approved by the ethics committee. Informed consent was not required given the retrospective design.

Venom immunotherapy and follow-up

The updosing phase of VIT was administered according to a 3-day rush protocol with Pharmalgen yellow jacket venom (ALK-Abelló, Nieuwegein, The Netherlands). This VIT protocol was the same as for HVA patients without mastocytosis. Ten milligrams cetirizine was routinely given as pre-medication one hour before each dose. On day 1 doses of 0.01 µg, 0.1 µg, 1 µg and 2 µg were given. On the second day, 5 µg, 10 µg, 20 µg and 40 µg were given. On the last day two doses of 50 µg were given. The treatment was continued with 100 µg Alutard SQ 802 (ALK-Abelló, Nieuwegein, The Netherlands). The interval between injections was gradually increased to 6 weeks after the first year and to 8 weeks after the second year. Patients were admitted for the 3-day rush VIT to our inpatient clinic. The patients were continuously monitored for local and systemic symptoms by trained personnel. Maintenance therapy was given in our outpatient clinic for at least the first year. Subsequent maintenance treatment was given by the referring specialist or by the general practitioner in the case of patients residing far away from the clinic.
Safety was evaluated by carefully assessing any local or systemic allergic symptoms. All patients were supplied with emergency medication including an epinephrine auto-injector, prednisolone and antihistamines. Patients were re-evaluated annually.

Literature review

A thorough review of the literature was conducted. The PubMed database was searched using the following terms: mastocytosis, immunotherapy, urticaria pigmentosa, hymenoptera venom allergy. We specifically searched for patients with (any type of) mastocytosis with sensitization and immunotherapy for wasp venom.

Results

Patient characteristics

Nine patients, four female and five male, with mastocytosis and WA were included (**table 3**). All had had a severe systemic reaction with cardiovascular symptoms within 15 minutes of a wasp sting. Six of the patients had cutaneous mastocytosis only, two had combined indolent systemic and cutaneous mastocytosis, and one had indolent systemic mastocytosis only. The median specific IgE at baseline was 18 kU/L (range < 0.35 - >100 kU/L) and positive in 7/8 patients (missing in one patient). An intracutaneous test with yellow jacket venom was positive in all patients tested. Honeybee venom allergy (HA) was excluded in all patients. For details see **table 3**.

Safety of immunotherapy

The median duration of immunotherapy was 6.1 years (range 0.1-19 years). All patients are still on immunotherapy. Patient 9 had a systemic reaction on one occasion at a dose of 40 µg/ml, during the up-dosing phase. Symptoms started with erythema on the chest, which subsequently spread over the arms, followed by chest pain, palpitations, dyspnea, nausea and a decrease in blood pressure (from 120/80 to 99/53) with a tachycardia of 97 beats per minute. The patient responded rapidly to treatment. VIT was continued after dose adjustment, without any further systemic side effects during follow-up. There were no systemic side effects in any of the patients during the maintenance phase.

Efficacy of VIT

Two patients had a field sting during the maintenance phase of venom immunotherapy, in both cases 2 years after the start of VIT treatment. They experienced a local reaction for which treatment was unnecessary. Both had been diagnosed with a severe WA with respiratory as well as cardiovascular symptoms including loss of consciousness, within 15 minutes of a yellow jacket sting, requiring treatment (before the start of VIT) with epinephrine, prednisolone and antihistamines in the ambulance and in the emergency department.

Discussion

We report the successful treatment of nine patients with WA and mastocytosis, using WA IT with a rush protocol. Most patients had no side effects at all. One patient had a systemic

Table 3 *- Safety and efficacy of IT in our study population.*

							sIgE		Tryptase			
No	**Age**	**Sex**	**Type mastocytosis**	**BM**	**SRS**	**IC[1] t = 0**	**t = 0**	**t = IT**	**t = 0**	**t = IT**	**Duration IT (years)**	**Symptoms IT**
1	59	m	CM	-	4	0.0001	20.4	5.3	28	22	5	-
2	54	f	CM	nd	4	0.0001	> 100	29.3	5	6.3	4	-
3	71	m	CM	nd	4	0.0001	11	5	nd	nd	19	-
4	67	m	CM	nd	4	nd	26	nd	nd	26	3	-
5	64	f	CM+SM	pos	4	0.0001	6	1.3	13	31,7	6	Numbness hand/feet/tongue
6	39	m	CM	neg	4	0.0001	3.8	nd	12.5	nd	0.1	Nausea, headache
7	56	f	SM	pos	4	0.01	0.4	< 0.35	38	31	3	-
8	41	f	CM	neg	4	0.01	< 0.35	< 0.35	22.3	20.1	2	-
9	67	m	CM+SM	pos	4	0.01	1.17	nd	nd	43	7	Urticaria, oedema, erythema, dyspnea, drop blood pressure

IT: immunotherapy; BM: bone marrow biopsy; SRS: systemic reaction score; CM: cutaneous mastocytosis; SM: systemic mastocytosis; nd: not done; NA: not applicable.

[1]positive at dilution (in mcg/ml).

reaction following one injection, during the up-dosing phase, with a rapid response to treatment. After dose adjustment VIT was continued without problems. Ruëff et al. found that the two greatest risk factors for a systemic reaction during VIT were elevated BTC, bee venom allergy and (ultra) rush VIT (13). When evaluating the literature with respect to VIT in patients with mastocytosis and WA, we calculated that 23% of the WA patients had a systemic reaction during the up-dosing phase (see **table 1** and **2**). Note that we excluded the studies of Dubois et al. and Ruëff et al. from our review, since it was not clear which patients had HA or WA (2,8). However, as most of their patients had WA, Dubois et al. questioned the safety of VIT given their findings of a systemic reaction during VIT in 6/7 patients with mastocytosis (8). This high number of side effects might be explained by differences in dosing schemes or patient selection. Ruëff et al. supported the relative safety of VIT. They found a systemic reaction during VIT in 9/48 patients (2).

The WA VIT rush protocol seems to be associated with a higher percentage of systemic side effects compared to conventional protocols (table 1) (14). This is consistent with the findings of Przybilla and Ruëff in patients with HA and WA. There are however no comparative studies between HA and WA in mastocytosis patients. Our study revealed systemic side effects in one patient (11%) despite the fact that we used a rush protocol, thus contrasting with previous studies (table 1). This might be due to the fact that all our patients had pre-treatment with antihistamines. The beneficial effect of pre-treatment with antihistamines in VIT has previously been reported (5). The other studies gave no indication of any pre-treatment (table 1 and 2). With regard to the potential influence of the type of mastocytosis on the occurrence of side effects, the only systemic reaction occurred in one of the three patients diagnosed with systemic mastocytosis. In the literature systemic side effects were reported in 30% of patients with SM (table 2), which is a significantly higher percentage than that observed in patients without mastocytosis. The group of patients with CM and WA reported in the literature to date is small (n = 4, table 2). No systemic side effects of VIT were recorded in this group (table 2). Although the patient numbers are small, the results suggest that patients with systemic mastocytosis are at greater risk for systemic reactions.

Two patients had a field sting during the maintenance phase of VIT, 2 years after the start of VIT treatment, while still on therapy. They experienced a local reaction, without the need for treatment, illustrating the efficacy of the protocol, although patient numbers are limited. The efficacy of VIT in patients with mastocytosis has been debated, especially since two patients died after VIT for WA (7). In both cases this occurred following the cessation of VIT, respectively 1½ and 5 years previously. To date no fatalities have been reported in mastocytosis patients while still on VIT. Only 2/9 patients were re-stung during VIT without any systemic reaction. This supports the findings of other studies (table 1 and 2).

In conclusion, (rush) VIT in patients with WA and cutaneous mastocytosis is safe, while extra caution has to be made in patients with systemic mastocytosis. VIT was effective in two patients who were re-stung. Efficacy might be lower than that in patients without mastocyosis, and might disappear upon discontinuation. Therefore, lifelong treatment should be considered, as well as prescription of an epinephrine auto-injector.

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Â. Gaspar[1], N. Santos[2], S. Piedade[1], C. Santa-Marta[1], G. Pires[1], G. Sampaio[1], C. Arêde[1], L.M. Borrego[1,3], M. Morais-Almeida[1,4]

One-year survey of paediatric anaphylaxis in an allergy department

[1]Immunoallergy Department, CUF Descobertas Hospital, Lisbon, Portugal
[2]Immunoallergy Department, Centro Hospitalar de São João, Porto, Portugal
[3]CEDOC, NOVA Medical School, Lisbon, Portugal
[4]CINTESIS, Center for Research in Health Technologies and Information Systems, Porto, Portugal

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Anaphylaxis; children; epidemiology; epinephrine; management; notification.

Corresponding author
Ângela Gaspar
Immunoallergy Department
CUF Descobertas Hospital
1998-018 Lisbon, Portugal
E-mail: angela.gaspar@sapo.pt

Summary
Aim. *To determine the frequency of anaphylaxis in an allergy outpatient department, allowing a better understanding regarding aetiology, clinical manifestations and management, in children and adolescents.* ***Methods.*** *From among 3646 patients up to 18 years old observed during one-year period, we included those with history of anaphylaxis reported by allergists.* ***Results.*** *Sixty-four children had history of anaphylaxis (prevalence of 1.8%), with mean age 8.1 ± 5.5 years, 61% being male. Median age of the first anaphylactic episode was 3 years (1 month - 17 years). The majority of patients had food-induced anaphylaxis (84%): milk 22, egg 7, peanut 6, tree nuts 6, fresh fruits 6, crustaceans 4, fish 4 and wheat 2. Food-associated exercise-induced anaphylaxis was reported in 2 adolescents. Drug-induced anaphylaxis occurred in 8%: 4 non-steroidal anti-inflammatory drugs and 1 amoxicillin. Three children had cold-induced anaphylaxis, one adolescent had anaphylaxis to latex and one child had anaphylaxis to insect sting. The majority (73%) had no previous diagnosis of the etiologic factor. Symptoms reported were mainly mucocutaneous (94%) and respiratory (84%), followed by gastrointestinal (42%) and cardiovascular (25%). Fifty-one patients were admitted to the emergency department, although only 33% were treated with epinephrine. Recurrence of anaphylaxis occurred in 26 patients (3 or more episodes in 14).* ***Conclusions.*** *In our paediatric population, the main triggering agent of anaphylaxis was IgE-mediated food allergy. Epinephrine is underused, as reported by others. Often, children have several episodes before being assessed by an allergist. We stress the importance of systematic notification and improvement of educational programmes in order to achieve a better preventive and therapeutic management of this life-threatening entity.*

Introduction

According to the World Allergy Organization (WAO) (1), anaphylaxis is defined as an acute, multi-organ system, potentially life-threatening hypersensitivity reaction caused by the release of chemical mediators from mast cells and basophils. It can be triggered by immune mechanisms (allergic anaphylaxis) mediated by immunoglobulin E (IgE) (allergic IgE-mediated anaphylaxis) or other immune mechanisms (non-IgE-mediated allergic anaphylaxis) or non-immunologic mechanisms (non-allergic anaphylaxis).

It is a clinical emergency, being the most severe form of allergic disease. A practical and clinically based criteria definition, irrespective of the underlying mechanisms, that would allow the easy recognition of anaphylaxis both at the hospital and at the clinic levels, was one of the main objectives of a multidisciplinary symposium sought to standardize the diagnostic approach and treatment of this entity (2,3).

In 2006, Sampson *et al.* (4) revised and published the criteria for the diagnosis of anaphylaxis that included, in addition to the mucocutaneous, cardiovascular and respiratory symptoms,

Appendix 1 *- Clinical criteria for the diagnosis of anaphylaxis (Adapted from Sampson et al.)[4].*

Anaphylaxis is highly likely when any one of the following three criteria is fulfilled:
1. Sudden onset of an illness (minutes to several hours) with **involvement of the skin, mucosal tissue or both** (e.g. generalized hives, itching or flushing, swollen lips-tongue-uvula) **and at least one of the following**: a. **Sudden respiratory compromise** (e.g. dyspnea, wheeze-bronchospasm, stridor, reduced FEV_1/ PEF, hypoxemia) b. **Sudden reduced BP or associated symptoms** of end-organ dysfunction (e.g. hypotonia-collapse, syncope, incontinence)
2. **Two or more of the following**, that occur suddenly after exposure **to a likely allergen or other trigger for that patient** (minutes to several hours): a. Sudden skin or mucosal symptoms and signs (e.g. generalized hives, itching or flushing, swollen lips-tongue-uvula) b. Sudden respiratory symptoms and signs (e.g. dyspnea, wheeze-bronchospasm, stridor, reduced FEV_1/ PEF, hypoxemia) c. Sudden reduced BP or symptoms of end-organ dysfunction (e.g. hypotonia-collapse, syncope, incontinence) d. Sudden gastrointestinal symptoms (e.g. crampy abdominal pain, vomiting)
3. **Reduced BP after exposure to a known allergen** for that patient (minutes to several hours): a. Infants and children: low systolic BP (age-specific) or greater than 30% decrease in systolic BP[1]

FEV_1: Forced expiratory volume in 1 second; PEF: Peak expiratory flow; BP: Blood pressure.

[1]Low systolic blood pressure for children is defined as less than 70 mmHg from 1 month to 1 year, less than [70 mmHg + (2x age)] from 1 to 10 years, and less than 90 mmHg from 11 to 17 years.

gastrointestinal symptoms when occurring upon the exposure to a likely allergen or trigger, allowing the standardization of the definition of anaphylaxis. These criteria, initially proposed by the American Academy of Allergy, Asthma & Immunology (AAAAI), were later accepted by the European Academy of Allergy and Clinical Immunology (EAACI) (5) and the WAO (6).
The prevalence of anaphylaxis during life is estimated to be from 0.05 to 2% (7), with a recent review of European studies pointing to an estimated prevalence of 0.3%, meaning that 1 in every 300 persons suffers an episode of anaphylaxis during their lifetime (8).
The incidence of anaphylaxis was estimated at between 8.4 per 100 000 persons/year in population studies in the UK (9) to 50 individuals per 100 000/year in the United States (10) with a mortality of 1 to 3 cases per million inhabitants/year (11).
There has been an increase in the prevalence of anaphylaxis over time, especially in paediatric age groups (8,11,12) and an increase in the number of hospital admissions for anaphylaxis in preschool children (8,12-15), being food the most often implied cause of anaphylaxis in this age group (8,11,15,16).
In Portugal, the prevalence or incidence of anaphylaxis in the general population are not known to date. A study performed by Morais-Almeida *et al.* in 2006 reported a prevalence of 1.3% of anaphylaxis in patients observed at an outpatient allergy clinic for one year, half of them belonging to the paediatric age group (17).

Aim

The aim of this study was to contribute to a better knowledge of the epidemiology of anaphylaxis, based on the voluntary notification by allergy specialists, in order to estimate the prevalence of anaphylaxis in the paediatric age groups in a specialized allergy department, over a one year period, and identify the main clinical manifestations and etiological agents of anaphylaxis in these children and adolescents.

Material and methods

Population

A systematic reporting of anaphylaxis was implemented in the allergy department of CUF Descobertas Hospital from the 1st of January to the 31st of December 2011. All allergists (twelve) of the department were invited to participate in the study and a meeting was organized in order to promote the voluntary notification of all observed cases. All accepted to participate and joined this study. Episodes of anaphylaxis occurring in children and adolescents under 18 years old followed at the outpatient clinic of our department, described by the patient or caregiver, were reported.
During the one-year period 3646 patients aged less than 18 years old were observed, having been included all children with episodes fulfilling criteria of severe anaphylaxis. The diagnosis of anaphylaxis was made when "at least one episode of severe systemic reaction" occurred, as defined by the consensus, when in the presence of at least one of the three clinical criteria outlined in the **appendix 1** (4). A questionnaire with demographic and clinical data was completed. Etiological investigation was conducted by the attending allergy specialist

using appropriate diagnostic tests in each case, including skin tests with the suspected etiologic agent and/or assays of serum specific IgE, or other methods such as ice cube testing when appropriate.

Questionnaire

A questionnaire was carried out by the allergist to all patients with history of anaphylaxis in order to characterize the following parameters:

- Demographics, including age, gender and place of residence;
- Family history of allergic disease;
- Personal history of asthma or other allergic disease;
- Date of the first anaphylactic reaction and detailed description of the clinical manifestations: mucocutaneous, respiratory, gastrointestinal and cardiovascular; elapsed time between exposure to the causal factor and the onset of symptoms; description of the performed treatment, including information about the use of epinephrine; place of anaphylaxis occurrence; attendance to the emergency department and hospitalization;
- Previous prescription and use of a self-injectable epinephrine from an auto-injector device;
- Number of episodes of anaphylaxis, reproducibility and reasons for recurrence;
- Characterization of the causative factor involved and the date of diagnosis; in case of a previous diagnosis, assessment of the context of exposure: accidental contact or challenge test.

Etiological investigation

The skin prick tests with the suspected allergen(s) (food allergens, latex, antibiotics or hymenoptera venom) were performed on the anterior surface of the forearm with a minimum distance of 2 cm between each allergen extract and using metal lancets applied perpendicularly to the skin with a 1 mm penetration (PrickLancet®, Stallergenes, Antony, France), taking into account the recommended eviction timings for medications and using standard methodology (18,19). Histamine hydrochloride 10 mg/mL was used as a positive control and a solution of 0.5% phenol as a negative control. The reading was performed after 15 minutes. Tests with a mean wheal diameter ≥ 3 mm were considered positive. In patients with suspected food allergy, skin prick tests with the food were performed whenever the test with the allergenic extract was negative or unavailable. For suspected allergy to antibiotics and hymenoptera venom, prick and intradermal tests were performed according to international guidelines (20,21), after obtaining informed consent and at least six weeks after the anaphylactic reaction.
When available, the assay for specific IgE (sIgE) was performed through the UniCAP® method to the suspected allergen (Thermo Fisher Scientific, Uppsala, Sweden). Results were considered positive for sIgE ≥ 0.35 kU/L.
The ice cube test was performed by applying a cold stimulus (0 to 4°C) on the anterior surface of the forearm by a sequential time of 3, 5, 10 and 20 minutes to obtain a positive response (wheal). This test was considered negative if a wheal did not appear after 20 minutes of exposure (22).

Atopy

Atopy was defined as positive test for at least one allergen from a panel of aeroallergens (Bial-Aristegui® extracts, Bilbao, Spain) adapted according to the age of the patient: mites (*Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blomia tropicalis* and *Lepidoglyphus destructor*), pollens (grass mix, *Parietaria judaica, Artemisia vulgaris, Plantago lanceolata, Olea europaea, Cupressus spp* and *Platanus spp*), fungi (*Alternaria alternata*), dog and cat dander.

Statistical analysis

The results are presented as absolute and relative frequencies. Quantitative variables with normal distribution are expressed as mean ± standard deviation. Variables not normally distributed are expressed as median (minimum-maximum). The Chi-square test and calculation of odds ratios with confidence interval of 95% was used to test association between qualitative variables and considered significant for a p-value < 0.05.

Results

Over the one year period, 64 patients were reported with history of anaphylaxis (prevalence of 1.8%), with a mean age (standard deviation (SD)) of 8.1 ± 5.5 years and a median of 7 years (5 months to 17 years old) at the time of observation, including 19 adolescents (aged 12 years or older); 39 (61%) were male.
Atopy, personal and family history of allergic disease is presented in **table 1**. Only two children had no personal or family history of allergic disease. The majority (91%) had personal history of allergic disease, and 44% had asthma as co-morbidity.
The median age of the first anaphylaxis episode was of 3 years (1 month to 17 years old). In 14 children (22%) the first episode occurred in the first year of life and in 50 (78%) up to 5 years of age. Only 6 (9%) had the first episode in adolescence.

Clinical manifestations

Reported symptoms are shown in **figure 1**. In 51 patients (80%) both skin and respiratory symptoms occurred. Four children aged 1 to 2 years did not have mucocutaneous manifestations. The number of respiratory symptoms was similar in patients

***Table 1** - Frequency of atopy and personal and family history of allergic disease.*

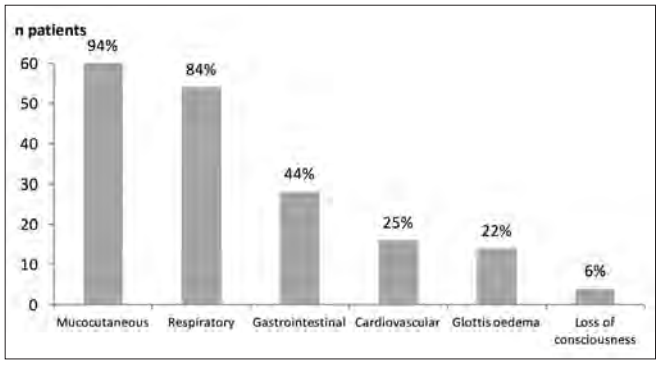
	n	(%)
Atopy	**44**	**(75)**[1]
Personal history of allergic disease	**58**	**(91)**
Rhinitis	47	(73)
Eczema	37	(58)
Asthma	28	(44)
Food allergy	14	(22)
Family history of allergic disease	**50**	**(78)**

[1]Percentage of children on whom skin prick tests with aeroallergens were performed (n = 59).

with and without asthma (84% vs. 83%, p = 1000). In all cases with gastrointestinal complaints, food was the suspected etiological agent, and in 25 (89%) cases it occurred in children aged less than 3 years. Cardiovascular manifestations, glottis oedema or loss of consciousness occurred in 25 patients (39%); the diagnosis of asthma was not a risk factor for these symptoms (p = 0.114).

The majority (86%) of the reactions started within 30 minutes after exposure to the etiological agent. The 5 delayed reactions began after 2 to 3 hours of exposure and included 4 children aged less than 2 years with food-induced anaphylaxis and a 3 year-old boy with insect sting anaphylaxis.

***Figure 1** - Number and percentage of patients according to clinical manifestations at the first episode of anaphylaxis.*



Emergency department attendance and treatment

Thirty-six (57%) of the first episodes of anaphylaxis occurred at home, 11 (17%) in restaurants, 11 (17%) on vacation or recreational sites, 4 (6%) at the hospital and 2 (3%) at school.

Fifty-one patients (80%) resorted to the emergency department. However, only 21 (33%) were treated with epinephrine; from these, 13 (62%) had cardiovascular events, glottis oedema or loss of consciousness. Three (5%) patients were hospitalized for more than 24 hours with no need of mechanical ventilation and there were no fatalities. There were no differences in the use of the emergency department or epinephrine administration in patients with and without asthma (p = 0.872 and p = 0.331, respectively).

Previous diagnosis, recurrence and use of self-injectable epinephrine

Seventeen patients (27%) had a prior diagnosis of allergy: in 15 children, anaphylaxis occurred after accidental contact with the causative agent and in 2 during an oral food challenge with cow's milk. In 47 patients (73%) the diagnosis of allergy was performed after the episode of anaphylaxis. Epinephrine for self-administration was prescribed to all but 6 children, who could maintain complete eviction (anaphylaxis to drugs) or who weighed less than 7.5 kg.

In 26 patients (41%) anaphylaxis occurred more than once: 12 patients with 2 episodes, 9 with 3 to 4 episodes and 5 patients with 5 or more episodes. Three patients had successfully used the self-injectable epinephrine from an auto-injector device.

Etiological study

In 54 patients (84%) anaphylaxis was food-induced. The remaining causes are specified in **table 2**. Two adolescents had more than one cause of anaphylaxis, accounting for a total of 66 reports of etiological agents: anaphylaxis to shrimp and acetylsalicylic acid (ASA), and anaphylaxis to cow's milk with subsequent food dependent exercise-induced anaphylaxis (FDEIA).

The foods implicated in anaphylaxis according to the age at the first episode are specified in **figure 2** and the results of further study in **table 3**. Three children had anaphylaxis with two different food groups (fish, peanut or milk associated with anaphylaxis to egg).

Milk was the most frequent cause of food anaphylaxis, with the highest incidence in children below 2 years of age. Eight of the 10 children with anaphylaxis to milk in the first year of life had no previous diagnosis of milk allergy, while 11 of the 12 children older than one year had a previous diagnosis of allergy to cow's milk proteins (CMP), with 82% of the first episodes of anaphylaxis in this age group occurring in the context of accidental ingestion: food with trace amounts of milk in restaurants and at school; dairy products, such as yogurt, butter and cheese; milk-containing cookies and bread. A 5 year-old child diagnosed with allergy to CMP had anaphylaxis after goat milk ingestion at home by rec-

Table 2 - *Causes and patients' characteristics of non food-induced anaphylaxis.*

Etiological agent	Age[1]	Sex	Atopy	Complementary study
Drug-induced anaphylaxis (n = 5)				
NSAID: paracetamol	8 years	M	Yes	negative sIgE, SPT, ID and CAST
ibuprofen	3 years	M	Yes	n.p.
ibuprofen	5 years	F	No	n.p.
ASA	16 years	F	Yes	n.p.
Amoxicillin	3 years	F	Yes	sIgE MDM = 0.13kU/L; positive ID[2]
Cold-induced anaphylaxis (n = 3)				
Cold drink	14 years	F	Yes	positive ICT at 10 minutes
Plunge in the sea	14 years	M	Yes	positive ICT at 3 minutes
Plunge in the sea	2 years	F	No	positive ICT at 3 minutes
FDEIA (n = 2)				
Soy and green bean	11 years	M	Yes	positive SPT to soy and green bean
Cow's milk	17 years	M	Yes	positive SPT and sIgE to cow's milk, alpha-lactalbumin, beta-lactoglobulin and casein[3]
Latex allergy (n = 1)				
Peri-operative, chestnut and kiwi	6 years	F	Yes	Positive SPT to latex, chestnut and kiwi; positive sIgE to latex, rHev b 1, rHev b 3, rHev b 5, rHev b 6.01 and rHev b 6.02[4]
Allergy to insect sting (n = 1)				
Mosquito (3 reproducible reactions)	3 years	M	Yes	negative sIgE to mosquito

[1]Age at the first reaction.

[2]Second anaphylaxis episode during the intradermal test with amoxicillin (2.5 mg/mL), performed after negative skin prick tests to amoxicillin, PPL, MDM and penicillin G.

[3]Adolescent who underwent oral tolerance induction to cow's milk and tolerates cow's milk at rest.

[4]Patient with history of 22 surgeries for spina bifida and latex-fruit syndrome, currently under sublingual latex immunotherapy.

M: male; F: female; NSAID: non-steroidal anti-inflammatory drug; ASA: acetylsalicylic acid; SPT: skin prick tests; ID: intradermal tests; sIgE: specific immunoglobulin E; CAST: cellular antigenic stimulation test; n.p.: not performed; MDM: penicillin allergens (*minor* determinants); ICT: ice cube test; FDEIA: food-dependent exercise-induced anaphylaxis.

ommendation of a non-allergist doctor. In those eight children with anaphylaxis to milk in the first year of life who had no previous diagnosis of milk allergy, the anaphylactic reaction occurred: after the first intake of milk-containing puree (between 4 to 6 months age) in 4; after the first intake of adapted milk formula (between 4 to 11 months age) in 3 (2 with previous exclusive breastfeeding and 1 with previous partially hydrolysed formula); and 1 one-month-old hospitalized child after adopted milk formula intake.

At the time of the study, children with CMP anaphylaxis had a median age of 8 years (5 months to 17 years old). Thirteen (59%) underwent an oral tolerance induction protocol, which was effective in all cases, with current tolerance of 200 ml of cow's milk per day and the possibility of free diet. In these children submitted to oral tolerance induction the median milk-specific IgE level at the time of diagnosis was of 25.8 kU/L (1.43 to > 100 kU/L).

Of children with anaphylaxis to egg, only one had a previous diagnosis of allergy. Of the 7 children, 3 (43%) acquired natural tolerance to the whole egg, 2 children aged 4 and 6 years are in absolute egg avoidance and 2 children aged 2 and 6 years are avoiding egg white while tolerating egg yolk.

Of the total of patients studied, 53 (83%) were concluded to have had an IgE-mediated reaction, corresponding to 51 (94%) of patients with food-induced anaphylaxis.

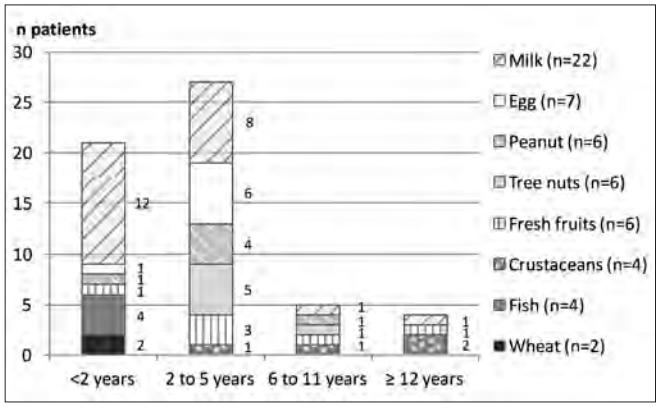
Table 3 *- Foods implied in food-induced anaphylaxis.*

Food	Age at the first reaction[1]	IgE-mediated n (%)	
Milk (41%) (cow's milk 21, goat's milk 1)	1 year (1 month - 15 years)	21	(95%)
Egg (13%) (whole egg 4, raw egg 2, egg white 1)	3 years (10 months - 4 years)	7	(100%)
Peanut (11%) (n = 6)	4 years (10 months - 6 years)	6	(100%)
Tree nuts (11%) (pine nut 2, walnut 2, cashew 1, hazelnut 1)	3 years (2-6 years)	6	(100%)
Fresh fruit (11%) (apple 1, banana 1, kiwi 1, melon 1, papaya 1, pineapple 1)	4 years (1-17 years)	5	(83%)[2]
Crustaceans (7%) (shrimp 3, barnacle 1)	11 years (5-16 years)	4	(100%)
Fish (7%) (n = 4)	1 year	2	(50%)
Wheat (4%) (n = 2)	6 months	2	(100%)

[1]Median (minimum-maximum).

[2]Skin prick test (with the extract and the fresh fruit) and specific IgE negative to kiwi.

Figure 2 *- Causes of food-induced anaphylaxis according to the age at the first reaction*



Discussion

This study, conducted in an outpatient allergy department, confirms IgE-mediated food allergy as the leading cause of anaphylaxis in children, accounting for more than three-quarters of reported cases. Other identified causes were drug allergy (anti-inflammatory and beta-lactam antibiotics), cold, exercise, latex and insect bite.

Compared to the study by Morais-Almeida *et al.* (17), performed with a similar methodology and duration, we observed an increase in the prevalence of anaphylaxis from 1.3% to 1.8% over a 5-year interval. Bearing in mind that notification was based on voluntary participation, it is admissible that some cases may not have been reported, thus, any deviation from our estimation will be by default.

Regarding the causes of anaphylaxis, the results are in agreement with previous studies conducted in outpatient allergy clinics in Portugal (17,23) and in a paediatric emergency department in Australia (16). In these studies, the main causes of anaphylaxis were foods in 71 to 85%, drugs in 6 to 11%, and insects in 3 to 6%. In children hospitalized for anaphylaxis in Israel (24), foods were also the most frequent cause (43%), although allergy to drugs (22%) and to insect venom (14%) were more frequent. In the anaphylaxis survey carried out by the Latin American Society of Asthma, Allergy and Immunology (SLAAI), by applying the OLASA survey (Online Latin American Survey of Anaphylaxis) in children, the foods were also the most frequent cause, although with a higher reported frequency for drugs (28%) and insect sting (26%) allergy (25).

Among foods, milk was the most often implicated cause of anaphylaxis, as reported in previous studies (43-53%) (16,17,23),

being more frequent in the first years of life (24). According to Silva *et al.* (23), milk was the causative food in 47% of children with food anaphylaxis, followed by fish and shellfish (23%), cereals and nuts (14%) and egg (9%). According to Morais-Almeida *et al.* (17), the implicated food was milk in 53% of children under the age of 15, fish in 19%, egg in 14%, crustaceans in 14%, peanut in 6%, fresh fruits in 6% and tree nuts in 3%. In the present study, we observed an increased frequency of anaphylaxis to peanut and tree nuts (11% each), with an approximation to the values found in the Australian study (18% to peanut and 17% for tree nuts) (16).

We underline the absence of anaphylaxis episodes during allergen immunotherapy protocols, confirming the good current safety of this treatment (26).

We also emphasize that, in the current study, it was possible to conclude the cause of anaphylaxis in all patients, in contrast to previous studies, where idiopathic anaphylaxis was reported with a frequency of 5 to 7% of children (16,24). This is probably related to the fact that patients were evaluated in a specialized department and to the greater availability of differentiated means of diagnosis.

The possible occurrence of systemic reactions during the diagnostic procedures, as reported in the child with immediate-onset anaphylaxis induced by intradermal test with amoxicillin (**table 2**), also reinforce the need of referring these patients to specialized centers. These tests must always be performed based on the consensus published by the European Network of Drug Allergy (ENDA)/EAACI (21), beginning with a more diluted concentration. Even though, in this child the intradermal test was accompanied by respiratory and cutaneous systemic symptoms, which was immediately treated with intramuscular epinephrine, followed by anti-histamine and corticosteroid, with total resolution.

Several studies suggest that the diagnosis of asthma is a risk factor for the occurrence of severe, potentially fatal, anaphylactic reactions to food (5,27). One limitation of this study was the fact that reactions were not categorized according to the degree of severity. However, we observed that patients with asthma had no increased frequency of respiratory symptoms or of symptoms considered more severe, such as glottis oedema, cardiovascular symptoms or loss of consciousness, and that there were no differences in attendance to the emergency department or to epinephrine administration. Nonetheless, we were not able to assess the level of asthma control before the episode, which could be the determining factor for more severe anaphylaxis.

Regarding gastrointestinal symptoms, Rudders *et al.* (28) have described that, although uniformly present in all age groups, nausea and vomiting are more common in children, occurring in 53% of children up to 2 years, 34% of preschool aged children, 29% of school aged children and only 17% of adolescents. In the present study, 89% of gastrointestinal manifestations occurred in children aged less than 3 years, which reinforces the importance of including these symptoms in the diagnostic criteria of anaphylaxis, especially at younger ages. Moreover, the absence of cutaneous symptoms, as occurred in 4 patients, does not exclude the diagnosis of anaphylaxis.

The high frequency of anaphylaxis caused by accidental exposure, in restaurants, recreational activities and at school, starting in the first 30 minutes after contact, as well as the high number of recurrences (17,23,25,29), emphasizes the importance of education of teachers, catering professionals and the general public for the rapid identification and action in this situation. In the present study recurrence of anaphylaxis happened in 41% of cases, with five children having suffered more than 4 episodes. This frequency is similar to that reported in the OLASA survey (42%) (25), but higher to the one observed by Cianferoni *et al.* (29) in a 7-year follow-up survey of children with anaphylaxis.

Moreover, the education of patients and caregivers is crucial, for the correct evaluation of the ingredients listed on labels, especially in situations of food allergy with potentially severe reactions that might occur even with trace amounts of the responsible food, such as milk, peanut and tree nuts. All patients and caregivers should be given a document containing the agents to avoid and possible alternatives, as well as the treatment to be carried out in case of accidental contact. The recognition of the signs of anaphylaxis and early and proper use of epinephrine from an auto-injector device should also be reinforced.

Despite epinephrine being recommended as the first-line treatment in anaphylaxis consensus and guidelines (5,6,27,30), and its non-utilization or delayed administration being a risk factor for biphasic, more severe anaphylaxis reactions and death (5,6,31), it remains underused in the emergency department. In our study performed 5 years earlier, the use of epinephrine was reported in 26% of patients, with an increase to 33% in the present study, similar to that reported by Solé *et al.* in the OLASA survey (34.6%) (25), but still falling short in relation to data presented in other countries (72 to 76%) (16,24). This emphasizes the importance of continuing the work started in undergraduate and postgraduate medical education and the incorporation of anaphylaxis diagnosis and treatment protocols in emergency departments. Also, the implementation of digital reports of allergy, with exchangeable information between health facilities, will be important to improve the recognition and treatment of anaphylaxis (32).

In children, there is no absolute contraindication for the administration of epinephrine, although the risk-benefit ratio should be weighed in cases of known cardiac disease (5). Self-injectable epinephrine, currently available in doses of 0.15 mg and 0.30 mg, may be prescribed in children from 7.5 kg, since apparently there is no risk of administering a higher dose than recom-

mended in a healthy child and the availability of an auto-injector epinephrine device can be life-saving (5,27). Education for the patient and caregivers on when and how to use the device is essential.

The occurrence of anaphylaxis can have a profound effect on the quality of life of the children and their family. Finally, we reinforce the importance of an adequate and streamlined referral to allergy specialists in order to improve the correct diagnosis, investigate triggers and cofactors, adopt effective preventive measures such as allergen avoidance, structure a management plan with an emergency action plan, offer alternatives, namely to foods or drugs, and implement a treatment with allergen immunotherapy (hymenoptera venom, latex) or tolerance induction (food, drug) when appropriate. Database networks promoted by scientific societies, such as the Portuguese Society of Allergology and Clinical Immunology (SPAIC), and national reporting systems such as the recently implemented Portuguese Catalog of Allergies and other Adverse Reactions (CPARA) (32) will allow the improvement of knowledge of this disease and to delineate better strategies for prevention and treatment.

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J. Liippo, K. Lammintausta

An oral challenge test with carmine red (E120) in skin prick test positive patients

Department of Dermatology and Allergy Unit, Turku University Hospital and University of Turku, Hämeentie 11, 20520 Turku, Finland

Key words

Carmine red (E120); food additive; IgE-mediated allergy; single-blind placebo-controlled oral challenge test; skin prick test.

Corresponding author
Jussi Liippo
Turku University Hospital
University of Turku
Department of Dermatology, Allergy Unit
P.O. Box 52, 20521 Turku, Finland
Phone: +358-2-313 1644
E-mail:jussi.liippo@utu.fi

Summary

Background. *Positive skin prick test reactions to carmine red (E120) occur in approximately 3% of the patients studied for food allergy. Carmine ingestion associated systemic symptoms are occasionally suspected, but sufficient information of proven carmine allergy is not available.* ***Patients and methods.*** *To analyse carmine related symptoms in skin prick test positive patients a cohort of 23 patients with suspected allergy to carmine red was subjected to a single-blind placebo-controlled oral challenge test with carmine red.* ***Results.*** *Five patients developed clinical symptoms during the placebo-controlled oral challenge. As a result, the overall frequency of clinical carmine allergy is estimated to be 0.7% in general dermatology patients studied for food-associated symptoms.* ***Conclusions.*** *Oral challenge test provides a valuable in vivo tool to better inform patients with positive skin prick tests to additives to avoid false allergy diets.*

Introduction

Carmine red is a natural food additive (E120) and a cosmetic colorant (CI 75470) that is derived as an extraction product of the cochineal insect (Dactylopius coccus Costa) (1). Immediate IgE-mediated allergic reactions (urticaria, angio-oedema, anaphylaxis and asthma) have been reported to occur following oral carmine exposure (2-6). In a majority of cases positive skin prick test (SPT) reactions to carmine red seem to occur as immunologic cross-reactions concurrently with reactions to house dust and / or storage mites (7).

We have previously shown that approximately 25% of carmine red sensitive patients have no house dust or storage mite reactions in SPTs (7). Some patients thus seem to have developed primary carmine sensitization. Carmine ingestion associated symptoms seem to occur in approximately 10-20% of patients with positive SPT results (**table 1** and ref. 7). In addition, patients experience clinical symptoms at similar frequency regardless of their IgE reactivity to dust mites, and the nature of carmine associated symptoms seems to be comparable in both patient groups.

To better analyse the degree and also true individual susceptibility of carmine-related allergic symptoms, we have subjected 23 patients with positive SPT to oral challenge test (OCT) with carmine red.

Materials and Methods

Patients and skin prick tests

During 2007-2011, 2926 patients were tested at the Department of Dermatology Turku University Hospital with carmine red (5 mg/ml) E120 (Celego, Dr. Marcus) for suspected food

Table 1 *- Oral challenge test to patients with positive skin prick test to Carmine red (E120).*

Patient	Sex/age	Carmine SPT (mm)	Histamine SPT (mm)	D.pt. / D.far. SPT	Storage mites SPT	Active Carmine avoidance before oral challenge	Symptoms	Results of oral challenge to Carmine	S-IgE [kU/l]	Other SPT reactions (=/> than histamine)
1	F/61	3	5	neg	< hist	no	none[1]	neg	53	pollen & cross-reactions
2	M/30	4	5	neg	< hist	no	none	neg	NT	pollen & cross-reactions
3	M/39	4	5	< hist	NT	no	none	neg	NT	no other reactions
4	F/42	15	4	< hist	> hist	yes	probable	neg	300	nutmeg
5	F/39	10	5	> hist	= hist	yes	suspected	pos	62	pollen
6	F/48	5	5	neg	NT	yes	possible[1]	neg	1126	pollen, animals
7	F/67	4	4	neg	< hist	no	none	neg	548	pollen & cross-r., animals, soybean
8	F/21	4	4	neg	= hist	yes	none	neg	NT	no other reactions
9	F/50	5	5	< hist	= hist	no	none	neg	59	pollen
10	F/62	5	5	= hist	NT	yes	yes2[1]	pos	NT	pollen, animals
11	F/57	5	4	neg	> hist	yes	yes3[1]	pos	NT	pollen
12	M/26	7	6	neg	= hist	yes	none	neg	81	pollen & cross-reactions, soybean
13	M/46	5	5	< hist	< hist	no	none	neg	NT	gliadin, wheat
14	M/47	4	4	< hist	NT	no[2]	yes	neg	NT	pollen & cross-r., animals
15	F/33	8	6	neg	< hist	no	yes	pos	54	no other reactions
16	M/20	4	5	< hist	< hist	no	none	neg	231	soybean
17	M/33	5	5	< hist	= hist	no	none	neg	NT	pollen & cross-r., animals
18	F/57	6	5	= hist	NT	no	none	neg	348	pollen & cross-r., soybean, animals
19	M/19	5	5	neg	< hist	partially	probable	pos	NT	pollen & cross-r., animals
20	M/30	4	4	= hist	neg	no	none	neg	25	no other reactions
21	M/33	3	4	neg	< hist	no	none	neg	NT	pollen & cross-reactions
22	M/61	4	4	< hist	> hist	no	none	neg	419	rapeseed
23	M/20	9	5	< hist	> hist	yes	none	neg	NT	no other reactions

1) Three years after oral provocation: gastrointestinal symptoms associated with carmine red containing lipstick.
2) Facial flush, stomach pain, diarrhea and tachycardia.
3) Oral mucosa associated symptoms and general itch.
[1]No symptoms after carmine red avoidance.
[2]The patient had avoided one specific carmine containing yoghurt that had caused oral symptoms
SPT: skin prick test; NT: not tested; hist: histamine hydrochloride.

allergy. The patient records were retrospectively analysed and a cohort of 23 patients (11 females; average age 48.8 years and 12 males; average age 33.7 years) having carmine red SPT reaction 3 mm or more and suspected carmine allergy were invited for an oral challenge test with carmine red solution.

All these patients had been tested with the standard series of pollen (birch, alder, timothy, mugwort) and animal (cat and dog) as well as with Dermatophagoides pteronyssinus (D. pt.) and/or Dermatophagoides farinae (D. far.) allergens (Soluprick SQ allergens; ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark). Allergy to carmine red was studied using SPT panels containing various food allergens. 18/23 patients had also been tested with storage mites (Acarus siro, Tyrophagus putrescentiae and Lepidoglyphus destructor). SPTs were carried out using positive (histamine dihydrochloride 10 mg/ml, ALK) and negative (saline, Soluprick, ALK) controls. The two largest perpendicular diameters of the wheal were measured at 15 min to calculate the mean value representing the size of SPT reaction.

Single-blind placebo-controlled oral challenge with carmine red

Oral challenge tests were performed after patients' informed consent. The study protocol was approved by the ethics committee and was in line with the ethical standards of the Helsinki declaration. A placebo control solution with similar red colour as the carmine red test doses was produced using boiled beetroot extract dissolved in water. As a food additive carmine was found to be used in 5 mg / ml average concentration in food items. **Table 2** presents the protocol of the oral challenge test. Patients had avoided antihistamines 5 days before the challenge and they had no ongoing infections. SPT with carmine red was repeated before the challenge. The challenge was started with a placebo solution, and if any subjective or objective symptoms appeared, placebo doses were repeated. Blood pressure values and peak expiratory flow (PEF) rates were measured at each step. Patients were asked to report any symptoms experienced in the mouth, lip, tongue, nasopharynx, airways or in the skin. Patient-reported subjective symptoms and objective findings were registered during the challenge. Patients were followed 1-2 hours after the challenge and they were asked to contact the clinic if any later reactions occurred. OCT positive patients were also contacted 1/2 to 4 years later. Those, who were still motivated to avoid carmine, and had remained symptom free, were regarded as OCT positive cases.

Table 2 *- Protocol of oral challenge test with carmine red (E120). Intervals between challenge steps were 20 minutes, and a final 1-hour follow up was performed after the last dose. Oral doses were 1 ml and 2 ml and the 5 mg / ml concentration of carmine red was titrated from 1:100 to 1:2.*

1	Red placebo solution (boiled beetroot extract)
2	Red placebo solution (boiled beetroot extract)
3	1:100 (carmine solution 5 mg / ml) / vol. 2 ml
4	1:10 (carmine solution 5 mg / ml) / vol. 1 ml
5	1:10 (carmine solution 5 mg / ml) / vol. 2 ml
6	1:5 (carmine solution 5 mg / ml) / vol. 1 ml
7	1:5 (carmine solution 5 mg / ml) / vol. 2 ml
8	1:2 (carmine solution 5 mg / ml) / vol. 1 ml
9	1:2 (carmine solution 5 mg / ml) / vol. 2 ml

Results

The size of the carmine reaction reached or exceeded the positive control wheal size in 18/23 patients and 15/18 of them had equal or larger carmine reactions than any of the obtained mite reactions. After the previous SPT results 8/23 patients reported to have experienced carmine ingestion associated symptoms and in 5 of them the challenge test was interpreted as positive. Majority of patients (21/23) had SPT reactions to common IgE allergens, too. 13/23 patients reacted to D. far. or D. pt. and 17/18 to storage mites in SPTs.

Cases of positive challenge results

Patient no. 5 had been remitted for relapsing urticarial rash. She had experienced palmar itch and mouth tingling associated with food ingestion. She had not experienced any mucosal symptoms, although she had positive SPT reactions to birch and mites (**table 1**). This 39-year-old female worked with fresh food products in a grocery market. During the OCT she experienced facial and palmar itch for about half an hour. Symptoms disappeared following antihistamine intake. After a six-month follow-up she had remained symptom free when avoiding carmine red.

Patient no. 10 was a 62-year-old female and she was referred to the clinic with recurrent, occasional facial rash, gastrointestinal pain and diarrhoea following food ingestion. After the SPT with carmine red was positive, the patient started to avoid carmine red and no symptoms appeared during a 6-month follow-up. Once, by chance she ingested a piece of cake containing carmine red, which resulted in facial flush, stomach pain, diarrhoea and general discomfort. During the challenge she reported tingling in the lips followed by stomach pain in about 1 hour.

Three other patients reported mild symptoms during the test. Patient no. 11 was studied for hand eczema, but she had occasionally suffered from urticarial rash. Carmine red containing candies had caused mild oral symptoms, and she had actively avoided carmine. She reported tingling in the tongue after the third (1 mg) dose in the provocation, but the latter steps remained symptomless. Patient no. 15 reported that red coloured candies and red lip stick had caused swelling in lips and gastrointestinal symptoms earlier. She developed general itch during the OCT. Patient no. 19 had suffered from recurrent urticaria and stomach pain associated with food ingestion. She experienced upper body itch during the challenge. In addition to patients no. 5 and no. 10, also these three patients reported no carmine associated symptoms following an at least 2 years' follow-up.

The measured values of PEF and blood pressure remained unchanged in all the challenged patients. All the patients received a 12.1 mg cumulative dose of carmine red (starting dose 0.1 mg and maximum dose 5 mg) regardless of the symptoms during the provocation.

Discussion

Hypersensitivity reactions and subjective intolerance symptoms to food additives are commonly reported. True IgE-mediated

type I allergy to additives is far less common. It may however also appear as immunologic cross-reaction between conserved antigenic structures (8). The prevalence of intolerance to food additives seems not to exceed the level of 4% in western countries (9-12). Oral provocations or challenge tests can be used to differentiate true allergy from suspected reactions (13,14). Among urticaria patients previous oral challenge studies have shown 0.63% prevalence of allergy to food additives and carmine allergy seems to account for one half of those allergies (12). While the prevalence of positive SPTs to carmine has been reported to be 3% (7), the frequency of carmine allergy can be estimated to be approximately 0.7% among patients studied for suspected food allergy.

SPT with carmine red were done 2 to 24 months before the OCT. Still 9/23 patients, including the challenge positive cases, reported to have actively avoided carmine red containing food before the challenge, since ingestion associated symptoms were suspected. All these patients had carmine SPT reactions the size of which reached that of the positive control wheal. In OCT 5 patients developed positive symptoms and those patients had a history of corresponding symptoms. In the follow up they remained symptom free by partial avoidance. Following the negative OCT, 4/9 patients abandoned their carmine free diet. None of them reported symptoms, although they were encouraged to report if any carmine associated symptoms appeared. Our material suggests that challenge positive cases may have primary carmine sensitization, since mite reactions were smaller in all the cases with positive OCT. However, strong mite reactions may also lead to carmine ingestion related symptoms. Unfortunately, cross-inhibition studies, that may have helped to more accurately study the importance of immunologic cross-reaction between mite and carmine red epitopes, were not carried out in this study.

Like patient no. 15, patient no. 1 had used carmine (C.I. 75470) containing red lipstick at least three times preceding her symptoms. She had experienced local swelling and angioedema in the face, but in OCT she did not experience any symptoms. Only local symptoms after repeated applications are expected in sensitized subjects. According to the registry of the Helsinki Asthma and Allergy Association 40 lip sticks that are marketed in Finland contain carmine red as well as some make up creams. Immediate type symptoms in the face and lips are probably regarded as irritation in most cases.

There are no standardized methods to study allergy to food additives using oral challenges. If strong reactions are unlikely and broadening of the diet is desired, it is easier to start with higher concentrations and amounts of the allergen. Still, the interpretation of mainly subjective and often mild symptoms is difficult when provocation tests are being planned. In our previous report (7), carmine-associated symptoms were suspected in 8/94 patients at the time of initial skin testing. Two of them had developed anaphylaxis, while the others had suffered from urticaria or angioedema. The size of the carmine reaction in SPT was not less than histamine wheal in those who experienced carmine ingestion associated symptoms. The present report supports the importance of oral provocation test if suspected food allergy causes restrictions and constraint in normal life. As patients with history of anaphylaxis were not included this study, severe reactions were not found in the oral challenge.

Unspecific non-immunologic reactions are likely to generate food additive related symptoms via unknown mechanisms. Still, the amount of patients with challenge-proven clinical symptoms due to food additives appears to be rather low as earlier shown for e.g. tartrazine and sodium benzoate (15,16). Our patient cohort was chosen based on positive SPT results for carmine red. To our knowledge there is no regulation concerning the amount of E120 in food items. It can be argued that a proportion of our patients may have required additional steps with higher carmine concentrations or a larger cumulative dose (exceeding the used 12.1 mg) to reach the symptoms-eliciting allergen dose. On the other hand, false negative findings might partially be influenced by a specific oral tolerance induction (SOTI) that may appear during an oral provocation analysis with increasing concentrations of the orally administered antigen (17). The possibility of real and more gradual tolerance induction can neither be excluded between the initial SPTs and OCTs. Still, the total amount of carmine red used in our provocation test was almost 10-fold more compared to the amount (1.3 mg) that was enough to cause anaphylaxis in a patient reported earlier (2).

Potential concomitant intake of acetylsalicylic acid (ASA) has been reported to increase the risk of food-dependent severe allergic reactions following exercise (18). Also other non-steroidal anti-inflammatory drugs and alcohol intake increase the risk of anaphylaxis in patients prone to develop severe food-induced immediate allergic reactions (19). Although carmine ingestion associated exercise-induced anaphylaxis or urticaria has not been reported in the literature, a combination of ASA intake with carmine ingestion could improve the accuracy of carmine OCT or lower the reaction threshold in subjects who have no ASA hypersensitivity.

Conclusions

Oral challenge test combined to preceding SPT provides a useful tool to discriminate between true symptomatic allergy and other cases having mere SPT reactivity or milder reactions resulting from either immunologic cross-reaction or other unspecific hypersensitivity conditions. As a result, number of unnecessary or even false elimination diets can hopefully be reduced.

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Conflict of interest

The authors have neither economic nor any other type of conflicts of interest. The authors have received no special funding for the study.

R. Asero

Detection of risk factors for systemic adverse reactions to SCIT with natural depot allergen extracts: a retrospective study

Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI), Italy

Key words

Allergen specific immunotherapy; subcutaneous immunotherapy; systemic reactions; airborne allergy; grass pollen; ragweed pollen.

Corresponding author
Riccardo Asero
Ambulatorio di Allergologia,
Clinica San Carlo
Via Ospedale 21
20037 Paderno Dugnano (MI), Italy
Phone: +39 02 990 38 470
Fax: +39 02 990 38 223
E-mail: r.asero@libero.it

Summary

Background. Some patients seem to show a particular propensity to experience systemic reactions (SR) when undergoing SCIT. This study looked at their features. Methods. 423 adults submitted to subcutaneous immunotherapy (SCIT) with 583 depot allergens extracts were studied. A "slow" build-up schedule was followed, and maintenance doses were given monthly. No mixtures of allergens were employed; multi-sensitized patients were treated with two extracts at the same time. IgE to pollen allergen components were measured. Patients experiencing several SR and those showing repeated large local reactions preventing up dosing were analyzed. Results. Altogether, 14% of patients experienced at least 2 SR to SCIT and further 13% repeated local reactions. All SR involved the skin. Eight treatments were stopped. No reactor was using beta-blockers. SR were not associated with pollen season, use of freshly prepared vials, administration of 2 allergens, or extract producer, nor were preceded by large local reactions. Reactors were younger than tolerant subjects ($p < 0.05$), and females were less frequently fully tolerant than males ($p < 0.001$). The multiple regression analysis showed that both ragweed and grass SCIT were significantly associated with adverse reactions ($p < 0.001$). Specific IgE to Amb a 1 or Phl p 1 did not differ statistically between reactors and tolerant subjects, whereas grass pollen-allergic reactors showed higher levels of IgE to Phl p 5. Intolerance did not depend on the number of primary sensitizations or on hypersensitivity to pollen pan-allergens. Conclusion. Young patients or women hypersensitive to grass and ragweed pollen seem at higher risk for SR during SCIT.

Introduction

Allergen-specific immunotherapy (SIT) is presently the only treatment able to change the natural history of respiratory allergic disease (1,2). Subcutaneous immunotherapy (SCIT) reduces rhino-conjunctivitis and asthma symptoms induced by allergen exposure, improves the quality of life, and may prevent the progression of the disease towards asthma (3). The only major concerns with allergen SCIT are adverse reactions. In previous studies, the frequency of systemic reactions induced by SCIT has been largely variable, depending on the allergen administered, treatment schedule, dose given, allergen standardization and clinical conditions before the start of the treatment (4-7). A recent survey carried out in Italian allergy centers concluded that SCIT is quite safe, as systemic reactions occurred only in 3.6% of patients and 0.15% of injections in more than 2000 courses (8) in accordance with other European studies (9-11). Nonetheless, a fraction of systemic reactions still remains and seems unavoidable and unpredictable. Particularly, in the clinical practice, along with adverse reactions that may occur in otherwise SCIT-tolerant patients possibly as the result of administration or of dosing errors, there are some patients showing a special, persisting intolerance to the treatment characterized by repeated adverse reactions even at low doses. The present study

analyzed retrospectively the outcome of SCIT in a large number of patients with the aim to investigate the clinical features of this latter population.

METHODS

Patients

The study involved mainly adult patients with respiratory allergy submitted to SCIT according to ARIA and WHO recommendations (1,2) for at least 2 years during the last 8 years. A minimum of 2 years of treatment duration was chosen in order to exclude from the analysis all patients that dropped out due to poor compliance short after starting SCIT without experiencing any adverse events, as these patients would have altered the overall prevalence of adverse reactions in the population studied.
No patient included in the present study had undergone allergen immunotherapy before. Respiratory allergy was diagnosed in the presence of an unequivocal clinical history of seasonal and or perennial rhinitis with or without conjunctivitis and/or asthma associated with a positive reaction on skin prick tests (SPT) with one or more commercial extracts out of a large panel of seasonal and perennial airborne allergens (Allergopharma, Reinbeck, Germany). The panel tested included timothy, mugwort, short ragweed, pellitory, plantain, birch, olive and cypress pollen (all 50000 BU/ml), house dust mite (HDM), *Alternaria tenuis* (10000 BU/ml), cat and dog dander (both 50000 BU/ml). SPT were carried out and read at 15' following EAACI guidelines. Wheals exceeding 3 mm in mean diameter were considered positive. All asthmatic patients prescribed allergen specific immunotherapy had a controlled disease at the start of SCIT and throughout the whole treatment period; further, no patient was using beta blockers during SCIT course.
Four-hundred-twenty-three patients with respiratory allergy (M/F 207/216; mean age 39.6 years, range 12-78 years) entered the study.

In-vitro diagnostics

The measurement of IgE specific both for markers of primary sensitization (rPhl p 1, rPhl p 5, rArt v 1, rAmb a 1, rPar j2, rBet v 1, rOle e 1, and rCup a 1) and for markers of sensitization to cross-reacting pollen pan-allergens (rPhl p 7 for polcalcin, and rPhl p 12 for profilin) has become available during the last 5 years in our Clinic. Patients showing skin reactivity to > 3 seasonal allergen sources (12) and willing to undergo allergen specific immunotherapy underwent these in-vitro tests in order to decide the most correct treatment(s). In case of IgE reactivity to multiple allergens the SCIT treatments were chosen on the basis of both clinical severity of symptoms and correspondence between positive allergen sources and seasonality of symptoms. Specific IgE were also measured in some patients showing few sensitizations on SPT, particularly in those with late summer symptoms hypersensitive to both ragweed and mugwort on SPT in order to discriminate between co-sensitization to and co-recognition of these two allergen sources (13). Specific IgE were measured by ImmunoCAP EIA (ThermoFisher Scientific, Uppsala, Sweden) following producer's recommendations and were expressed as kUA/L. Values < 0.35 kUA/L were considered negative.

Subcutaneous immunotherapy

All patients were treated with extracts of natural unmodified allergens in depot formulation (adsorbed on aluminum hydroxide or calcium phosphate). Standardized commercial allergen extracts from the following producers were used: Allergopharma, Reinbeck, Germany; Stallergenes, Anthony, France; Lofarma Allergeni, Milan, Italy; Hal Allergy, Leiden, The Netherlands; ALK, Horsholm, Denmark; Abellò, Madrid, Spain. Treatments and allergens given are summarized in **table 1**.
During the build-up phase, weekly injections were administered with the aim to reach the maximum tolerated dose (the so-called "optimal dose") within the upper limit recommended by the producer. Maintenance doses were given on a monthly basis. In pollen-allergic patients maintenance doses were reduced (14) by 50% during the pollen season of this geographical area (from mid-February to mid-April for cypress; from the beginning of March to mid-May for birch; from the end of April to the end of June for both Grass and Parietaria; and from mid-August to the end of September for both ragweed and mugwort). The dosage was properly reduced also in case of systemic adverse reactions. No patient did pre-medication before SCIT injections. After each injection, patients were kept under medical surveillance for 30 min. All data, including allergen extract dosage, local and systemic reactions were recorded. The same physician (R.A.) gave all shots and was also responsible for the treatment of all SCIT-induced adverse reactions. Patients allergic to several allergen sources and requiring more than one SCIT were treated with two distinct extracts who were administered one per arm at the same time. Allergen mixtures of non-homologous allergen sources were not employed for the treatment: the only mixtures used were grass pollen mix, a birch-hazel-alder pollen mix, and a mixture of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. All patients gave an informed written consent before the start of treatment. Since the study was retrospective and based on routine clinical activity, a formal approval by the local Ethical Committee was not required. The internal Review Board approved the study.

Table 1 *- SCIT courses, allergen extracts used, and producers.*

	Total	Allergopharma	Abellò	ALK	Lofarma	Stallergenes	Hal
Total SCIT	583	403	17	21	32	100	10
Grass	143	99	3	7	19	9	6
Ragweed	270	175	8	3	6	78	0
Birch	80	67	1	6	1	4	1
Mugwort	13	11	0	0	0	2	0
Pellitory	15	10	0	0	5	0	0
Cypress	4	0	0	1	0	3	0
HDM	38	26	3	3	1	3	2
Alternaria	8	8	0	0	0	0	0
Cat	9	7	1	1	0	0	0
Dog	3	0	1	0	0	1	1

Grading of adverse reactions and patients classification

Both immediate (occurring within 30 min) and delayed (occurring after 30 min) systemic adverse reactions, were graded following the recent World Allergy Organization Subcutaneous Systemic Reactions Grading System (15). Briefly, Grade 1 includes symptoms/signs of one organ system (either cutaneous, upper respiratory, or conjunctival); Grade 2 includes either symptoms/signs of more than one organ system or lower respiratory, or gastrointestinal, or uterine cramps; Grade 3 includes asthma not responding to inhaled bronchodilator or laryngeal, uvular, or tongue angioedema; Grade 4 includes respiratory failure or hypotension with or without loss of consciousness; Grade 5 corresponds to death. Only patients experiencing more than one systemic reaction during the SCIT course were considered in this study. In some cases, SCIT had to be withdrawn due to repeated systemic reactions. The occurrence of repeated, severe large local reactions that prevented the up dosing of the SCIT throughout the whole treatment course was recorded as well.

Statistics

Means were compared non-parametrically by the Mann-Whitney U-test. Proportions were compared by the chi-square test with Yates' correction. Multiple stepwise logistic regression was applied to evaluate possible risk factors for systemic SCIT-induced reactions. Adjusted odds ratio (OR) and its 95% confidence interval (95% CI) were calculated. STATA 12.1 (StataCorp LP, College Station, Texas, USA) was used for this analysis. Probability values less than 5% were considered significant.

RESULTS

General findings

583 SCIT treatments were administered to the 423 patients included (i.e., 160 patients underwent SCIT with two distinct allergen sources at the same time). Allergens administered, extract producers, as well as standardization and major allergen concentration of the extracts are shown in **tables 1** and **2**. Altogether, 60/423 (14%) patients experienced at least 2 systemic adverse reactions to SCIT; 29 patients had Grade 1 reactions only, 18 Grade 2 reactions only, 12 both Grade 1 and Grade 2 reactions, and 1 patient experienced Grade 1, 2 and 4 reactions. Most systemic reactions were characterized by urticaria/angioedema only (Grade 1 reaction after WAO classification); in some cases, skin symptoms were associated with slight rhinitis or asthma (Grade 2 reactions). Both Grade 1 and 2 reactions were quite easily controlled by the use of injection antihistamines and short-acting beta-agonists. No cases of severe asthma occurred. One patient experienced one episode of hypotension associated with skin symptoms that responded promptly to epinephrine (Grade 4). The severity and/or recurrence of systemic adverse reactions led to stop the treatment in 8 cases. Adverse reactions did not occur preferentially during the pollen season, were not associated with the use of freshly changed vials or with new batches of allergen, and were not preceded by large local reactions in most cases. Further 54 patients (13%) experienced repeated large local reactions upon SCIT administration that, though not compromising efficacy, prevented the up dosing throughout the whole therapy course of 2-4 years. Altogether 309/423 patients did

Table 2 *- Standardization and major allergen concentration (if available) of the maintenance vial of the commercial extracts for SCIT used in study patients.*

	Allergopharma (Novo Helisen Depot)	**Abellò (Pangramin)**	**ALK (Alutard SQ)**	**Lofarma (AlOH Retard)**	**Stallergenes (Phostal)**	**HAL Allergy (DepotHAL)**
Grass	5000 UT/ml	1000 STU/ml	100000 USQ/ml	10000 U/ml	10 IR/ml	20000 AU/ml
(Phl p 5 µg/ml)	(n.a.)	(2.5)	(20.2)	(n.a.)	(0.7)	(n.a.)
Ragweed	2500 PNU/ml	1000 STU/ml	100000 USQ/ml	10000 U/ml	10 IR/ml	-
(Amb a 1 µg/ml)	(n.a.)	(9.0)	(n.a.)	(n.a.)	(10.0)	
Birch	5000 UT/ml	1000 STU/ml	100000 USQ/ml	10000 U/ml	10 IR/ml	20000 AU/ml
(Bet v 1 µg/ml)	(20.0)[1]	(22.5)	(12.3)	(n.a.)	(5.0)	(n.a.)
Mugwort	5000 UT/ml	-	-	-	10 IR/ml	-
(Art v 1 µg/ml)	(n.a.)				(n.a.)	
Pellitory	5000 UT/ml	-	-	10000 U/ml	-	-
(Par j 1 µg/ml)	(n.a.)			(n.a.)		
Cypress	-	-	100000 USQ/ml	-	10 IR/ml	-
(Jun a 1 µg/ml)			(n.a.)		(10.0)	
HDM	5000 UT/ml	1000 STU/ml	100000 USQ/ml	10000 U/ml	10 IR/ml	20000 AU/ml
(Der p 1 µg/m)	(n.a.)	(4.0)	(9.8)	(n.a.)	(2.0)	(n.a.)
(Der p 2 µ/ml)	(n.a.)	(2.0)	(0.7)	(n.a.)	(0.4)	(n.a.)
Alternaria	2500 PNU/ml	-	-	-	-	-
(Alt a 1 µg/ml)	(n.a.)					
Cat	2500 PNU/ml	1000 STU/ml	100000 USQ/ml	-	-	-
(Fel d 1 µg/ml)	(n.a.)	(2.0)	(14.6)			
Dog	-	1000 STU/ml	-	-	10 IR/ml	20000 AU/ml
(Can f 1 µg/ml)		(n.a.)			(2.0)	(n.a.)

n.a. = information not available. [1] = personal communication.

not experience systemic reactions nor repeated local reactions and reached the scheduled recommended doses.

The age analysis showed that patients experiencing systemic adverse reactions were significantly younger than those who did not (mean age 35.4 years [range 13-70] vs 40.2 years [12-78], respectively; $p < 0.05$). Such difference increased if patients who never experienced systemic reactions were divided into local reactors (mean age 37.3 years [range 13-75]; p = NS vs SCIT reactors) and fully tolerant subjects (mean age 40.8 years [range 12-78]; $p < 0.01$ vs SCIT reactors).

The gender analysis demonstrated that male patients were more frequently fully tolerant to SCIT than female patients (170/207 [82%] vs 138/ 216 [64%], respectively; $p < 0.001$), although this was due more to a lower prevalence of local reactions (13/207 [6%] vs 41/216 [20%], respectively; $p < 0.001$) than to a difference of systemic reactions (24/207 [12%] vs 36/216 [17%], respectively; p = NS).

The effect of sex and age was further investigated by multiple regression analysis, which confirmed that both younger age and female sex were associated with adverse reactions induced by SCIT ($p < 0.001$).

Looking at the possible link between systemic reactions and the number of SCIT administered it was found that patients treated with one or two extracts did not show any differences (43/263 [16%] vs 17/160 [11%], respectively; p = NS).

Table 3 shows SCIT tolerance in the whole study group. There was a marked prevalence of SCIT treatments with ragweed and grass pollen, which strictly reflected the frequencies of airborne allergies in this geographic area. Grass and ragweed were also the two allergens that caused most cases of adverse reactions as a whole and were characterized by the highest frequencies of adverse reaction. The multiple regression analysis showed that both ragweed and grass SCIT (adjusted by age and gender) were significantly associated with adverse reactions (OR 3.6, CI 95%

Table 3 *- Tolerance of allergen specific immunotherapy administered to the study population.*

	Total	Tolerated	Local reactions	Systemic reactions
Total SCIT	583	449 (77%)	58 (10%)	77 (13%)
Grass	143	101 (70%)	16 (11%)	26 (18%)[1]
Ragweed	270	197 (72%)	35 (13%)	38 (14%)[1]
Birch	80	70 (88%)	4 (5%)	6 (8%)
Mugwort	13	11 (85%)	0 (0%)	2 (15%)
Pellitory	15	12 (80%)	1 (7%)	2 (13%)
Cypress	4	4 (100%)	0 (0%)	0 (0%)
HDM	38	36 (93%)	1 (3%)	1 (3%)
Alternaria	8	8 (100%)	0 (0%)	0 (0%)
Cat	9	7 (78%)	1 (11%)	1 (11%)
Dog	3	2 (66%)	0 (0%)	1 (33%)

P < 0.01 for grass + ragweed vs all other treatments.

Table 4 *- Mean maximum tolerated doses of SCIT.*

	Tolerant	Local reactions	Systemic reactions
Ragweed (n = 270)	0.76 [0.4 - 1]	0.41 [0.1 - 0.75]	0.26 [0.02 - 0.35][1]
Grass (n = 143)	0.7 [0.3 - 1]	0.3 [0.05 - 1]	0.24 [0.02 - 0.7][1]
Mugwort (n = 13)	0.78 [0.6 - 1]		0.08 [0.02 - 0.15][1]
Pellitory (n = 15)	0.8 [0.3 - 1]	0.05	0.12 [0.05 - 0.2][1]
Birch (n = 807)	0.79 [0.25 - 1]	0.13 [0.05 - 0.2]	0.38 [0.08 - 0.7][1]
HDM (n = 38)	0.8 [0.5 - 1]		0.3[1]

Doses are expressed as ml of final vial.

[1]p < 0.001 vs tolerant patients.

2.0-6.4; p < 0.001 for ragweed) (OR 3.1, CI 95% 1.7-5.8; p < 0.001 for grass). In contrast, perennial airborne allergens other than animal dander (i.e., house dust mite and *Alternaria tenuis*) were very rarely involved in adverse reactions.

The 8 patients who stopped the treatment due to severe and repeated systemic adverse reactions were being treated with 12 extracts: grass (n = 6), ragweed (n = 2), pellitory (n = 2), birch and mite (n = 1 each), although, notably, both pellitory and the mite treatments were being given in association with grass pollen SCIT. In one patient treated with two extracts, ragweed SCIT was withdrawn due to repeated systemic adverse reactions while grass pollen SCIT was continued without any further problem.

Not surprisingly, the mean maximum tolerated doses of SCIT were significantly lower in patients who experienced systemic adverse reactions than in tolerant patients (p < 0.001 in all cases; **table 4**).

In order to detect possible differences between allergen extracts from different producers, the adverse reactions induced by SCIT with ragweed and grass pollen extract were re-analyzed based on the commercial extract used for the treatment but the statistical analysis did not show any difference in the prevalence of systemic adverse reactions between extracts from different producers. Further, the analysis of the SCITs carried out using the extracts from the most frequently employed producer (Allergopharma) confirmed the significant prevalence of seasonal allergens as a cause of systemic adverse reactions (systemic reactions: p < 0.025 for seasonal vs perennial allergens).

Specific IgE measurements

The possible association between specific IgE levels and adverse reactions upon SCIT administration was investigated for grass and ragweed pollen, the two allergens inducing the majority of systemic adverse reactions.

a. Ragweed pollen SCIT

IgE measurements were available for 65 patients. Baseline Amb a 1-specific IgE levels ranged between 3.9 and > 100 kU/L (median 42.5 kU/L), and did not show any difference between patients who experienced systemic reactions upon SCIT administration (n = 5; median 58.9 kU/L, range 6.1-94.1), those who experienced repeated local reactions (n = 7; median 39.0 kU/L, range 7.1 - 100), and those who tolerated the treatment well (n = 53; median 41.6 kU/L, range 3.92 - 100). Patients with and without a history of SCIT-induced systemic reactions did not differ in the mean number of primary sensitizations to allergen sources other than ragweed, nor in the prevalence of sensitization to any specific allergen source other than ragweed (data not shown). Further, SCIT-induced systemic adverse reactions were not influenced by the presence or absence hypersensitivity to the pollen pan-allergens, profilin and/or polcalcin.

b. Grass pollen SCIT

IgE measurements were available for 48 patients submitted to SCIT with grass pollen extract. Baseline IgE levels ranged between 2.5 and > 100 kU/L for Phl p 1 and 0 and > 100 kU/L for Phl p 5, respectively. Two out of the 9 patients who experienced systemic reactions had to stop SCIT due to their severity. The levels of IgE specific for Phl p 1 did not statistically differ between patients with a history of systemic reactions (n = 9; median 40.0 kU/L, range 9.1 - 94.8), local reactions (n = 6; median 25.8 kU/L; range 14.3 - 44.8), or good tolerance to the treatment (median 19.3 kU/L; range 2.5 - 100). In contrast, IgE specific for Phl p 5 were higher in subjects with a history of systemic reaction to SCIT (median 42.4 kU/L; range 14.6 - 100), than in those with a history of local reactions (median 28.4 kU/L; range 6.04 - 67.4), or those who tolerated SCIT well (median 8.7 kU/L; range 0 - 100). The difference was statistically significant ($p < 0.05$). Patients with and without a history of SCIT-induced systemic reactions did not differ in the mean number of primary sensitizations to allergen sources other than grass pollen, nor in the prevalence of sensitization to any specific pollen source other than grass pollen. Finally, grass pollen SCIT-induced adverse reactions were not influenced by co-recognition of the pollen pan-allergens, profilin and/or polcalcin.

DISCUSSION

Systemic reactions are considered to a certain extent an unavoidable risk associated with SCIT (7). Specific risk factors associated with systemic reactions include poor asthma control, concomitant medication (particularly beta-blockers), lack of dose adjustment during the pollen season, type of build-up protocol, and both administration and dosing errors (16). In the clinical practice, some patients seem to show an unexplainable propensity to react repeatedly and severely to SCIT in the absence of any of the risk factors summarized above. A second group of patients shows large local reactions even at low doses that prevent up dosing of the treatment; these patients also would probably experience systemic reactions if higher doses were given. Finally, the majority of patients submitted to SCIT experience slight local reactions, more rarely mild urticaria or asthma episodes, in most cases during the build-up phase, but eventually tolerate high doses of allergen extract for a long time without further problems. The present retrospective study tried to better characterize the clinical features of the patients belonging to the former group in order to detect risk factors for systemic reactions to SCIT. Notably, none of the patients with systemic reactions had severe asthma attacks following the shots; this is in keeping with the fact that no patient was taking beta-blocking agents, showed a poor asthma control, or was given high doses of allergen during the pollen season, all conditions that have been associated with respiratory adverse reactions. The analysis of our data ruled out some potential risk factors such as the number of extracts administered at the same time, the producer of the allergen extract, the number of baseline primary sensitizations to different allergen sources, hypersensitivity to the pollen pan-allergens profilin and polcalcin and, importantly, also the level of IgE specific for the major allergen of the extract administered (Amb a 1 and Phl p 1 for ragweed and grass pollen, respectively). However, interestingly, patients with a history of systemic and local reactions to grass pollen SCIT showed significantly higher levels of IgE to Phl p 5, another major allergen, than tolerant subjects. Previous studies found that a high degree of allergen sensitivity represents a risk factor for systemic adverse reactions (17-19). It is therefore possible that in the case of grass pollen allergy, IgE to allergens other than Phl p 1 play a role in increasing patients' reactivity to the treatment. In this study only IgE to Phl p 1 and Phl p 5 were measured; it cannot be excluded that IgE reactivity to one of the other currently available specific grass pollen allergens (i.e.; Phl p 2, Phl p 4, Phl p 6 or Phl p 11) may be also a risk factor for SCIT intolerance. In effect, studies carried out in children showed that the IgE response to grass pollen develops from Phl p 1 and only in a subsequent stage spreads to other allergens (20). It is therefore probable that high levels of IgE to allergens other than Phl p 1 are a marker of a heavier immune response to this allergen source. Further, allergen specific nasal/ocular provocations, along with quantitative measurement of SPT, would have theoretically provided two alternative means to assess a hyper-reactive state to be plotted against SCIT tolerance/intolerance but, unfortunately, such measurements were not carried out.

In this study, female gender was associated with a worse tolerance of SCIT, and systemic adverse reactions occurred more

frequently in younger patients as well as in subjects submitted to SCIT with seasonal allergens, particularly ragweed and grass. The lower tolerance to SCIT by female patients is in keeping with a number of other clinical conditions of allergological interest characterized by mast cell degranulation where a clear female prevalence can be observed, including chronic spontaneous urticaria, food allergy, respiratory allergy, and hypersensitivity to non-steroidal anti-inflammatory drugs (21-25). The higher rate of reactivity to pollen allergens (particularly grass and ragweed) than to perennial allergens is a novel finding and is not easy to explain. The possibility that commercial extracts of perennial allergens for SCIT may contain a relatively lower concentration of allergen proteins or of major allergens seems unlikely as each producer adopts the same standardization procedures in-vivo and in-vitro for all the allergens. Further, hypersensitive patients show equally elevated specific IgE levels for either seasonal or perennial allergens. Altogether, one gets the impression that grass and ragweed pollen allergens may possess an intrinsically higher ability to induce histamine release from mast cells and basophils of hypersensitive patients than allergens from house dust mite or molds although, clearly, further studies are needed to confirm this hypothesis.

In conclusion, young patients, and women hypersensitive to grass and ragweed pollen seem subsets at higher risk for systemic adverse reactions during SCIT. In grass-allergic patients, IgE to allergens other than Phl p 1 seem one further risk factor for SCIT adverse reactions.

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C. Nonotte-Varly

Allergenicity of Artemisia contained in bee pollen is proportional to its mass

Centre hospitalier Marie-José Treffot, avenue du Maréchal Juin, Hyeres, France

Key words

Allergenicity; Artemisia pollen; bee pollen; biological potency; melissopalynology.

Corresponding author
C. Nonotte-Varly
Centre hospitalier Marie-José Treffot
Avenue du Maréchal Juin
BP 50082, 83407 Hyeres cedex, France
Phone: +33 49 408 6182
E-mail: allergologue@limperialsante.net

Summary

Bee product mugwort is identified as being at the origin of allergic accidents but the biological potency of Artemisia contained in bee pollen is not well known. In this experiment, Artemisia mass was identified in bee pollen mass and after having calculated the proportion of Artemisia using the bee pollen melissopalynology spectrum. Skin reactivity to Artemisia was assessed by measuring wheal diameters (W) from skin prick tests using three serial dilutions of bee pollen on 11 allergic patients to Artemisia, in order to calculate the relationship between Artemisia mass ($Mass_{artemisia}$) in bee pollen and skin reactivity.

The dose-response power regression curve ($W_{artemisia}$) = 3.328 ($Mass_{artemisia}$)$^{0.297}$ (R2 = 0.9947) and the linear function Log_{10} ($W_{artemisia}$) = 0.297 (Log10 ($Mass_{artemisia}$) + 0.520 (R = 0.9974)) were established using a bee pollen sample with 0.246 mg of Artemisia pollen per mg. Mugwort allergens seem to be little or not altered by bee secretions and bee pollen retains its allergenic capacity.

To our knowledge this is the first time it has been shown that skin reactivity of patients allergic to mugwort is proportional to the absolute mugwort mass contained in the bee pollen.

Introduction

Pollen is flower sperm. It is the only source of certain macronutrients collected by worker honeybees. Collected from floral anthers at the tips of stamens, flower pollen grains stick to bee secretions. They are then assembled by the bee in loads placed in the baskets of the hind legs of the insect. Each load has a weight of 5 to 10 mg (1) and has several hundred thousand grains of a single floral species. Each load requires a visit to at least 80 flowers of the same plant type. The mixture of floral pollen into pellets is what is commonly called "bee pollen". It consists of various loads from different plant species.

Bees visit numerous plant flowers. There are, for example, more than 268 species and varieties of plants in England (2). G. Ricciardelli D'Albore and F. Intoppa have listed of all families of plants in Europe visited by bees (3).

Some floral pollen in bee products is responsible for allergies. Anaphylactic accidents related to the use of bee products are on the increase. There is substantial literature supporting this observation (4,5,6).

But, mugwort pollen and, more generally, the Asteraceae family are implicated in the origin of such accidents (7). A small proportion of Artemisia pollen in beehive products (only a few percent) is, however, enough to cause allergic symptoms. Asteraceae mainly includes genera Achillea, Artemisia, Carduus, Cichorium, Circium, Solidago and Taraxacum.

Artemisia's allergenic proteins appear to retain their allergenic properties in bee pollens from the time they enter the beehive to the harvesting by the beekeeper and their use by the end consumer (8).

To our knowledge, however, there is currently no technical definition of the allergenic potential of Artemisia in bee pollen. The

purpose of this study is to define the biological potency of Artemisia pollen in bee pollen *in vivo* by skin prick tests on patients allergic to Artemisia pollen.

Material and Methods

1) Analysis of bee pollen spectrum

A pollen analysis of bee products is usually performed by a specialist laboratory by analyzing the beehive products. In our case, we used Honey Expertise Laboratory (Naturalim France Miel, 39330 Port-Lesney, France). Such analysis defines the type and frequency of each botanical genera or family floral pollen and determines the total mass of floral pollen. Bee pollen is thoroughly cleaned when intended for human consumption. Cleaning the pollen separates pollen dust balls, bee body fragments, wax or propolis. Melissopalynology is based on the European Maurizio and Louveaux standard without acetolysis, as recommended by the International Commission for Bee Botany (9).
Counting and identifying floral pollen grains are made by examination under a microscope. Identification of pollen types is based on the laboratory pollen reference collection for local plants or on guides specialized in the pollen morphology of floral species.
Ten grams of well-homogenized bee pollen were dissolved and washed in distilled water, centrifuged, then prepared using aqueous glycerine and paraffin for smear preparations. Each smear was studied under a microscope to identify 500 floral pollen grains, in order to determine the percentage of each type of flower pollen. With bee pollen, the floral pollen mass is equated with the bee pollen mass, because it is accepted that the bee pollen pellets only contain kneaded floral pollen grains.

2) Calculation of the floral pollen allergen mass Mass $_{p\text{-}allergen}$ in bee pollen is as follows:

2.1) Calculate the volume "Vpn" of each of the 1 to n types of floral pollen from the bee pollen spectrum using the formula $Vpn = 4/3\ \pi\ r^3$ if the pollen grain is spherical or using the formula $Vpn = 4/3\ \pi\ e^2\ l$ if the floral pollen has an ellipsoidal shape.
The values of the radius r and of the mid-equatorial and longitudinal axes e and l are obtained from the literature from observations made on bee product pollen, including bee pollen (2). It is important to take into account changes in volumes of flower pollen due to orthodox or recalcitrant pollen qualities when pollen grains come into contact with aqueous bee fluids.

2.2) Calculate the proportion of volume $P_{p\text{-}allergene}$ of flower pollen allergen «p-allergen»

$$P_{p\text{-}allergene} = (V_{p\text{-}allergene} \times \%_{p\text{-}allergene}) / ((V_{p\text{-}allergene} \times \%_{p\text{-}allergene}) + (V_{p2} \times \%_{p2}) + \ldots + (V_{pn} \times \%_{pn}))$$

(%pn is the percentage of flower pollen pn observed in the bee pollen analysis).

2.3) Calculate the mass of floral pollen allergen Mass $_{p\text{-}allergen}$

$$Mass_{p\text{-}allergen} = P_{p\text{-}allergen} \times Mass_{pollens}$$

3) Calculation using the equation defining the allergenic potential of flower pollen allergen in bee pollen

Before applying this equation, it is necessary to:
- Use bee pollen with only one floral pollen allergen,
- Calculate the mass of floral pollen allergen as indicated above,
- Use a bee pollen without any floral pollen allergen as a "bee pollen negative control" to eliminate a skin sensitization to bee specific allergens.

3.1) Preparation of bee pollen extracts. Samples were prepared with the two types of bee pollen defined above. Five grams of fresh or frozen bee pollen was well homogenized on a glass plate and 450 mg of bee pollen was diluted in 4.5 ml of isotonic 0.4% phenol diluent (dilution weight/volume: 100 mg of bee pollen/ml of diluent) and was homogenized with a stirrer at a maximum speed for one minute. Samples were stored at room temperature for 24 hours and homogenized one more time with the stirrer before being removed, then 0.5 ml of the 100 mg/ml diluted solution was diluted in 4.5 ml of isotonic 0.4% phenol diluent to produce a 10 mg/ml diluted solution. These steps were repeated one more time to produce at least three samples of bee pollen, i.e. 100 mg/ml, 10 mg/ml and 1 mg/ml.
The allergen pollen floral mass contained per milliliter of each sample was deduced using the mass of floral pollen allergen in the bee pollen. Samples were kept at 5°C and were used within five days.

3.2) Measurement of skin reactivity to floral pollen allergen contained in bee pollen. Skin prick tests were duplicated on the inner side of the forearms of 11 subjects. Patients (seven women/four men) aged between 19 and 46 (mean: 29.7), who had been referred for seasonal symptoms (rhino-conjunctivitis and/or asthma) produced in July and August, were recruited in Hyères, in the south of France. They were not hyposensitized and were positive skin prick tested with a commercially available Artemisia vulgaris extract (Stallergenes) and sensitized to Art-v1 by testing for specific IgE-antibodies (> 0,27 kui/l). In addition to mugwort, they were sensitive to grasses (5), cats (3), cypress (4), olive (2), mites (7) and fungi mould (1) but none had a history of bee sting reactions. Informed consent was obtained from each patient.

Skin reactivity was assessed by geometric measuring of the two largest wheal diameters observed twenty minutes after the pollen sample prick tests, positive (histamine 10 mg/ml) and negative (glycerinated saline) controls and commercial extract tests (Stallergenes Artemisia vulgaris 100 IR/ml). $W_{p\text{-allergen}}$ was defined by geometric measuring of skin reactivity to floral pollen allergen contained in bee pollen.

3.3) Analysis of the relationship between skin reactivity to floral pollen allergen in bee pollen $W_{p\text{-allergen}}$ and floral pollen allergen mass $Mass_{p\text{-allergen}}$. If the model curve was a power regression.

$$(W_{p\text{-allergen}}) = b\ (Mass_{p\text{-allergen}})a$$

then the linear function was calculated as follows:

$$Log_{10}\ (W_{p\text{-allergen}}) = a\ (Log_{10}\ (Mass_{p\text{-allergen}})) + B$$

where A and B are specific pollen allergen constants.

Variances analysis was performed by calculating R^2, which records the results of the value dispersions associated with regression. The closer R^2 is to 1, the more the total variance is explained by the linear regression.

Results

1) Calculation of $Mass_{artemisia}$ of bee pollen

Our bee pollen has a floral pollen allergen: Artemisia. It was collected in August 2012 in Eguisheim (France) at the GPS location: X 48.0428, Y 7.3062. Its spectrum includes 43.1% Artemisia, 25.7% Mercurialis, 16.0% Lythrum salicaria and 14.7% Brassicaceae (< 0,5% undetermined). Artemisia, Mercurialis and Brassicaceae are spherical pollens. Their respective diameters are 20, 25 and 20 micrometers. Lythrum pollen is ellipsoid in shape, the equatorial and longitudinal axes are respectively 26 and 36 microns. Indeterminate fractions were ignored.

1.1) Calculation of V_{pn} volumes

Artemisia. $V_{artemisia} = 4/3\ \pi\ (20/2)3 = 4187\ \mu^3$

Mercurialis. $V_{mercurialis} = 4/3\ \pi\ (25/2)3 = 8177\ \mu^3$

Lythrum. $V_{lythrum} = 4/3\ \pi\ (26/2).(36/2)2 = 17643\ \mu^3$

Brassicaceae. $V_{brassicaceae} = 4/3\ \pi\ (20/2)3 = 4187\ \mu^3$

1.2) Calculation of $P_{artemisia}$ proportion

$$P_{artemisia} = (V_{artemisia} \times \%_{artemisia}) / ((V_{artemisia} \times \%_{artemisia}) + (V_{mercurialis} \times \%_{mercurialis}) + (V_{lythrum} \times \%_{lythrum}) + (V_{brassicaceae} \times \%_{brassicaceae})) = (4187 \times 43.1\ \%) / ((4187 \times 43.1\ \%) + (8177 \times 25.7\ \%) + (17643 \times 16.0\ \%) + (4187 \times 14.7\ \%)) = 180460 / 734446 = 0.246$$

1.3) Calculation of $Mass_{artemisia}$

$Mass_{artemisia} = P_{artemisia} \times Mass_{pollens} = 0.246 \times 1\ mg = 0.246\ mg$

There was 0.246 mg of Artemisia pollen per mg of bee pollen.

2) Calculation of $Mass_{hedera\ helix}$ of bee pollen

Our bee pollen is a pure, unique, floral pollen, Hedera Helix (99%; indeterminate percentage < 0,9%). It was collected in September 2013 in Thezillieu (France) at GPS location. X 45.8833, Y 5.6. This is a spherical pollen with a diameter of 25 micrometers.

2.1) Calculation of volume V_{pn}

Hedera helix. $V_{hedera\ helix} = 4/3\ \pi\ (25/2)3 = 8177\ \mu^3$

2.2) Calculation of proportion $P_{hedera\ helix}$

$$P_{hedera\ helix} = (V_{hedera\ helix} \times \%_{hedera\ helix}) / (V_{hedera\ helix} \times \%_{hedera\ helix}) = (8177 \times 99\%) / (8177 \times 99\%) = 1$$

2.3) Calculation of $Mass_{hedera\ helix}$

$$Mass_{hedera\ helix} = P_{hedera\ helix} \times Mass_{pollens} = 1 \times 1\ mg = 1\ mg$$

There was 1 mg of Hedera helix pollen per mg of bee pollen.

3) Measurements of skin reactivity to Artemisia and Hedera helix pollen and analysis of the relationship between $W_{p\text{-allergen}}$ and $Mass_{p\text{-allergen}}$

Out of the 11 patients sensitized to Artemisia, one was excluded because of a positive control test of less than 3 mm. Skin prick test results with three 10-fold dilutions of bee pollen with 0.246 mg of Artemisia pollen per milligram or with 1 mg of Hedera helix pollen per milligram are shown in **table 1**.

The model dose-response curve of Artemisia bee pollen is a power regression.

$$(W_{artemisia}) = 3.328\ (Mass_{artemisia})^{0.297} \qquad R2 = 0.9947$$

The dose-response curve power regression is shown in **figure 1** and the linear function is:

$$Log_{10}\ (W_{artemisia}) = 0.297\ (Log_{10}\ (Mass_{artemisia})) + 0.520 \qquad R = 0.9974$$

The dose-response curve linear function is shown in **figure 2**.

The model dose-response curve of Hedera helix bee pollen is not a power regression.

$$(W_{hedera\ helix}) = 0.27\ (Mass_{hedera\ helix})^{0.033} \qquad R2 = 0.0292$$

Table 1 *- Skin prick test results with three 10 fold dilution of bee pollen with 0.246 mg of artemisia pollen per milligram or with 1 mg of hedera helix pollen per milligram.*

	ARTEMISIA[1]				CONTROL[1]		HEDERA HELIX[2]		
Patient	**Artemisia 24.6 mg/ml**	**Artemisia 2.46 mg/ml**	**Artemisia 0.25 mg/ml**	**Artemisia commercial extract**	**Positive control**	**Negative control**	**Hedera helix 100 mg/ml**	**Hedera helix 10 mg/ml**	**Hedera helix 1 mg/ml**
P1	22.97	11	8.94	8.48	6.48	0	0	0	0
P2	4.90	2.83	1.73	4.9	7.93	0	0.5	0	0
P3	6.93	3.87	1	7.93	6.48	0	0.5	0.5	0
P4	11.96	9	4.90	9.48	3.46	0	0	0	0.5
P5	17.97	6	1.41	10.48	6.48	0	0	1	1
P6	6.93	1.73	1	3	4.24	0	0.5	0	1
P7	8	5.92	3.87	3.87	3.46	0	1	0	0.5
P8	8.94	6	2.83	9.38	3.87	0	0	0	0
P9	6.93	3.87	1.73	3.87	8.48	0	0	0	0
P10	3	2.83	1	3.87	6.92	0	0.5	0	0.5
Mean wheal	8.39	4.61	2.14	5.93	5.49	0	0.3	0.15	0.35

[1]geometric mean wheal (mm). [2]mean wheal (mm).

Figure 1 *- Dose-response curve power regression between* $W_{artemisia}$ *and* $Mass_{artemisia}$.

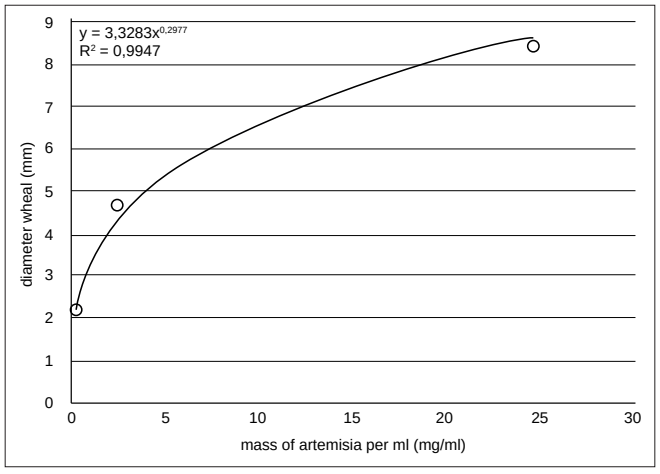
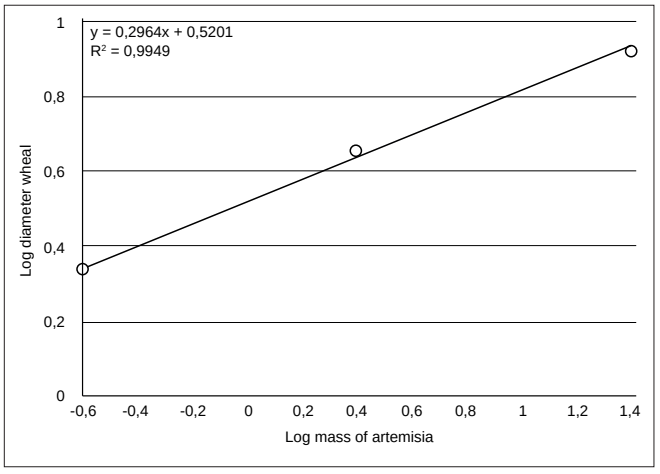


Figure 2 *- Dose-response curve linear function between log* $W_{artemisia}$ *and Log* $Mass_{artemisia}$.



Discussion

Artemisia is one of plant species that provide bees with pollen but not nectar. Patients sensitized to Artemisia pollen who ingested bee products (honey, royal jelly, bee pollen) may experience an immediate allergic reaction because of cross-reaction between Artemisia and Compositae bee product pollen and airborne Artemisia pollen (11,12,13,14,15).

However, patients who are allergic to bee products may be also sensitized to honeybee secretion proteins, pollen proteins contained in bee products (16) or bee venom components (7). This is why we tested our patients with bee pollen not containing

airborne pollen allergens, which was used as a bee pollen negative control. This was to eliminate skin sensitization to allergens other than Artemisia allergens (i.e., bee specific component allergens). Our bee pollen negative control was 100% Hedera helix bee pollen. Hedera helix pollen is not a common allergic pollen. In some rare cases, it might be responsible for cross-reaction to pollen panallergens (e.g., olea, quercus, fraxinus, alnus and lolium, but not mugwort) among Mexican allergic patients with dermatitis (17).

None of our patients had positive skin prick reactions to Hedera helix bee pollen. No relationship was established between Hedera helix bee pollen and skin reactivity.

A honeybee collects pollen grains at maturity from the male organs of flowers in order to obtain certain proteins or lipids. It gathers using an elaborate strategy based on pollen research of the highest quality for optimal protein and nutrient collecting. It takes advantage of the plant fertilization mechanisms in order to attain its objective, which is why the bee is not interested in wet pollen. As with floral nectar, wet pollen swells on contact with the secretions of sugar-water pollen grains that then release the soluble nutrient content. Based on comparisons between hand and bee-collected pollens, it appears that half or more of the mass of bee-collected pollens can be attributed to the addition of nectar-derived sugars to the pollen (18). The protein content of the grain decreases and this causes a leakage of the proteins in the external environment (19).

It seems that our Artemisia bee pollen sample contains protein allergens that are exclusively Artemisia

Mercurialis, Brassicaceae and Lythrum pollens were contained in our bee pollen sample. To our knowledge they are common in bee pollens. Literature searches in Medline were performed and no paper has described these pollens as being allergic pollens when they are included in bee pollens. This fact should be compared with what we know of the allergen qualities of these pollens.

- Lythrum is a strictly entomophilous pollen and is not known as an allergenic pollen.
- Mercurialis belongs to the Euphorbiaceae family. This family contains strongly sensitizing allergens (eg, latex, ricinus). Mercurialis allergens have shown allergenic cross-reactivity observed *in vitro* with profilins of other Euphorbiaceae and other families (e.g., Oleaceae, Asteraceae) (20), but the clinical significance is not well known (21). Furthermore, this cross-reactivity seems to be low (20) and the incidence of sensitivity to Mercurialis pollen is less than 0.9% among Italian patients with pollinosis (22).
- Brassicaceae pollen allergens are well known in cabbage, oilseed rape or mustard (e.g., profilin, calcium-binding protein, lipid transfer protein). They might be responsible for cross-reactivity between foods and pollens (23,24), e.g. mugwort. The prevalence of sensitization of rapeseed pollen is correlated to exposure level and is higher (11.8%) among French atopic patients (25). In contrast, the prevalence of oilseed rape pollen allergy is very low (between 0.2% and 2%) in the United Kingdom, even in areas of high production (26,27), and the symptoms may be due to both allergens and irritant potentials of oilseed rape (28). In addition, our bee pollen was harvested in a vineyard monoculture area where there is no rapeseed or mustard cultivation and where the Brassicaceae genus, wild white rocket (Diplotaxis), is very common. The prevalence of sensitization to Diplotaxis pollen is low (14/410, i.e. 3.4%) and allergy even lower (3/410, i.e. 0.7%). It may be an occupational allergy in vineyard workers. In addition, as patients sensitized to mugwort do not report reactive symptoms to wild rocket pollen, there appears to be a biological cross-reactivity (29). In addition our patients are not winemakers.

Furthermore, there is no Mercurialis, Brassicaceae or Lythrum pollen in the analysis of the contents of the pollen traps of the French aerobiology network in the area neighboring to Hyères.

It seems that our bee pollen sample with Artemisia contains Artemisia protein allergens

In the literature, a strong correlation has been noted between cutaneous reactivity to bee pollen containing mugwort pollen and the cutaneous reactivity in patients with a positive skin prick test to an Artemisia commercial extract (8). Pitsios et al. found that approximately 40% of patients were sensitized to both bee pollen and floral Artemisia pollen. They considered that it might be due to Asteraceae pollen in their samples, which contained 20 mg of bee pollen per ml of solution.

This correlation was observed in their five bee pollen samples, but only two melissopalynology analyses of bee pollen samples have shown Compositae pollen. This might be due to the qualitative and quantitative methods used to analyse bee pollen. Only five spherules of different tinges were chosen from each bee pollen sample. Tinge loads are subjective. Colours change with time, if the loads are dry or are exposed to sunlight (30). Many plant species have pollen loads with very similar colours and sometimes up to three colours are observed for a single genus (2). Compositae pollen is often a minor bee pollen and choosing five pellets can raise the risk of non-homogenized samples.

On the contrary, our bee pollen was analyzed using the standard European melissopalynologycal method recommended by the International Commission for Bee Botany (9). This method is based on the study of 10 grams of well-homogenized bee pollen and 10 grams composed of more than 1.000 pellets. Our bee pollen sample is rich in Artemisia pollen, with 43.1% and 0.246

mg of Artemisia pollen per mg. Quantifying the absolute mass of Artemisia pollen with bee pollen per gram requires knowing the pollen spectrum of bee pollen and measuring pollen grain sizes. More particularly, this requires knowing pollen sizes when in contact with aqueous fluids. In contact with water, the pollen grain is in osmotic shock. Hydrated grain results in a change of its volume and opens pores and fissures (18) depending on the recalcitrance and orthodoxy of the pollen (31). Furthermore, two pollens of the same genus can have different reactions, e.g. Helianthus annuus pollen is orthodox and swells in contact with water, whereas Helianthus tuberosus pollen maintains the same volume (31).

A strong relationship was established between the absolute mass of mugwort pollen in bee pollen and skin reactivity despite our patient group including a small number of individuals sensitized to Artemisia and Art v1. The dose-response curve was a power regression curve:

$$(W_{artemisia}) = 3.328\ (Mass_{artemisia})^{0.297}\ \ (R2 = 0.9947)$$

from which we were able to deduce the linear curve.

$$Log_{10}(W_{artemisia}) = 0.297\ (Log_{10}(Mass_{artemisia})) + 0.520\ (R = 0.9974).$$

Mugwort allergens in bee pollen appears to be little or not altered by bee secretions and the allergens retain their allergenic capacity. In fact, the bee secretions contain digestive enzyme sugars (32) but are devoid of proteases. There is no protein digestion, as salivary and hypopharyngeal glands do not produce proteolytic enzymes (33).

Conclusion

To our knowledge this is the first time it has been shown that the skin reactivity of patients who are allergic to mugwort is proportional to the absolute mugwort mass contained in bee pollen. Further studies are needed to determine how mugwort allergens retain their allergenic qualities.

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E. Dias de Castro[1], A. Leblanc[1], A. Sarmento[2], J. R. Cernadas[1]

An unusual case of delayed-type hypersensitivity to ceftriaxone and meropenem

[1]Allergy and Immunology Division, Hospital S. João, E.P.E., Porto, Portugal
[2] Infecciology Division, Hospital S. João, E.P.E., Porto, Portugal

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Ceftriaxone; meropenem; delayed-type hypersensitivity; cross-reactivity.

Corresponding author
Eunice Dias de Castro
Serviço de Imunoalergologia
Hospital S. João
Alameda Prof. Hernâni Monteiro
4200-319 Porto, Portugal
E-mail: eunicediascastro@gmail.com

Summary
Recent studies have demonstrated a low cross-reactivity between β-lactam antibiotics and carbapenems in IgE-mediated reactions. There are no studies on cross-reactivity of meropenem in patients with non-immediate hypersensitivity to cephalosporins.
We describe a case of a 13-year-old male, admitted in Neurosurgery with a severe extradural empyema complicating frontal sinusitis, submitted to an emergent bifrontal craniotomy. A generalized maculopapular exanthema, fever and malaise, appeared by the 7th day of meningeal doses of ceftriaxone, clindamycin and vancomycin. Those were replaced by meropenem, with posterior worsening of the reaction and mucosal involvement. A new scheme with amikacin, metronidazole and linezolid was done with improvement. Skin prick, intradermal and patch tests to penicillins, ceftriaxone and meropenem were negative. Lymphocyte transformation test was positive to ceftriaxone and negative to meropenem.
Non-immediate T cell mechanism seems to be involved. Diagnosis work-up couldn't exclude cross-reactivity between ceftriaxone and meropenem.

Introduction

Depending on their chemical structure, β-lactam (BL) antibiotics are classified into 2 major classes, penicillins and cephalosporins, and 4 minor classes, monobactams, carbapenems, oxacephems and clavams (1). Cephalosporins and penicillins are the most widely used antibiotics for the treatment of common infections. Each one has a 4-membered β-lactam ring, but the 5-membered dihydrothiazine ring of penicillins is replaced by the 6-membered dihydrothiazine ring in the cephalosporins nucleus. Monobactams contain a monocyclic ring structure, whereas carbapenems have a bicyclic nucleus comprised of β-lactam ring with an association 5-membered ring (2).
Meropenem is a broad-spectrum carbapenem with potent antimicrobial activity against a broad range of Gram-negative, Gram-positive and anaerobic bacteria. The second parental carbapenem to be introduced worldwhile, meropenem has been in clinical use since 1994 and showed a favorable safety profile (3).
β-lactam (BL) antibiotics are referred as the most frequent elicitors of drug hypersensitivity reactions. The skin is the organ most frequently involved in hypersensitivity reactions to BLs, sometimes accompanied by systemic symptoms (1).
The frequency of carbapenem associated hypersensitivity in the general population is estimated to be in maximum 3% (0.3 to 3%) (4,5,6,7,8), mostly reported as rash, pruritus or urticaria (4).
The structural similarity between penicillin and carbapenem antibiotics is the bicyclic core, composed of a 5-membered ring attached to the β-lactam ring, which is generally believed to be responsible for the cross-reactivity between these classes of antibiotics. However, there is no consensus on the rate of hypersensitivity in individuals also allergic to penicillins. Several studies have evaluated the cross-sensitivity between carbapenems and penicillins on IgE-mediated reactions. The results range widely, from 0.9 to 47.4% (4-13), mainly due to different studies methodologies. Recent pro-

spective studies, that confirmed penicillin allergy by standardized procedures and tested for carbapenem allergy by administering a full therapeutic dose to carbapenem skin test-negative patients, showed rates of cross-reactivity around 1% (11,12,13).
Studies concerning the tolerability of carbapenems in subjects with hypersensitivity to cephalosporins are lacking, with exception of a prospective study that pointed to cross-reactivity between cephalosporins and carbapenems inferior to 5%, in IgE mediated-reactions (2).
Cross-reactivity between carbapenems and other β-lactams has been poorly investigated in patients with delayed-type cell-mediated allergy to β-lactams, with a recent prospective study showing a rate of 5.5% of cross-reactivity between imipenem-cilastatin and other β-lactams (14).
As far as we know, there are no studies on cross-reactivity and tolerability of meropenem in patients with delayed-type, cell-mediated hypersensitivity to cephalosporins.

Case Report

The authors report a case of a 13-year-old non-atopic adolescent male, admitted in Neurosurgery Department with a severe extradural empyema complicating frontal sinusitis, despite amoxicillin and acid clavulanic oral treatment. He was submitted to an emergent bifrontal craniotomy in order to drain empyema and to a simultaneous ethmoidectomy by ENT. Meningeal doses of intravenous antibiotic with ceftriaxone, clindamycin and vancomycin were prescribed. By the 7th day, he presented a pruriginous generalized maculopapular exanthema, fever and malaise, with no analytical changes like leucocitosis, neutrophilia or eosinophilia and with PCR reducing values. Antibiotherapy was replaced by meropenem without further treatment, namely corticosteroids. An initial improvement of the symptoms occurred, followed by posterior reappearance of the malaise and fever, worsening of the cutaneous lesions (without blistering) and appearance of oral mucosal lesions, at the third day of treatment. No analytical changes were found, also at this stage. Meropenem withdrawn and β-lactams eviction was advised. None of the cutaneous reactions were compatible with a Steven Johnson Syndrome. The absence of analytical changes excluded a DRESS Syndrome (Drug rash and eosinophilic systemic syndrome).
A new antibiotherapy scheme with amikacin, metronidazole and linezolid was done during the following week, with good clinical response and resolution of mucocutaneous lesions.
The allergy diagnosis work-up was performed 8 weeks after hospital discharge, in the Drug Allergy Unit, according to ENDA guidelines (15,16) and after a patient's legal responsible signed informed consent. Skin prick tests (SPT) and intradermal tests (ITD) to penicillins and ceftriaxone, including delayed reading at 48 hours, were negative. Meropenem at 1 mg/ml was tested beginning with SPT and followed by ITD. SPT (1 mg/ml) and ITD (1/1000 - 1/10 dilutions) to meropenem were negative (immediate and delayed reading), but ITD at 1 mg/ml was positive in immediate reading (15 mm medium diameter wheal, with surrounding erythema). Patch tests were negative to all antibiotics.
In *vitro* tests were performed, namely lymphocyte transformation test (LTT), with positive results to ceftriaxone (3.1 mcg/ml) and negative to meropenem. Specific IgE to meropenem (CAP-FEIA) performed at *Phadia,* Uppsala, Sweden, was negative (< 0.10 KU/L).
Due to the severity of the reaction, drug provocation tests with beta-lactam antibiotics weren't performed.
SPT (pure drug) and ITD (1/1000 - 1/1 dilutions) to meropenem were repeated one year after. As in the first time, all the tests were negative, with exception of IDT with pure drug, which remained positive in immediate reading (8.5 mm papule diameter).

Discussion

The clinical presentation of the reaction and the time of occurrence are suggestive of non-immediate T cell mechanism, supported by LTT positive result to ceftriaxone. The negative LTT to meropenem doesn't allow the exclusion of this mechanism to this antibiotic, since the LTT has a sensitivity of just 74% to BLs (16,17).
The absence of published standardized concentrations to meropenem skin tests was also a difficulty in this case. In a case report, SPT and ITD were done with maximum concentration of meropenem 25 mg/ml (8). In more recent prospective studies with larger series of patients (104, 108 and 98 respectively) meropenem was used at a concentration of 1 mg/ml of normal saline, but with no reference to the used dilutions (11,12,13). Based on those larger series, we decided to perform SPT and ITD tests with meropenem at 1 mg/ml (dilutions from 1/1000 to 1/1). The positivity in ITD with pure meropenem could be irritative, since the mechanism didn't seem to be IgE-mediated. To clarify this result, SPT and ITD tests to meropenem were performed in 10 controls with the described concentrations with negative results, except in one patient previous exposed to meropenem. This result could be a sign of exposure rather than a sensitization. The result of the specific IgE to meropenem and the reduction on the wheal size on the test performed one year after, also suggest that. This last result could also be in consonance with the decrease of sensitivity of the skin tests to penicillins over time (1,16,18,19).
The negative results of skin tests (SPT and ITD at delayed reading, and patch tests) to penicillins and ceftriaxone don't exclude a cell-mediated mechanism to these antibiotics. For non-immediate allergic reactions to BLs, skin tests appear to be less sensitive than for immediate allergic reactions (16). Delayed reading of intradermal and/or patch tests have been used for many years in the evaluation of non-immediate reactions to BLs, particularly to penicillins. ENDA recommendations advise a combined approach (16), since sensitivity to these procedures

ranging from 2.6% (patch tests) (20) to 37.8% (patch tests and/or delayed reading IDTs) (21).

The severity of the reaction in our patient contra-indicated a provocation diagnostic test, which remains the gold standard in the drug allergy diagnosis (although the known limitations in non-immediate reactions). In this case, cross-reactivity between ceftriaxone and meropenem couldn't be clearly established, in spite of the allergy diagnosis work-up performed according to recommendations. Moreover, the described limitations during allergy diagnosis procedures and the particularities of this unusual case became an interesting challenge.

Published data show a very low incidence of carbapenem-associated hypersensitivity in general population, which is estimated to be less than 3% (4,5,6,7,8) and low cross-reactivity between carbapenems and other BLs (2,11,12,13,14).

Although first studies showed an important cross-reactivity between carbapenems and penicillins in IgE-mediated reactions (5,6,9,10), recent prospective studies, that confirmed penicillin allergy by standardized procedures and tested for carbapenem allergy by administering a full therapeutic dose to carbapenem skin test-negative patients, showed low rates of cross-reactivity (around 1%), with all carbapenem skin test-negative patients tolerating the challenge (11,12,13).

Studies concerning the tolerability of carbapenems in subjects with hypersensitivity to cephalosporins are lacking, with exception of a prospective study, which demonstrated the tolerability of meropenem in 97 of a total of 98 patients with well-demonstrated, IgE-mediated hypersensitivity to cephalosporins (2).

Cross-reactivity between carbapenems and other β-lactams has been poorly investigated in patients with delayed-type cell-mediated allergy to β-lactams, with a recent prospective study showing a rate of 5.5% of cross-reactivity between imipenem-cilastatin and other β-lactams (14).

As far as we know, there are no studies on cross-reactivity and tolerability of meropenem in patients with delayed-type, cell-mediated hypersensitivity to cephalosporins.

This case reports an unusual case of hypersensitivity to ceftriaxone and meropenem that seems to be cell-mediated, although the diagnosis work-up performed didn't establish clearly cross-reactivity between them. However, the severity of the reaction combined with a suggestive history, still advice the eviction of these ATB in this patient.

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W. Lee[1], F.S.W. Teo[2,4], A. Santosa[3,4], G.G. Teng[3,4]

Eosinophilic Granulomatosis with Polyangiitis preceding allergic bronchopulmonary aspergillosis

[1] University Medicine Cluster, Internal Medicine Residency, National University Health System, Singapore
[2] University Medicine Cluster, Division of Respiratory and Critical Care Medicine, National University Health System, Singapore
[3] University Medicine Cluster, Division of Rheumatology, National University Health System, Singapore
[4] Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, Singapore

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Corresponding author:
Gim Gee Teng
University Medicine Cluster
Division of Rheumatology
National University Health System,
Singapore

Summary
A 61-year-old Chinese man with long-standing, stable Eosinophilic Granulomatosis with Polyangiitis (EGPA) and asthma, presented with acute hypoxemia and declining obstructive pulmonary function. Elevated serum IgE levels, positive Aspergillus fumigatus specific IgE and CT findings of central bronchiectasis with small airway mucoid impaction confirmed new development of Allergic Bronchopulmonary Aspergillosis (ABPA). The maintenance therapy for EGPA, azathioprine, was discontinued. Prednisolone 0.5mg/kg/day and Itraconazole improved his symptoms and IgE levels. To our knowledge, ABPA occurring in a patient with EGPA has not been reported. Differentiation of EGPA with asthmatic flare vs ABPA vs asthma with aspergillus hypersensitivity is discussed. Heightened Th2 immunity where eosinophils play a central role may link these conditions.

Manuscript

Introduction

Eosinophilic Granulomatosis with Polyangiitis (EGPA) is a small-vessel vasculitis which was first described as "allergic granulomatosis and angiitis" in patients with asthma and eosinophilia (1). There exist many overlapping features between EGPA and ABPA. ABPA is an allergic pulmonary disorder characterized by chronic asthma, recurrent pulmonary infiltrates, and bronchiectasis caused by hypersensitivity to *Aspergillus fumigatus*. Coexistence of EGPA and ABPA is extremely rare and to our knowledge, ABPA occurring in a patient with EGPA has not been reported. Differentiation between EGPA, ABPA and asthma exacerbation is crucial in directing subsequent management, as illustrated by our case.

Case Description

A 61-year-old Chinese man with mild intermittent bronchial asthma and rhino-sinusitis since the age of 30 years and chronic hepatitis B, was evaluated eight years ago for hypereosinophilia. He presented with anorexia and weight loss, lethargy and fever. Besides intermittent nasal congestion and post-nasal drip, he had no facial fullness, hyposmia, diplopia or severe headache. He denied any new cough or dyspnea, weakness, numbness, diarrhea or rashes and had not been taking any new medications. Examination was unremarkable, he had no focal neurologic or cutaneous signs, his lungs were clear and there was no organomegaly. Investigations revealed leucocytosis with absolute eosinophil count (AEC) 22.9 x 10^9/L (0.00 - 0.60 x 10^9/L). Work-up ruled out parasitic infection and lymphoproliferative diseases. Bone marrow biopsy showed eosinophilic hyperplasia without clonality. Immunoglobulin E (IgE) were highly elevated at 7610

(0 - 87 IU/mL). Anti-neutrophil cytoplasmic antibody (ANCA) was negative. High Resolution Computed tomography (HRCT) thorax and abdomen and echocardiography were normal. CT sinuses showed mild mucosal thickening of the maxillary and frontal sinuses with good aeration, absence of polyposis or abscess, confirming uncomplicated chronic rhinosinusitis. There were no recent symptoms suggestive of asthma exacerbations and no other organ-systems involvement. He was treated with prednisolone for hypereosinophilic syndrome, concurrent with lamivudine. However, the patient did not return for follow-up visits and stopped taking his medications.

Four years later, he developed skin rash and neuropathies affecting the left common peroneal and right median nerves. His AEC was 14.67 x 10^9/L and erythrocyte sedimentation rate 87 mm/hr. He had palpable purpura mixed with hyperpigmented macules scattered on both lower limbs up to the thighs, as well as some petechial rash on the dorsum of his right hand. Skin biopsy showed fibrinoid necrosis of small vessels with surrounding neutrophils, nuclear dust and extravasated red blood cells, consistent with leucocytoclastic vasculitis (**figure 1**). Anti-proteinase-3 and myeloperoxidase antibodies were negative. Based on the progression of clinical findings, he was diagnosed with EGPA, despite stable asthma and normal chest radiograph. In addition to prednisolone, he received oral cyclophosphamide 50 mg/day for a year for the indication of peripheral neuropathy as an organ-threatening manifestation (2) and steroid sparing effect, he recovered with no residual neurological deficit and treatment was maintained with azathioprine.

A year into his remission of vasculitis, he presented with more frequent symptoms of dyspnea, productive cough and wheezing, from once monthly to weekly. He was started on Formoterol/Budesonide (4.5/160 units) inhaler and theophylline, with prompt improvement. In the following year, however, he developed acute asthmatic exacerbations with hypoxemia. His white cell count was 11.97 x 10^9/L and AEC 0.52 - 1.88 x 10^9/L. Serial pulmonary function tests (**table 1**) showed significant deterioration with obstructive physiology. Lung volume and diffusion capacity remained normal. CT thorax (**figure 2**) showed predominantly upper lobe central bronchiectasis.

Bronchoscopic lavage revealed 8470 nucleated cells with neutrophil predominance. Microbiological investigations were negative. Transbronchial biopsy showed no evidence of infection, granuloma or vasculitis. A skin prick test (SPT) was conducted on the volar aspect of the patient's forearm. Histamine (1 mg/ml) served as positive control, while physiological saline served as negative control (both from Allergopharma®); the SPT was considered to be positive if the wheal diameter was larger than 3 mm. SPT showed a small wheal diameter (< 3mm) to several tested allergens, including *Aspergillus* and *Penicillium* (10000 U/ml, both from Allergopharma®). We therefore proceeded with an intradermal test to *Aspergillus* and *Penicillium* (both intradermal test solutions at 2500 U/ml from Allergopharma®, undiluted), which was positive to *Aspergillus* (6 mm wheal) but negative to *Penicillium* (0 mm wheal). *Aspergillus fumigatus* specific IgE level was raised at 1.85 kU/L. On a clinical diagnosis of Allergic Bronchopulmonary Aspergillosis (ABPA), azathioprine was discontinued and prednisolone was increased to 0.5 mg/kg daily for 3 weeks, with improvement in clinical symptoms and laboratory parameters. Prednisolone was decreased over 5 months based on symptoms, IgE and eosinophil levels, then maintained between 7.5 - 10 mg daily thereafter. Serum IgE levels declined by more than 50%, but remained at high levels (538 - 1889 IU/mL) over the next two years. Itraconazole was subsequently initiated to allow further tapering of prednisolone. CT evidence of bronchiectasis remained largely stable, with decreased mucus plugging.

Figure 1 - *Hematoxylin and eosin (H&E) stained sections showed (**A**) perivascular and interstitial inflammatory cellular infiltrate within the dermis at 10x magnification; and (**B**) neutrophils and nuclear dust (long arrows) and extravasated red blood cells (short arrow) at 40x magnification.*

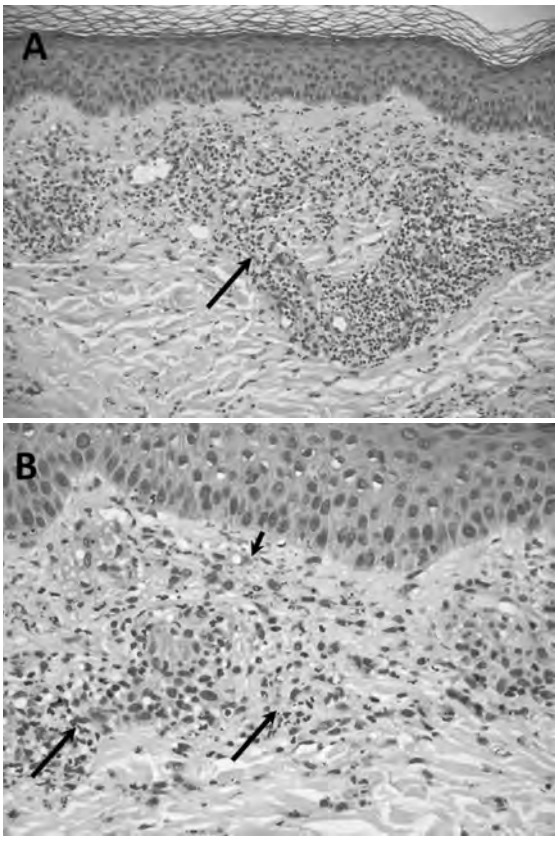
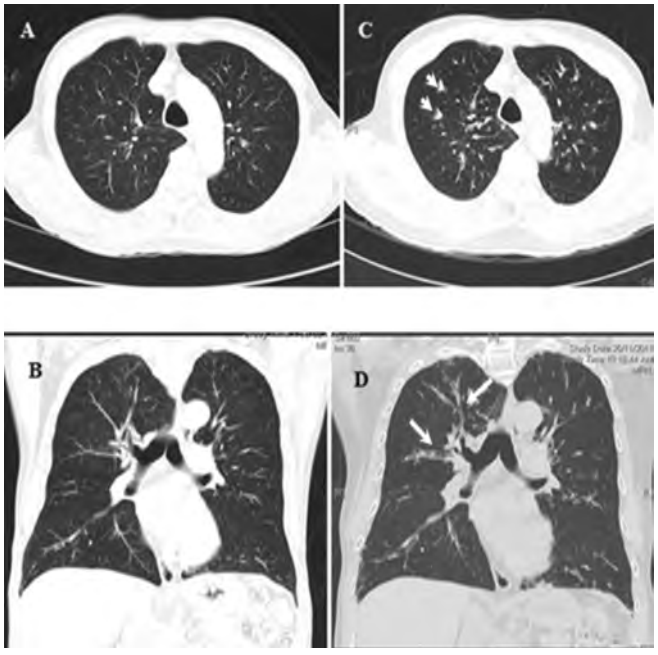


Table 1 - *Results of spirometry at clinical remission of Eosinophilic Granulomatosis with Polyangiitis (EGPA) and at diagnosis of Allergic bronchopulmonary aspergillosis (ABPA).*

	Jan 2011 (EGPA remission)		Dec 2011 (ABPA diagnosis)	
	Pre-Bronchodilator	**Post-Bronchodilator**	**Pre-Bronchodilator**	**Post-Bronchodilator**
FVC (L) (% of predicted)	2.85 (102%)	3.11 (111%)	2.52 (91%)	2.35 (85%)
FEV1 (L) (% of predicted)	1.34 (64%)	1.70 (81%)	0.83 (40%)	0.83 (40%)
FEV1/FVC	47	55	33	35

FVC: Forced vital capacity; FEV1: Forced expiratory volume in one second.

Figure 2 - *Computed tomography of the lung with high resolution cuts showing normal lung fields (**A** and **B**) compared with lung parenchymal changes after two years (**C** and **D**). There is interval development of predominantly central bronchiectasis (long arrows), with evidence of small airway mucoid impaction (short arrows), consistent with a diagnosis of allergic bronchopulmonary aspergillosis.*



Discussion

This patient has EGPA but developed ABPA many years later. A diagnostic concern in a patient with sensitization to *Aspergillus* is whether a proportion of asthmatic patients merely have asymptomatic *Aspergillus* sensitization rather than true ABPA, which can be difficult to differentiate from each other. Patients with sensitization only typically have a positive SPT to *Aspergillus* antigens without other accompanying laboratory indices associated with ABPA, specifically excessively elevated serum IgE levels (> 1000 IU/mL) and a positive *Aspergillus* specific antibody (> 0.35 kUA) (3). Sensitization to recombinant *Asp f 4* and *Asp f 6* allergens are more specific for the diagnosis of ABPA (4); however, these tests are not readily available in our center.

Arguably, chronic persistent asthma symptoms (with or without EGPA) may have led to airway remodelling in our patient, with irreversible obstructive physiology and even central bronchiectasis. However, the dramatic fall in FEV1 with radiologic evolution of bronchiectasis over one year, together with serological evidence of significant immuno-reactivity to *Aspergillus fumigatus*, favours a diagnosis of ABPA over Aspergillus hypersensitivity (AH) with chronic asthma. By the Rosenberg-Patterson criteria, our patient fulfils 6 out of 8 major criteria, namely asthma, positive intradermal test (type 1), elevated serum IgE, elevated serum *Aspergillus*-specific IgE, hyper-eosinophilia and central bronchiectasis.

EGPA has recently replaced the eponym Churg Strauss Syndrome (CSS) (5). The patient's background diagnosis was consistent with EGPA despite the patient's normal CXR and stable asthma, because it is well-recognized that one-third of patients may have normal chest radiographs and attenuation of asthma during the vasculitic phase (6,7). Although the characteristic granulomatous reaction associated with eosinophilic infiltration of tissues was absent in our patient, the cutaneous and subcutaneous lesions in EGPA often lack diagnostic specificity, with biopsies revealing only nonspecific inflammatory features of leukocytoclastic vasculitis (7,8). At least two other cases of ABPA and EGPA have been reported (9,10). A 67 year-old woman with intermittent wheeze with histological diagnosis of ABPA developed EGPA 17 years later (10). Another woman with long-standing bronchiectasis and asthma who was first diagnosed with anti-MPO positive EGPA with peripheral neuropathy was found to have ABPA during the same hospitalization

(9). The authors concluded that radiographic evidence of ABPA was present seven years prior (9). This patient responded to systemic glucocorticoid but was given itraconazole subsequently. ANCA became negative after 2 weeks (9). Other fungi, including *C. albicans* (11) and *Fusarium* (12) may produce clinical presentations similar to ABPA and have been reported to predate the onset of EGPA. To our knowledge, our case may be the first of EGPA antecedent to ABPA.

EGPA and ABPA share many common features, such as asthma, rhinosinus involvement, eosinophilia, raised IgE levels and radiographic evidence of pulmonary involvement. An antigenic stimulus drives the Th2 immunity (driven by interleukin (IL)-5, IL-4 and IL-13), leading to increased production and activation of eosinophils (7,13) in EGPA. However, the extrapulmonary manifestations as well as the additional mechanisms, which trigger leucocyte infiltration into vessel walls and tissues to cause systemic vasculitis are not found in ABPA. In our patient, it is possible that fungal sensitization occurred during the period of more intense immunosuppression, which may have masked the manifestations of ABPA until the steroid doses were tapered. On the other hand, Aspergillus colonization as the etiologic factor of EGPA may be hypothesized. However, a study showed that a minority of EGPA was linked to specific allergic responses to common allergens and even then, *Aspergillus* was not one of the identified allergens (14), suggesting that *Aspergillus* exposure is an unlikely trigger of EGPA.

Our case report highlights that a change in asthma control in a patient with EGPA may not merely be attributable to a flare of asthma or pulmonary vasculitis. Once remission of the systemic vasculitic phase of EGPA is achieved with treatment, asthma exacerbation constitutes the majority (70%) of first flares in a large prospective cohort (2). Therefore, other etiologies for asthma may sometimes be overlooked. Due to the significant immunosuppressive burden inherent to the treatment of vasculitis, physicians treating EGPA must be cognizant of possible intercurrent infective processes.

Long term oral glucocorticoid therapy is often required for ABPA and its dose and duration is guided by IgE levels. As long term glucocorticoid use is associated with significant side effects, itraconazole or voriconazole may be used in steroid dependent cases to reduce fungal burden. Biologics such as anti-IgE (omalizumab) and anti-IL5 (mepolizumab) may have a role in the future management of refractory cases of ABPA, but their use is best reserved for exceptional cases (13,15).

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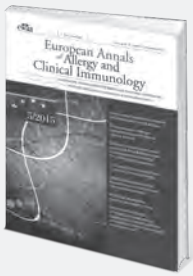

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Riassunto delle caratteristiche del prodotto

1. DENOMINAZIONE DEL MEDICINALE DuoResp Spiromax 160 microgrammi/4,5 microgrammi polvere per inalazione. **2. COMPOSIZIONE QUALITATIVA E QUANTITATIVA** Ogni dose erogata (dose che fuoriesce dal boccaglio di Spiromax) contiene 160 microgrammi di budesonide e 4,5 microgrammi di formoterolo fumarato diidrato. Ciò equivale a una dose preimpostata da 200 microgrammi di budesonide e 6 microgrammi di formoterolo fumarato diidrato. Eccipienti con effetti noti: ogni dose contiene circa 5 milligrammi di lattosio (monoidrato). Per l'elenco completo degli eccipienti, vedere paragrafo 6.1. **3. FORMA FARMACEUTICA** Polvere per inalazione. Polvere bianca. Inalatore bianco con un cappuccio protettivo semitrasparente di colore bordeaux. **4. INFORMAZIONI CLINICHE 4.1 Indicazioni terapeutiche** DuoResp Spiromax è indicato esclusivamente negli adulti di età pari o superiore ai 18 anni. ***Asma*** DuoResp Spiromax è indicato per il regolare trattamento dell'asma quando è appropriato l'uso di un'associazione (corticosteroide per via inalatoria e $ß_2$-adrenocettori agonisti a lunga durata d'azione): • in pazienti non adeguatamente controllati con corticosteroidi per via inalatoria e con $ß_2$-adrenocettori agonisti a breve durata d'azione usati "al bisogno" o • in pazienti già adeguatamente controllati sia con corticosteroidi per via inalatoria sia con $ß_2$-adrenocettori agonisti a lunga durata d'azione. **Broncopneumopatia cronica ostruttiva (BPCO)** Trattamento sintomatico di pazienti con BPCO grave (FEV_1 < 50% del normale) e anamnesi di ripetute riacutizzazioni, con sintomi significativi nonostante la terapia regolare con broncodilatatori a lunga durata d'azione. **4.2 Posologia e modo di somministrazione** DuoResp Spiromax è indicato esclusivamente negli adulti di età pari o superiore ai 18 anni. DuoResp Spiromax non è indicato per l'uso nei bambini di età pari o inferiore ai 12 anni o negli adolescenti dai 13 ai 17 anni di età. **Posologia *Asma*** DuoResp Spiromax non è destinato alla gestione iniziale dell'asma. DuoResp Spiromax non è indicato per il trattamento del paziente adulto che presenta solo asma lieve non adeguatamente controllata con un corticosteroide per via inalatoria e $ß_2$-adrenocettori agonisti a rapida azione "al bisogno". Il dosaggio di DuoResp Spiromax è individuale e deve essere adattato in relazione alla gravità della malattia. Ciò deve essere tenuto in considerazione non solo quando si inizia un trattamento con combinazioni di medicinali, ma anche quando la dose di mantenimento viene modificata. Se un singolo paziente necessita di una combinazione di dosi diversa da quelle disponibili nell'inalatore combinato, si devono prescrivere dosi appropriate di $ß_2$-adrenocettori agonisti e/o corticosteroidi con inalatori separati. Una volta raggiunto il controllo dei sintomi dell'asma, si potrà prendere in considerazione una riduzione graduale della dose di DuoResp Spiromax. I pazienti devono essere rivalutati regolarmente dal medico prescrittore/personale sanitario in modo che la dose di DuoResp Spiromax rimanga ottimale. La dose deve essere ridotta gradualmente al livello di dose più basso che consente di mantenere un efficace controllo dei sintomi. Se si ritiene di poter effettuare una riduzione graduale a un dosaggio inferiore a quello disponibile per DuoResp Spiromax, è necessario passare a una combinazione a dose fissa di budesonide e formoterolo fumarato alternativa contenente una dose inferiore del corticosteroide per via inalatoria. Quando il controllo dei sintomi viene mantenuto nel lungo periodo con la dose più bassa raccomandata, come fase successiva si potrebbe provare il solo corticosteroide per via inalatoria. Nella pratica corrente, quando viene raggiunto il controllo dei sintomi con il regime posologico di due volte al giorno con un prodotto con dosaggio inferiore, la riduzione graduale alla dose efficace più bassa potrebbe includere un dosaggio di una volta al giorno, nel caso in cui, nell'opinione del medico, sia richiesto un broncodilatatore a lunga durata d'azione per il mantenimento del controllo piuttosto che per il trattamento con solamente un corticosteroide inalatorio. Per DuoResp Spiromax ci sono due modalità di trattamento: **Terapia di mantenimento con DuoResp Spiromax:** DuoResp Spiromax viene utilizzato come regolare trattamento di mantenimento con un altro broncodilatatore a rapida azione da utilizzare come inalatore di sollievo. **Terapia di mantenimento e sollievo con DuoResp Spiromax:** DuoResp Spiromax viene utilizzato come regolare trattamento di mantenimento e al bisogno in risposta ai sintomi. ***Terapia di mantenimento con DuoResp Spiromax*** Ai pazienti si deve consigliare di tenere sempre a disposizione l'inalatore broncodilatatore di sollievo a rapida azione da utilizzare al bisogno. ***Dosi raccomandate:*** Adulti (di età pari o superiore ai 18 anni): 1-2 inalazioni due volte al giorno. Alcuni pazienti potrebbero richiedere fino a un massimo di 4 inalazioni due volte al giorno. Un ricorso crescente a un altro broncodilatatore a rapida azione indica un peggioramento della condizione di base e richiede una rivalutazione della terapia per l'asma. ***Terapia di mantenimento e sollievo con DuoResp Spiromax*** I pazienti assumono una dose di mantenimento giornaliera di DuoResp Spiromax, oltre a utilizzare DuoResp Spiromax al bisogno in risposta ai sintomi. Ai pazienti si deve consigliare di tenere sempre a disposizione DuoResp Spiromax da utilizzare al bisogno. La terapia di mantenimento e sollievo con DuoResp Spiromax deve essere presa in considerazione soprattutto in pazienti con: • controllo inadeguato dell'asma e necessità frequente di un inalatore di sollievo; • riacutizzazioni dell'asma che hanno richiesto intervento medico in passato. Nei pazienti che assumono un elevato numero di inalazioni al bisogno con DuoResp Spiromax è necessario un attento monitoraggio delle reazioni avverse correlate alla dose. ***Dosi raccomandate:*** Adulti (di età pari o superiore ai 18 anni): la dose di mantenimento raccomandata è di 2 inalazioni al giorno, un'inalazione mattina e sera o 2 inalazioni al mattino o alla sera. Per alcuni pazienti potrebbe essere indicata una dose di mantenimento di 2 inalazioni due volte al giorno. I pazienti devono assumere 1 ulteriore inalazione al bisogno in risposta ai sintomi. Se i sintomi persistono dopo alcuni minuti, si deve assumere un'ulteriore inalazione. Non si devono superare le 6 inalazioni contemporaneamente. Una dose giornaliera totale superiore alle 8 inalazioni di norma non è necessaria; tuttavia, per un periodo di tempo limitato si potrebbe ricorrere a una dose giornaliera totale fino a 12 inalazioni. Ai pazienti che effettuano più di 8 inalazioni al giorno si deve fortemente raccomandare di consultare un medico. Tali pazienti dovranno essere rivalutati e si dovrà riconsiderare la loro terapia di mantenimento. ***Broncopneumopatia cronica ostruttiva (BPCO) Dosi raccomandate:*** Adulti (di età pari o superiore ai 18 anni): 2 inalazioni due volte al giorno ***Popolazioni speciali: Anziani (≥65 anni di età)*** Non vi sono requisiti particolari per quanto riguarda il dosaggio nei pazienti anziani. ***Pazienti con compromissione renale o epatica*** Non vi sono dati disponibili sull'uso di una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato in pazienti con compromissione epatica o renale. Poiché budesonide e formoterolo sono eliminati principalmente tramite metabolismo epatico, nei pazienti con grave cirrosi epatica ci si può attendere una maggiore esposizione. ***Popolazione pediatrica*** La sicurezza e l'efficacia di DuoResp Spiromax nei bambini di età pari o inferiore ai 12 anni e negli adolescenti dai 13 ai 17 anni di età non sono state ancora stabilite. Non ci sono dati disponibili. Non è raccomandato l'uso di questo medicinale nei bambini e negli adolescenti di età inferiore ai 18 anni. **Modo di somministrazione Uso inalatorio** L'inalatore Spiromax è azionato dalla respirazione e dal flusso inspiratorio, il che significa che i principi attivi vengono erogati nelle vie respiratorie quando il paziente inala attraverso il boccaglio. È stato dimostrato che i pazienti moderatamente e gravemente asmatici sono in grado di generare un flusso inspiratorio sufficiente affinché Spiromax eroghi la dose terapeutica (vedere paragrafo 5.1). Per ottenere un trattamento efficace, DuoResp Spiromax deve essere utilizzato correttamente. Per questa ragione, i pazienti devono essere invitati a leggere attentamente il foglio illustrativo e a seguire le istruzioni per l'uso descritte in dettaglio al suo interno. L'uso di DuoResp Spiromax segue tre semplici passaggi, di seguito illustrate: aprire, respirare e chiudere. **Aprire:** tenere lo Spiromax con il cappuccio protettivo in basso e aprire il cappuccio piegandolo verso il basso finché non risulta completamente aperto e si avverte un clic. **Respirare:** posizionare il boccaglio tra i denti chiudendolo fra le labbra; non mordere il boccaglio dell'inalatore. Respirare vigorosamente e profondamente attraverso il boccaglio. Rimuovere lo Spiromax dalla bocca e trattenere il respiro per 10 secondi o finché possibile per il paziente. **Chiudere:** espirare delicatamente e richiudere il cappuccio protettivo. È anche importante consigliare ai pazienti di non agitare l'inalatore prima dell'uso, a non espirare attraverso lo Spiromax e a non ostruire le prese d'aria quando si stanno preparando alla fase del "Respirare". Si deve inoltre consigliare ai pazienti di sciacquarsi la bocca con acqua dopo l'inalazione (vedere paragrafo 4.4). Il paziente potrebbe avvertire un certo sapore dovuto all'eccipiente lattosio quando utilizza DuoResp Spiromax. **4.3 Controindicazioni** Ipersensibilità ai principi attivi o ad uno qualsiasi degli eccipienti elencati al paragrafo 6.1. **4.4 Avvertenze speciali e precauzioni d'impiego Informazioni generali** Si raccomanda una diminuzione graduale della dose quando si pone fine al trattamento, che non deve essere interrotto bruscamente. Se i pazienti rilevano inefficacia del trattamento o eccedono la dose massima raccomandata di DuoResp Spiromax, si deve richiedere un parere medico (vedere paragrafo 4.2). Un improvviso e progressivo peggioramento nel controllo dell'asma o della BPCO rappresenta un potenziale pericolo di vita e il paziente deve sottoporsi a una valutazione medica d'urgenza. In tale situazione, si deve considerare la necessità di aumentare la terapia con corticosteroidi, per esempio con un ciclo di corticosteroidi per via orale o un trattamento antibiotico in caso di infezione. Ai pazienti si deve consigliare di tenere sempre a disposizione l'inalatore da utilizzare al bisogno, che si tratti di DuoResp Spiromax (per i pazienti asmatici che utilizzano DuoResp Spiromax come terapia di mantenimento e sollievo) o un altro broncodilatatore a rapida azione (per i pazienti asmatici che utilizzano DuoResp Spiromax solo come terapia di mantenimento). Si deve ricordare ai pazienti di assumere la dose di mantenimento di DuoResp Spiromax secondo la prescrizione medica, anche in assenza di sintomi. L'uso profilattico di DuoResp Spiromax, per esempio prima dell'esercizio fisico, non è stato studiato. Le inalazioni al bisogno di DuoResp Spiromax devono essere effettuate in risposta ai sintomi, ma non sono destinate al regolare uso profilattico, per esempio prima dell'esercizio fisico. A questo fine, si deve prendere in considerazione un altro broncodilatatore a rapida azione. **Sintomi dell'asma** I pazienti devono essere regolarmente riesaminati dal medico prescrittore/personale sanitario in modo che la dose di DuoResp Spiromax resti ottimale. La dose deve essere ridotta gradualmente al livello di dose più basso che consente di mantenere un efficace controllo dei sintomi. Una volta raggiunto il controllo dei sintomi dell'asma, si potrà prendere in considerazione una riduzione graduale della dose di DuoResp Spiromax. Se è appropriato effettuare una riduzione graduale a un dosaggio inferiore a quello disponibile per DuoResp Spiromax, è necessario passare a un'associazione a dose fissa di budesonide e formoterolo fumarato alternativa contenente una dose inferiore del corticosteroide per via inalatoria. È importante esaminare regolarmente i pazienti durante la riduzione graduale della dose. I pazienti non devono iniziare il trattamento con DuoResp Spiromax durante una riacutizzazione o qualora presentino un peggioramento significativo o deterioramento acuto dell'asma. Durante il trattamento con DuoResp Spiromax possono verificarsi gravi reazioni avverse e riacutizzazioni correlate all'asma. Ai pazienti deve essere richiesto di proseguire il trattamento, ma di consultare un medico se i sintomi dell'asma rimangono incontrollati o peggiorano dopo l'inizio del trattamento con DuoResp Spiromax. Dopo la somministrazione si può osservare broncospasmo paradosso, con un aumento immediato di sibilo e affanno. Se il paziente manifesta broncospasmo paradosso, DuoResp Spiromax deve essere immediatamente interrotto, il paziente deve essere valutato e, se necessario, si deve istituire una terapia alternativa. Il broncospasmo paradosso risponde all'inalazione di broncodilatatori a rapida azione e deve essere trattato immediatamente (vedere paragrafo 4.8). **Effetti sistemici** Effetti sistemici possono verificarsi con qualsiasi corticosteroide per via inalatoria, soprattutto a dosi elevate prescritte per lunghi periodi. La comparsa di questi effetti è molto meno probabile con il trattamento per via inalatoria che con i corticosteroidi per via orale. I possibili effetti sistemici comprendono sindrome di Cushing, caratteristiche cushingoidi, soppressione surrenale, ritardi della crescita nei bambini e negli adolescenti, diminuzione della densità minerale ossea, cataratta e glaucoma e, più raramente, una gamma di effetti psicologici e comportamentali che includono iperattività psicomotoria, disturbi del sonno, ansia, depressione o aggressività (in particolare nei bambini) (vedere paragrafo 4.8). Si raccomanda di controllare periodicamente la statura dei bambini in trattamento prolungato con corticosteroidi per via inalatoria. Se la crescita risulta rallentata, la terapia deve essere rivalutata al fine di ridurre la dose di corticosteroide inalatorio fino alla dose più bassa alla quale si ha un effettivo controllo dell'asma, se possibile. I benefici della terapia con corticosteroidi e i possibili rischi di soppressione della crescita devono essere attentamente ponderati. Inoltre, si deve prendere in considerazione l'eventualità di rinviare il paziente a uno specialista in pneumologia pediatrica. Dati limitati provenienti da studi a lungo termine suggeriscono che la maggior parte dei bambini e degli adolescenti trattati con budesonide per via inalatoria, raggiunge un'adeguata statura da adulto. Tuttavia è stata osservata una piccola riduzione iniziale, ma transitoria, nell'accrescimento (circa 1 cm). Ciò si verifica in genere entro il primo anno di trattamento. **Effetti sulla densità ossea** Si devono prendere in considerazione i potenziali effetti sulla densità ossea, in particolare nei pazienti trattati a dosi elevate per periodi prolungati e con coesistenti fattori di rischio per l'osteoporosi. Studi a lungo termine con budesonide per via inalatoria nei bambini a dosi giornaliere medie da 400 microgrammi (dose preimpostata) o negli adulti a dosi giornaliere da 800 microgrammi (dose preimpostata) non hanno mostrato effetti significativi sulla densità minerale ossea. Non sono disponibili informazioni sull'effetto di una combinazione a dose fissa di budesonide/formoterolo fumarato diidrato a dosi più elevate. **Funzione surrenale** Se sussistono ragioni per supporre una compromissione della funzione surrenale causata da una precedente terapia sistemica con steroidi, si deve prestare attenzione quando si avviano i pazienti a una terapia di associazione a dose fissa di budesonide/formoterolo fumarato. I benefici della terapia con budesonide per via inalatoria dovrebbero di norma ridurre al minimo la necessità di steroidi orali, ma nei pazienti che provengono da una terapia con steroidi orali può permanere il rischio di compromissione della riserva surrenale per un periodo di tempo considerevole. La guarigione potrebbe richiedere una notevole quantità di tempo in seguito alla sospensione della terapia con steroidi orali e per questo nei pazienti steroido-dipendenti avviati a bude-

sonide per via inalatoria può permanere il rischio di compromissione della funzione surrenale per un periodo di tempo considerevole. In tali circostanze si deve effettuare il regolare monitoraggio della funzione dell'asse ipotalamico-pituitario-corticosurrenale (hypotalamic pituitary adrenocortical, HPA). **Corticosteroidi ad alte dosi** Il trattamento prolungato con dosi elevate di corticosteroidi per via inalatoria, soprattutto a dosi superiori a quelle raccomandate, può determinare anche una soppressione surrenale clinicamente significativa. In periodi di stress, come infezioni gravi o interventi chirurgici di elezione, deve quindi essere presa in considerazione una copertura addizionale con corticosteroidi sistemici. Una rapida riduzione della dose di steroidi può indurre una crisi surrenale acuta. I sintomi e i segni che si potrebbero osservare in una crisi surrenale acuta possono essere alquanto vaghi, ma possono includere anoressia, dolore addominale, perdita di peso, stanchezza, cefalea, nausea, vomito, ridotto livello di coscienza, convulsioni, ipotensione e ipoglicemia. Il trattamento con steroidi sistemici aggiuntivi o budesonide per via inalatoria non deve essere interrotto bruscamente. **Passaggio dalla terapia orale** Durante il passaggio da una terapia orale a una terapia combinata a dose fissa con budesonide/formoterolo fumarato, si osserverà un'azione sistemica degli steroidi generalmente inferiore, che potrà dar luogo all'insorgenza di sintomi allergici o artritici quali rinite, eczema e dolore muscolare e articolare. In queste condizioni si deve iniziare un trattamento specifico. Si deve sospettare un effetto generale insufficiente dei glucocorticoidi qualora, in rari casi, dovessero verificarsi sintomi quali stanchezza, cefalea, nausea e vomito. In questi casi è talvolta necessario un aumento temporaneo della dose dei glucocorticoidi orali. **Infezioni del cavo orale** Per ridurre al minimo il rischio di infezione da candida nel tratto orofaringeo, si deve istruire il paziente a sciacquarsi la bocca con acqua dopo l'inalazione della dose. In caso di mughetto, il paziente deve sciacquarsi la bocca con acqua anche dopo le inalazioni effettuate al bisogno. **Interazioni con altri medicinali** Il trattamento concomitante con itraconazolo, ritonavir o altri potenti inibitori del CYP3A4 deve essere evitato (vedere paragrafo 4.5). Se ciò non fosse possibile, l'intervallo di tempo tra le somministrazioni dei medicinali che interagiscono tra loro deve essere il più lungo possibile. La combinazione di budesonide/formoterolo fumarato a dose fissa non è raccomandata nei pazienti che utilizzano potenti inibitori del CYP3A4. **Precauzioni con malattie speciali** Una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato deve essere somministrata con cautela nei pazienti con tireotossicosi, feocromocitoma, diabete mellito, ipopotassiemia non trattata, cardiomiopatia ipertrofica ostruttiva, stenosi subvalvolare aortica idiopatica, ipertensione grave, aneurisma o altri gravi disturbi cardiovascolari quali cardiopatia ischemica, tachiaritmia o insufficienza cardiaca grave. Deve essere osservata cautela nel trattamento di pazienti con prolungamento dell'intervallo QTc. Il formoterolo stesso può indurre un prolungamento dell'intervallo QTc. La necessità e la dose di corticosteroidi per via inalatoria devono essere rivalutate nei pazienti con tubercolosi polmonare attiva o quiescente, e infezioni micotiche e virali delle vie aeree. Nei pazienti diabetici devono essere presi in considerazione controlli supplementari della glicemia. **ß$_2$-adrenocettori agonisti** Dosi elevate di ß$_2$-adrenocettori agonisti possono determinare un'ipopotassiemia potenzialmente grave. L'effetto di un trattamento concomitante con ß$_2$-adrenocettori agonisti e medicinali che possono causare ipopotassiemia o potenziare un effetto ipopotassiemico, per esempio derivati xantinici, steroidi e diuretici, può sommarsi a un possibile effetto ipopotassiemico del ß$_2$-adrenocettore agonista. Il trattamento con ßß$_2$-adrenocettori agonisti può determinare un aumento dei livelli ematici di insulina, acidi grassi liberi, glicerolo e corpi chetonici. Si raccomanda particolare cautela nell'asma instabile che necessita di un uso variabile di broncodilatatori per l'uso al bisogno, nell'asma acuta grave, in quanto il rischio associato può essere aumentato dall'ipossia, e in altre condizioni in cui la probabilità di ipopotassiemia è maggiore. Si raccomanda in tali circostanze di monitorare i livelli di potassio sierico. **Eccipienti** Questo medicinale contiene lattosio. I pazienti affetti da rari problemi ereditari di intolleranza al galattosio, deficit di lattasi di Lapp o malassorbimento di glucosio-galattosio non devono assumere questo medicinale. L'eccipiente lattosio contiene piccole quantità di proteine del latte che possono causare reazioni allergiche. **4.5 Interazione con altri medicinali ed altre forme d'interazione Interazioni farmacocinetiche** È probabile che potenti inibitori del CYP3A4 (es. ketoconazolo, itraconazolo, voriconazolo, posaconazolo, claritromicina, telitromicina, nefazodone e inibitori dell'HIV-proteasi) aumentino notevolmente i livelli plasmatici di budesonide e l'uso concomitante deve essere evitato. Se ciò non fosse possibile, l'intervallo di tempo tra la somministrazione dell'inibitore e quella di budesonide deve essere il più lungo possibile (vedere paragrafo 4.4). La terapia di mantenimento e sollievo con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato non è raccomandata nei pazienti che utilizzano potenti inibitori del CYP3A4. Il ketoconazolo, un potente inibitore del CYP3A4, 200 mg una volta al giorno, ha aumentato in media di sei volte i livelli plasmatici di budesonide somministrata in concomitanza per via orale (dose singola da 3 mg). Se somministrato 12 ore dopo budesonide, la concentrazione di ketoconazolo è risultata aumentata in media solo di tre volte, il che dimostra che la separazione dei tempi di somministrazione può ridurre l'aumento dei livelli plasmatici. Dati limitati riguardo a questa interazione relativi a budesonide per via inalatoria a dosi elevate indicano che può verificarsi un notevole aumento dei livelli plasmatici (in media di quattro volte) se itraconazolo, 200 mg una volta al giorno, viene somministrato in concomitanza con budesonide per via inalatoria (dose singola da 1000 microgrammi). **Interazioni farmacodinamiche** I ß-bloccanti adrenergici possono indebolire o inibire l'effetto del formoterolo. Pertanto, una terapia con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato non deve essere somministrata contemporaneamente ai beta-bloccanti adrenergici (inclusi i colliri) salvo in casi di necessità. Il trattamento concomitante con chinidina, disopiramide, procainamide, fenotiazine, antistaminici (terfenadina), inibitori delle monoamino ossidasi e antidepressivi triciclici può prolungare l'intervallo QTc e aumentare il rischio di aritmie ventricolari. Inoltre, L-Dopa, L-tiroxina, ossitocina e l'alcool possono indebolire la tolleranza cardiaca nei confronti dei ß$_2$-simpaticomimetici. Il trattamento concomitante con inibitori delle monoamino ossidasi, compresi medicinali con proprietà simili come furazolidone e procarbazina, può scatenare reazioni ipertensive. Sussiste un rischio elevato di aritmie in pazienti sottoposti contemporaneamente ad anestesia con idrocarburi alogenati. L'uso concomitante di altri medicinali ß-adrenergici e anticolinergici può avere un potenziale effetto broncodilatatorio additivo. L'ipopotassiemia può accrescere la tendenza alle aritmie nei pazienti trattati con glicosidi digitalici. Non sono state osservate interazioni di budesonide e formoterolo con altri medicinali utilizzati nel trattamento dell'asma. **Popolazione pediatrica** Sono stati effettuati studi d'interazione solo negli adulti. **4.6 Fertilità, gravidanza e allattamento Gravidanza** Per una terapia con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato o il trattamento concomitante con formoterolo e budesonide non sono disponibili dati clinici relativi a gravidanze esposte. Dati di uno studio sullo sviluppo embrio-fetale nel ratto non hanno evidenziato prove di ulteriori effetti dovuti all'associazione. Non sono disponibili dati adeguati sull'uso del formoterolo nelle donne in gravidanza. In studi su animali, il formoterolo ha causato reazioni avverse in relazione alla riproduzione a livelli di esposizione sistemica molto elevati (vedere paragrafo 5.3). Dati su circa 2000 gravidanze esposte indicano che non vi è alcun aumento del rischio di teratogenicità associato all'uso di budesonide per via inalatoria. In studi su animali, è stato dimostrato che i glucocorticoidi inducono malformazioni (vedere paragrafo 5.3), fatto probabilmente non rilevante per l'uomo alle dosi raccomandate. Studi su animali hanno inoltre rilevato la correlazione fra un eccesso di glucocorticoidi in età prenatale e l'aumento del rischio di crescita intrauterina ritardata, malattia cardiovascolare nell'adulto e modifiche permanenti nella densità dei recettori dei glucocorticoidi nonché nel turnover e nel comportamento dei neurotrasmettitori a esposizioni inferiori all'intervallo di dose teratogenico. Una terapia con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato deve essere usata in gravidanza solo se i benefici sono superiori ai potenziali rischi. La budesonide deve essere utilizzata alla dose efficace più bassa necessaria per il mantenimento di un adeguato controllo dell'asma. **Allattamento** La budesonide viene escreta nel latte materno. Tuttavia, alle dosi terapeutiche non sono attesi effetti sul lattante. Non è noto se il formoterolo passi nel latte materno umano. Nei ratti, piccole quantità di formoterolo sono state riscontrate nel latte materno. La somministrazione di una terapia con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato in donne in allattamento deve essere presa in considerazione solo se il beneficio atteso per la madre è maggiore di ogni possibile rischio per il bambino. **Fertilità** Non sono disponibili dati sulla fertilità. **4.7 Effetti sulla capacità di guidare veicoli e sull'uso di macchinari** DuoResp Spiromax non altera o altera in modo trascurabile la capacità di guidare veicoli o di usare macchinari. **4.8 Effetti indesiderati Riassunto del profilo di sicurezza** Poiché DuoResp Spiromax contiene sia budesonide sia formoterolo, può verificarsi lo stesso quadro di reazioni avverse riportato per queste sostanze. Non sono stati osservati aumenti nell'incidenza di reazioni avverse in seguito alla somministrazione concomitante dei due composti. Le reazioni avverse più comuni sono reazioni avverse farmacologicamente prevedibili della terapia con ß$_2$-adrenocettori agonisti, come tremore e palpitazioni. Tali reazioni tendono a essere di grado lieve e solitamente scompaiono entro pochi giorni di trattamento. In una sperimentazione clinica di 3 anni con budesonide per la BPCO, si sono verificate ecchimosi e polmonite con una frequenza rispettivamente del 10% e del 6%, rispetto al 4% e al 3% del gruppo placebo (rispettivamente $p<0,001$ e $p<0,01$). DuoResp Spiromax non è indicato nei bambini e negli adolescenti di età inferiore ai 18 anni (vedere paragrafo 4.2). **Tabella delle reazioni avverse** Le reazioni avverse associate a budesonide o formoterolo sono riportate di seguito ed elencate in base alla classificazione per sistemi e organi e frequenza. Le frequenze sono definite come: molto comune (≥1/10), comune (≥1/100, <1/10), non comune (≥1/1.000, <1/100), raro (≥1/10.000, <1/1.000), molto raro (<1/10.000) e non nota (la frequenza non può essere definita sulla base dei dati disponibili).

Classificazione per sistemi e organi	Frequenza	Reazione avversa
Infezioni e infestazioni	Comune	Infezioni da candida del tratto orofaringeo
Disturbi del sistema immunitario	Raro	Reazioni di ipersensibilità immediate e ritardate, es. esantema, orticaria, prurito, dermatite, angioedema e reazione anafilattica
Patologie endocrine	Molto raro	Sindrome di Cushing, soppressione surrenale, ritardi nella crescita, riduzione della densità minerale ossea
Disturbi del metabolismo e della nutrizione	Raro	Ipopotassiemia
	Molto raro	Iperglicemia
Disturbi psichiatrici	Non comune	Aggressività, iperattività psicomotoria, ansia, disturbi del sonno
	Molto raro	Depressione, modificazioni comportamentali (prevalentemente nei bambini)
Patologie del sistema nervoso	Comune	Cefalea, tremore
	Non comune	Capogiri
	Molto raro	Disturbi del gusto
Patologie dell'occhio	Molto raro	Cataratta e glaucoma

Patologie cardiache	Comune	Palpitazioni
	Non comune	Tachicardia
	Raro	Aritmie cardiache, es. fibrillazione atriale, tachicardia sopraventricolare, extrasistoli
	Molto raro	Angina pectoris. Prolungamento dell'intervallo QTc
Patologie vascolari	Molto raro	Variazione della pressione arteriosa
Patologie respiratorie, toraciche e mediastiniche	Comune	Lieve irritazione alla gola, tosse, raucedine
	Raro	Broncospasmo
	Molto raro	Broncospasmo paradosso
Patologie gastrointestinali	Non comune	Nausea
Patologie della cute e del tessuto sottocutaneo	Non comune	Ecchimosi
Patologie del sistema muscoloscheletrico e del tessuto connettivo	Non comune	Crampi muscolari

Descrizione di reazioni avverse selezionate L'infezione da candida nel tratto orofaringeo è dovuta al deposito dei principi attivi. Consigliando al paziente di sciacquarsi la bocca con acqua dopo ogni dose si ridurrà al minimo tale rischio. L'infezione da candida nel tratto orofaringeo solitamente risponde al trattamento topico con antimicotici senza la necessità di sospendere i corticosteroidi per via inalatoria. La possibilità di broncospasmo paradosso è molto rara - interessa 1 persona su 10.000 - con un aumento immediato del sibilo e dell'affanno. Il broncospasmo paradosso risponde ai broncodilatatori per via inalatoria a rapida azione e deve essere trattato immediatamente. DuoResp Spiromax deve essere immediatamente interrotto, il paziente deve essere valutato e, se necessario, si deve istituire una terapia alternativa (vedere paragrafo 4.4). Effetti sistemici dei corticosteroidi per via inalatoria si possono verificare soprattutto a dosi elevate prescritte per lunghi periodi. La comparsa di questi effetti è molto meno probabile che con i corticosteroidi per via orale. I possibili effetti sistemici includono sindrome di Cushing, caratteristiche cushingoidi, soppressione surrenale, ritardi della crescita nei bambini e negli adolescenti, diminuzione della densità minerale ossea, cataratta e glaucoma. Può inoltre verificarsi una maggiore suscettibilità alle infezioni e una compromissione della capacità di adattarsi allo stress. Gli effetti probabilmente dipendono da dose, tempo di esposizione, esposizione concomitante e precedente a steroidi e sensibilità individuale. Il trattamento con $ß_2$-adrenocettori agonisti può determinare un aumento dei livelli ematici di insulina, acidi grassi liberi, glicerolo e corpi chetonici. **Segnalazione delle reazioni avverse sospette** La segnalazione delle reazioni avverse sospette che si verificano dopo l'autorizzazione del medicinale è importante, in quanto permette un monitoraggio continuo del rapporto beneficio/rischio del medicinale. Agli operatori sanitari è richiesto di segnalare qualsiasi reazione avversa sospetta tramite il sistema nazionale di segnalazione all'indirizzo: http://www.agenziafarmaco.gov.it/it/responsabili. **4.9 Sovradosaggio** Un sovradosaggio di formoterolo produrrebbe effetti tipici dei $ß_2$-adrenocettori agonisti: tremore, cefalea, palpitazioni. Sono stati riportati casi isolati di sintomi di tachicardia, iperglicemia, ipopotassiemia, prolungamento dell'intervallo QTc, aritmia, nausea e vomito. Può essere indicato il trattamento sintomatico e di supporto. Una dose da 90 microgrammi somministrata nell'arco di tre ore in pazienti con ostruzione bronchiale acuta non ha destato preoccupazioni circa la sicurezza. Non si prevede che un sovradosaggio acuto di budesonide, anche a dosi eccessive, possa essere un problema clinico. L'uso cronico a dosi eccessive può dar luogo a effetti sistemici dovuti ai glucocorticoidi, come ipercorticismo e soppressione surrenale.Qualora si renda necessaria una sospensione della terapia con DuoResp Spiromax a causa di un sovradosaggio del formoterolo, un componente del medicinale, si deve prendere in considerazione una adeguata terapia con un corticosteroide per via inalatoria. **5. PROPRIETÀ FARMACOLOGICHE 5.1 Proprietà farmacodinamiche** Categoria farmacoterapeutica: adrenergici ed altri farmaci per le sindromi ostruttive delle vie respiratorie.Codice ATC: R03AK07 **Meccanismo d'azione ed effetti farmacodinamici** DuoResp Spiromax contiene formoterolo e budesonide, che presentano un meccanismo d'azione diverso e mostrano effetti additivi in termini di riduzione delle riacutizzazioni dell'asma. Le proprietà specifiche di budesonide e formoterolo consentono di utilizzarne l'associazione come terapia di mantenimento e sollievo o come trattamento di mantenimento per l'asma. I meccanismi di azione delle due sostanze rispettivamente sono descritti di seguito. **Budesonide** Budesonide è un glucocorticoide che, quando inalato, esercita un'azione antinfiammatoria dose-dipendente sulle vie respiratorie, con conseguente riduzione dei sintomi e diminuzione delle riacutizzazioni dell'asma. La budesonide per via inalatoria comporta meno reazioni avverse gravi rispetto ai corticosteroidi per via sistemica. L'esatto meccanismo responsabile dell'effetto antinfiammatorio dei glucocorticoidi non è noto. **Formoterolo** Formoterolo è un $ß_2$-adrenocettore agonista che, quando inalato, induce un rapido e prolungato rilassamento della muscolatura bronchiale liscia nei pazienti con ostruzione reversibile delle vie aeree. L'effetto broncodilatatore è dose-dipendente e si manifesta entro 1-3 minuti. La durata dell'effetto è di almeno 12 ore dopo una singola dose. **Efficacia e sicurezza clinica *Asma* *Terapia di mantenimento con budesonide/formoterolo*** Studi clinici condotti su soggetti adulti hanno dimostrato che l'aggiunta di formoterolo a budesonide migliora i sintomi dell'asma e la funzione polmonare, oltre a ridurre le riacutizzazioni. In due studi di 12 settimane, l'effetto di budesonide/formoterolo sulla funzione polmonare è risultato uguale a quello della libera combinazione di budesonide e formoterolo e ha superato quello della sola budesonide. Tutti i bracci di trattamento prevedevano l'utilizzo al bisogno di un $ß_2$-adrenocettore agonista a rapida azione. Non sono emersi segni di attenuazione dell'effetto antiasmatico nel corso del tempo. Sono stati effettuati due studi pediatrici di 12 settimane, nei quali 265 bambini di età compresa tra i 6 e gli 11 anni sono stati trattati con una dose di mantenimento di budesonide/formoterolo (2 inalazioni da 80 microgrammi/4,5 microgrammi/inalazione due volte al giorno) e un $ß_2$-adrenorecettore agonista a breve durata d'azione al bisogno. In entrambi gli studi, la funzione polmonare è risultata migliorata e il trattamento ben tollerato rispetto alla dose corrispondente di sola budesonide. ***Terapia di mantenimento e sollievo con budesonide/formoterolo per l'asma*** Un totale di 12.076 pazienti asmatici sono stati inclusi in 5 studi clinici condotti in doppio cieco (4447 sono stati randomizzati per la terapia di mantenimento e sollievo con budesonide/formoterolo) per 6 o 12 mesi. I pazienti dovevano essere sintomatici nonostante l'uso di glucocorticoidi per via inalatoria. La terapia di mantenimento e sollievo con budesonide/formoterolo ha prodotto riduzioni statisticamente significative e clinicamente importanti delle riacutizzazioni gravi per tutti i casi confrontati nei 5 studi. In particolare un confronto con budesonide/formoterolo a una dose di mantenimento più alta con terbutalina per il sollievo dei sintomi (studio 735) e budesonide/formoterolo alla stessa dose di mantenimento con formoterolo o terbutalina per il sollievo dei sintomi (studio 734) (vedere tabella seguente). Nello Studio 735, funzione polmonare, controllo dei sintomi e utilizzo del medicinale di sollievo sono risultati simili in tutti i gruppi di trattamento. Nello Studio 734, i sintomi e l'uso del medicinale di sollievo sono apparsi ridotti e la funzione polmonare migliorata, rispetto a entrambi i trattamenti di confronto. Nei 5 studi combinati, i pazienti che hanno ricevuto la terapia di mantenimento e sollievo con budesonide/formoterolo non hanno fatto ricorso a inalazioni di sollievo in media nel 57% dei giorni di trattamento. Non sono emersi segni di sviluppo di tolleranza nel corso del tempo.

Panoramica delle riacutizzazioni gravi negli studi clinici

Numero dello studio, durata	**Gruppi di trattamento**	**N**	**Riacutizzazioni gravi[a]**	
			Eventi	**Eventi/paziente-anno**
Studio 735 6 mesi	**Budesonide/formoterolo fumarato diidrato** 160/4,5 µg BD + al bisogno	**1103**	**125**	**0,23[b]**
	Budesonide/formoterolo fumarato diidrato 320/9 µg BD + terbutalina 0,4 mg al bisogno	1099	173	0,32
	Salmeterolo/fluticasone 2 x 25/125 µg BD + terbutalina 0,4 mg al bisogno	1119	208	0,38
Studio 734 12 mesi	**Budesonide/formoterolo fumarato diidrato** 160/4,5 µg BD + al bisogno	1107	194	0,19[b]
	Budesonide/formoterolo fumarato diidrato 160/4,5 µg BD + formoterolo 4,5 mg al bisogno	1137	296	0,29
	Budesonide/formoterolo fumarato diidrato 160/4,5 µg BD + terbutalina 0,4 mg al bisogno	1138	377	0,37

[a] Trattamento in regime di ospedalizzazione/pronto soccorso o trattamento con steroidi per via orale
[b] La riduzione del tasso di riacutizzazioni è statisticamente significativa (valore P<0,01) per entrambi i confronti

In altri 2 studi su pazienti che si sono rivolti al medico per sintomi acuti dell'asma, budesonide/formoterolo hanno fornito un sollievo rapido ed efficace della broncocostrizione, simile a salbutamolo e formoterolo. **Broncopneumopatia cronica ostruttiva (BPCO)** In due studi di 12 mesi si è valutato l'effetto sulla funzione polmonare e il tasso di riacutizzazioni (definito come cicli di steroidi per via orale e/o cicli di antibiotici e/o ospedalizzazioni) nei pazienti con BPCO grave. La FEV1 mediana all'inclusione nelle sperimentazioni era pari al 36% del valore normale previsto. Il numero medio di riacutizzazioni per anno (come definito sopra) è risultato significativamente ridotto con budesonide/formoterolo rispetto al trattamento con formoterolo da solo o placebo (tasso medio 1,4 rispetto all'1,8-1,9 nel gruppo placebo/formoterolo). Il numero medio di giorni di trattamento con corticosteroidi orali/paziente durante i 12 mesi è apparso leggermente ridotto nel gruppo con budesonide/formoterolo (7-8 giorni/paziente/anno rispetto a 11-12 e 9-12 giorni, rispettivamente nei gruppi placebo e formoterolo). Per quanto concerne le variazioni nei parametri della funzione polmonare, come FEV1, la combinazione budesonide/formoterolo non è risultata superiore al trattamento con formoterolo da solo. **Picco di flusso inspiratorio mediante il dispositivo Spiromax** Per valutare il picco di flusso inspiratorio (Peak Inspiratory Flow Rate, PIFR) e altri parametri di inalazione correlati, è stato effettuato uno studio con placebo, randomizzato e in aperto, su bambini e adolescenti con asma (età 6-17 anni), adulti con asma (età 18-45 anni), adulti con broncopneumopatia cronica ostruttiva (BPCO) (età >50 anni) e volontari sani (età 18-45 anni), attraverso il confronto tra l'inalazione con il dispositivo Spiromax (contenente placebo) e l'inalazione con un inalatore a polvere secca multidose reperibile in commercio (contenente placebo). In questi gruppi di soggetti è stato valutato anche l'impatto del potenziamento della formazione nella tecnica di inalazione mediante inalatore a polvere secca sulla velocità e sul volume di inalazione. I dati dello studio indicano che, a prescindere dall'età e dalla gravità della patologia sottostante, i bambini, gli adolescenti e gli adulti con asma nonché i pazienti con BPCO erano in grado di generare un flusso inspiratorio sufficiente attraverso il dispositivo Spiromax simile a quello generato attraverso il dispositivo di inalazione a polvere secca multidose reperibile in commercio. Il PIFR medio generato dai pazienti con asma o BPCO era superiore ai 60 l/min, un tasso di flusso con il quale entrambi i dispositivi studiati sono noti per erogare ai polmoni quantità paragonabili di farmaco. Pochissimi pazienti hanno avuto valori PIFR inferiori a 40 l/min; nei casi in cui i PIFR sono risultati inferiori a 40 l/min non è stato evidenziato alcun raggruppamento per età o gravità della malattia. **5.2 Proprietà farmacocinetiche Assorbimento** La combinazione a dose fissa di budesonide e formoterolo e i corrispondenti monoprodotti hanno dimostrato di essere bioequivalenti per quanto riguarda l'esposizione sistemica rispettivamente di budesonide e formoterolo. Nonostante ciò, un leggero aumento della soppressione del cortisolo è stato osservato dopo la somministrazione della combinazione a dose fissa rispetto ai singoli prodotti. La differenza è considerata priva di impatto sulla sicurezza clinica. Non vi è alcuna evidenza di interazioni farmacocinetiche tra budesonide e formoterolo. I parametri farmacocinetici per le rispettive sostanze sono risultati comparabili dopo la somministrazione di budesonide e formoterolo singolarmente o in combinazione a dose fissa. Per la budesonide, la AUC è risultata leggermente più elevata, la velocità di assorbimento più rapida e la concentrazione plasmatica massima più alta dopo la somministrazione della combinazione fissa. Per il formoterolo, la concentrazione plasmatica massima è risultata simile dopo la somministrazione della combinazione fissa. Budesonide per via inalatoria viene assorbita rapidamente e la concentrazione plasmatica massima viene raggiunta entro 30 minuti dall'inalazione. Negli studi, la deposizione polmonare media di budesonide dopo l'inalazione tramite l'inalatore a polvere variava dal 32% al 44% della dose erogata. La biodisponibilità sistemica è pari a circa il 49% della dose erogata. Nei bambini di 6-16 anni di età, la deposizione polmonare rientra nel medesimo intervallo di valori degli adulti a parità di dose somministrata. Le concentrazioni plasmatiche derivanti non sono state determinate. Formoterolo per via inalatoria viene assorbito rapidamente e la concentrazione plasmatica massima viene raggiunta entro 10 minuti dall'inalazione. Negli studi, la deposizione polmonare media del formoterolo dopo l'inalazione tramite l'inalatore a polvere variava dal 28% al 49% della dose erogata. La biodisponibilità sistemica è pari a circa il 61% della dose erogata. **Distribuzione** Il legame alle proteine plasmatiche è di circa il 50% per il formoterolo e del 90% per la budesonide. Il volume di distribuzione è di circa 4 l/kg per il formoterolo e 3 l/kg per la budesonide. Il formoterolo è inattivato tramite reazioni di coniugazione (si formano metaboliti O-demetilati e deformilati, per lo più rilevati come coniugati inattivi). Budesonide subisce un notevole livello (circa il 90%) di biotrasformazione in metaboliti a bassa attività glucocorticoide al primo passaggio epatico. L'attività glucocorticoide dei metaboliti principali, 6-beta-idrossi-budesonide e 16-alfa-idrossi-prednisolone, è inferiore all'1% rispetto a quella di budesonide. Non esistono indicazioni di interazioni metaboliche o reazioni da sostituzione tra formoterolo e budesonide. **Eliminazione** La maggior parte di una dose di formoterolo viene trasformata tramite il metabolismo epatico seguito da eliminazione renale. Dopo l'inalazione, dall'8% al 13% della dose erogata di formoterolo viene escreta non metabolizzata nelle urine. Formoterolo ha un elevato livello di clearance sistemica (circa 1,4 l/min) e l'emivita terminale è in media di 17 ore. Budesonide viene eliminata per via metabolica principalmente catalizzata dall'enzima CYP3A4. I metaboliti della budesonide vengono eliminati nelle urine come tali o in forma coniugata. Nelle urine sono stati riscontrati solo livelli trascurabili di budesonide immodificata. Budesonide ha un'elevata eliminazione sistemica (circa 1,2 l/min) e l'emivita di eliminazione plasmatica dopo somministrazione EV è in media di 4 ore. **Relazioni farmacocinetiche/farmacodinamiche** La farmacocinetica di budesonide o formoterolo nei bambini e nei pazienti con insufficienza renale non è nota. L'esposizione di budesonide e formoterolo può risultare aumentata nei pazienti con epatopatia. **Profilo farmacocinetico di DuoResp Spiromax** In studi farmacocinetici con e senza blocco con carbone, DuoResp Spiromax è stato valutato attraverso un confronto con un prodotto di combinazione per via inalatoria a dose fissa autorizzato alternativo contenente gli stessi principi attivi (budesonide e formoterolo), dimostrandone l'equivalenza in termini sia di esposizione sistemica (sicurezza) sia di deposizione polmonare (efficacia). **5.3 Dati preclinici di sicurezza** La tossicità osservata negli studi condotti su animali con budesonide e formoterolo somministrati in combinazione o separatamente si è dimostrata sotto forma di effetti associati ad attività farmacologica esagerata. Negli studi di riproduzione su animali, i corticosteroidi come budesonide hanno dimostrato di indurre malformazioni (palatoschisi, malformazioni scheletriche). Tuttavia, tali risultati sperimentali nell'animale non sembrano rilevanti nell'uomo alle dosi raccomandate. Gli studi di riproduzione su animali con formoterolo hanno dimostrato una certa riduzione della fertilità nei ratti maschi dopo elevata esposizione sistemica e perdite degli impianti embrionali, così come sono state osservate, ad una esposizione molto più elevata rispetto a quella osservata durante l'uso clinico, una riduzione della sopravvivenza post-natale e del peso alla nascita. Tuttavia, tali risultati sperimentali nell'animale non sembrano rilevanti nell'uomo. **6. INFORMAZIONI FARMACEUTICHE 6.1 Elenco degli eccipienti** Lattosio monoidrato. **6.2 Incompatibilità** Non pertinente. **6.3 Periodo di validità** 2 anni. Dopo l'apertura dell'involucro di alluminio: 6 mesi. **6.4 Precauzioni particolari per la conservazione** Non conservare a temperatura superiore ai 25°C Tenere chiuso il cappuccio protettivo dopo la rimozione dell'involucro di alluminio. **6.5 Natura e contenuto del contenitore** L'inalatore è bianco, con un cappuccio protettivo semitrasparente di colore bordeaux ed è costituito da acrilonitrile butadiene stirene (ABS), polietilene tereftalato (PT) e polipropilene (PP). Ogni inalatore contiene 120 dosi ed è avvolto in un involucro di alluminio. Confezioni multiple contenenti 1, 2 o 3 inalatori. È possibile che non tutte le confezioni siano commercializzate. **6.6 Precauzioni particolari per lo smaltimento e la manipolazione** Nessuna istruzione particolare. **7. TITOLARE DELL'AUTORIZZAZIONE ALL'IMMISSIONE IN COMMERCIO** Teva Pharma B.V. - Computerweg 10, 3542 DR Utrecht - Paesi Bassi **8. NUMERO(I) DELL'AUTORIZZAZIONE ALL'IMMISSIONE IN COMMERCIO** EU/1/14/920/001 - EU/1/14/920/002 - EU/1/14/920/003 **9. DATA DELLA PRIMA AUTORIZZAZIONE/RINNOVO DELL'AUTORIZZAZIONE** Data della prima autorizzazione: 28 Aprile 2014. Data del rinnovo più recente: **10. DATA DI REVISIONE DEL TESTO** Luglio 2014. Informazioni più dettagliate su questo medicinale sono disponibili sul sito web dell'Agenzia europea dei medicinali: http://www.ema.europa.eu Classe A - RR - Prezzo al pubblico 2015: € 49,31

Riassunto delle caratteristiche del prodotto

1. DENOMINAZIONE DEL MEDICINALE DuoResp Spiromax 320 microgrammi/9 microgrammi polvere per inalazione 2. COMPOSIZIONE QUALITATIVA E QUANTITATIVA Ogni dose erogata (dose che fuoriesce dal boccaglio di Spiromax) contiene 320 microgrammi di budesonide e 9 microgrammi di formoterolo fumarato diidrato. Ciò equivale a una dose preimpostata di 400 microgrammi di budesonide e 12 microgrammi di formoterolo fumarato diidrato. Eccipienti con effetti noti: ogni dose contiene circa 10 milligrammi di lattosio (monoidrato). Per l'elenco completo degli eccipienti, vedere paragrafo 6.1. **3. FORMA FARMACEUTICA** Polvere per inalazione. Polvere bianca. Inalatore bianco con un cappuccio protettivo semitrasparente di colore bordeaux. **4. INFORMAZIONI CLINICHE 4.1 Indicazioni terapeutiche** DuoResp Spiromax è indicato esclusivamente negli adulti di età pari o superiore ai 18 anni. ***Asma*** DuoResp Spiromax è indicato per il regolare trattamento dell'asma quando è appropriato l'uso di un'associazione (corticosteroide per via inalatoria e β_2-adrenocettori agonisti a lunga durata d'azione): • in pazienti non adeguatamente controllati con corticosteroidi per via inalatoria e con β_2-adrenocettori agonisti a breve durata d'azione usati "al bisogno" o • in pazienti già adeguatamente controllati sia con corticosteroidi per via inalatoria sia con β_2-adrenocettori agonisti a lunga durata d'azione. **Broncopneumopatia cronica ostruttiva (BPCO)** Trattamento sintomatico di pazienti con BPCO grave (FEV_1 < 50% del normale) e anamnesi di ripetute riacutizzazioni, con sintomi significativi nonostante la terapia regolare con broncodilatatori a lunga durata d'azione. **4.2 Posologia e modo di somministrazione** DuoResp Spiromax è indicato esclusivamente negli adulti di età pari o superiore ai 18 anni. DuoResp Spiromax non è indicato per l'uso nei bambini di età pari o inferiore ai 12 anni o negli adolescenti dai 13 ai 17 anni di età. **Posologia *Asma*** DuoResp Spiromax non è destinato alla gestione iniziale dell'asma. DuoResp Spiromax non è indicato per il trattamento del paziente adulto che presenta solo asma lieve non adeguatamente controllata con un corticosteroide per via inalatoria e β_2-adrenocettori agonisti a rapida azione "al bisogno". Il dosaggio di DuoResp Spiromax è individuale e deve essere adattato in relazione alla gravità della malattia. Ciò deve essere tenuto in considerazione non solo quando si inizia un trattamento con combinazioni di medicinali, ma anche quando la dose di mantenimento viene modificata. Se un singolo paziente necessita di una combinazione di dosi diversa da quelle disponibili nell'inalatore combinato, si devono prescrivere dosi appropriate di β_2-adrenocettori agonisti e/o corticosteroidi con inalatori separati. Una volta raggiunto il controllo dei sintomi dell'asma, si potrà prendere in considerazione una riduzione graduale della dose di DuoResp Spiromax. I pazienti devono essere rivalutati regolarmente dal medico prescrittore/personale sanitario in modo che la dose di DuoResp Spiromax rimanga ottimale. La dose deve essere ridotta gradualmente al livello di dose più basso che consente di mantenere un efficace controllo dei sintomi. Se è appropriato effettuare una riduzione graduale a un dosaggio inferiore a quello disponibile per DuoResp Spiromax, è necessario passare a un'associazione a dose fissa di budesonide e formoterolo fumarato alternativa contenente una dose inferiore del corticosteroide per via inalatoria. Quando il controllo dei sintomi viene mantenuto nel lungo periodo con la dose più bassa raccomandata, come fase successiva si potrebbe provare il solo corticosteroide per via inalatoria. Nella pratica corrente, quando viene raggiunto il controllo dei sintomi con il regime posologico di due dosi al giorno con un prodotto con dosaggio inferiore, la riduzione graduale alla dose efficace più bassa potrebbe includere un dosaggio di una volta al giorno, nel caso in cui, nell'opinione del medico, sia richiesto un broncodilatatore a lunga durata d'azione per il mantenimento del controllo piuttosto che per il trattamento con solamente un corticosteroide inalatorio. Ai pazienti deve essere consigliato di tenere sempre a disposizione l'inalatore broncodilatatore di sollievo a rapida azione da utilizzare "al bisogno". ***Dosi raccomandate:*** Adulti (di età pari o superiore ai 18 anni): 1 inalazione due volte al giorno. Alcuni pazienti potrebbero richiedere fino a un massimo di 2 inalazioni due volte al giorno. Un ricorso crescente a un altro broncodilatatore a rapida azione indica un peggioramento della condizione di base e richiede una rivalutazione della terapia per l'asma. DuoResp Spiromax 320 microgrammi/9,0 microgrammi deve essere utilizzato esclusivamente come terapia di mantenimento. La dose inferiore di DuoResp Spiromax è disponibile per il regime terapeutico di mantenimento e sollievo. ***Broncopneumopatia cronica ostruttiva (BPCO) Dosi raccomandate:*** Adulti (di età pari o superiore ai 18 anni): 1 inalazione due volte al giorno. ***Popolazioni speciali: Anziani (≥65 anni di età)*** Non vi sono requisiti particolari per quanto riguarda il dosaggio nei pazienti anziani. ***Pazienti con compromissione renale o epatica*** Non vi sono dati disponibili sull'uso di una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato in pazienti con compromissione epatica o renale. Poiché budesonide e formoterolo sono eliminati principalmente tramite metabolismo epatico, nei pazienti con grave cirrosi epatica ci si può attendere una maggiore esposizione. ***Popolazione pediatrica*** La sicurezza e l'efficacia di DuoResp Spiromax nei bambini di età pari o inferiore ai 12 anni e negli adolescenti dai 13 ai 17 anni di età non sono state ancora stabilite. Non ci sono dati disponibili. Non è raccomandato l'uso di questo medicinale nei bambini e negli adolescenti di età inferiore ai 18 anni. **Modo di somministrazione Uso inalatorio** L'inalatore Spiromax è azionato dalla respirazione e dal flusso inspiratorio, il che significa che i principi attivi vengono erogati nelle vie respiratorie quando il paziente inala attraverso il boccaglio. È stato dimostrato che i pazienti moderatamente e gravemente asmatici sono in grado di generare un flusso inspiratorio sufficiente affinché Spiromax

eroghi la dose terapeutica (vedere paragrafo 5.1). Per ottenere un trattamento efficace, DuoResp Spiromax deve essere utilizzato correttamente. Per questa ragione, i pazienti devono essere invitati a leggere attentamente il foglio illustrativo e a seguire le istruzioni per l'uso descritte in dettaglio al suo interno. L'uso di DuoResp Spiromax segue tre semplici passaggi, di seguito illustrate: aprire, respirare e chiudere. **Aprire:** tenere lo Spiromax con il cappuccio protettivo in basso e aprire il cappuccio piegandolo verso il basso finché non risulta completamente aperto e si avverte un clic. **Respirare:** posizionare il boccaglio tra i denti chiudendolo fra le labbra; non mordere il boccaglio dell'inalatore. Respirare vigorosamente e profondamente attraverso il boccaglio. Rimuovere lo Spiromax dalla bocca e trattenere il respiro per 10 secondi o finché possibile per il paziente. **Chiudere:** espirare delicatamente e richiudere il cappuccio protettivo. È anche importante consigliare ai pazienti di non agitare l'inalatore prima dell'uso, non espirare attraverso lo Spiromax e non ostruire le prese d'aria quando si stanno preparando alla fase del "Respirare". Si deve inoltre consigliare ai pazienti di sciacquarsi la bocca con acqua dopo l'inalazione (vedere paragrafo 4.4). Il paziente potrebbe avvertire un certo sapore dovuto all'eccipiente lattosio quando utilizza DuoResp Spiromax. **4.3 Controindicazioni** Ipersensibilità ai principi attivi o ad uno qualsiasi degli eccipienti elencati al paragrafo 6.1. **4.4 Avvertenze speciali e precauzioni d'impiego Informazioni generali** Si raccomanda una diminuzione graduale della dose quando si pone fine al trattamento, che non deve essere interrotto bruscamente. Se i pazienti rilevano inefficacia del trattamento o eccedono la dose massima raccomandata di DuoResp Spiromax, si deve richiedere un parere medico (vedere paragrafo 4.2). Un improvviso e progressivo peggioramento nel controllo dell'asma o della BPCO rappresenta un potenziale pericolo di vita e il paziente deve sottoporsi a una valutazione medica d'urgenza. In tale situazione, si deve considerare la necessità di aumentare la terapia con corticosteroidi, per esempio con un ciclo di corticosteroidi per via orale o un trattamento antibiotico in caso di infezione. Ai pazienti si deve consigliare di tenere sempre a disposizione l'inalatore da utilizzare al bisogno, che si tratti di DuoResp Spiromax (per i pazienti asmatici che utilizzano DuoResp Spiromax come terapia di mantenimento e sollievo) o un altro broncodilatatore a rapida azione (per i pazienti asmatici che utilizzano DuoResp Spiromax solo come terapia di mantenimento). Si deve ricordare ai pazienti di assumere la dose di mantenimento di DuoResp Spiromax secondo la prescrizione medica, anche in assenza di sintomi. L'uso profilattico di DuoResp Spiromax, per esempio prima dell'esercizio fisico, non è stato studiato. Le inalazioni al bisogno di DuoResp Spiromax devono essere effettuate in risposta ai sintomi, ma non sono destinate al regolare uso profilattico, per esempio prima dell'esercizio fisico. A questo fine, si deve prendere in considerazione un altro broncodilatatore a rapida azione. **Sintomi dell'asma** I pazienti devono essere regolarmente riesaminati dal medico prescrittore/personale sanitario in modo che la dose di DuoResp Spiromax resti ottimale. La dose deve essere ridotta gradualmente al livello di dose più basso che consente di mantenere un efficace controllo dei sintomi. Una volta raggiunto il controllo dei sintomi dell'asma, si potrà prendere in considerazione una riduzione graduale della dose di DuoResp Spiromax. Se è appropriato effettuare una riduzione graduale a un dosaggio inferiore a quello disponibile per DuoResp Spiromax, è necessario passare a un'associazione a dose fissa di budesonide e formoterolo fumarato alternativa contenente una dose inferiore del corticosteroide per via inalatoria. È importante esaminare regolarmente i pazienti durante la riduzione graduale della dose. I pazienti non devono iniziare il trattamento con DuoResp Spiromax durante una riacutizzazione o qualora presentino un peggioramento significativo o deterioramento acuto dell'asma. Durante il trattamento con DuoResp Spiromax possono verificarsi gravi reazioni avverse e riacutizzazioni correlate all'asma. Ai pazienti deve essere richiesto di proseguire il trattamento, ma di consultare un medico se i sintomi dell'asma rimangono incontrollati o peggiorano dopo l'inizio del trattamento con DuoResp Spiromax. Dopo la somministrazione si può osservare broncospasmo paradosso, con un aumento immediato di sibilo e affanno. Se il paziente manifesta broncospasmo paradosso, DuoResp Spiromax deve essere immediatamente interrotto, il paziente deve essere valutato e, se necessario, si deve istituire una terapia alternativa. Il broncospasmo paradosso risponde all'inalazione di broncodilatatori a rapida azione e deve essere trattato immediatamente (vedere paragrafo 4.8). **Effetti sistemici** Effetti sistemici possono verificarsi con qualsiasi corticosteroide per via inalatoria, soprattutto a dosi elevate prescritte per lunghi periodi. La comparsa di questi effetti è molto meno probabile con il trattamento per via inalatoria che con i corticosteroidi per via orale. I possibili effetti sistemici comprendono sindrome di Cushing, caratteristiche cushingoidi, ritardi della crescita nei bambini e negli adolescenti, soppressione surrenale, diminuzione della densità minerale ossea, cataratta e glaucoma e, più raramente, una gamma di effetti psicologici e comportamentali che includono iperattività psicomotoria, disturbi del sonno, ansia, depressione o aggressività (in particolare nei bambini) (vedere paragrafo 4.8). Si raccomanda di controllare periodicamente la statura dei bambini in trattamento prolungato con corticosteroidi per via inalatoria. Se la crescita risulta rallentata, la terapia deve essere rivalutata al fine di ridurre la dose di corticosteroide inalatorio fino alla dose più bassa alla quale si ha un effettivo controllo dell'asma, se possibile. I benefici della terapia con corticosteroidi e i possibili rischi di soppressione della crescita devono essere attentamente ponderati. Inoltre, si deve prendere in considerazione l'eventualità di rinviare il paziente a uno specialista in pneumologia pediatrica. Dati limitati provenienti da studi a lungo termine suggeriscono che la maggior parte dei bambini e degli adolescenti trattati con budesonide per via inalatoria, raggiunge un'adeguata statura da adulto. Tuttavia è stata osservata una piccola riduzione iniziale, ma transitoria, nell'accrescimento (circa 1 cm). Ciò si verifica in genere entro il primo anno di trattamento. **Effetti sulla densità ossea** Si devono prendere in considerazione i potenziali effetti sulla densità ossea, in particolare nei pazienti trattati a dosi elevate per periodi prolungati e con coesistenti fattori di rischio per l'osteoporosi. Studi a lungo termine con budesonide per via inalatoria nei bambini a dosi giornaliere medie da 400 microgrammi (dose preimpostata) o negli adulti a dosi giornaliere da 800 microgrammi (dose preimpostata) non hanno mostrato effetti significativi sulla densità minerale ossea. Non sono disponibili informazioni sull'effetto di una combinazione a dose fissa di budesonide/formoterolo fumarato diidrato a dosi più elevate. **Funzione surrenale** Se sussistono ragioni per supporre una compromissione della funzione surrenale causata da una precedente terapia sistemica con steroidi, si deve prestare attenzione quando si avviano i pazienti a una terapia di associazione a dose fissa di budesonide/formoterolo fumarato. I benefici della terapia con budesonide per via inalatoria dovrebbero di norma ridurre al minimo la necessità di steroidi orali, ma nei pazienti che provengono da una terapia con steroidi orali può permanere il rischio di compromissione della riserva surrenale per un periodo di tempo considerevole. La guarigione potrebbe richiedere una notevole quantità di tempo in seguito alla sospensione della terapia con steroidi orali e per questo nei pazienti steroido-dipendenti avviati a budesonide per via inalatoria può permanere il rischio di compromissione della funzione surrenale per un periodo di tempo considerevole. In tali circostanze si deve effettuare il regolare monitoraggio della funzione dell'asse ipotalamico-pituitario-corticosurrenale (hypotalamic pituitary adrenalcortical, HPA). **Corticosteroidi ad alte dosi** Il trattamento prolungato con dosi elevate di corticosteroidi per via inalatoria, soprattutto a dosi superiori a quelle raccomandate, può determinare anche una soppressione surrenale clinicamente significativa. In periodi di stress, come infezioni gravi o interventi chirurgici di elezione, deve quindi essere presa in considerazione una copertura addizionale con corticosteroidi sistemici. Una rapida riduzione della dose di steroidi può indurre una crisi surrenale acuta. I sintomi e i segni che si potrebbero osservare in una crisi surrenale acuta possono essere alquanto vaghi, ma possono includere anoressia, dolore addominale, perdita di peso, stanchezza, cefalea, nausea, vomito, ridotto livello di coscienza, convulsioni, ipotensione e ipoglicemia. Il trattamento con steroidi sistemici aggiuntivi o budesonide per via inalatoria non deve essere interrotto bruscamente. **Passaggio dalla terapia orale** Durante il passaggio da una terapia orale a una terapia combinata a dose fissa con budesonide/formoterolo fumarato, si osserverà un'azione sistemica degli steroidi generalmente inferiore, che potrà dar luogo all'insorgenza di sintomi allergici o artritici quali rinite, eczema e dolore muscolare e articolare. In queste condizioni si deve iniziare un trattamento specifico. Si deve sospettare un effetto generale insufficiente dei glucocorticoidi qualora, in rari casi, dovessero verificarsi sintomi quali stanchezza, cefalea, nausea e vomito. In questi casi è talvolta necessario un aumento temporaneo della dose dei glucocorticoidi orali. **Infezioni del cavo orale** Per ridurre al minimo il rischio di infezione da candida nel tratto orofaringeo, si deve istruire il paziente a sciacquarsi la bocca con acqua dopo l'inalazione della dose. In caso di mughetto, il paziente deve sciacquarsi la bocca con acqua anche dopo le inalazioni effettuate al bisogno. **Interazioni con altri medicinali** Il trattamento concomitante con itraconazolo, ritonavir o altri potenti inibitori del CYP3A4 deve essere evitato (vedere paragrafo 4.5). Se ciò non fosse possibile, l'intervallo di tempo tra le somministrazioni dei medicinali che interagiscono tra loro deve essere il più lungo possibile. La combinazione di budesonide/formoterolo fumarato a dose fissa non è raccomandata nei pazienti che utilizzano potenti inibitori del CYP3A4. **Precauzioni con malattie speciali** Una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato deve essere somministrata con cautela nei pazienti con tireotossicosi, feocromocitoma, diabete mellito, ipopotassiemia non trattata, cardiomiopatia ipertrofica ostruttiva, stenosi subvalvolare aortica idiopatica, ipertensione grave, aneurisma o altri gravi disturbi cardiovascolari quali cardiopatia ischemica, tachiaritmia o insufficienza cardiaca grave. Deve essere osservata cautela nel trattamento di pazienti con prolungamento dell'intervallo QTc. Il formoterolo stesso può indurre un prolungamento dell'intervallo QTc. La necessità e la dose di corticosteroidi per via inalatoria devono essere rivalutate nei pazienti con tubercolosi polmonare attiva o quiescente, e infezioni micotiche e virali delle vie aeree. Nei pazienti diabetici devono essere presi in considerazione controlli supplementari della glicemia. **$ß_2$-adrenocettori agonisti** Dosi elevate di $ß_2$-adrenocettori agonisti possono determinare un'ipopotassiemia potenzialmente grave. L'effetto di un trattamento concomitante con $ß_2$-adrenocettori agonisti e medicinali che possono causare ipopotassiemia o potenziare un effetto ipopotassiemico, per esempio derivati xantinici, steroidi e diuretici, può sommarsi a un possibile effetto ipopotassiemico del $ß_2$-adrenocettore agonista. Il trattamento con $ß_2$-adrenocettori agonisti può determinare un aumento dei livelli ematici di insulina, acidi grassi liberi, glicerolo e corpi chetonici. Si raccomanda particolare cautela nell'asma instabile che necessita di un uso variabile di broncodilatatori per l'uso al bisogno, nell'asma acuta grave, in quanto il rischio associato può essere aumentato dall'ipossia, e in altre condizioni in cui la probabilità di ipopotassiemia è maggiore. Si raccomanda in tali circostanze di monitorare i livelli di potassio sierico. **Eccipienti** Questo medicinale contiene lattosio. I pazienti affetti da rari problemi ereditari di intolleranza al galattosio, deficit di lattasi di Lapp o malassorbimento di glucosio-galattosio non devono assumere questo medicinale. L'eccipiente lattosio contiene piccole quantità di proteine del latte che possono causare reazioni allergiche. **4.5 Interazione con altri medicinali ed altre forme d'interazione Interazioni farmacocinetiche** È probabile che potenti inibitori del CYP3A4 (p.es. ketoconazolo, itraconazolo, voriconazolo, posaconazolo, claritromicina, telitromicina, nefazodone e inibitori dell'HIV-proteasi) aumentino notevolmente i livelli plasmatici di budesonide e l'uso concomitante deve essere evitato. Se ciò non fosse possibile, l'intervallo di tempo tra la somministrazione dell'inibitore e quella di budesonide deve essere il più lungo possibile (vedere paragrafo 4.4). Il ketoconazolo, un potente inibitore del CYP3A4, 200 mg una volta al giorno, ha aumentato in media di sei volte i livelli plasmatici di budesonide somministrata in concomitanza per via orale (dose singola da 3 mg). Se somministrato 12 ore dopo budesonide, la concentrazione di ketoconazolo è risultata aumentata in media solo di tre volte, il che dimostra che la separazione dei tempi di somministrazione può ridurre l'aumento dei livelli plasmatici. Dati limitati riguardo a questa interazione relativi a budesonide per via inalatoria a dosi elevate indicano che può verificarsi un notevole aumento dei livelli plasmatici (in media di quattro volte) se itraconazolo, 200 mg una volta al giorno, viene somministrato in concomitanza con budesonide per via inalatoria (dose singola da 1000 microgrammi). **Interazioni farmacodinamiche** I beta-bloccanti adrenergici possono indebolire o inibire l'effetto del formoterolo. Pertanto, una terapia con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato non deve essere somministrata contemporaneamente ai beta-bloccanti adrenergici (inclusi i colliri) salvo in casi di necessità.Il trattamento concomitante con chinidina, disopiramide, procainamide, fenotiazine, antistaminici (terfenadina), inibitori delle monoamino ossidasi e antidepressivi triciclici può prolungare l'intervallo QTc e aumentare il rischio di aritmie ventricolari. Inoltre, L-Dopa, L-tiroxina, ossitocina e l'alcool possono indebolire la tolleranza cardiaca nei confronti dei $ß_2$-simpaticomimetici. Il trattamento concomitante con inibitori delle monoamino ossidasi, compresi medicinali con proprietà simili come furazolidone e procarbazina, può scatenare reazioni ipertensive. Sussiste un rischio elevato di aritmie in pazienti sottoposti contemporaneamente ad anestesia con idrocarburi alogenati. L'uso concomitante di altri medicinali beta-adrenergici e anticolinergici può avere un potenziale effetto broncodilatatorio additivo. L' ipopotassiemia può accrescere la tendenza alle aritmie nei pazienti trattati con glicosidi digitalici. Non sono state osservate interazioni di budesonide e formoterolo con altri medicinali utilizzati nel trattamento dell'asma. **Popolazione pediatrica** Sono stati effettuati studi d'interazione solo negli adulti. **4.6 Fertilità, gravidanza e allattamento Gravidanza** Per una terapia con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato o il trattamento concomitante con formoterolo e budesonide non sono disponibili dati clinici relativi a gravidanze esposte. Dati di uno studio sullo sviluppo embrio-fetale nel ratto non hanno evidenziato prove di ulteriori effetti dovuti all'associazione. Non sono disponibili dati adeguati sull'uso del formoterolo nelle donne in gravidanza. In studi su animali, il formoterolo ha causato reazioni avverse in relazione alla riproduzione a livelli di esposizione sistemica molto elevati (vedere paragrafo 5.3). Dati su circa 2000 gravidanze esposte indicano che non vi è alcun aumento del rischio di teratogenicità associato all'uso di budesonide per via inalatoria. In studi su animali, è stato dimostrato che i glucocorticoidi inducono malformazioni (vedere paragrafo 5.3). Ciò non sembra rilevante per l'uomo se somministrato alle dosi raccomandate. Studi su animali hanno inoltre rilevato la correlazione fra un eccesso di glucocorticoidi in età prenatale e l'aumento del rischio di crescita intrauterina ritardata, malattia cardiovascolare nell'adulto e modifiche permanenti nella densità dei recettori dei glucocorticoidi nonché nel turnover e nel comportamento dei neurotrasmettitori a esposizioni inferiori all'intervallo di dose teratogenico. Una terapia con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato deve essere usata in gravidan-

za solo se i benefici sono superiori ai potenziali rischi. La budesonide deve essere utilizzata alla dose efficace più bassa necessaria per il mantenimento di un adeguato controllo dell'asma. **Allattamento** La budesonide viene escreta nel latte materno. Tuttavia, alle dosi terapeutiche non sono attesi effetti sul lattante. Non è noto se il formoterolo passi nel latte materno umano. Nei ratti, piccole quantità di formoterolo sono state riscontrate nel latte materno. La somministrazione di una terapia con una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato in donne in allattamento deve essere presa in considerazione solo se il beneficio atteso per la madre è maggiore di ogni possibile rischio per il bambino. **Fertilità** Non sono disponibili dati sulla fertilità. **4.7 Effetti sulla capacità di guidare veicoli e sull'uso di macchinari** DuoResp Spiromax non altera o altera in modo trascurabile la capacità di guidare veicoli o di usare macchinari. **4.8 Effetti indesiderati Riassunto del profilo di sicurezza** Poiché DuoResp Spiromax contiene sia budesonide sia formoterolo, può verificarsi lo stesso quadro di reazioni avverse riportato per queste sostanze. Non sono stati osservati aumenti nell'incidenza di reazioni avverse in seguito alla somministrazione concomitante dei due composti. Le reazioni avverse più comuni sono reazioni avverse farmacologicamente prevedibili della terapia con β_2-adrenocettori agonisti, come tremore e palpitazioni. Tali reazioni tendono a essere di grado lieve e solitamente scompaiono entro pochi giorni di trattamento. In una sperimentazione clinica di 3 anni con budesonide per la BPCO, si sono verificate ecchimosi e polmonite con una frequenza rispettivamente del 10% e del 6%, rispetto al 4% e al 3% del gruppo placebo (rispettivamente $p<0,001$ e $p<0,01$). DuoResp Spiromax non è indicato nei bambini e negli adolescenti di età inferiore ai 18 anni (vedere paragrafo 4.2). **Tabella delle reazioni avverse** Le reazioni avverse associate a budesonide o formoterolo sono riportate di seguito ed elencate in base alla classificazione per sistemi e organi e frequenza. Le frequenze sono definite come: molto comune (≥1/10), comune (≥1/100, <1/10), non comune (≥1/1.000, <1/100), rare (≥1/10.000, <1/1.000), molto rare (<1/10.000) e non nota (la frequenza non può essere definita sulla base dei dati disponibili).

Classificazione per sistemi e organi	Frequenza	Reazione avversa
Infezioni e infestazioni	Comune	Infezioni da candida del tratto orofaringeo
Disturbi del sistema immunitario	Raro	Reazioni di ipersensibilità immediate e ritardate, es. esantema, orticaria, prurito, dermatite, angioedema e reazione anafilattica
Patologie endocrine	Molto raro	Sindrome di Cushing, soppressione surrenale, ritardi nella crescita, riduzione della densità minerale ossea
Disturbi del metabolismo e della nutrizione	Raro	Ipopotassiemia
	Molto raro	Iperglicemia
Disturbi psichiatrici	Non comune	Aggressività, iperattività psicomotoria, ansia, disturbi del sonno
	Molto raro	Depressione, modificazioni comportamentali (prevalentemente nei bambini)
Patologie del sistema nervoso	Comune	Cefalea, tremore
	Non comune	Capogiri
	Molto raro	Disturbi del gusto
Patologie dell'occhio	Molto raro	Cataratta e glaucoma
Patologie cardiache	Comune	Palpitazioni
	Non comune	Tachicardia
	Raro	Aritmie cardiache, es. fibrillazione atriale, tachicardia sopraventricolare, extrasistoli
	Molto raro	Angina pectoris. Prolungamento dell'intervallo QTc
Patologie vascolari	Molto raro	Variazione della pressione arteriosa
Patologie respiratorie, toraciche e mediastiniche	Comune	Lieve irritazione alla gola, tosse, raucedine
	Raro	Broncospasmo
	Molto raro	Broncospasmo paradosso
Patologie gastrointestinali	Non comune	Nausea
Patologie della cute e del tessuto sottocutaneo	Non comune	Ecchimosi
Patologie del sistema muscoloscheletrico e del tessuto connettivo	Non comune	Crampi muscolari

Descrizione di reazioni avverse selezionate L'infezione da candida nel tratto orofaringeo è dovuta al deposito dei principi attivi. Consigliando al paziente di sciacquarsi la bocca con acqua dopo ogni dose si ridurrà al minimo tale rischio. L'infezione da candida nel tratto orofaringeo solitamente risponde al trattamento topico con antimicotici senza la necessità di sospendere i corticosteroidi per via inalatoria. La possibilità di broncospasmo paradosso è molto rara - interessa 1 persona su 10.000 - con un aumento immediato del sibilo e dell'affanno. Il broncospasmo paradosso risponde ai broncodilatatori per via inalatoria a rapida azione e deve essere trattato immediatamente. DuoResp Spiromax deve essere immediatamente interrotto, il paziente deve essere valutato e, se necessario, si deve istituire una terapia alternativa (vedere paragrafo 4.4). Effetti sistemici dei corticosteroidi per via inalatoria si possono verificare soprattutto a dosi elevate prescritte per lunghi periodi. La comparsa di questi effetti è molto meno probabile che con i corticosteroidi per via orale. I possibili effetti sistemici includono sindrome di Cushing, caratteristiche cushingoidi, soppressione surrenale, ritardi della crescita nei bambini e negli adolescenti, diminuzione della densità minerale ossea, cataratta e glaucoma. Può inoltre verificarsi una maggiore suscettibilità alle infezioni e una compromissione della capacità di adattarsi allo stress. Gli effetti probabilmente dipendono da dose, tempo di esposizione, esposizione concomitante e precedente a steroidi e sensibilità individuale. Il trattamento con β_2-adrenocettori agonisti può determinare un aumento dei livelli ematici di insulina, acidi grassi liberi, glicerolo e corpi chetonici. **Segnalazione delle reazioni avverse sospette** La segnalazione delle reazioni avverse sospette che si verificano dopo l'autorizzazione del medicinale è importante, in quanto permette un monitoraggio continuo del rapporto beneficio/rischio del medicinale. Agli operatori sanitari è richiesto di segnalare qualsiasi reazione avversa sospetta tramite il sistema nazionale di segnalazione all'indirizzo: http://www.agenziafarmaco.gov.it/it/responsabili. **4.9 Sovradosaggio** Un sovradosaggio di formoterolo produrrebbe effetti tipici dei β_2-adrenocettori agonisti: tremore, cefalea, palpitazioni. Sono stati riportati casi isolati di sintomi di tachicardia, iperglicemia, ipopotassiemia, prolungamento dell'intervallo QTc, aritmia, nausea e vomito. Può essere indicato il trattamento sintomatico e di supporto. Una dose da 90 microgrammi somministrata nell'arco di tre ore in pazienti con ostruzione bronchiale acuta non ha destato preoccupazioni circa la sicurezza. Non si prevede che un sovradosaggio acuto di budesonide, anche a dosi eccessive, possa essere un problema clinico. L'uso cronico a dosi eccessive può dar luogo a effetti sistemici dovuti ai glucocorticoidi, come ipercorticismo e soppressione surrenale. Qualora si renda necessaria una sospensione della terapia con DuoResp Spiromax a causa di un sovradosaggio del formoterolo, un componente del medicinale, si deve prendere in considerazione una adeguata terapia con un corticosteroide per via inalatoria. **5. PROPRIETÀ FARMACOLOGICHE 5.1 Proprietà farmacodinamiche** Categoria farmacoterapeutica: adrenergici ed altri farmaci per le sindromi ostruttive delle vie respiratorie. Codice ATC: R03AK07 **Meccanismo d'azione ed effetti farmacodinamici** DuoResp Spiromax contiene formoterolo e budesonide, che presentano un meccanismo d'azione diverso e mostrano effetti additivi in termini di riduzione delle riacutizzazioni dell'asma. I meccanismi d'azione delle due sostanze sono descritti di seguito. ***Budesonide*** Budesonide è un glucocorticoide che, quando inalato, esercita un'azione antinfiammatoria dose-dipendente sulle vie respiratorie, con conseguente riduzione dei sintomi e diminuzione delle riacutizzazioni dell'asma. La budesonide per via inalatoria comporta meno reazioni avverse gravi rispetto ai corticosteroidi per via sistemica. L'esatto meccanismo responsabile dell'effetto antinfiammatorio dei glucocorticoidi non è noto. ***Formoterolo*** Formoterolo è un β_2-adrenocettore agonista che,

quando inalato, induce un rapido e prolungato rilassamento della muscolatura bronchiale liscia nei pazienti con ostruzione reversibile delle vie aeree. L'effetto broncodilatatore è dose-dipendente e si manifesta entro 1-3 minuti. La durata dell'effetto è di almeno 12 ore dopo una singola dose. **Efficacia e sicurezza clinica** ***Asma Terapia di mantenimento con budesonide/formoterolo*** Studi clinici condotti su soggetti adulti hanno dimostrato che l'aggiunta di formoterolo a budesonide migliora i sintomi dell'asma e la funzione polmonare, oltre a ridurre le riacutizzazioni. In due studi di 12 settimane, l'effetto di budesonide/formoterolo sulla funzione polmonare è risultato uguale a quello della libera combinazione di budesonide e formoterolo e ha superato quello della sola budesonide. Tutti i bracci di trattamento prevedevano l'utilizzo al bisogno di un $ß_2$-adrenocettore agonista a rapida azione. Non sono emersi segni di attenuazione dell'effetto antiasmatico nel corso del tempo. Sono stati effettuati due studi pediatrici di 12 settimane, nei quali 265 bambini di età compresa tra i 6 e gli 11 anni sono stati trattati con una dose di mantenimento di budesonide/formoterolo (2 inalazioni da 80 microgrammi/4,5 microgrammi/inalazione due volte al giorno) e un $ß_2$-adrenorecettore agonista a breve durata d'azione al bisogno. In entrambi gli studi, la funzione polmonare è risultata migliorata e il trattamento ben tollerato rispetto alla dose corrispondente di sola budesonide. ***Broncopneumopatia cronica ostruttiva (BPCO)*** In due studi di 12 mesi si è valutato l'effetto sulla funzione polmonare e il tasso di riacutizzazioni (definito come cicli di steroidi per via orale e/o cicli di antibiotici e/o ospedalizzazioni) nei pazienti con BPCO grave. La FEV1 mediana all'inclusione nelle sperimentazioni era pari al 36% del valore normale previsto. Il numero medio di riacutizzazioni per anno (come definito sopra) è risultato significativamente ridotto con budesonide/formoterolo rispetto al trattamento con formoterolo da solo o placebo (tasso medio 1,4 rispetto all'1,8-1,9 nel gruppo placebo/formoterolo). Il numero medio di giorni di trattamento con corticosteroidi orali/paziente durante i 12 mesi è apparso leggermente ridotto nel gruppo con budesonide/formoterolo (7-8 giorni/paziente/anno rispetto a 11-12 e 9-12 giorni, rispettivamente nei gruppi placebo e formoterolo). Per quanto concerne le variazioni nei parametri della funzione polmonare, come FEV_1, la combinazione budesonide/formoterolo non è risultata superiore al trattamento con formoterolo da solo. **Picco di flusso inspiratorio mediante il dispositivo Spiromax** Per valutare il picco di flusso inspiratorio (Peak Inspiratory Flow Rate, PIFR) e altri parametri di inalazione correlati, è stato effettuato uno studio con placebo, randomizzato e in aperto, su bambini e adolescenti con asma (età 6-17 anni), adulti con asma (età 18-45 anni), adulti con broncopneumopatia cronica ostruttiva (BPCO) (età >50 anni) e volontari sani (età 18-45 anni), attraverso il confronto tra l'inalazione con il dispositivo Spiromax (contenente placebo) e l'inalazione con un inalatore a polvere secca multidose reperibile in commercio (contenente placebo). In questi gruppi di soggetti è stato valutato anche l'impatto del potenziamento della formazione nella tecnica di inalazione mediante inalatore a polvere secca sulla velocità e sul volume di inalazione. I dati dello studio indicano che, a prescindere dall'età e dalla gravità della patologia sottostante, i bambini, gli adolescenti e gli adulti con asma nonché i pazienti con BPCO erano in grado di generare un flusso inspiratorio sufficiente attraverso il dispositivo Spiromax simile a quello generato attraverso il dispositivo di inalazione a polvere secca multidose reperibile in commercio. Il PIFR medio generato dai pazienti con asma o BPCO era superiore ai 60 l/min, un tasso di flusso con il quale entrambi i dispositivi studiati sono noti per erogare ai polmoni quantità paragonabili di farmaco. Pochissimi pazienti hanno avuto valori PIFR inferiori a 40 l/min; nei casi in cui i PIFR sono risultati inferiori a 40 l/min non è stato evidenziato alcun raggruppamento per età o gravità della malattia. **5.2 Proprietà farmacocinetiche Assorbimento** La combinazione a dose fissa di budesonide e formoterolo e i corrispondenti monoprodotti hanno dimostrato di essere bioequivalenti per quanto riguarda l'esposizione sistemica rispettivamente di budesonide e formoterolo. Nonostante ciò, un leggero aumento della soppressione del cortisolo è stato osservato dopo la somministrazione della combinazione a dose fissa rispetto ai singoli prodotti. La differenza è considerata priva di impatto sulla sicurezza clinica. Non vi è alcuna evidenza di interazioni farmacocinetiche tra budesonide e formoterolo. I parametri farmacocinetici per le rispettive sostanze sono risultati comparabili dopo la somministrazione di budesonide e formoterolo singolarmente o in combinazione a dose fissa. Per budesonide, la AUC era leggermente più elevata, la velocità di assorbimento più rapida e la concentrazione plasmatica massima più alta dopo la somministrazione della combinazione fissa. Per il formoterolo, la concentrazione plasmatica massima è risultata simile dopo la somministrazione della combinazione fissa. Budesonide per via inalatoria viene assorbita rapidamente e la concentrazione plasmatica massima viene raggiunta entro 30 minuti dall'inalazione. Negli studi, la deposizione polmonare media di budesonide dopo l'inalazione tramite l'inalatore a polvere variava dal 32% al 44% della dose erogata. La biodisponibilità sistemica è pari a circa il 49% della dose erogata. Nei bambini di 6-16 anni di età, la deposizione polmonare rientra nel medesimo intervallo di valori degli adulti a parità di dose somministrata. Le concentrazioni plasmatiche derivanti non sono state determinate. Formoterolo per via inalatoria viene assorbito rapidamente e la concentrazione plasmatica massima viene raggiunta entro 10 minuti dall'inalazione. Negli studi, la deposizione polmonare media del formoterolo dopo l'inalazione tramite l'inalatore a polvere variava dal 28% al 49% della dose erogata. La biodisponibilità sistemica è pari a circa il 61% della dose erogata. **Distribuzione** Il legame alle proteine plasmatiche è di circa il 50% per il formoterolo e del 90% per la budesonide. Il volume di distribuzione è di circa 4 l/kg per il formoterolo e 3 l/kg per la budesonide. Il formoterolo è inattivato tramite reazioni di coniugazione (si formano metaboliti O-demetilati e deformilati, per lo più rilevati come coniugati inattivi). Budesonide subisce un grado esteso (circa il 90%) di biotrasformazione in metaboliti a bassa attività glucocorticoide al primo passaggio epatico. L'attività glucocorticoide dei metaboliti principali, 6-beta-idrossi-budesonide e 16-alfa-idrossi-prednisolone, è inferiore all'1% rispetto a quella di budesonide. Non esistono indicazioni di interazioni metaboliche o reazioni da sostituzione tra formoterolo e budesonide. **Eliminazione** La maggior parte di una dose di formoterolo viene trasformata tramite il metabolismo epatico seguito da eliminazione renale. Dopo l'inalazione, dall'8% al 13% della dose erogata di formoterolo viene escreta non metabolizzata nelle urine. Formoterolo ha un elevato livello di clearance sistemica (circa 1,4 l/min) e l'emivita terminale è in media di 17 ore. Budesonide viene eliminata per via metabolica principalmente catalizzata dall'enzima CYP3A4. I metaboliti della budesonide vengono eliminati nelle urine come tali o in forma coniugata. Nelle urine sono stati riscontrati solo livelli trascurabili di budesonide immodificata. Budesonide ha un'elevata eliminazione sistemica (circa 1,2 l/min) e l'emivita di eliminazione plasmatica dopo somministrazione EV è in media di 4 ore. **Relazioni farmacocinetiche/farmacodinamiche** La farmacocinetica di budesonide o formoterolo nei bambini e nei pazienti con insufficienza renale non è nota. L'esposizione di budesonide e formoterolo può risultare aumentata nei pazienti con epatopatia. **Profilo farmacocinetico di DuoResp Spiromax** In studi farmacocinetici con e senza blocco con carbone, DuoResp Spiromax è stato valutato attraverso un confronto con un prodotto di combinazione per via inalatoria a dose fissa autorizzato alternativo contenente gli stessi principi attivi (budesonide e formoterolo), dimostrandone l'equivalenza in termini sia di esposizione sistemica (sicurezza) sia di deposizione polmonare (efficacia). **5.3 Dati preclinici di sicurezza** La tossicità osservata negli studi condotti su animali con budesonide e formoterolo somministrati in combinazione o separatamente si è dimostrata sotto forma di effetti associati ad attività farmacologica esagerata. Negli studi di riproduzione su animali, i corticosteroidi come budesonide hanno dimostrato di indurre malformazioni (palatoschisi, malformazioni scheletriche). Tuttavia, tali risultati sperimentali nell'animale non sembrano rilevanti nell'uomo alle dosi raccomandate. Gli studi di riproduzione su animali con formoterolo hanno dimostrato una certa riduzione della fertilità nei ratti maschi dopo elevata esposizione sistemica e perdite degli impianti embrionali, così come sono state osservate, ad una esposizione molto più elevata rispetto a quella osservata durante l'uso clinico, una riduzione della sopravvivenza post-natale e del peso alla nascita. Tuttavia, tali risultati sperimentali nell'animale non sembrano rilevanti nell'uomo. **6. INFORMAZIONI FARMACEUTICHE 6.1 Elenco degli eccipienti** Lattosio monoidrato. **6.2 Incompatibilità** Non pertinente. **6.3 Periodo di validità** 2 anni. Dopo l'apertura dell'involucro di alluminio: 6 mesi. **6.4 Precauzioni particolari per la conservazione** Non conservare a temperatura superiore ai 25°C. Tenere chiuso il cappuccio protettivo dopo la rimozione dell'involucro di alluminio. **6.5 Natura e contenuto del contenitore** L'inalatore è bianco, con un cappuccio protettivo semitrasparente di colore bordeaux ed è costituito da acrilonitrile butadiene stirene (ABS), polietilene tereftalato (PT) e polipropilene (PP). Ogni inalatore contiene 60 dosi ed è avvolto in un involucro di alluminio. Confezioni multiple contenenti 1, 2 o 3 inalatori. È possibile che non tutte le confezioni siano commercializzate. **6.6 Precauzioni particolari per lo smaltimento e la manipolazione** Nessuna istruzione particolare. **7. TITOLARE DELL'AUTORIZZAZIONE ALL'IMMISSIONE IN COMMERCIO** Teva Pharma B.V. - Computerweg 10, 3542 DR Utrecht - Paesi Bassi. **8. NUMERO(I) DELL'AUTORIZZAZIONE ALL'IMMISSIONE IN COMMERCIO** EU/1/14/920/004 - EU/1/14/920/005 - EU/1/14/920/006 **9. DATA DELLA PRIMA AUTORIZZAZIONE/RINNOVO DELL'AUTORIZZAZIONE** Data della prima autorizzazione: 28 Aprile 2014. 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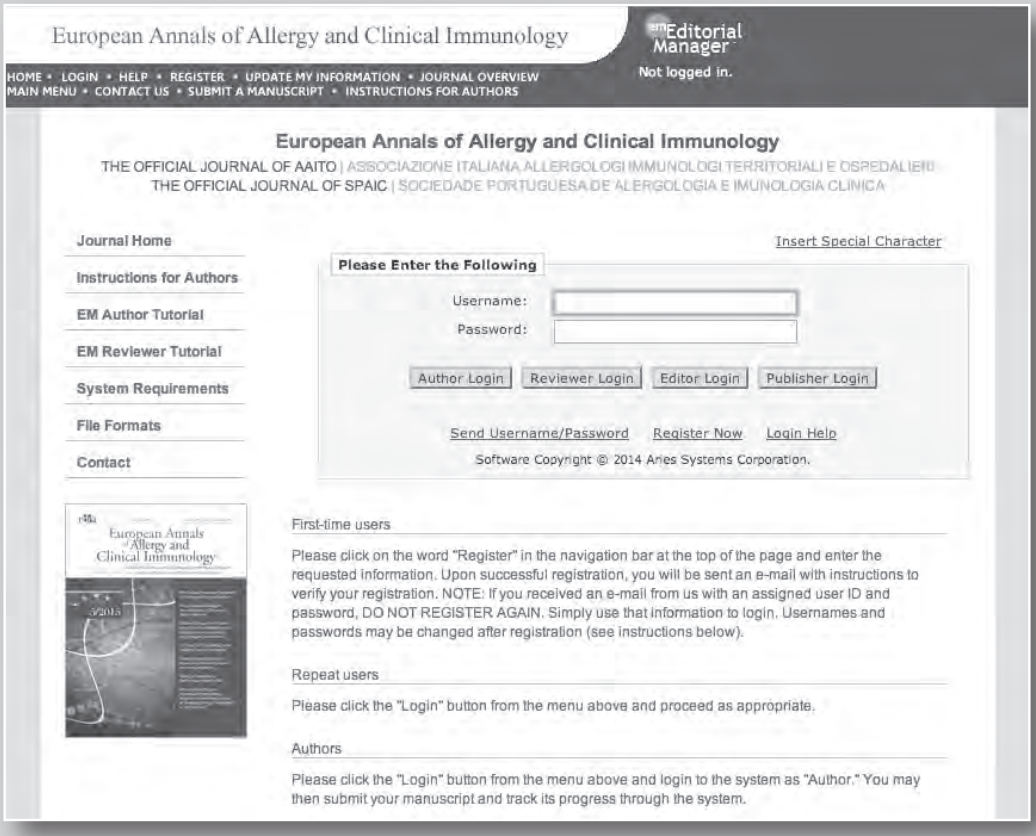

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