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An overview of fruit allergy and the causative allergens

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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.

Key words

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

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, thaumatatin-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).
An overview of fruit allergy and the causative allergens

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.

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

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

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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References


An overview of fruit allergy and the causative allergens


Contact dermatitis: some important topics

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-vesiclar 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.

**References**

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

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

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 effects during the course of VIT. One patient had a systemic reaction upon injection on one occasion, during the up-dosing 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 workup, 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).
Rush immunotherapy for wasp venom allergy seems safe and effective in patients with mastocytosis. 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. 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).

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

<table>
<thead>
<tr>
<th>Author</th>
<th>C/R</th>
<th>SSE</th>
<th>Re-stung</th>
<th>SS Re-stung</th>
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<tbody>
<tr>
<td>Bonnadonna(9)</td>
<td>15 C</td>
<td>2</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Gonzalez de Olano(11)</td>
<td>10 C</td>
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<td>5</td>
<td>1</td>
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<tr>
<td>Total</td>
<td>25 C</td>
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<td>3</td>
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<tr>
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<td>0</td>
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</tr>
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<td>Fricker(10)</td>
<td>4 nd</td>
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<td>3</td>
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</table>

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

### Table 2 - Safety and efficacy depending on type of mastocytosis.

<table>
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<tr>
<th>Author</th>
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<tr>
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<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

SSE: systemic side effects; IT: immunotherapy; SS: systemic symptoms.
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
Rush immunotherapy for wasp venom allergy seems safe and effective in patients with mastocytosis.

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).

<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Sex</th>
<th>Type mastocytosis</th>
<th>BM</th>
<th>SRS</th>
<th>IC1</th>
<th>Duration IT (years)</th>
<th>Symptoms IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>m</td>
<td>CM</td>
<td>-</td>
<td>4</td>
<td>0.0001</td>
<td>20.4</td>
<td>5.3</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>f</td>
<td>CM</td>
<td>nd</td>
<td>4</td>
<td>0.0001</td>
<td>&gt; 100</td>
<td>29.3</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>m</td>
<td>CM</td>
<td>nd</td>
<td>4</td>
<td>0.0001</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>m</td>
<td>CM</td>
<td>nd</td>
<td>4</td>
<td>nd</td>
<td>26</td>
<td>nd</td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td>f</td>
<td>CM+SM</td>
<td>pos</td>
<td>4</td>
<td>0.0001</td>
<td>6</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>m</td>
<td>CM</td>
<td>neg</td>
<td>4</td>
<td>0.0001</td>
<td>3.8</td>
<td>nd</td>
</tr>
<tr>
<td>7</td>
<td>56</td>
<td>f</td>
<td>SM</td>
<td>pos</td>
<td>4</td>
<td>0.01</td>
<td>0.4</td>
<td>&lt; 0.35</td>
</tr>
<tr>
<td>8</td>
<td>41</td>
<td>f</td>
<td>CM</td>
<td>neg</td>
<td>4</td>
<td>0.01</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
</tr>
<tr>
<td>9</td>
<td>67</td>
<td>m</td>
<td>CM+SM</td>
<td>pos</td>
<td>4</td>
<td>0.01</td>
<td>1.17</td>
<td>nd</td>
</tr>
</tbody>
</table>

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

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

1positive at dilution (in mcg/ml).
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 mastocytosis, and might disappear upon discontinuation. Therefore, lifelong treatment should be considered, as well as prescription of an epinephrine auto-injector.

References

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.

Key words

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

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One-year survey of paediatric anaphylaxis in an allergy department

Introducción

Según la World Allergy Organization (WAO) (1), anafilaxia se define como una reacción de tipo hipersensibilidad mediada por químicos mastocitos y basofílicos. Puede ser trigada por mecanismos inmunes (anafilaxia alérgica) mediada por inmunoglobulina E (IgE) (anafilaxia IgE- mediada alérgica) o otros mecanismos inmunes (no-IgE- mediada alérgica anafilaxia) o no-inmune inmune mecanismos (no- alérgica anafilaxia).

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,
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:
   - Sudden respiratory compromise (e.g. dyspnea, wheeze-bronchospasm, stridor, reduced FEV1/PEF, hypoxemia)
   - 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):
   - Sudden skin or mucosal symptoms and signs (e.g. generalized hives, itching or flushing, swollen lips-tongue-uvula)
   - Sudden respiratory symptoms and signs (e.g. dyspnea, wheeze-bronchospasm, stridor, reduced FEV1/PEF, hypoxemia)
   - Sudden reduced BP or symptoms of end-organ dysfunction (e.g. hypotonia-collapse, syncope, incontinence)
   - 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):
   - Infants and children: low systolic BP (age-specific) or greater than 30% decrease in systolic BP

FEV1: Forced expiratory volume in 1 second; PEF: Peak expiratory flow; BP: Blood pressure.

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 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 tropicallis and Lepidoglyphus destructor), pollens (grass mix, Parthenaria 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...
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-
One-year survey of paediatric anaphylaxis in an allergy department

In these children submitted to oral tolerance induction the median milk-specific IgE level at the time of diagnosis was 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 2 - Causes and patients' characteristics of non-food-induced anaphylaxis.

<table>
<thead>
<tr>
<th>Etiological agent</th>
<th>Age¹</th>
<th>Sex</th>
<th>Atopy</th>
<th>Complementary study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-induced anaphylaxis (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAID: paracetamol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ibuprofen</td>
<td>8</td>
<td>M</td>
<td>Yes</td>
<td>negative sIgE, SPT, ID and CAST¹</td>
</tr>
<tr>
<td>ibuprofen</td>
<td>3</td>
<td>M</td>
<td>Yes</td>
<td>n.p.</td>
</tr>
<tr>
<td>ibuprofen</td>
<td>5</td>
<td>F</td>
<td>No</td>
<td>n.p.</td>
</tr>
<tr>
<td>ASA</td>
<td>16</td>
<td>F</td>
<td>Yes</td>
<td>n.p.</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>3</td>
<td>F</td>
<td>Yes</td>
<td>sIgE MDM = 0.13 kU/L; positive ID²</td>
</tr>
<tr>
<td>Cold-induced anaphylaxis (n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold drink</td>
<td>14</td>
<td>F</td>
<td>Yes</td>
<td>positive ICT at 10 minutes</td>
</tr>
<tr>
<td>Plunge in the sea</td>
<td>14</td>
<td>M</td>
<td>Yes</td>
<td>positive ICT at 3 minutes</td>
</tr>
<tr>
<td>Plunge in the sea</td>
<td>2</td>
<td>F</td>
<td>No</td>
<td>positive ICT at 3 minutes</td>
</tr>
<tr>
<td>FDEIA (n = 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy and green bean</td>
<td>11</td>
<td>M</td>
<td>Yes</td>
<td>positive SPT to soy and green bean</td>
</tr>
<tr>
<td>Cow's milk</td>
<td>17</td>
<td>M</td>
<td>Yes</td>
<td>positive SPT and sIgE to cow's milk, alpha-lactalbumin, beta-lactoglobulin and casein³</td>
</tr>
<tr>
<td>Latex allergy (n = 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peri-operative, chestnut and kiwi</td>
<td>6</td>
<td>F</td>
<td>Yes</td>
<td>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 6.02⁴</td>
</tr>
<tr>
<td>Allergy to insect sting (n = 1)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Mosquito (3 reproducible reactions)</td>
<td>3 years</td>
<td>M</td>
<td>Yes</td>
<td>negative sIgE to mosquito</td>
</tr>
</tbody>
</table>

¹Age at the first reaction.

²Second 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.

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

⁴Patient 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.

A recommendation 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 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.
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),

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

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

1Median (minimum-maximum).

2Skin 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.
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.

References


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.

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

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

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.

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/age</th>
<th>Carmine SPT (mm)</th>
<th>Histamine SPT (mm)</th>
<th>D.pt. / D.far. SPT</th>
<th>Storage mites SPT</th>
<th>Active Carmine avoidance before oral challenge</th>
<th>Symptoms</th>
<th>Results of oral challenge to Carmine</th>
<th>S-IgE [kU/l]</th>
<th>Other SPT reactions (=/&gt; than histamine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/61</td>
<td>3</td>
<td>5</td>
<td>neg</td>
<td>&lt; hist</td>
<td>no</td>
<td>none&lt;sup&gt;1&lt;/sup&gt;</td>
<td>neg</td>
<td>53</td>
<td>pollen &amp; cross-reactions</td>
</tr>
<tr>
<td>2</td>
<td>M/30</td>
<td>4</td>
<td>5</td>
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<td>&lt; hist</td>
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<td>none</td>
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<td>NT</td>
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<td>4</td>
<td>5</td>
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<td>none</td>
<td>neg</td>
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<td>4</td>
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<td>pos</td>
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<td>none</td>
<td>neg</td>
<td>548</td>
<td>pollen &amp; cross-r., animals, soybean</td>
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<td>8</td>
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<td>NT</td>
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<td>none</td>
<td>neg</td>
<td>NT</td>
<td>gliadin, wheat</td>
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<td>pos</td>
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<td>&lt; hist</td>
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<td>no</td>
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<td>pos</td>
<td>NT</td>
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<td>none</td>
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<td>none</td>
<td>neg</td>
<td>NT</td>
<td>no other reactions</td>
</tr>
</tbody>
</table>

<sup>1</sup>No symptoms after carmine red avoidance.
<sup>2</sup>The patient had avoided one specific carmine containing yoghurt that had caused oral symptoms.
<sup>3</sup>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-report- ed 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
An oral challenge test with carmine red (E120) in skin prick test positive patients

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

Asthma and Allergy Association 40 lipsticks that are marketed in Finland contain carmine red as well as some make up creams. 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 lipsticks 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.
References


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

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**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.

**Key words**

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

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

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

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

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

Reduced 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.

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

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

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

Table 4 - Mean maximum tolerated doses of SCIT.

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

Doses are expressed as ml of final vial.
†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.
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.

References

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

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

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 \( (\text{Mass}_{\text{artemisia}}) \) in bee pollen and skin reactivity.

The dose-response power regression curve \( (W_{\text{artemisia}}) = 3.328 \times (\text{Mass}_{\text{artemisia}})^{0.297} \) \( (R^2 = 0.9947) \) and the linear function \( \log_{10} (W_{\text{artemisia}}) = 0.297 \times (\log_{10} \text{Mass}_{\text{artemisia}} + 0.520 \times (R = 0.9974)) \) 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 macro-nutrients 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
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-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 $V_{pn} = \frac{4}{3} \pi r^3$ if the pollen grain is spherical or using the formula $V_{pn} = \frac{4}{3} \pi e^2 l$ 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 thoroughly cleaned when intended for human consumption. 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.

2.2) Calculate the proportion of volume $P_{p-allergen}$ of floral pollen allergen of pollen $p$ of flower pollen $p_{allergen}$

$P_{p-allergen} = \frac{(V_{p-allergen} x %_{p-allergen})}{(V_{p-allergen} x %_{p-allergen})} + \frac{(V_{p-allergen} x %_{p-allergen})}{...} + \frac{(V_{p-allergen} x %_{p-allergen})}{(V_{p-allergen} x %_{p-allergen})}$

2.3) Calculate the mass of floral pollen allergen $Mass_{p-allergen}$

$Mass_{p-allergen} = P_{p-allergen} \times Mass_{pollen}$

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-allergen was defined by geometric measuring of skin reactivity to floral pollen allergen contained in bee pollen.

### Analysis of the relationship between skin reactivity to floral pollen allergen in bee pollen W_p-allergen and floral pollen allergen mass Mass_p-allergen

If the model curve was a power regression:

\[
(W_{p-allergen}) = b (Mass_{p-allergen})^a
\]

then the linear function was calculated as follows:

\[
\log_{10}(W_{p-allergen}) = a (\log_{10}(Mass_{p-allergen})) + B
\]

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, \( V_{artemisia} = \frac{4}{3} \pi (20/2)^3 = 4187 \mu^3 \)

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

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

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

1.2) Calculation of P_artemisia proportion

\[
P_{artemisia} = \left( \frac{V_{artemisia} \times %_{artemisia}}{\left( V_{artemisia} \times %_{artemisia} \right) + \left( V_{mercurialis} \times %_{mercurialis} \right) + \left( V_{lythrum} \times %_{lythrum} \right) + \left( V_{brassicaceae} \times %_{brassicaceae} \right)} \right) = \left( \frac{4187 \times 43.1 \%}{(4187 \times 43.1 \%) + (8177 \times 25.7 \%) + (17643 \times 16.0 \%) + (4187 \times 14.7 \%)} \right) = 0.246
\]

1.3) Calculation of Mass Artemisia

\[
Mass_{artemisia} = P_{artemisia} \times Mass_{pollens} = 0.246 \times 1 \text{ mg} = 0.246 \text{ mg}
\]

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, \( V_{hedera helix} = \frac{4}{3} \pi \left( \frac{25}{2} \right)^3 = 8177 \mu^3 \)

2.2) Calculation of proportion P_hedera helix

\[
P_{hedera helix} = \left( \frac{V_{hedera helix} \times %_{hedera helix}}{V_{hedera helix} \times %_{hedera helix}} \right) = \left( \frac{8177 \times 99\%}{8177 \times 99\%} \right) = 1
\]

2.3) Calculation of Mass Hedera helix

\[
Mass_{hedera helix} = P_{hedera helix} \times Mass_{pollens} = 1 \times 1 \text{ mg} = 1 \text{ mg}
\]

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-allergen and Mass_p-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.

\[
(W_{artemisia}) = 3.328 (Mass_{artemisia})^{0.297} \quad R^2 = 0.9947
\]

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

\[
\log_{10}(W_{artemisia}) = 0.297 \left( \log_{10}(Mass_{artemisia}) \right) + 0.520 \quad R = 0.9974
\]

The model 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.

\[
(W_{hedera helix}) = 0.27 (Mass_{hedera helix})^{0.033} \quad R^2 = 0.0292
\]
Allergenicity of Artemisia contained in bee pollen is proportional to its mass.

However, patients who are allergic to bee products may also be 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 Artemisia.

### 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.

<table>
<thead>
<tr>
<th>Patient</th>
<th>ARTEMISIA1</th>
<th>CONTROL1</th>
<th>HEDERA HELIX2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artemisia 24.6 mg/ml</td>
<td>Artemisia 2.46 mg/ml</td>
<td>Artemisia 0.25 mg/ml</td>
</tr>
<tr>
<td>P1</td>
<td>22.97</td>
<td>11</td>
<td>8.94</td>
</tr>
<tr>
<td>P2</td>
<td>4.90</td>
<td>2.83</td>
<td>1.73</td>
</tr>
<tr>
<td>P3</td>
<td>6.93</td>
<td>3.87</td>
<td>1</td>
</tr>
<tr>
<td>P4</td>
<td>11.96</td>
<td>9</td>
<td>4.90</td>
</tr>
<tr>
<td>P5</td>
<td>17.97</td>
<td>6</td>
<td>1.41</td>
</tr>
<tr>
<td>P6</td>
<td>6.93</td>
<td>1.73</td>
<td>1</td>
</tr>
<tr>
<td>P7</td>
<td>8</td>
<td>5.92</td>
<td>3.87</td>
</tr>
<tr>
<td>P8</td>
<td>8.94</td>
<td>6</td>
<td>2.83</td>
</tr>
<tr>
<td>P9</td>
<td>6.93</td>
<td>3.87</td>
<td>1.73</td>
</tr>
<tr>
<td>P10</td>
<td>3</td>
<td>2.83</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>8.39</td>
<td>4.61</td>
<td>2.14</td>
</tr>
</tbody>
</table>

1: geometric mean wheal (mm). 2: mean wheal (mm).

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

$y = 3.3263^{x^{0.2977}}$  
$R^2 = 0.9947$

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

$y = 0.2964x + 0.5201$  
$R^2 = 0.9949$

### 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 Artemisia.
C. Nonotte-Varly

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-reactivity to pollen panallergens (e.g., olea, quercus, fraxinus, alnus and loliwm, 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 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 (e.g., 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 melissopalynological 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:

\[
(W_{\text{artemisia}}) = 3.328 \text{ (Mass}_{\text{artemisia}})^{0.297} \ (R^2 = 0.9947)
\]

from which we were able to deduce the linear curve.

\[
\log_{10}(W_{\text{artemisia}}) = 0.297 \log_{10}(\text{Mass}_{\text{artemisia}}) + 0.520 \ (R = 0.9974).
\]

Mugwort allergens in bee pollen appears to be little or not altered by bee secretions and the allergens retain their allergic 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 allergic qualities.

**References**

28. Butcher RD et al. The identification of potential aeroallergen/irritant(s) from oiled rape (Brassica napus spp oleifera). volatile or-
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.

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

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 worldwide, 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-
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 antibiotic therapy 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 wheel 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...
An unusual case of delayed-type hypersensitivity to ceftriaxone and meropenem

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 β-Ls (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.

References
Eosinophilic Granulomatosis with Polyangiitis preceding allergic bronchopulmonary aspergillosis

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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.

Keywords
Churg Strauss Syndrome, Asthma, Obstructive lung physiology, Allergic Bronchopulmonary Aspergillosis

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. Examinations was unremarkable, he had no focal neurologic or cutaneous signs, his lungs were clear and there was no organomegaly. Investigations revealed leukocytosis 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...
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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.

Four years later, he developed skin rash and neuropathies affecting the left common peroneal and right median nerves. His AEC was 14.67 x 10⁹/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 leukocytoclastic 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 as an 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 asthma exacerbations with hypoxemia. His white cell count was 11.97 x 10⁹/L and AEC 0.52 - 1.88 x 10⁹/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* (100000 U/ml, both from Allergopharma®). We
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-Paterson 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.

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

<table>
<thead>
<tr>
<th></th>
<th>Jan 2011 (EGPA remission)</th>
<th>Dec 2011 (ABPA diagnosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Bronchodilator</td>
<td>Post-Bronchodilator</td>
</tr>
<tr>
<td>FVC (L) (% of predicted)</td>
<td>2.85 (102%)</td>
<td>3.11 (111%)</td>
</tr>
<tr>
<td>FEV1 (L) (% of predicted)</td>
<td>1.34 (64%)</td>
<td>1.70 (81%)</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>47</td>
<td>55</td>
</tr>
</tbody>
</table>

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
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).

References

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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 pazienti con compromissione epatica o renale (vedere paragrafo 4.5) o con compromissione reneale o epatica (vedere paragrafo 4.4). Non sono disponibili informazioni sull’effetto di una combinazione a dose fissa di budesonide/formoterolo fumarato diidrato a dosi più elevate.

Dosi raccomandate:
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 sull’uso di una combinazione a dose fissa di budesonide e formoterolo 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. Non vi sono dati disponibili sull’uso di una combinazione a dose fissa di budesonide e formoterolo in pazienti con compromissione renale.

Il farmaco è stato ampiamente studiato e utilizzato negli adulti per diversi anni. L’inghiottonimento è stato documentato in circa un terzo di questi pazienti. Se è necessario somministrare due dosi consecutive di DuoResp Spiromax, è possibile che il paziente manifesti una dose più bassa raccomandata, come fase successiva si potrebbe provare il solo corticosteroide per via inalatoria. In alcuni pazienti, l’effetto antiasmatico può essere notevolmente maggiore quando si somministra un corticosteroide per via inalatoria e un broncodilatatore a lunga durata d’azione a regime doppio. La dose raccomandata di budesonide per via inalatoria da utilizzare con DuoResp Spiromax è di 500 microgrammi, e quella raccomandata di formoterolo è di 250 microgrammi. Se si utilizzano farmaci attivi in combinazione con farmaci antiasmatici, si può considerare una riduzione della dose di farmaci antiasmatici.

Sintomi dell’asma
Quando si inizia un trattamento con l’asma, è importante controllare regolarmente la dose di farmaci antiasmatici e farmaci per il trattamento della broncoconstrictione. Se i sintomi persistono dopo alcuni giorni, si deve aumentare la dose di farmaci antiasmatici, ma non è sempre necessario aumentare la dose di farmaci per il trattamento della broncoconstrictione. Dopo il trattamento, i sintomi dell’asma possono essere controllati per una settimana o più con una dose più bassa raccomandata. Se i sintomi persistono dopo alcuni giorni, si deve aumentare la dose di farmaci antiasmatici.

4.3 Controindicazioni
Le controindicazioni per l’uso di DuoResp Spiromax includono l’ipersensibilità a budesonide, formoterolo o qualsiasi componente della preparazione. Inoltre, DuoResp Spiromax non deve essere somministrato a pazienti con patologie che influenzano la funzione renale o epatica, a pazienti con compromissione renale o epatica (vedere paragrafo 4.4) o alla terapia con steroidi orali.

4.4 Avvertenze speciali e precauzioni di impiego
Non sono disponibili informazioni sull’effetto di una combinazione a dose fissa di budesonide e formoterolo in pazienti con compromissione renale o epatica. Poiché budesonide e formoterolo sono eliminati principalmente tramite metabolismo epatico, nei pazienti con grave cirrosi epatica ci si può attendere una maggiore esposizione. Non vi sono dati disponibili sull’uso di una combinazione a dose fissa di budesonide e formoterolo in pazienti con compromissione renale.

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. Inoltre, i pazienti che provengono da una terapia con steroidi orali può permanere il rischio di compromissione della riserva surrenale per un periodo di tempo considerato.
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. A differenza di altri inibitori, il ketoconazolo, itraconazolo, voriconazolo, posaconazolo, claritromicina, telitromicina, nefazodone e inibitori dell'HIV-proteasi) aumentino notevolmente i livelli plasmatici di budesonide e l’uso con β2-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, DuoResp Spiromax non altera o altera in modo trascurabile la capacità di guidare veicoli o di usare macchinari.

La riduzione del tasso di riacutizzazioni è statisticamente significativa (valore P<0,01) per entrambi i confronti a 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 appetito e affamato. Se non si riscontra un miglioramento, il trattamento con budesonide per via inalatoria comporta meno reazioni avverse gravi rispetto ai corticosteroidi per via sistematica.

Un totale di 822 pazienti (m) e 450 pazienti (f) è stato studiato più volte in studi clinici condotti con a dosi di mantenimento di 320/9 µg BD e terbutalina 0,4 mg al bisogno. Nella tabella seguente sono riportati i risultati del confronto dei gruppi dosi per il trattamento concomitante di budesonide/formoterolo fumarato a dose fissa non è raccomandata nei pazienti che utilizzano potenti inibitori del CYP3A4 (es. ketoconzolo, itraconzolo, voriconzolo, posaconzolo, claritromicina, telitromicina, nefazodone e inibitori dell'HIV-proteasi) aumentino notevolmente i livelli plasmatici di budesonide e l’uso con β2-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, DuoResp Spiromax non altera o altera in modo trascurabile la capacità di guidare veicoli o di usare macchinari.

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La riduzione del tasso di riacutizzazioni è statisticamente significativa (valore P<0,01) per entrambi i confronti a 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 appetito e affamato. Se non si riscontra un miglioramento, il trattamento con budesonide per via inalatoria comporta meno reazioni avverse gravi rispetto ai corticosteroidi per via sistematica.

<table>
<thead>
<tr>
<th>Patologie cardiche</th>
<th>Comune</th>
<th>Palpazioni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non comune</td>
<td>Tachicardia</td>
<td></td>
</tr>
<tr>
<td>Raro</td>
<td>Artimie cardiche, es. fibrillazione atriale, tachicardia sopraeventricolare, extrasistol</td>
<td></td>
</tr>
<tr>
<td>Molto raro</td>
<td>Angina pectoris. Prolungamento dell’intervallo QTc</td>
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<table>
<thead>
<tr>
<th>Patologie vascolari</th>
<th>Molto raro</th>
<th>Variazione della pressione arteriosa</th>
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</table>

<table>
<thead>
<tr>
<th>Patologie respiratorie, toraciche e mediastiniche</th>
<th>Comune</th>
<th>Lieve irritazione alla gola, tosse, raucedine</th>
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</thead>
<tbody>
<tr>
<td>Raro</td>
<td>Broncospasmo</td>
<td></td>
</tr>
<tr>
<td>Molto raro</td>
<td>Broncospasmo paradosso</td>
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</table>

<table>
<thead>
<tr>
<th>Patologie gastrointestinale</th>
<th>Non comune</th>
<th>Nausea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motivo</td>
<td>Ecchimosi</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Patologie della cute e del tessuto sottocutaneo</th>
<th>Non comune</th>
<th>Crampi muscolari</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Patologie del sistema muscoloschelettrico e del tessuto connettivo</th>
<th>Non comune</th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Studio 735 6 mesi</th>
<th>Gruppi di trattamento</th>
<th>N</th>
<th>Eventi</th>
<th>Eventi/paziente-anno</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budesonide/formoterolo fumarato diidratato 160/4,5 μg BD + al bisogno</td>
<td>1103</td>
<td>125</td>
<td>0,23^a</td>
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<tr>
<td>Budesonide/formoterolo fumarato diidratato 320/9 μg BD + terbutalina 0,4 mg al bisogno</td>
<td>1099</td>
<td>173</td>
<td>0,32</td>
<td></td>
</tr>
<tr>
<td>Salmeterolo/fluticasone 2 x 25/125 μg BD + terbutalina 0,4 mg al bisogno</td>
<td>1119</td>
<td>208</td>
<td>0,38</td>
<td></td>
</tr>
<tr>
<td>Budesonide/formoterolo fumarato diidratato 160/4,5 μg BD + al bisogno</td>
<td>1107</td>
<td>194</td>
<td>0,19b</td>
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</table>

<table>
<thead>
<tr>
<th>Studio 734 12 mesi</th>
<th>Gruppi di trattamento</th>
<th>N</th>
<th>Eventi</th>
<th>Eventi/paziente-anno</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budesonide/formoterolo fumarato diidratato 160/4,5 μg BD + formoterolo 4,5 mg al bisogno</td>
<td>1137</td>
<td>296</td>
<td>0,29</td>
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<tr>
<td>Budesonide/formoterolo fumarato diidratato 160/4,5 μg BD + terbutalina 0,4 mg al bisogno</td>
<td>1138</td>
<td>377</td>
<td>0,37</td>
<td></td>
</tr>
</tbody>
</table>

^a Trattamento in regime di ospedalizzazione/pronto soccorso o trattamento con steroidi per via orale

^b La riduzione del tasso di riadmissione è statisticamente significativa (valore P<0,01) per entrambi i confronti
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 preimposta 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 di funzione pulmonare, la FEV1 del gruppo con budesonide/formoterolo non è risultata superiore al trattamento con formoterolo da solo. Pillole di flusso inspiratorio mediate mediante il dispositivo Spiraxom 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 anni). Non vi è alcuna evidenza di interazioni farmacocinetiche tra budesonide e formoterolo. I parametri farmacologici 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 del budesonide dopo l’inalazione con il boccaglio dipende da soli il 2% 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 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 varia dal 28% al 49% della dose erogata. La biodisponibilità sistemica è pari a circa il 45% 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.

MODALITÀ D’USO

Il formoterolo in inalatore può essere utilizzato in qualsiasi momento della giornata, in base alle necessità personali e alle manifestazioni dei sintomi. L’efficacia e la sicurezza del trattamento possono variare in base alla dose e alla frequenza dell’uso. Il miglior risultato può essere ottenuto utilizzando la droga per un periodo di tempo sufficientemente lungo, in modo da ottenere un controllo adeguato dei sintomi.

POPOLAZIONE Pediatrica

Sono stati effettuati studi su bambini e adolescenti in età minore di 18 anni per determinare l’efficacia e la sicurezza del farmaco. Tuttavia, è necessario controllare attentamente gli effetti collaterali e i possibili rischi associati. È importante che la somministrazione sia agevolata e che i bambini compiano correttamente l’inalazione.

INTERAZIONI FARMACOCINETICHE/FARMACODINAMICHE

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. In questi pazienti, l’effetto del formoterolo può essere aumentato, rendendo necessaria una riduzione graduale del dosaggio del corticosteroide. Inoltre, le interazioni farmacocinetiche tra budesonide e formoterolo devono essere considerate in pazienti con insufficienza renale o epatica.

4.6 Fertilità, gravidanza e allattamento

La somministrazione di budesonide per via inalatoria può influire sulla capacità di riproduzione. Non esiste alcuna evidenza di effetti collaterali sulle funzioni spermatogene e ovogene. Tuttavia, è necessario tenere presente che il farmaco può essere somministrato in gravidanza solo se l’antidoto di gravità della condizione supera il rischio per il feto. In caso di allattamento, la somministrazione di budesonide per via inalatoria non è consigliata.

4.7 Disposizione dei rifiuti

Una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato è stata sperimentata e la sua efficacia e sicurezza sono state stabilite in pazienti con broncopneumopatia cronica ostruttiva. Tuttavia, ε’ importante sottolineare che l’efficacia e la sicurezza del trattamento possono variare in base alla dose e alla frequenza dell’uso. Il miglior risultato può essere ottenuto utilizzando la droga per un periodo di tempo sufficientemente lungo, in modo da ottenere un controllo adeguato dei sintomi.

RIASSUNTO DELLE CARATTERISTICHE DEL PRODOTTO

1. DENOMINAZIONE DEL MEDICINALE Duodrop Spiraxom 520 microgrammi/9 microgrammi polvere per inalazione 2. COMPOSIZIONE QUALITATIVA E QUANTITATIVA

Ogni dose erogata (dose che fuoriesce dal boccaglio di Spiraxom) contiene 320 microgrammi di budesonide e 9 microgrammi di formoterolo fumarato diidrato. Ciò equivale a una dose preimposta di 400 microgrammi di budesonide e 12 microgrammi di formoterolo fumarato diidrato. Le dosi somministrate contengono 120 dosi e sono avvolte in un involucro di alluminio. Confezioni multiple contenenti 1, 2 o 3 inalatori. È possibile che non tutte le dosi contenute in ogni confezione siano utilizzate.

4. INFORMAZIONI CLINICHE

4.1 Indicazioni terapeutiche

DuoResp Spiraxom è indicato per il controllo e il trattamento della broncopneumopatia cronica ostruttiva (BPCO) e per il trattamento dei sintomi dell’asma acuto. È indicato per il trattamento dei sintomi dell’asma acuto, incluso il broncospasmo paradosso, nella prevenzione dei sintomi dell’asma e negli episodi acuti. Il farmaco è efficace per il trattamento di sintomi acuti e cronici dell’asma.

4.2 Posologia e modo di somministrazione

La somministrazione di duo Resp Spiraxom deve essere condotta in base alle necessità del paziente. È importante che il trattamento venga iniziato da un medico specializzato in medicina respiratoria. La somministrazione deve essere effettuata correttamente, in base alle istruzioni fornite dal medico o dal farmacista.

4.3 Interventi in caso di multimedicazione

DuoResp Spiraxom deve essere somministrato con cautela in caso di multimedicazione. È importante che il farmaco venga somministrato in modo corretto e in conformità con le istruzioni fornite dal medico o dal farmacista.

4.4 Informazioni al paziente

Il paziente deve essere informato sulla somministrazione corretta di DuoResp Spiraxom. È importante che il paziente sappia quando e come utilizzare correttamente il prodotto.

4.5 Concentrazione plasmatica massima

La somministrazione di duo Resp Spiraxom può causare una maggiore concentrazione plasmatica massima. Tuttavia, ε’ importante sottolineare che l’efficacia e la sicurezza del trattamento possono variare in base alla dose e alla frequenza dell’uso. Il miglior risultato può essere ottenuto utilizzando la droga per un periodo di tempo sufficientemente lungo, in modo da ottenere un controllo adeguato dei sintomi.

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La somministrazione di budesonide per via inalatoria può influire sulla capacità di riproduzione. Non esiste alcuna evidenza di effetti collaterali sulle funzioni spermatogene e ovogene. Tuttavia, ε’ importante sottolineare che il farmaco può essere somministrato in gravidanza solo se l’antidoto di gravità della condizione supera il rischio per il feto. In caso di allattamento, la somministrazione di budesonide per via inalatoria non è consigliata.

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Una combinazione a dose fissa di budesonide e formoterolo fumarato diidrato è stata sperimentata e la sua efficacia e sicurezza sono state stabilite in pazienti con broncopneumopatia cronica ostruttiva. Tuttavia, ε’ importante sottolineare che l’efficacia e la sicurezza del trattamento possono variare in base alla dose e alla frequenza dell’uso. Il miglior risultato può essere ottenuto utilizzando la droga per un periodo di tempo sufficientemente lungo, in modo da ottenere un controllo adeguato dei sintomi.

4.8 Informazione al paziente

Il paziente deve essere informato sulla somministrazione corretta di DuoResp Spiraxom. È importante che il paziente sappia quando e come utilizzare correttamente il prodotto.

4.9 Studio e valutazione della qualità

La qualità del prodotto è stata valutata in base alle normative vigenti. Tuttavia, ε’ importante sottolineare che l’efficacia e la sicurezza del trattamento possono variare in base alla dose e alla frequenza dell’uso. Il miglior risultato può essere ottenuto utilizzando la droga per un periodo di tempo sufficientemente lungo, in modo da ottenere un controllo adeguato dei sintomi.

4.10 Revisione

Tutti i dati specifici riguardanti la somministrazione di duo Resp Spiraxom sono stati utilizzati per valutare la sicurezza e l’efficacia del prodotto. Tuttavia, ε’ importante sottolineare che l’efficacia e la sicurezza del trattamento possono variare in base alla dose e alla frequenza dell’uso. Il miglior risultato può essere ottenuto utilizzando la droga per un periodo di tempo sufficientemente lungo, in modo da ottenere un controllo adeguato dei sintomi.

4.11 Contattare il farmacista

In caso di dubbi o questioni riguardanti la somministrazione di duo Resp Spiraxom, è possibile contattare il farmacista per ricevere assistenza in base alle istruzioni fornite dal medico o dal farmacista.
effogli la dose terapeutica (vedere paragrafo 5.1). Per ottenere un buon risultato, 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 illustrati:

1. Aprire: 
   - Rimuovere lo Spiromax dalla bocca e trattenere il respiro per 10 secondi o finché possibile per il paziente.

2. Inalare: 
   - Proprio dopo aver trattenuto il respiro, inalare il Spiromax in profondità e controllare il flux di espirazione per evitare di soffocare.

3. Chiudere: 
   - Una volta terminata l’inalazione, rilasciare lentamente il respiro e tenere il regolatore (vedere paragrafo 5.1) pressato per evitare un’eventuale inalazione residua.

Il trattamento con ß2-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 situazioni in cui la probabilità di ipopotassiemia è maggiore. Si raccomanda in tali circostanze di monitorare i livelli di potassio sierico.

In caso di mughetto, il paziente deve sciacquarsi la bocca con acqua anche dopo le inalazioni effettuate al bisogno.

Infezioni del cavo orale

Per ridurre al minimo il rischio di infezioni da candida nel tratto orofaringeo, si deve istituire una terapia alternativa. Il broncospasmo paradosso risponde all’inalazione di broncodilatatori a rapida azione e deve essere trattato immediatamente (vedere paragrafo 4.5). Se ciò non fosse possibile, l’intervallo di tempo tra le somministrazioni dei medicinali che interagiscono con il broncospasmo paradosso potrebbe essere prolungato per evitare una cattiva tolleranza.

Intolleranza ai principi attivi o ad uno qualsiasi degli eccipienti elencati al paragrafo 6.1.

Precauzioni con malattie speciali

Lealtà de ß2-adrenocettori agonisti possono determinare un’ipopotassiemia potenzialmente grave. L’effetto di un trattamento concomitante con ß2-adrenocettori agonisti e medicinali che possono causare iperossalizzazione (per esempio derivati stantici, steroidi e diuretici) può sembrare in un effetto soppressore di un’eventuale iperossalizzazione.

Conclusione

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/formoterolo fumarato nel trattamento dell’asma non devono essere compromessi da una diminuzione acuta della dose di steroidi inaspettatamente, in particolare durante l’assunzione del farmaco. Da tale decremento può essere necessario, si deve istituire una terapia concomitante con corticosteroidi sistemici.

Il broncospasmo paradosso risponde all’inalazione di broncodilatatori a rapida azione e deve essere trattato immediatamente (vedere paragrafo 4.5). Se ciò non fosse possibile, l’intervallo di tempo tra le somministrazioni dei medicinali che interagiscono con il broncospasmo paradosso potrebbe essere prolungato per evitare una cattiva tolleranza.

Intolleranza ai principi attivi o ad uno qualsiasi degli eccipienti elencati al paragrafo 6.1.

Precauzioni con malattie speciali

La combinazione a dose fissa di budesonide/formoterolo fumarato può essere effettuata su di un dosaggio inferior 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 devono iniziare il trattamento con DuoResp Spiromax durante una ricaduta o qualora presentino un peggioramento significativo o deterioramento acuto dell’asma. Durante il trattamento con DuoResp Spiromax può essere necessario un aumento dei dosaggi con altri broncodilatatori a rapida azione ed è necessario prendere in considerazione un altro broncodilatatore a rapida azione.

Il trattamento comunque deve essere iniziato con una 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 dosaggio di DuoResp Spiromax devono essere effettuate su di un dosaggio inferior 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 devono iniziare il trattamento con DuoResp Spiromax durante una ricaduta o qualora presentino un peggioramento significativo o deterioramento acuto dell’asma. Durante il trattamento con DuoResp Spiromax può essere necessario un aumento dei dosaggi con altri broncodilatatori a rapida azione ed è necessario prendere in considerazione un altro broncodilatatore a rapida azione.

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Budesonide è un glucocorticoide che, quando inalato, esercita un'azione antinfiammatoria dose-dipendente e precedente a steroidi e sensibilità individuale. Il trattamento con ß2-adrenocettori agonisti può determinare un aumento dei livelli ematici di insulina, acidi grassi liberi, glicerolo e corpi chetovatici 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, caratterizzato da aumento di peso, acné, rarietà dell’ovario e amenorrea. L'incidenza di questi effetti è molto più alta rispetto a quelle che si osservano con i corticosteroidi per via orale. I rischi sistemici possono essere ridotti se si inizia il trattamento con inalatore per due o tre giorni prima di iniziare l'uso orale dei farmaci steroidi.

La tossicità osservata negli studi condotti su animali con budesonide è stata minore rispetto a quella osservata con altri glucocorticoide, con un'efficacia di dose minore e un'incidenza di effetti avversi più piccola. La tossicità sistemica del budesonide è molto minore rispetto a quella osservata con altri glucocorticoide, in particolare con i corticosteroidi per via orale. I glucocorticoide, in particolare con i corticosteroidi per via orale.

DuoResp Spiromax contiene formoterolo e budesonide, che presentano un meccanismo d'azione complementare e mostrano effetti additivi in termini di riduzione della riacutizzazione. Le reazioni avverse più rilevanti sono correlate alla somministrazione del formoterolo, in particolare quando utilizzato in combinazione con glucocorticoide. Le reazioni avverse più frequenti sono correlate alla somministrazione del formoterolo, in particolare quando utilizzato in combinazione con glucocorticoide.

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quello della sola budesonide. Tutti i bracci di trattamento prevedevano l’utilizzo al bisogno di un ß2-adrenocettore agonista a rapida azione. Non sono emersi segni di attenuazione dell’effetto moterolo 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-dipenden-

Sono stati effettuati due studio 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 mante-
nimento di budesonide/formoterolo (2 inalazioni da 80 microgrammi/4,5 microgrammi/inalazione due volte al giorno) e un ß2-adrenocettore agonista a breve durata d’azione al bisogno. In

numero medio di giorni di trattamento con corticosteroidi orali/paziente durante i 12 mesi è approssimativamente ridotto nel gruppo con budesonide/formoterolo (7-8 giorni/paziente/anno rispetto a 11-12 e 9-12 giorni, rispettivamente nei gruppi placebo e,pertanto). Per quanto concerne le variazioni nei parametri della funzione polmonare, come FEV1, la combinazione bude-

sonide/formoterolo non è risultata superiore al trattamento con formoterolo da solo. Piccoli di flusso inspiratorio mediante il dispositivo Spiromax per valutare il picco di flusso inspirato-

Distribuzione

Il legame alle proteine plasmatiche è di circa il 50% e la concentrazione plasmatica massima viene raggiunta entro 10 minuti dall’inalazione. Negli studi, la deposizione polmonare media del formoterolo dopo l’inalazione a polvere secca multidose reperibile in commercio è stata di circa il 25% del peso alla nascita. Tuttavia, tali risultati sperimentali nell’animale non sembrano rilevanti nell’uomo.

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