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Immunoglobulin G in IgE-mediated allergy and allergen-specific immunotherapy

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Summary
Allergen-specific IgG antibodies play a significant role in allergen-specific tolerance, either naturally induced or generated by specific immunotherapy. Nevertheless, the underlying mechanisms are still debated, and allergen-specific IgG determinations are not recommended as a diagnostic tool in IgE-mediated allergy. This review summarizes the latest findings on the immunological and diagnostic role of IgG antibodies in respiratory and food allergies, and during allergen-specific immunotherapy.

Allergen-specific IgG antibodies in respiratory allergy

Type-1 allergy is mainly based on the production and effects of IgE antibodies (1); however, other immunoglobulin classes such as IgG (2,3) with its subclasses and IgA (4,5) have also gained considerable attention in allergy research. Allergen-specific IgG antibodies appear increasingly within the course of and after immunotherapy (6,7) and gained the attribute of "blocking antibodies" (8,9) against antigens involved in IgE-mediated allergy. On the other hand, allergen-specific IgG may also play a role in the occurrence of anaphylactic events (10). This broadens the line-up for possible functions of IgG and its subclasses in type-1 allergy.
The evidence that IgE deficient mice could still develop anaphylaxis (11,12) introduced IgG in the group of allergy promoting factors. Recent studies on mouse models confirmed the role of the high-affinity human IgG receptors FcγRIIA (CD32) and FcγRI (CD64) in IgG-mediated allergic inflammation and anaphylaxis (10,13). As the binding affinity of IgG antibodies to the antigen is much lower than that of IgE (14), their blocking function seems to be based mainly on the sheer quantity of antibodies able to bind the allergen before it reaches the surface of the mast cells. This higher concentration of antibodies is among other factors promoted by the significantly longer half-life of IgG compared to IgE (15,16). Although various studies point out a contributing effect of allergen-specific IgG in the pathogenesis of allergic disease (17,18), the overall results remain controversial and vary according to the antigen and exposure levels (19). For example, the appearance and protective role of IgG antibodies in cat allergy may be related to the dose of exposure to the major allergen Fel d 1 (20). By contrast, although the correlation between cat ownership and higher IgG levels, especially of the subclass IgG4, could also be shown in a study on 412 Swedish children (21), no significant protective effect of these antibodies could be demonstrated. Finally, among 227

children aged 12 to 14 years (22), those exposed to higher antigen concentrations showed a higher risk of being sensitized to house dust mite or cat (OR 4.0, 99% CI 1,49-10,00). Within this group, only high concentrations of IgG antibodies to Fel d 1 correlated with a decreased prevalence of sensitization. Other studies among children reported a relation of lower IgG4 levels with positive skin prick test (SPT) (5), an increased risk of rhinoconjunctivitis (23), and a modifying effect of IgG (not IgG4) on the association between cat-specific IgE and childhood wheezing, with decreasing symptoms related to higher IgG levels (24). This goes along with the results of the German Multicentre Allergy Study (MAS), which reported a low risk of wheezing in children with high IgG levels to cat (25). However, these specific IgG levels were only protective in the absence of IgE and not in children with IgE-mediated sensitization. Serum levels of mouse related IgG or IgG4 were initially suggested as markers for clinical tolerance among 23 laboratory animal workers (26), but following tests among an increased number of probands (n = 110) could not confirm this evidence (27). Various studies on the above-mentioned antigens (28,29) and on Malassezia (30) or Alternaria (31), report on parallel trends in the appearance of IgE, IgG and IgG4 antibodies, suggesting a complementary role. In addition to these findings, Jenmalm et al. repeatedly discovered a strong correlation between elevated IgG4 serum levels and atopic sensitization to birch, egg and cat allergens in childhood (32,33).

Allergen-specific IgG antibodies in food allergy

Food-specific IgG antibodies can be found in most children at the age of three months, independently from their atopic status (34). In a trial on 89 food-allergic children with eczema, the levels of serum and salivary antibodies were examined as potential biomarkers predicting safe reintroduction of previously eliminated foods (35). Interestingly, high pre-diet serum IgG4 levels and IgG4/IgE ratios correlated to established allergen-specific tolerance. The importance of allergen-specific IgE/IgG4 ratios in tolerance induction has been repeatedly underlined (36-38) and recently confirmed in 107 egg-allergic children (39) undergoing an oral food challenge with baked egg. While children with a low IgE/IgG4 ratio to ovomucoid and/or ovalbumin were able to tolerate baked egg, higher levels of this ratio were related to a positive challenge and even anaphylactic reactions. Then, a low IgE/IgG4 to ovalbumin and ovomucoid has been suggested as a marker for tolerance to baked egg in egg-allergic children. Similarly, tolerance was associated in cow's milk-allergic children with a decrease in epitope binding by IgE in combination with an opposed increase in IgG4 binding to the corresponding epitopes (40,41). Among 95 infants with eczema, low serum IgG4 levels to ß-lactoglobulin differentiated those with a clear from those with only suspected cow's milk allergy (4). Accordingly, various clinical trials showed that the efficacy of oral immunotherapy for different antigens, such as peanut (42,43), milk (44) and egg (45), is related to a significant increase of IgG and IgG4 concentrations. By contrast, some studies reported elevated IgG levels in IgE sensitized children to peanut, milk and egg (46,47), warning that the role of IgG in food allergy or tolerance has not been fully determined yet.

Allergen-specific IgG antibodies in drug allergy

Although food allergens are more frequently the cause of anaphylactic events, hypersensitivity to drugs can also lead to severe and potentially life threatening allergic reactions (48). Especially adverse reactions to penicillins are reported by patients and can be observed in daily clinical practice, which made their immunological base a matter of interest already in the 1990s. After reporting on diverse isotypes and specificities of IgG and IgE antibodies to penicillins at individual level (49), a Spanish research group evaluated the role of IgG antibodies in immediate allergic reactions to different determinants of benzylpenicillin, amoxicillin, and ampicillin (50). The study on 59 patients could not confirm its hypothesis of a protective role of allergen-specific IgG in the development of anaphylaxis. A later study on 249 patients with penicillin allergy (51) reported on higher IgG levels specific to various allergen components in allergic subjects, also in patients with negative skin tests but typical symptoms. These findings underline the role of allergen-specific IgG antibodies in the development of drug hypersensitivity, but further research on this topic and on reactions to other drugs is still needed.

The role of IgG antibodies in allergen-specific immunotherapy

To date, allergen-specific immunotherapy (SIT) is the only recognized disease modifying and clinical effective treatment for allergic rhinitis and allergic asthma, as well as IgE-mediated venom allergy. Unlike symptomatic pharmacotherapy for allergy, SIT can reduce both, symptoms and use of medication, prevent sensitization to new allergens, and induce prolonged allergen-specific tolerance after discontinuation of the treatment (52-54). However, the immunological mechanisms underlying SIT still remain incompletely understood. Successful SIT has been associated with several immunological changes, including reduction in mucosal recruitment of basophils and eosinophils, suppression of peripheral Th2 effector cells, immune deviation of cytokine responses from an allergic Th2 to a Th1 pattern, and induction of regulatory T-cells, which suppress the specific Th2 response to allergens through cell-to-cell contact and release of immunosuppressive cytokines (such as TGF-β and interleukin IL-10) (55,56). In addition, there is increasing evidence that clinically effective SIT is associated with an increase in allergen-specific IgG antibodies, particularly the IgG4 subclass. Several studies, involving either

sublingual immunotherapy (SLIT) with aeroallergens (57-60) or subcutaneous immunotherapy (SCIT) with aeroallergens (60-64) and hymenoptera venoms (65,66), have documented an induction of allergen-specific IgG and IgG4 in sera. Furthermore, the duration of clinical reactivity (67) or tolerance (68) has also been shown to be related to the level of specific IgG4. It should be stressed that an increase in IgG and IgG4 antibodies has been related not only to a "naturally" acquired food tolerance, but also to the development of tolerance induced by oral immunotherapy (OIT or SOTI) (69-73). Additionally, the specific IgG4/IgG1 ratio as well as the IgG4/IgE ratio have been proposed in some studies as predictive parameters of a beneficial response to SIT (74, 75). However, there is no consensus on using these antibodies as biomarkers to predict the clinical response to SIT (76). It is still a matter of debate whether the efficacy of SIT could depend on allergen-specific IgG induction. According to the same studies, the induction of allergen-specific IgG antibodies during SIT is mainly an "epiphenomenon", reflecting the development of favorable conditions for tolerance such as the appearance of IL-10 producing regulatory T-cells, which also increase IgG4 production (77,78). Furthermore, a link between increased allergen-specific IgG4 titers and favorable response to SIT has not always been found, particularly with hymenoptera venoms immunotherapy (79).

A possible explanation for the lack of correlation in some studies is that successful SIT seems to induce changes not only in allergen-specific-IgG concentrations, but also in their biological activity, which require qualitative rather than quantitative assays for the detection (2). SIT-induced IgG4 antibodies have been shown to act as "blocking antibodies", which prevent both immediate and late-phase responses by inhibiting IgE-mediated basophils and mast cells degranulation, and allergen presentation to T-cells (3,7-9,80,81). Noteworthy, these blocking activities do not solely depend on allergen-specific-IgG concentrations. Changes in the antigenic reactivity and specificity of SIT-induced IgG antibodies have been reported (82). Moreover, it has been shown that long-term clinical tolerance after discontinuation of SIT is associated with persistence of the IgG4-associated blocking activities (particularly after SIT with aeroallergens). In contrast, SIT-induced allergen-specific IgG4 levels tend to decrease after withdrawal of immunotherapy (83,84). Therefore, the measurement of the IgG inhibitory activities with functional assays, rather than IgG serum titers with quantitative assays, seems a more reliable biomarker to predict the clinical response to SIT (76,85).

In light of these evidences, an effective role of allergen-specific IgG antibodies in the induction and maintenance of the beneficial effects of SIT has been reconsidered. In a very recent experimental study in mouse models, the potential therapeutic and preventive effects of passive immunization with allergen-specific IgG antibodies on allergy have been tested, showing promising results (86).

Lack of diagnostic value of allergen-specific IgG in routine clinical practice

Especially in food allergy, an accurate diagnostic procedure is fundamental to avoid potentially life-threatening reactions (87,88); hence, the clinical history, a controlled food challenge or skin prick testing and serum IgE determinations should be used as a combination of diagnostic tools. When the diagnosis of IgE-mediated allergy cannot be established and serologically confirmed, it is not rare that patients seek for alternative test methods to meet their expectations for results. These procedures often include the determination of allergen-specific IgG antibodies and subclasses offered by commercial laboratories and pharmacies. These measures are not only expensive for the patient and a burden for any health system but do also lack sufficient scientific background. In 2008, a task force of the European Academy of Allergology and Clinical Immunology (89) comprehensively discussed the use of IgG4 testing against foods in allergy, and got to the clear conclusion that it cannot be recommended as a diagnostic tool. This opinion has been also expressed by the American Academy of Allergy Asthma and Immunology (90). Since then, various studies have been conducted to further investigate the role of IgG antibodies in allergy diagnosis. Among 150 hen's egg-allergic children, neither IgG nor IgG4 measurements added any valuable information to the diagnostic procedure of hen's egg allergy (91), thus supporting the position that neither IgG nor IgG4 assays should be included in the diagnostic routine for allergy testing. Similarly, no diagnostic value of IgG and IgA antibodies could be found for cow's milk allergic patients (92). This unanimity against IgG antibodies and subclasses in allergy diagnosis does not rule out the hypothesis of potential other roles of this serological parameter such as e.g. a predictive value. In the early 1990s, an observational study from the Netherlands showed that high IgG1 levels to food allergens were related to the development of allergy to airborne allergens later in life (93). About 50% of the children with a high IgG1 anti-food score developed an IgE response to grass pollen and/or cat dander, which suggested a predictive value of IgG antibodies to food allergens. Although a cross-sectional approach by the same group could confirm this trend (94), a final prospective study on 397 children was not able to reproduce the results and described the determination of allergen-specific IgG levels as not very useful for the identification of patients at risk in clinical practice (95). A randomized double-blind allergy prevention trial from Finland also reported on an increased risk of egg allergy in relation to elevated serum IgA and IgG levels to ovalbumin, but could not confirm this trend for other allergens or as a valid predictive tool (96). Thus, although IgG antibodies, especially the subclass IgG4, are certainly of importance in allergy and tolerance induction, they are nowadays still not of value for clinical practice.

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On the behalf of the Italian Association of Hospital and Territorial Allergologists (AAITO - Campania district)

Sensitization to cockroach allergens in the urban atopic populations living in Campania district (southern Italy). A multicenter study

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Key words

Allergic rhinitis; allergic sensitization; bronchial asthma; Campania district; cockroach; cockroach allergy; hypersensitivity

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Summary

Background. *Although cockroach (CR) is an important cause of allergic sensitization worldwide, only a few data are available in Italy and in a previous study we have observed, in Naples area, a low prevalence of CR sensitization.* ***Objectives.*** *We sought to perform a prospective study for assessing the prevalence of allergic sensitization to CR in a sample of atopic population living in Campania district area (Southern Italy).* ***Methods.*** *Ten Allergy Units or Centres of Campania district participated in this cross-sectional study. Each centre was required to collect the results of at least 100 allergy consultations in consecutive outpatients referred for actual or suspected respiratory allergy. We registered demographic data, type and duration of respiratory symptoms, pets ownership, possible exposure to CR allergens, results of the skin prick tests (SPTs).* ***Results.*** *A total of 1477 patients were examined, 985 (66.68%) had a SPTs positivity to at least one allergen. In this context, ninety were sensitized to CR, thus the overall sensitization prevalence in subjects with respiratory allergy was 6.09% ranging between 0-11% and only five patients were mono-sensitized. Thirteen patients reported rhinitis (R) + bronchial asthma (A), twenty-one R + A + conjunctivitis (C), thirty-seven R + C, five only A and eleven individuals only R. Sixty-seven patients exhibited persistent and twenty-three intermittent symptoms. Dust mite constituted the first cause of associated sensitization to CR.* ***Conclusions.*** *The prevalence of allergic sensitization to CR is not negligible in population living in Campania district and shows a higher trend in comparison to that found recently and some years ago in Naples area. Finally, we suggest atopic individuals and especially those highly sensitized to mite allergens or those living in low-income areas to be tested by SPTs / evaluation of serum specific IgE to CR allergens to exclude the occurrence of CR allergic sensitization.*

Introduction

Although cockroach (CR) is an important cause of allergic sensitization worldwide (1, 2) only a few data are available in Italy. In adults, a mean 13% prevalence of allergic sensitization to CR has been shown in only one multicentre Italian study (3) while 4.58% and a peak of 20% have been found respectively in a study carried out in Naples area (4) and in another on the immigrants of Northern Italy (5). In children the prevalence is lower, ranging from 0.45 to 12.7% (6-8). It has been demonstrated that allergic sensitization to cockroach increases the risk of developing sensitization also to shrimp and house dust mite, because of the presence of IgE-binding cross-reactive epitopes between respective tropomyosins (9).
Recently, we have shown that the prevalence of allergic sensitization to CR is still low (3.62%) in an atopic population living in urban area of Naples (10) and confirms the low trend found seventeen years ago (4.58%) (4).
However, since this low value does not necessarily reflect the true value of a larger territory such as the district area in which Naples is the chief town, we sought to perform a prospective study for assessing the prevalence of allergic sensitization, clinical characteristics and modality of exposure to CR in a sample of atopic population without occupational exposure living in Campania district area (Southern Italy).

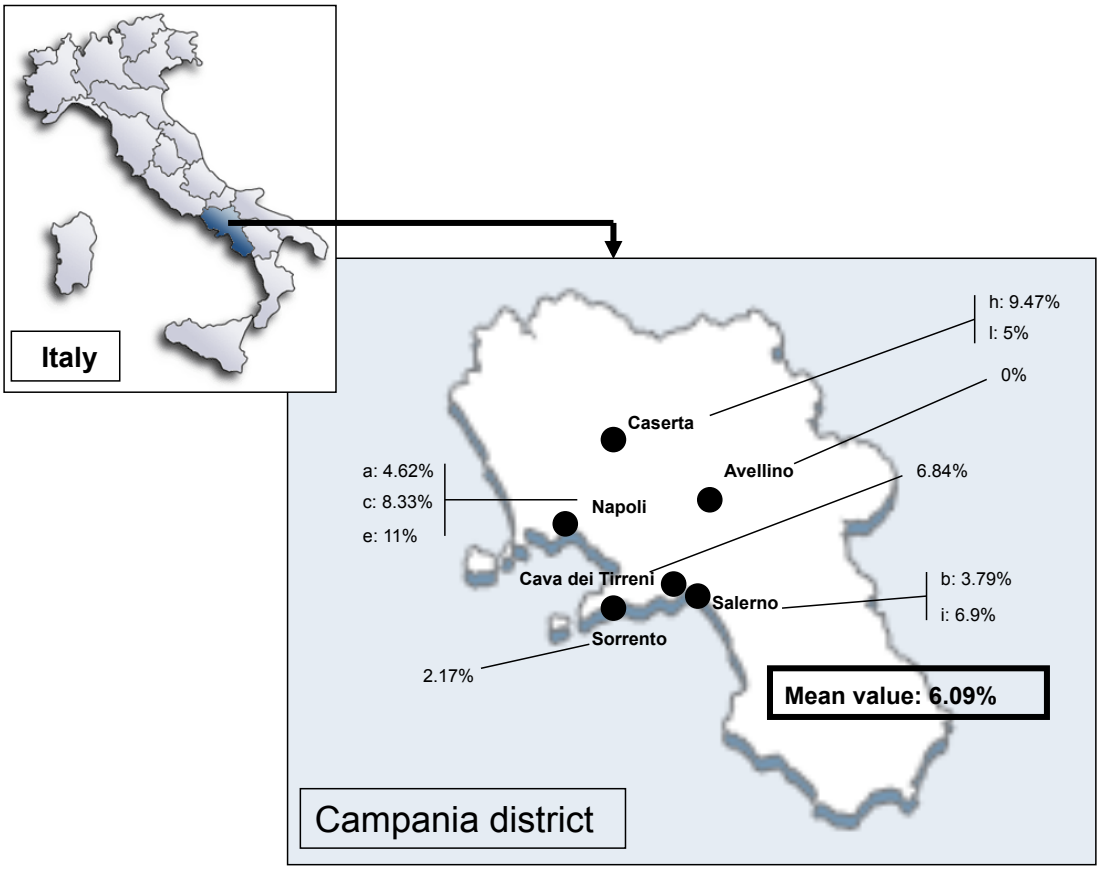
Methods

Ten Allergy Units or Centres, uniformly distributed over the whole territory of Campania district (13.595 Km^2, 6.074.882 inhabitants) participated in this cross-sectional study. Each centre was required to collect the results of at least 100 allergy consultations in consecutive outpatients referred for actual or suspected respiratory allergy (asthma and/or rhinitis), starting from January 1 to June 30, 2011. 1477 subjects aged between 3 and 79 years (mean age 31.2) were examined.
All centres followed the same protocol. A case report form (CRF) containing all information and specifically designed for this study was completed during the screening consultation of each patient. The standardized form reported: demographic data, type and duration of respiratory symptoms, pets ownership, possible exposure to CR allergens assessed by some predictors (such as evidence of CR presence, poor housing conditions, etc.) (11), results of the skin prick tests (SPTs). The diagnosis of respiratory allergy was carried out according to the International Guidelines (12,13).
The commercial allergen extracts used for screening SPTs were provided by ALK-Abello Group, Milan, Italy. We used a standard panel of allergens including: *Dermatophagoides pteronyssinus* and *D. farinae, Alternaria alternata, Cladosporium herbarum,* cat and dog dander, *Parietaria,* grass mix, *Artemisia vulgaris, Olea europaea, Betula pendula, Cupressus sempervirens* and *Corylus avellana.* These allergens cover the majority of causative agents of respiratory allergy in Italy. In addition we used allergenic extracts of cockroach (mix) provided by ALK-Abello Group, Milan, Italy. Positive (10 mg/ml histamine HCl) and negative (saline solution in glycerine-phenol solution) controls were used as well. SPTs were carried out and interpreted according to International Guidelines (14). The result was read after 15 minutes and expressed as the mean of the major wheal diameter plus its orthogonal. A 3 mm skin reaction or greater was considered positive. The profile of the wheals was outlined using a fine-point marking pen and transferred by adhesive tape onto patient's form.
Patients with chronic infectious diseases, malignancies or dysmetabolic diseases, severe cutaneous disorders, negative skin reaction to histamine, or in treatment with drugs interfering with skin response were excluded as well (15,16).

Results

A total of 1477 patients were examined. In this context 985 (66.68%) had a SPTs positivity to at least one allergen and were diagnosed as having respiratory allergy. The 1477 subjects had a mean age of 31.2 years (range 3-79) and 834 (56.46%) of them were females. 90 patients were sensitized to CR, 42 were females and 48 males. Thus, the overall sensitization prevalence in subjects with respiratory allergy was 6.09% ranging between 0-11% (**figure 1**). Only 5 patients were mono-sensitized to CR. 13 patients reported rhinitis (R) + bronchial asthma (A), 25 R + A + conjunctivitis (C), 36 R + C, 5 individuals only A and 11 only R. 67 patients exhibited persistent and 23 intermittent symptoms. 26 out of 90 patients reported some indoor conditions that constitute predictors for the presence of CR allergens. In 9 of these individuals we found the higher levels of cutaneous sensitization to CR, the remaining patients exhibited low/moderate degree of SPT positivity. Since the majority (85/90) of CR-sensitized patients showed cutaneous positivity to other common allergens (mites, pollens, moulds and pets) we could not quantify the role of CR sensitization in eliciting symptoms. The most common sensitizing allergens associated in CR allergic individuals are reported in **figure 2**. Dust mite constitutes the first cause of associated sensitization followed by *Parietaria*, grasses, *Artemisia vulgaris, Olea europaea* and pet danders. These findings are substantially similar to that found in Naples area in previous surveys (4,10). Since monoclonal antibody-based methods to measure the amount of CR allergens in the dust of indoor environments are not available in Italy, we have no information about the levels of indoor exposure to these allergens. However, Curtis-Brosnan et al. (11) have shown that patients' report on the presence of rodents at home and some predictors such as cockroach infestation and poor housing conditions may be sufficient to hypothesize CR allergen exposure in indoor environments. The main characteristics of the patients sensitized to CR are summarized in **table 1**.

Figure 1 *- Geographic distribution of the Campania district centers with the percentages of subjects having positive skin reactions to cockroach allergens*



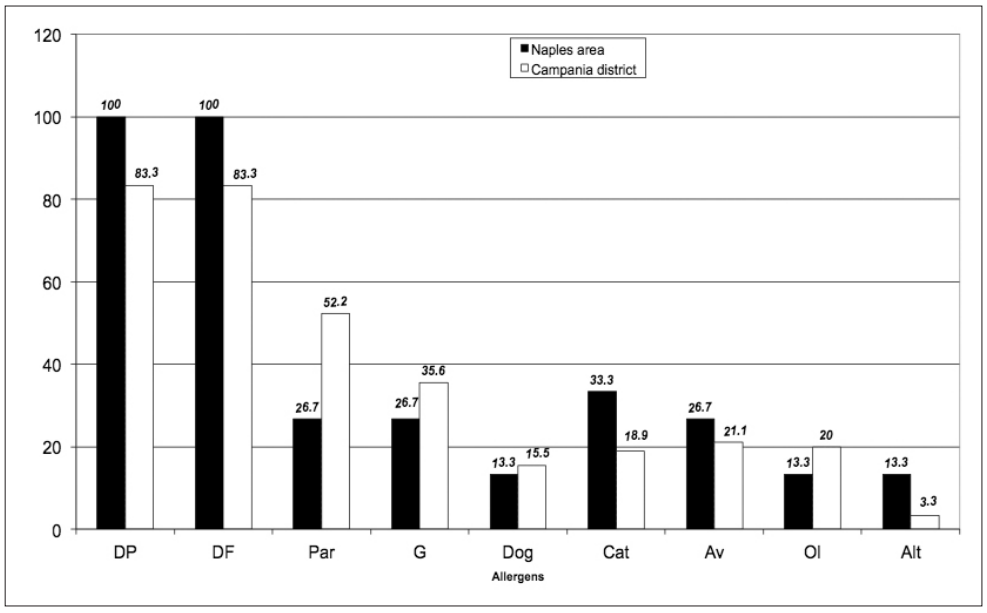


Figure 2 *- Associated sensitizations*

DP = Dermatophagoides pteronyssinus;
DF = Dermatophagoides farinae;
Par = Parietaria;
G = Grasses;
Av = Artemisia vulgaris;
Ol = Olea europaea
Alt = Alternaria

Table 1 - *Characteristics of patients sensitized to cockroach allergens*

	N°	Percentage
SEX (M/F)	42/48	46.7/53.3
MEAN AGE	45	
AGE RANGE		
- 0-20	25	27.8
- 21-41	46	51.1
- 41-60	14	15.5
- > 60	5	5.6
+ VE. FAMILY HISTORY OF ALLERGY	51 yes/39 no	56.7/43.3
PET AT HOME		
- Cat	6	6.7
- Dog	11	12.2
- None	70	77.8
- Other animals	2	2.2
- Cat + Dog	1	1.1
MODALITY OF EXPOSURE TO CR		
- Positive contact	26	28.9
- Negative contact	64	71.1
SMOKING		
- YES	12	13.3
- NO	70	77.8
- EX	8	8.9
CLINICAL SYMPTOMS		
-Rinithis (R) only	11	12.2
-Asthma (A) only	5	5.6
-Rinithis + Asthma	13	14.4
-Rinithis + Conjunctivitis (C)	36	40
-R + C + A	25	27.8
SEASONALITY OF SYMPTOMS		
- Intermittent	23	25.6
- Persistent	67	74.4
ASTHMA SEVERITY		
- Mild	20	22.2
- Moderate/Severe	70	77.8
MONOSENSITIZED TO CR	5	5.6
ASSOCIATED SENSITIZATIONS		
- Parietaria	47	52.2
- Dermatophagoides pteronyssinus	75	83.3
- Grasses	32	35.6
- Olive	18	20
- Mugwort	19	21.1
- Alternaria	3	3.3
- Cladosporium	1	1.1
- Birch	5	5.6
- Hazelnut	5	5.6
- Dog	14	15.5
- Cat	17	18.9
- Other allergens	8	8.9
DIAMETERS OF CR - ALLERGEN-INDUCED WHEALS (SPTs)	< 6x6 mm (81) > 6x6 mm (9)	90 10

Discussion

CR allergens constitute a common cause of allergic sensitization and bronchial asthma in children and adult populations of the US living in inner cities (1). CR exposure, independent of IgE-mediated sensitization status, constitutes also a relevant risk factor for asthma hospitalization in inner city children (17). Among several allergens produced by CR, it has been shown that sensitization to Per a 2 of the American cockroach correlates with more clinical severity among airway allergic patients (2). Wada et al. (18) have found that CR induces inflammatory responses through protease-dependent pathways. Additionally, genetic factors may play an important role in conferring the susceptibility to CR sensitization (19).

The results of this study suggest that the prevalence of allergic sensitization to CR allergens is not negligible in urban atopic population living in Campania district area. This rate of sensitization is higher in comparison to that found in Naples area in previous reports (respectively 4.58 and 3.62%) (4,10). In the three studies the main characteristics of CR-sensitized individuals (prevalence of female sex, high rate of family history of allergy, periods and type of clinical symptoms) may be easily explained by associated sensitization to other common allergens involved in all individuals. However, no specific symptoms related to exposure to CR were found in patients with higher degree of cutaneous sensitization to CR and also in five patients mono-sensitized to CR. The low prevalence of allergic sensitization to CR allergens in our previous study (10) is probably due to the rarely reported presence (only in two cases - 13.3%) of environmental conditions commonly considered at high risk for CR allergens presence (11). In the present study 26 (28.9%) patients reported ideal conditions for the presence of CR in indoor environments (11).

The associated sensitization to mite allergens in CR-sensitized individuals is a common feature in this as well as in the previous reports. Since CR and dust mites usually share the same indoor environments as well as some of their allergens, it is likely that these mechanisms could explain the finding of such a high prevalence of associated sensitization. Moreover, our study suggests that performing a multicenter study at level of district area is more likely to reflect the real rate of allergic sensitization to CR in Southern Italy in comparison to the rate of single urban area of Naples.

It is important to outline that the majority of patients referring to Allergy Services of Campania district who participated to this survey don't live in low-income areas of respective towns. This limitation is similar to that found in previous studies of Naples area. As a consequence, we cannot exclude that a survey carried out specifically in low-income areas of Naples and other towns of Campania district could reveal a higher prevalence of sensitization to CR.

In conclusion, the prevalence of allergic sensitization to CR is not negligible in population living in Campania district and shows a higher trend in comparison to that found recently and

some years ago in Naples area. Finally, we suggest that atopic individuals, especially those highly sensitized to mite allergens or those living in low-income areas, are tested by SPTs / evaluation of serum specific IgE to CR allergens to exclude the occurrence of CR allergic sensitization. We are planning further studies examining exclusively allergic individuals living in some low-income areas of Campania district to verify a possible increase in the rate of allergic sensitization to CR.

Authorship

All authors contributed equally to the writing and revision of the manuscript.

Conflict of interest and financial resources

All authors declare that they have no conflict of interest and that the study has been carried out without any financial support.

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Evaluation of house dust mite allergy in real life: patients' characteristics and satisfaction with treatment

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Key words

House dust mite; respiratory allergy; treatment; satisfaction

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Summary

Background. *HDMs are a ubiquitous allergen source, with a very well defined biology, but their role in clinical settings and in everyday clinical practice is not well characterized. Aim of this cross-sectional, questionnaire-based study was to assess the clinical characteristics of HDM-related respiratory allergy in a large population of Italian patients.* ***Methods***. *A structured questionnaire was sent to allergists randomly chosen among those of the Italian Federation of Immunology, Allergy and Clinical Immunology (IFIACI). They were asked to fill it with the clinical data of 10-12 consecutive patients referred for respiratory allergy, positive to HDM skin prick test. The questionnaire assessed type and severity of allergy, demographics, yearly distribution of symptoms, treatment, and satisfaction with the therapy.* ***Results***. *45 allergists collected data from 499 patients. Within the evaluated population, 42% had rhinitis only, 45% asthma + rhinitis and 13% asthma alone. Rhinitis was moderate/severe in 51% of patients. Asthma was intermittent in 36% of patients, mild in 37% and moderate in 27%. Conjunctivitis was the most frequent comorbidity (36%), followed by rhinosinusitis (16%), adenoid hypertrophy (6%) and polyposis (5%). Out of the population, 56.2% of patients were not at all or partially not satisfied of their treatment for rhinitis, whereas the percentage of dissatisfied patients was about 53% for asthma therapy. 34% patients (n = 170) were monosensitized to HDM. It is confirmed that patients have more symptoms during the fall-winter periods.* ***Conclusion.*** *Patients with HDM allergy have frequently moderate-severe rhinitis, and about 50% of them are not satisfied with their treatment.*

Introduction

Sensitization to house dust mite (HDM) is probably the most frequent cause of IgE-mediated respiratory allergy all over the world (1,2). Indeed, the prevalence of sensitisation to HDM overcomes that of all other common inhalant allergens, with few exceptions, according to the geographical area (3). In addition, the IgE response to HDM allergens uniquely starts very early in life, and persists unchanged until adolescence and adulthood (4). HDM belongs to the *Arachnida* class, is ubiquitous under the altitude of 2000 m and proliferates better in humid and warm environments. The demonstration in asthmatic children of the beneficial effects of sojourning over 2.000 m is part of the history of allergy (5). The allergen components of HDM have been well characterized and cloned (6). The major allergens are Group 1 proteins (Der p 1 and Der f 1) and Group 2 proteins (Der p 2 and Der f 2). These are proteases that can *per se* favour the Th2 response (7). Another important allergen component is tropomyosin (Der p 10), a pan-allergen that has some relevance in food-inhalant allergies (8). This type of allergy is usually characterized by persistent symptoms (previously referred to as a perennial disease), since HDM aerodispersed allergens are present around all the year, with a lower burden during summer months. The continuous

exposure to allergens maintains a chronic inflammation, which is responsible for mucosal hyperreactivity and for symptoms. In particular, in the nose, the continuous exposure and inflammation account for nasal obstruction, which is the most bothersome manifestation (9). In the case of an environmental allergen load insufficient to elicit symptoms, a sub-clinical inflammation (minimal persistent inflammation) can be detected (10). As a consequence of the wide diffusion of the allergenic source and of the clinical and immunological effects, allergy to mite represents a huge socio-economical and healthcare burden.

Despite the biology and allergenic characteristics of HDM being well known, there is still room to investigate its impact on the life of allergic patients, including the treatments used to manage the mite-induced disease. In particular, the patients' viewpoint on the effectiveness of guideline-based pharmacological treatment has been so far poorly investigated.

Thus, the aim of this cross-sectional study was to assess the clinical characteristics (including co-morbidities and severity of disease) of HDM-related respiratory allergy in a large population of Italian patients, using a questionnaire-based method.

Methods

Overall design

This is a questionnaire-based survey involving Italian allergists distributed over the entire territory. The allergists were randomly chosen, in equal proportion, from the three Italian societies of allergy and clinical immunology, which currently convene in the Italian Federation of Immunology, Allergy and Clinical Immunology (IFIACI). The names of the allergists were selected from the member list of each Society. A structured questionnaire was e-mailed to each allergist, who had to fill it with the clinical data of 10-12 consecutive patients referred for respiratory allergy and who resulted positive to HDM skin prick test (SPT). Only patients suffering from respiratory allergy (asthma and/or rhinitis) were included. Each allergist used the same criteria for diagnosing rhinitis and asthma (11,12). SPTs were performed and read according to the recent European recommendations (13). A standard panel of allergens was used in addition to HDM, including: grass mix, C*ynodon dactylon, Parietaria*, birch, olive, ragweed, cypress, cat, dog, and *Alternaria*.

Each physician notified the survey to his/her referral Ethical Committee. The personal data of the patients were kept strictly anonymous. The questionnaires were returned, by e-mail again, to a central organization (IBIS Informatica, Milan, Italy) that performed the data entry and the statistical analyses.

Questionnaire

The questionnaire was set up by a panel of experts from IFIACI. For each physician, the essential demographic information was obtained: age, sex, specialty, region of Italy (North, Centre, South). In addition it was asked to estimate when they more frequently experienced symptoms, and in what proportion of them was specific immunotherapy (SIT) prescribed. The patient's questionnaire, filled by the physician according to clinical history and diagnostic procedures, included:

- Region of residence (North, Centre, South of Italy)
- age range (5≤ years <14, 14≤ years <18, ≥18 years)
- type of disease (rhinitis, asthma, both)
- severity of rhinitis (intermittent, persistent, mild, moderate/severe)
- severity of asthma (intermittent, mild persistent, moderate, severe)
- duration of symptoms (months/years)
- months of the year when symptoms are worse
- treatments for rhinitis (topical/systemic antihistamines, topical/oral/injected steroids, leukotriene modifiers, cromones, nose lavages)
- treatments for asthma (short/long acting bronchodilators, inhaled/oral/injected steroids, inhaled anticholinergics, leukotriene modifiers, theophylline, others)
- degree of satisfaction with treatments for asthma and/or and rhinitis (not satisfied at all, partially dissatisfied, partially satisfied, fully satisfied)
- presence of clinical symptoms following the ingestion of shrimps or snails
- additional positive SPTs (grass, *Parietaria*, birch, olive, ragweed, cypress, cat, dog, *Alternaria*, any other tested)
- comorbidities (conjunctivitis, rhinosinusitis, nasal polyps, adenoid hypertrophy, recurrent otitis).

Results

Forty-five allergists were involved in this survey (46% Northern Italy, 21% Central and 33% Southern Italy). The 45 specialists collected data from 499 patients, 48% adults, 30% children and 22% adolescents. Missing/unreported data varied, on average, from 0 to about 8% according to the questionnaire's items. The descriptive statistical analysis was done per each item only on entered data.

Within the evaluated population, 42% had rhinitis only, 45% asthma plus rhinitis and 13% asthma alone. Rhinitis was mild intermittent in 11%, moderate/severe intermittent in 15%, mild persistent in 38% and moderate/severe persistent in 36% of patients. Asthma was intermittent in 36% of patients, mild in 37% and moderate in 27% (**figure 1**). For rhinitis, oral antihistamines were prescribed in 76% of patients and topical steroids in 63%. Concerning asthma, inhaled steroids were used in 55% of patients, short acting beta2 agonists in 49%, long acting beta2 agonists in 34% and leukotriene modifiers in 41%. Conjunctivitis was the most frequent comorbidity, reported by

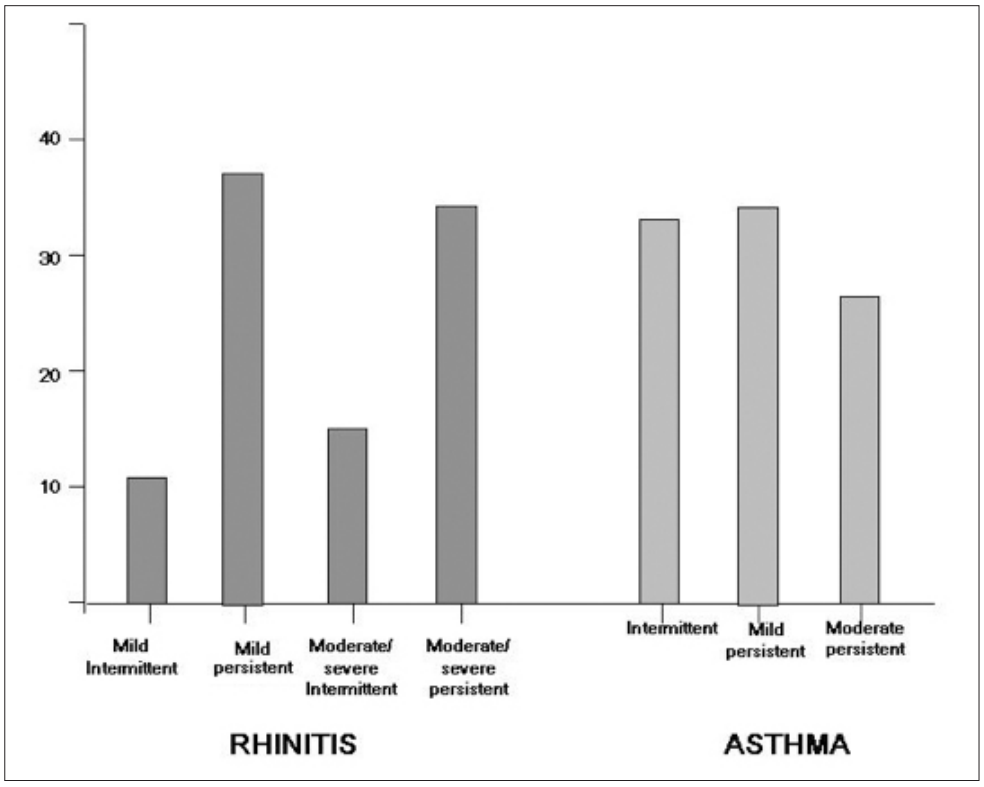

Figure 1 *- Percentage distribution of the severity/duration of asthma (right) and rhinitis (left)*

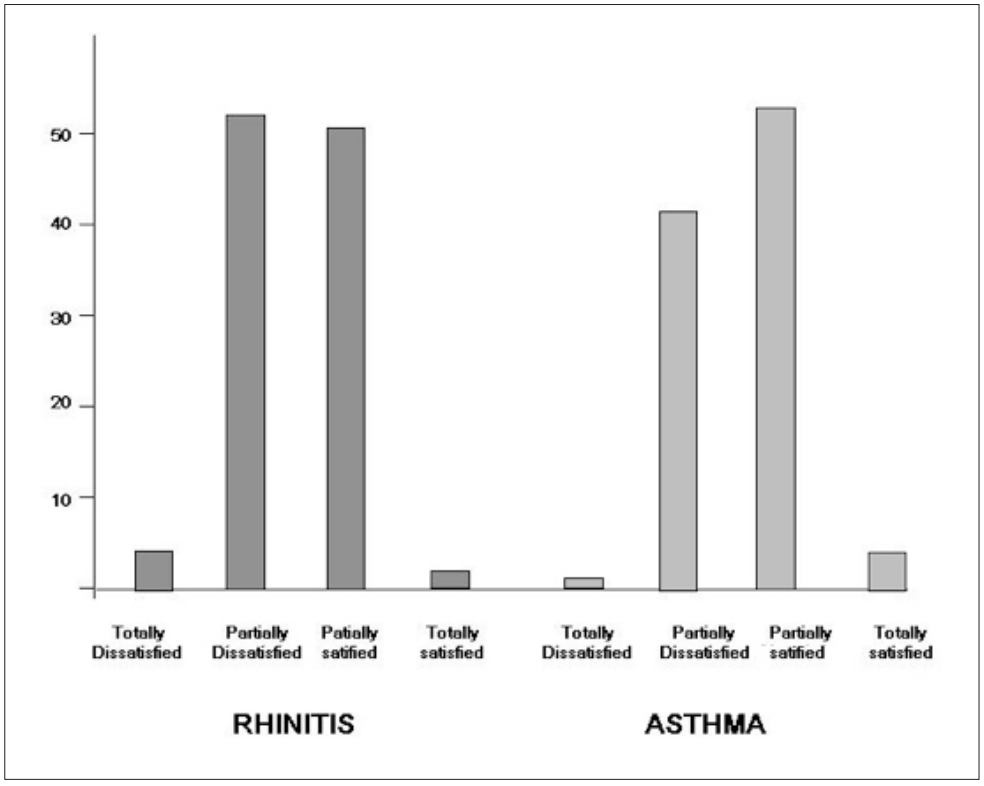

Figure 2 *- Percentage distribution of the satisfaction with treatment as reported by patients*

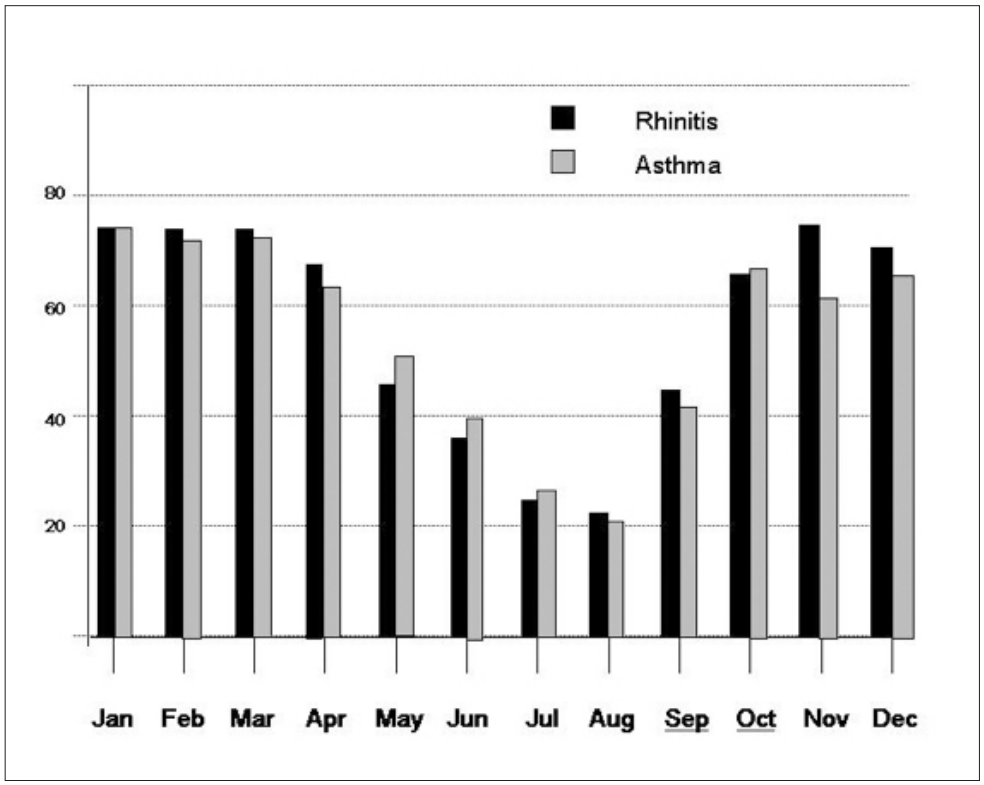

Figure 3 *- Yearly distribution of symptoms (% of patients indicating each month as that with worst symptoms)*

36% of patients, followed by rhinosinusitis (16%), adenoid hypertrophy (6%) and polyposis (5%).

Out of the population, 56.2% of patients were not at all or partially not satisfied of their treatment for rhinitis, whereas the percentage of dissatisfied patients was about 53% for asthma therapy (**figure 2**).

According to SPT results, performed with the standard panel used, 34% patients (n = 170) resulted to be monosensitized to HDM, whereas 66% (n = 329) were polysensitized, being the association grass + HDM the most frequently recorded (239/499, 48% patients). The yearly distribution of symptoms (worst period) is summarized in **figure 3**. It is confirmed that patients have more symptoms during the fall-winter periods.

Discussion

Despite the features of HDM-induced respiratory allergy being well described in literature, and the biology and molecular aspects of mites being well known, there are few data in the literature focused on patients with HDM allergy regarding their characteristics and response to therapy. In the recent past, it was believed that HDM allergy was a "perennial" disease (i.e. present all around the year) (14), whereas it has been recently acknowledged that symptoms and inflammation may vary, according to the changes in allergen burden and persistence. For this reason, the traditional classification of "perennial" and "seasonal" allergic rhinitis has been changed in the *Allergic Rhinitis and its Impact on Asthma* (ARIA) document into "persistent" and "intermittent" depending on symptoms' duration (11), with a subdivision according to the intensity of symptoms into "mild" and "moderate/severe".

HDMs are a ubiquitous allergen source, with a very well defined biology, but their role in clinical settings is usually the result of deductions, and few data are available on the aspects of mite-induced allergy in everyday clinical practice. For this reason, we undertook this cross-sectional study within a population of HDM sensitised patients to evaluate their main clinical characteristics and their feelings about the treatments received. The survey was questionnaire-based, and the database was filled by allergists who evaluated HDM-sensitized patients. According to the data collected, most of the patients sensitised to HDM resulted to have a severe and persistent rhinitis, frequently associated with conjunctivitis. In addition, it was observed that the worst periods, as far as clinical symptoms were concerned, were limited to autumn-winter, with a relevant improvement during the summer months. This increase in symptoms' severity during fall is in accordance with the already described "September epidemic" in allergic patients (15) and also with the

reported "seasonality" of HDM. In fact, a study from Australia found that the mite concentrations in beds had a two- to three-fold annual fluctuation during a 7-year period of observation, showing the highest values in late autumn and the lowest values in summer (16). HDM-allergic patients we studied seemed to have a more severe disease, with more frequent comorbidities and impairment of their quality of life, when compared to other kind of allergic sensitizations. Concerning the treatments, that were prescribed according to current guidelines, for rhinitis oral antihistamines were the most prescribed drugs, followed by topical steroids, while for asthma the most prescribed drugs were inhaled steroids, followed by short acting beta2 agonists, long acting beta2 agonists and anti-leukotrienes. However, analyzing patients' satisfaction with the treatment of the allergic disease, more than 50% of them reported to be not satisfied with the therapies. In particular, 53% of patients were dissatisfied (partially or completely) by asthma therapy, and the rate rose to 56.2% concerning the treatment for rhinitis. These cases of allergic rhinitis, mostly satisfying the criteria for severe chronic upper airways disease (SCUAD) (17) are of particular interest because there is a need to control a disease that causes a particularly important impairment of quality of life. In a controlled study by Frew et al. it was showed that patients with seasonal allergic rhinitis resistant to drug treatment had a clear benefit from specific immunotherapy, with symptom and medication scores resulting significantly better in actively than in placebo treated patients. This suggests that specific immunotherapy should be considered as an adjunct to standard therapy in patients with severe allergic rhinitis.

The main limitations of the study are the questionnaire-based method and the selection bias. On the other hand, the large population involved, with a clinically established diagnosis, warrants for a well-selected population.

In conclusion, we found that in a population of HDM-allergic patients a consistent part of the population suffered from moderate to severe allergic disease and that more than half of them were not satisfied by the prescribed drug treatment. This calls for specific studies to be addressed on mite-allergic patients, with the aim to improve the management of the disease and consequently their quality of life.

Funding and conflict of interest

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Detection of 20 kDa and 32 kDa IgE-binding proteins as the major allergens in Italian sesame seed allergic patients

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Key words

Food allergy; sesame allergy; allergens

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Summary

Background and objective. *Sesame seed allergy, a potentially very severe food allergy, seems on the rise worldwide but is still uncommon in Italy. The aim of the present study was to investigate the allergenic profile of Italian sesame seed-allergic patients.* ***Methods.*** *Patients with genuine sesame seed allergy diagnosed over one year in a large number of allergy centers scattered through Italy were considered for the study. Their IgE reactivity to sesame seed allergens was characterized by immunoblot analysis.* ***Results.*** *Eleven sesame seed allergic patients were detected and studied. 10/10 patients showed IgE reactivity against a sesame allergen at about 20 kDa, and 7/10 showed an extremely strong reactivity at about 32 kDa. The same 7 sera reacted also against a 28 kDa allergen, although such reactivity was significantly weaker in 6/7 cases. Eight patients showed IgE reactivity at about 48 kDa, and 5 sera reacted against higher m.w. proteins at about 67 kDa. Two sera showed IgE reactivity at about 43 kDa as well. Only one serum appeared to react to 2S-albumin.* ***Conclusions.*** *Italian sesame seed-allergic patients react mostly against allergens other than those described so far as major ones. A large number of recombinant sesame allergens will be needed for a comprehensive component-resolved diagnosis of allergy to this food.*

Introduction

Sesamum indicum (*Pedaliaceae* family) is widely cultivated in many countries in the Middle East, Asia, Latin America and also in the USA. Probably due to its widespread use in international fast food restaurants, in bakery products and in snacks and salad dressings, a global increase in sesame seed allergy has been recorded (1). Following some isolated case reports, the first case series was reported from Switzerland in 1993 (2). Subsequently, reports of sesame seed allergy in both adults and children from Israel, many European countries, USA, Canada, and Australia have appeared in the literature (3). In certain geographic areas where large amounts of sesame are traditionally present in the common diet, this type of food allergy may occur very early in life (4). To date, 8 allergens have been identified in sesame seeds: Ses i 1 and Ses i 2 (2S-albumin; m.w. 7-9 kDa), Ses i 3 (7S vicilin; m.w. 45 kDa), Ses i 4 and Ses i 5 (oleosins; m.w. 15-17 kDa), Ses i 6 and Ses i 7 (11S globulin, legumin; 52-57 kDa) (5-10) and, although not yet an official IUIS allergen, Ses i 8 (profilin, m.w. 14 kDa) (11). Sesame seed allergy does not seem

very common in Italy. In a recent survey on more than 1000 food allergic adults, only 4 were allergic to sesame seed (12). Notably, sesame seed allergic patients show a high prevalence of severe systemic reactions following the ingestion of foods containing the offending allergen (12,13). A recent Italian in-vitro study found a high prevalence of reactivity to the 11S globulins (14), whereas previous studies reported a prevalent reactivity to 2S-albumins (6). In the present study, sesame seed allergic subjects were sought over one year and their IgE was characterized by immunoblot analysis in order to assess the major sesame allergens in Italy.

Patients and methods

Patients

The study was carried out on outpatients diagnosed as having sesame seed allergy referred to 33 Italian allergy departments from January 1st to December 31st, 2011. The diagnosis of sesame seed allergy had to be based on a clear-cut clinical history of oral allergy syndrome, asthma, urticaria/angioedema, and/or anaphylaxis following the ingestion of sesame seed under any form (raw, cooked, roasted, ground, etc.) except in one case (see beyond) with an unequivocally positive SPT with fresh sesame seed and/or commercial sesame seed extract. Since the objective of this study was to investigate the IgE reactivity to specific sesame seed proteins, patients sensitized to cross-reacting plant-food allergens such as PR-10, profilin, and LTP were excluded. Admitted patients were thoroughly interviewed to detect their clinical reactivity to foods other than sesame seed, particularly walnut, hazelnut, almond, peanut, pine nut, Brazil nut, and sunflower seed. The study was carried out as a part of the routine clinical activity of all participating centers, hence no formal approval by an Ethical Committee was required. All study patients gave an informed consent to the serological analyses. Sesame seed hypersensitivity was detected by SPT with fresh seeds using the prick-prick technique. In some centers where commercial sesame seed extracts were available (Lofarma SpA, Milan, Italy; ALK-Abellò, Madrid, Spain), SPT with such extracts were used as well. Hypersensitivity to PR-10, profilin and LTP was excluded on the basis of negative SPT with commercial birch pollen extract, date palm pollen profilin (Pho d 2, 50 µg protein/ml; ALK-Abellò, Madrid, Spain) and commercial peach allergen (30 µg LTP/ml; ALK-Abellò). All skin tests, either skin prick tests or prick-prick tests, were carried out and read following established criteria (15). Only wheals showing a mean diameter exceeding 3 mm at 15 min were considered as a positive response.

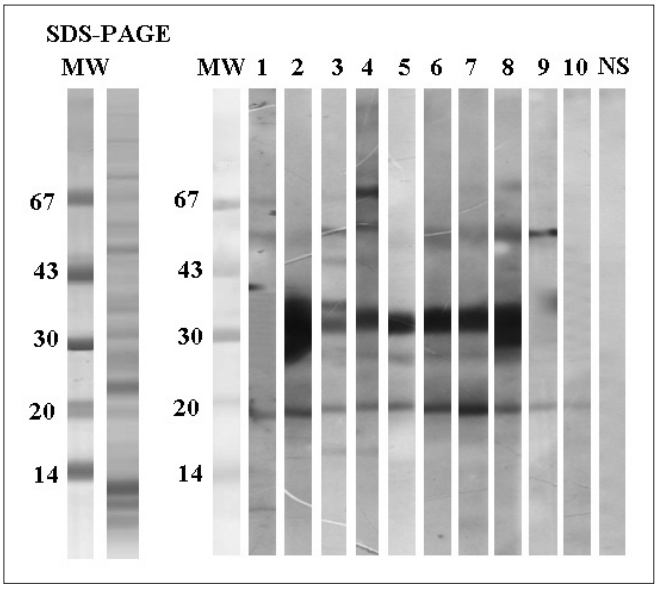
Immunoblot analysis

Eight grams of sesame seeds defatted with hexane were extracted for 1 hour in 100 ml of 0.9 M NaCl, at 4 °C under stirring. After centrifuging, the supernatant was harvested and dialyzed against the same buffer. The protein content, measured by Bradford's method (16), was 1.6 mg/ml. The electrophoresis of sesame seed extract (25 µg per lane) was carried out in a 10% polyacrylamide precast Nupage Bis-Tris gel according to manufacturer's instructions (Invitrogen, Milan, Italy) at 180 mA for 1 h under both reducing and non-reducing conditions. The resolved proteins were transferred onto a nitrocellulose membrane (Protran BA 85, Schleicher & Schuell, Milan, Italy) according to Towbin (17). The membrane was saturated in TBS (tris buffered saline) buffer containing 5% defatted dry milk (saturating buffer) and incubated with patient's serum or normal serum diluted 1:5 in saturating buffer. Specific IgE bound was detected by adding peroxidase-conjugated anti-human IgE from goat (diluted 1:8000, BioSpacific, Emeryville, CA, USA) and ECL western blotting kit (Amersham, Milan, Italy) as substrate.

Results

Eleven patients (aged 4-51; M/F ratio 7/4) diagnosed at 7 allergy centers fulfilled the admission criteria and were included in the study (**table 1**). Eight patients had a history of sesame seed allergy only, one had a clinical history of allergy to Brazil nut and another one to sunflower seed too, and one had a history of clinical allergy to multiple nuts and seeds, including walnut, hazelnut, almond, pine nut and Brazil nut. Sera from 10 pa-

Figure 1 *- Molecular weight markers, SDS-PAGE of white sesame seed extract, and immunoblot analysis. Lanes 1-10: sera from sesame-allergic patients, lane numbers correspond to patients' numbers in* **table 1**. *NS: normal serum*



tients (all but the 4 year old child, patient # 11 in **table 1**) were available for in-vitro testing. Immunoblot analysis results are shown in **figure 1**. All patients showed IgE reactivity against a sesame allergen at about 20 kDa, and 7/10 showed an extremely strong reactivity at about 32 kDa. The same 7 sera reacted also against a 28 kDa allergen, although such reactivity was significantly weaker in 6/7 cases. Eight patients showed IgE reactivity at about 48 kDa, and 5 sera reacted against higher m.w. proteins at about 67 kDa. Two sera showed IgE reactivity at about 43 kDa as well. Surprisingly, only one serum recognized (even if weakly) a zone corresponding to that of 2S-albumin. In order to rule out the possibility that reducing conditions used in SDS-PAGE might have destroyed IgE-binding epitopes of 2S-albumin, making them no more recognizable by sera in immunoblots, SDS-PAGE was also performed in non-reducing conditions, but IB profile against 2S-albumins profile (data not shown) did not change.

Table 1 *- Clinical features of study patients*

No.	Age	Sex	Other offending foods	Symptoms with s esame	Positive SPT other than sesame
1	22	F	None	Anaphylaxis	h, pn
2	20	M	None	Laryngeal oedema	None
3	58	M	sf	Anaphylaxis	sf, bn
4	39	M	None	Anaphylaxis	None
5	70	M	None	Anaphylaxis	None
6	56	M	None	Anaphylaxis	P
7	53	F	w, h, a, pn, bn	Urticaria	w, h, a, pn, bn
8	16	F	bn	Gastrointestinal	Bn
9	54	F	None	Urticaria	A
10	51	M	None	Urticaria	P
11	4	M	None	Urticaria/ angioedema	None

w = walnut; h = hazelnut; a = almond; pn = pine nut; bn = Brazil nut; sf = sunflower seed; p = peanut

Discussion

Sesame allergy is uncommon in Italy; in fact only 11 patients were diagnosed over one calendar year in 33 allergy clinics scattered throughout the country, where thousands of subjects suspected to be allergic are visited monthly. Not unexpectedly, most patients had a history of severe systemic allergic reactions following sesame intake. Surprisingly, few sera from study patients showed an IgE reactivity at molecular weights corresponding to those of sesame allergens described so far. All patients' sera recognized a protein at about 20 kDa and most of them showed an extremely strong IgE reactivity at about 32 kDa. Along with a protein at about 50 kDa (possibly 7S vicilin, Ses i 3 or 11S globulin-legumin, Ses i 7) these proteins seem to be the major allergens in this population. The specificity of recognition was confirmed by the lack of any IgE reactivity by a normal control serum (figure 1). Only one serum showed IgE response at the m.w. of the 2S-albumin (serum 1, **figure 1**), whereas 7 S vicilin recognition seemed weak and uncommon. A pattern of IgE recognition similar to that of our patients has been reported by Fremont et al. (18), whose patients reacted mainly to proteins at about 13 kDa and about 30 kDa. Similarly, in their study Beyer and co-workers found IgE reactivity against a number of proteins at different m.w. including 20 kDa, 32 kDa and 34 kDa (5). Since we did not carry out the N-terminal sequencing of the major allergens recognized by our patients' sera nor inhibition studies, we cannot exclude that the proteins recognized are polymers of lower m.w. allergens (e.g., 2S-albumin) or fragments of allergen proteins showing a higher molecular weight, although in previous studies (5,6) 2S-albumins showed a molecular weight of 7-9 kDa and vicilins a molecular weight of 45 kDa, respectively. We can exclude, however, that our study population was sensitized to PR-10 homologous proteins, profilin, and lipid transfer protein, the latter being the major food allergen in Mediterranean area even among tree nuts and seed-allergic subjects. This study suggests that sesame allergy shows some geographic variability, as is the case for other food allergies, and that probably our understanding of this type of food allergy is still incomplete. In the future, a large number of recombinant sesame allergens will be needed for a comprehensive component-resolved diagnosis of allergy to this food.

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OCCUPATION study (OCCUPationl Asthma: a naTIONal based study): A survey on occupational asthma awareness among italian allergists

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Key words

Occupational asthma; occupational rhinitis; diagnostic tools; treatment; medico-legal obligations

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Summary

Occupational asthma (OA) is the most common work-related respiratory disease. Case identification still remains underperformed. The present survey aimed at investigating the awareness about OA among Italian allergists. 538 Italian Allergists completed a web anonymous questionnaire concerning: patient profile, occupational history, disease features, diagnostic work-up, causal agents, management after diagnosis. 80 cases were registered by 14 members (2.4%). Patients were mostly between 30 and 62 years old; noteworthy, 19% were between 18 and 30. All the patients had a concomitant rhinitis, usually preceding asthma onset. Bakers, hairdressers and healthcare workers were more frequently involved. Diagnostic process included: skin prick test (85%), stop/resume test (57%), specific IgE dosage for occupational allergens (52.5%), peak expiratory flow monitoring (32.5%). Noteworthy, only 27,5% of patients underwent specific challenge. After the diagnosis 50% of patients did not change job. One third of the subjects were not referred to the national Workers Compensation Authority. Our data show that OA is quite neglected by Italian allergists, despite they have a pivotal role both in early identification and in primary prevention of OA. Thus, it is worth increasing awareness concerning OA and creating an easy-access network involving allergists and referral centers for Occupational respiratory diseases.

Introduction

Occupational asthma (OA) is the most common form of work-related lung disease, being about 9-15% of adult-onset asthma due to occupational exposure (1). Although its detection is relevant for medical and socioeconomic consequences, case identification and diagnosis still remains difficult (2). In order to evaluate in a real life setting the awareness of OA among Italian allergists, we performed a web survey concerning some relevant issues in OA, such as responsible agents, diagnostic work-up, treatment options, and medical legal decisions.

Methods

A web anonymous questionnaire was available on the website of the Association of Italian Allergists (www.aaito.it) for 60 days (from 11th April to 11th July). An invitation to participate to the survey was sent by e-mail twice to all 583 members of the Asso-

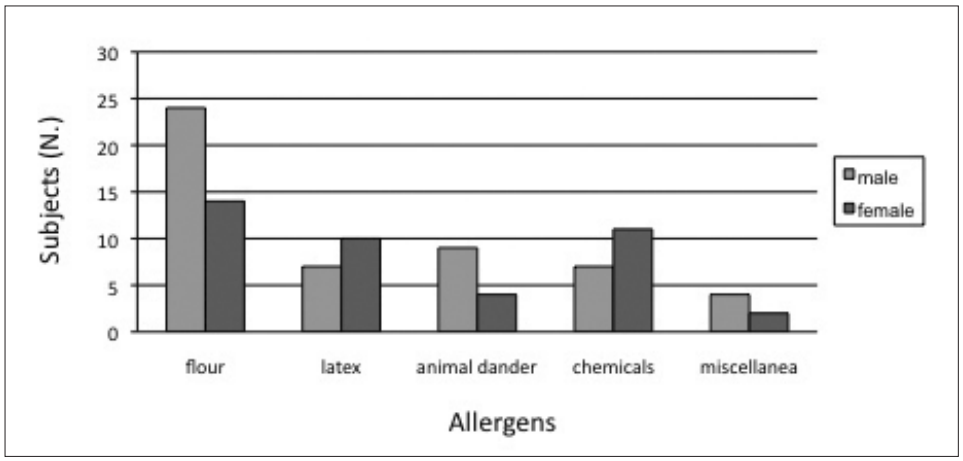

Figure 1 *- Causative allergens and male/female ratio*

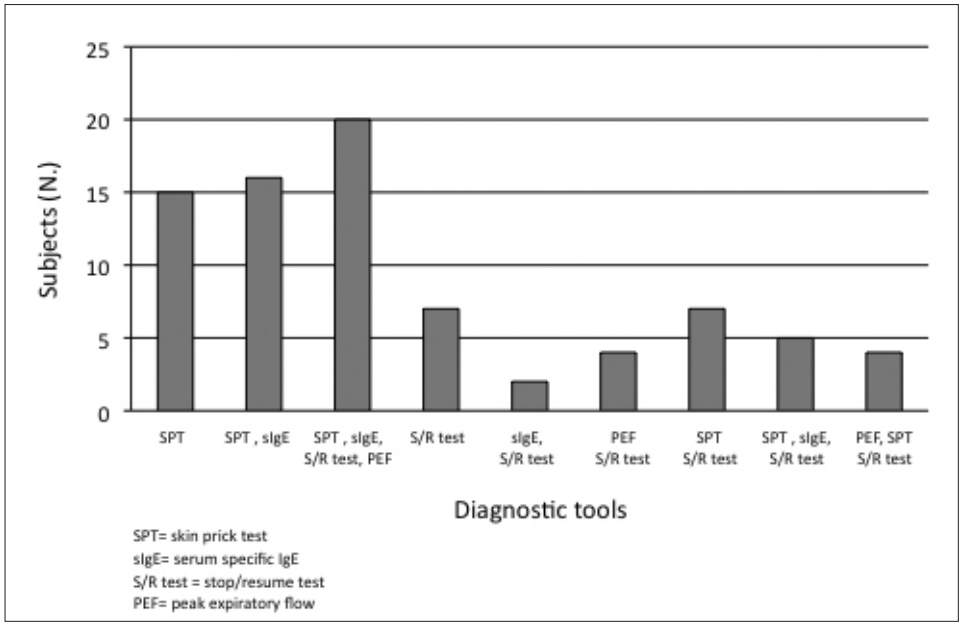

Figure 2 *- Diagnostic tools*

ciation, 30 days apart. Participants were asked to include information about all cases of OA observed in their clinic in the last two years. The 16 multiple-choice questions included the following items: patient profile (age, gender), occupational history (work, job duties), disease features (duration of employment at current job before symptoms onset, respiratory and associated symptoms, severity of the disease according to GINA(3) and ARIA(4) guidelines), diagnostic work-up (stop/resume test, skin prick tests, serum specific IgE to occupational allergens measurement, peak expiratory flow monitoring, specific bronchial/nasal/conjunctival challenge), causal agents, management (therapy, medico-legal management), occupational history after diagnosis (change in occupation, change in job but in the same workplace, no change, unemployment), number of followed-up patients, number of new OA cases per year.

Survey's results

1. Study population

Overall 80 cases were registered by 14 members (2.4% of all associated), belonging to different centers. Most of the cases (62.5%) were reported by three centers only. In the study population a slight prevalence of males (53.2%) was observed. The age ranged from 18 to 62 (mean: 39.89): 19 subjects were between 18 and 30 years old; 22 subjects were between 31 and 40 years old; 17 subjects were between 41 and 50 years old; 12 subjects were between 51 and 60 years old; 3 subjects were between 61 and 70 years old. No data are available for 7 patients. OA started in 53.3% of cases after more than 5 years of exposure to the occupational allergen, in 25% between 3-5 years and

in 19.7% within less than three years. Patients' jobs were distributed as follows: bakers (37.5%), hairdressers (15%), health care workers (15%), veterinaries (6%), industrial workers (5%), others (21,5%).

2. Allergens and clinical findings

Wheat flour was the most recurrent cause (47.2%) of OA, followed by natural rubber latex (23.6%), animal dander (17.8%), persulphates (11.1%) and diisocyanates (8.8%). A different male/female ratio was registered according to different allergens (**figure 1**). The severity of asthma according to GINA Guidelines (3) grading was as follows: mild intermittent in 19.7%, mild persistent in 30.3%, moderate persistent in 43.4% and severe persistent in 6.6%. A concomitant rhinitis was present in all patients, usually preceding the appearance of asthma. Its severity according to ARIA Guidelines (4) was as follows: mild intermittent 6.8%, mild persistent 31.5, moderate/severe intermittent 17% and moderate/severe persistent 38.4%. Various tools were used in the diagnostic process as reported in **figure 2**. Twenty-two patients (27,5%) underwent specific challenge with suspected professional agent. In 45,5% of cases bronchial challenge only was performed; in 9% specific challenge included both nasal and bronchial exposure; in 45,5% bronchial together with nasal and conjunctival challenge was set up. In one patient only nasal challenge was performed.

3. Management

Most of patients were treated for both asthma (98%) and rhinitis (87.5%). Nasal steroids were the most frequently used therapy (67%), alone (28.7%) or in combination with anti-histamines (40%). The combination treatment with a long acting beta 2 bronchodilator and an inhaled steroid was the most common therapy for asthma in agreement with a prevalence of moderate-persistent severity. However, comparing the level of severity and the treatment according to the GINA Guidelines (4) an over-treatment seems to be reported in moderate-persistent asthma, whereas an under-treatment was observed in mild and severe persistent asthma.
After the diagnosis of OA 50% of patients did not report any change in the job, 32% changed job in the same workplace, while 14% left work or changed occupation. One third of the subjects were not referred for an occupational disease to the national Workers Compensation Authority (INAIL).

Discussion

Despite being the most common work-related lung disease (5,6), few cases of OA have been identified by a minority of AAITO associates. It is noteworthy that most of the cases of OA (62.5%) have been reported by three centers only. Taken together, these data suggest that, excluding few referral national centers, Italian allergists don't seem to be focused on OA in daily practice. It has to be considered that allergists normally visit patients that are referred by GPs. It is therefore possible that GPs refer their patients with suspected occupational respiratory disease directly to specific reference centers (e.g. workplace health regional centers). On the other hand, as much as 25% of adult asthmatic patients are estimated to have work-related asthma (WRA), the possibility that non-occupational physicians, such as allergists or pneumologists, face WRA in daily practice is high (15). Thus, the overall prevalence of occupational asthma in the present survey may be underestimated. Actually, some aspects concerning case identification and diagnostic work-up of occupational respiratory allergy still remain problematic and unclear (7,8), despite recent guidelines (7,9). A detailed occupational and medical history collected by every physician would be helpful for identifying the subjects suspected of having work-related asthma, to address them to in-depth investigations in specialized centers (15). However, lack of standardized diagnostic tools and referral centers may account for weak awareness of allergists about OA. It can lead to a delayed diagnosis that is quite common among these patients (1). The high percentage of moderate and severe occupational asthma detected in this study may be explained by a long duration of symptomatic exposure period before diagnosis. OA affects working-aged population, which is consistent with causative role of occupational agents. Interestingly, in our survey 19% of cases are between 18 and 30 years old. This finding suggests that sensitization may take place during apprenticeship. The increasing prevalence of allergy and asthma in childhood in the last decades accounts for an increased number of young adults entering the workforce affected by respiratory allergy, that is a risk factor for developing sensitization to high molecular weight occupational agents. As pointed out by a recent EAACI position paper, allergists play a pivotal role in preventing occupational respiratory allergy, making their young allergic patients aware of potential work effects on rhinitis and asthma (10). From an epidemiological point of view, our results point out a slight prevalence of males (53.2%) affected by OA, and identify wheat flour as the most recurrent causative agent (47.2%). Our survey does not seem to confirm the epidemiological data in literature of the rising causative role of cleaning substances (11,12). According to our data, a different male/female ratio was registered according to different allergens. Male bakers and female hairdressers seem to have a higher risk of developing occupational respiratory disease. This probably reflects the different male/female ratio in the respective occupations. The model of United Airways Disease has been clearly confirmed in occupational field too (13). In most cases our patients suffering from OA have persistent moderate/severe, ac-

cording to ARIA classification. The severity of both allergic conditions is bothersome for patients. Moreover, these conditions seem to be independent of the type of causative agent involved. Occupational rhinitis should be always assessed and considered as a predictor of subsequent occupational asthma (14). As far as diagnostic work-up is concerned, our survey points out the lack of a homogeneous diagnostic approach. Surprisingly, specific challenge is not the main diagnostic tool, despite the fact that most of the cases of OA (62.5%) have been reported by referral centers for occupational allergy. Skin prick tests were the most frequently used diagnostic tools (85%). Implementation of current diagnostic guidelines in this field is mandatory to improve case identification and diagnostic work-up of occupational respiratory allergy (15). According to our data, most of patients did not change job or activity after the diagnosis of OA, despite the fact that the most common clinical presentation in our survey is moderate to severe asthma. A notification must be submitted to Workers Compensation Authority (INAIL - Istituto Nazionale Assicurazione contro gli Infortuni sul Lavoro) in Italy whenever a worker is diagnosed with OA. Our data show that one third of the subjects after the diagnosis of OA were not referred for an occupational disease to INAIL. This results in lack of compensation claims for the workers and affects INAIL's activity in the field of epidemiology and prevention of OA and rhinitis. It is thus mandatory to promote the knowledge of medico legal obligations among allergists.

In conclusion, it is well recognized that early diagnosis followed by early removal from exposure is the most important factor that determines a favorable prognosis of OA (7,10). Since many/most respiratory allergic diseases are evaluated by allergists as a first line approach, they have a key role in identifying suspected cases (15). Thus, it is worth increasing awareness concerning OA and creating an easy-access network involving allergists and referral centers for occupational respiratory diseases.

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Component resolved diagnosis in real life: the risk assessment of food allergy using microarray-based immunoassay

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Key words

Food allergy; component-resolved diagnostics; risk-assessment; food allergenic molecules

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Summary

Background. *The development of component-resolved diagnostics constitutes a potential breakthrough in food allergy testing, as detection of specific IgE (sIgE) to individual allergens may make it possible to establish the risk of a mild versus severe reaction.* ***Objective.*** *To compare allergists' risk assessment based on the current decision making process with that of virtual allergen-oriented risk assessment through microarray-based immunoassay.* ***Patients and Methods.*** *An observational, real-life study was performed on 86 adults with food allergy. The prescription of epinephrine was the surrogate marker of a severe reaction. In the same patients, the prescription of epinephrine based on the current decision making of the allergist and the independently established allergen-oriented risk assessment determined by microarray-based immunoassay were compared.* ***Results.*** *Fair degree of agreement between the specialists' risk assessment and that of the microarray-based immunoassay (k index 0.372 (95% CI: 0.185-0.559) $p < 0.001$) was documented. Three causes of discrepancy emerged: the poor sensitivity of the allergen microarray-immunoassay (51.9%), the differences in risk assessment established by the specialist and the microarray-immunoassay (33.3%), the non-inclusion of the causative allergen in the microarray-immunoassay platform (14.8%).* ***Conclusion.*** *Improvement of the diagnostic accuracy of microarray-immunoassay, combined with marrying its results to clinical information, could one day soon lead to changes in clinical practice in food allergy.*

Introduction

Food allergies impair quality of life and can culminate in life-threatening reactions, whose prevalence affects an estimated 2-4% of the general adult population (1, 2), with some studies suggesting that the last few decades have seen a rise in the number of cases in industrialized countries (3, 4).

Even more striking is the increase in the number of patients in industrialized countries who think they have a food allergy and seek medical assistance (5).

Diagnosis of food allergy is necessary both to prevent severe reactions and to avoid unnecessary dietary restrictions; unfortunately, the complex mixture of allergenic proteins of diagnostic extracts for skin and serological tests makes testing inaccurate and hence unable to predict the likelihood and, in particular, the severity of a future reaction (6).

As a result, in current clinical practice, in most patients combining their history with the results of skin testing or immunoassay is a necessary step towards reaching an accurate diagnosis, though controlled food challenge, which is time-consuming and labour intensive, remains the diagnostic gold standard and may sometimes be required (6).

The development of component-resolved diagnostics (CRD) is a potential breakthrough in food allergy testing, as the detection of specific IgE (sIgE) to individual allergens in diagnostic extracts could signify diagnostic improvement,

helping to establish the real risk of either a mild or severe reaction (7).

A further advancement in this field is the combination of CRD and microarray technology, which allows testing a panel of one hundred inhalant and food allergens, including recombinant and purified native allergens with very small quantities of serum (8,9). This technology holds promise for a significant change in the management of food allergic patients, depicting in a single step the virtual allergen-oriented risk assessment in less time and with fewer resources than the current diagnostic workup.

Possible changes in clinical practice in allergy through allergen microarray-immunoassay are currently under investigation both in food allergy (10) and in the prescription of specific immunotherapy (11).

In consideration of this, our aim was to compare the risk assessment that resulted from the allergist's current decision-making approach with that of the allergen-oriented approach reached by means of microarray-based immunoassay.

Methods

Study design

Observational, cross-sectional study performed in a real-life setting. Patients with a case history suggestive of food allergy to several food allergens or of respiratory and food allergy to several inhalant and food allergens were eligible for the study.

Diagnosis was carried out by four trained allergists involved in the study through their usual diagnostic workup, namely combining patient case history obtained using a standardized questionnaire with skin test results and, in case, the immunoassay.

Skin prick testing with commercial extracts (Alk Abellò, Madrid, Spain) was performed in all patients. The basic list of food allergens included almond, anisakis, apple, celery, cod, egg white, gliadin, hazelnut, milk, parsley, peanut, profilin, tomato, peach, sunflower, soy, shrimp, walnut and wheat. Any further skin tests used in current clinical practice (prick test for inhalant allergens, prick test with food allergens not on the basic list, prick by prick test with fresh food) were allowed if the physician deemed it necessary for the diagnosis. Histamine (10mg/ml) and glycerol-saline solution were used as controls. A skin test was considered positive if the mean wheal diameter ≥ to 3 mm was greater than the negative control.

Detection of sIgE, both for food extract or for allergenic molecules (ImmunoCAP, Phadia, Uppsala, Sweden) were added to the diagnostic workup if the physician judged that it was necessary for an accurate diagnosis.

The use of allergen microarray-immunoassay was disallowed to specialists. On completion of the diagnostic workup, the diagnosis and the decision whether or not to prescribe epinephrine were recorded. The allergist's decision was taken as the study's gold standard. The prescription of epinephrine was considered a surrogate marker of the risk assessment, as it is mandatory for severe, potentially life-threatening reactions, whereas an avoidance diet is prescribed for food allergy irrespective of the severity of the reaction.

Serum samples were collected from each participant and a commercial microarray-base immunoassay, which allows 103 airborne and food allergenic molecules (ISAC, Phadia, Uppsala, Sweden) to be tested at once, was performed in the university hospital's general laboratory, according to the manufacturer's recommendations.

Briefly, reaction sites were incubated with 20 mL of patient sera for two hours. After rinsing, washing and drying, allergen-specific IgE complexes were stained with a fluorescence-labelled anti-human IgE for one hour. After further washings, a laser scanner took fluorescence readings, and results were translated into numeric data by comparison with a reference serum standardized against ImmunoCAP IgE. As a consequence, the results, expressed as standardized ISAC units (ISU/L), are indirectly linked to the World Health Organization IRP 75/502 IgE standard.

Levels > 0.3 ISU/L were considered positive, according to the manufacturer's recommendations.

A physician, blinded to the allergist's decision making, prescribed epinephrine on the basis of the most recently published list of foods which trigger anaphylaxis in Italy (12) and of the available information on the risk of severe reactions associated with the allergenic molecules in the ISAC panel (7,9,13).

The prescription of epinephrine, for example, was allowed if a peach allergy was associated with the detection of Pru p 3 sIgE levels, or if a peanut allergy was associated with detection of Ara h1, Ara h2 sIgE levels, and so on (7,9,13).

To enhance the sensitivity of the virtual risk assessment, sIgE levels greater than a threshold level of 0.3 ISU/L were considered as significant.

On completion of the study, an independent referee compared the allergists' prescription of epinephrine against that suggested by the allergen microarray-immunoassay.

All data were collected between January 1st 2010 and June 30th 2010.

The study was approved by the local Ethics Committee of Azienda Ospedaliero-Universitaria "Ospedali Riuniti" of Ancona. Our patients underwent a routine medical examination and provided an oral informed consent before commencing the study and giving a blood sample.

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Science (SPSS) version 13.0 for Windows. Descriptive statistics were presented as numbers and percentages for qualitative variables. The agreement coefficient (k index) was used to

analyze the results. The degree of concordance between the two methods of epinephrine prescription was assessed using Landis and Koch's interpretive scale for k values: 0.81-1.00, almost perfect; 0.61-0.80, substantial; 0.41-0.60, moderate; 0.21-0.40, fair; 0.00-0.20, slight; and < 0.001, poor. Statistical significance was set at < 0.05.

Results

One hundred and four patients were enrolled, 18 of whom were excluded as they failed to meet the selection criteria. **Table 1** presents the main demographic and clinical features of the patients. In the diagnostic workup of the allergist, besides skin prick testing, almost two-thirds of the patients were tested for sIgE. In more than two-thirds of the patients, the risk assessment of the allergist and that of the microarray-based immunoassay agreed: in 23 cases neither suggested prescribing epinephrine, while in 36 both suggested its prescription (**table 2**). The k index was = 0.372 (95% CI: 0.185-0.559) p < 0.001. Three causes of discrepancy emerged (**table 2**). Poor sensitivity of the microarray-based immunoassay accounted for more than fifty percent of the divergent risk assessments: in 14 patients, allergists documented the role of food allergens and sometimes even of allergenic molecules in severe reactions, whereas ISAC yielded a negative score for the equivalent allergenic molecules in its platform (**table 3**). The discordance between the virtual risk assessment of microarray-based immunoassay and that of the physicians for the same allergenic molecule was the second cause of discrepancy. Consistent with the detection of Pru p 3 sIgE levels, the virtual risk assessment of ISAC suggested the need to prescribe epinephrine in six patients, whereas, consistent with their case histories (food-induced oral allergy syndrome or mild symptoms), the allergists did not take this decision. In the remaining three patients, the combination of their case histories (food-induced oral allergy and dyspnoea in one patient and food-induced oral allergy syndrome plus systemic urticaria in the other two) with skin test result in one patient, and skin test results plus sIgE for the allergen extracts in the other two, suggested epinephrine prescription to the doctor. The virtual ISAC risk assessment denied this decision, as sIgE against profilin, sIgE against Bet v1-like homologous allergens and sIgE against profilin plus sIgE against Bet v1-like homologous allergens were found in the first, second and third patient respectively.

The last cause of discordance arose from the non-inclusion of certain food allergens in the platform in the microarray-based immunoassay: while the ISAC test was negative, the diagnostic workup of the allergists revealed the role of bell pepper, pistachio, sesame and peanut (Ara h 9) respectively in four patients with severe reactions.

Table 1 *- Demographic and clinical profile of the patients (n = 86)*

Variable	
Gender	
Male	40 (46.5%)
Female	46 (53.5%)
Age (years)	
Mean	28
Range	15-67
Median	28
Symptoms	
Urticaria/angioedema	56 (64.4%)
Oral Allergic Symptom (OAS)	25 (28.7%)
Dyspnoea	17 (19.5%)
Gastrointestinal symptoms	9 (10.3%)
Anaphylactic shock	14 (16.1%)
Glottis oedema	2 (2.3%)
Respiratory allergy	
Yes	73 (84.8%)
No	13 (15.2%)
Diagnostic tests	
Commercial extracts skin prick test	86 (100%)
Prick by prick test	43 (49.4%)
Dosage specific IgE	59 (67.8%)
All diagnostic tests in a single patient	30 (34.5%)
Number of positive skin test for food	
<3	22 (19%)
≥3	64 (81%)

Table 2 *- Congruence between the physician work up and the virtual risk assessment of the microarray-base immunoassay and causes of disagreement*

Agreement in prescription of epinephrine	
Yes	59 (68.6%)
No	27 (31.4%)
Causes of disagreement	
Insufficient sensitivity of the microarray-based immunoassay	14 (51.9%)
Different risk assessment of the doctor and microarray-based immunoassay	9 (33.3%)
Allergen missing in the platform of the microarray-based immunoassay	4 (14.8%)

Table 3 *- Foods associated with severe allergic reactions detected by the diagnostic work up of the allergist but not by ISAC. The diagnostic tests used by allergist were indicated*

	Allergen	Skin Prick Test	sIgE for allergenic extract	sIgE for allergenic molecules
1	Egg, Cow milk	x	x	
2	Nuts, Peach	x	x	
3	Shrimp, Egg white, Cow milk	x	x	
4	Anisakis simplex	x	x	
5	Cod	x	x	
6	Peach, Apple, Celery, (Pru p 3)	x	x	x
7	Cow milk, Anisakis simplex	x	x	
8	Peach, Nuts	x		
9	Peach, Nuts, (Pru p 3)	x	x	x
10	Peach, apple, (Pru p 3)	x	x	x
11	Peach, nuts, (Pru p 3)	x	x	x
12	Nuts, fish	x	x	
13	Cow milk	x		
14	Peach	x	x	

Discussion

In a selected patient population with a case history suggestive of food allergy to several foods or of concomitant respiratory and food allergies, a weak but significant degree of agreement, 0.372 (95% CI: 0.185-0.559) $p < 0.001$, was found between the risk assessment of the allergists and that virtually established by the microarray-based immunoassay.

Whereas a weak result in a randomized clinical trial is often predictive of the failure of its translation to a real world setting, a weak result in the real world could nevertheless be promising, provided that the teething problems of a technology still in its infancy are addressed and overcome.

We found that the problems associated with virtual risk assessment of food allergy by ISAC were technological and conceptual. Technologically, the non-inclusion of certain allergenic molecules in the ISAC platform apart, we found that sometimes, for the same allergen, sIgE detection by ISAC was less sensitive than the combining of the skin and serological test results routinely used by the allergist; whether this results from the poor diagnostic ability of the allergenic molecules in the ISAC platform, from the high operator-dependent management of the instrument is an open question.

These difficulties will likely be overcome in time through technological improvements and adjustments, while the conceptual limitation of the risk assessment for a single allergenic molecule seems less easily surmountable.

Fundamentally, the allergenic molecule-oriented risk assessment arises from the fact that different degrees of risk for a severe reaction are associated with the detection of sIgE against different allergenic molecules coexisting in the same food (13).

Consistent with this scenario, in our model of virtual risk assessment the detection of Pru p 3 sIgE, which is associated with the most severe allergic reactions to peach, required the prescription of epinephrine: unfortunately decision making of this type reduced the specificity of the epinephrine prescription as, even though peach LTP-hypersensitivity is associated with the highest risk for anaphylaxis in Italy (22% of food-induced anaphylaxis), only 7% of peach LTP-hypersensitive patients manifest this syndrome (12).

Moreover, in the case of Pru p3, the serum level of sIgE seems unable to predict either the presence of the clinical allergy (14) or the severity of the reaction (15). Only association with other allergenic molecules seems indicative of a decreased risk for severe reactions (16).

Even though the risk assessment for other allergenic molecules appears to be more reliable (7,9), similar results were found for other allergenic molecules (Bet v1-homologous food allergy) of vegetal origin (17).

As a result, ISAC is currently able to provide an allergen-oriented risk assessment between different molecules in the same food, but the risk assessment of the single allergenic molecule needs to be supported by clinical information to improve reliability. We speculate that a software combining clinical information, obtained by standardized questionnaire, with ISAC results could partially overcome this problem.

Our study has several limitations, which include the small size of the patient sample and the over-simplified model of food allergy management.

Overall, the assumption that the allergists' prescription of epinephrine was the gold standard is questionable, as both the discussion on the appropriate use of adrenaline remains open (18,19), and the allergists' conclusions in regard to a severe food reaction might be erroneous, as they were not validated by a controlled food challenge.

Indeed, in the three patients whose results were discordant with those of ISAC, the prescription of epinephrine by allergists seems to derive from an incorrect diagnosis or from the allergists' defensive medicine.

In conclusion, our results suggest that the allergen-oriented risk assessment in food allergic patients by ISAC is still pre-

mature for current clinical practice. However, the combining of a robust improvement of its diagnostic accuracy and a close linking of its results with clinical information could lead to changes in clinical practice in food allergy, in the not too distant future.

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Hair dyes and temporary tattoos are a real hazard for adolescents?

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Key words

Paraphenylenediamine; contact sensitization; black henna tattoo; hair dye

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Summary

Temporary tattoos, especially those that contain black dyes, have become rampant among teenagers in recent years. Most of these tattoos, in addition to hair dyes include paraphenylenediamine (PPD). PPD is a well-known skin sensitizer, which causes allergic contact dermatitis. Allergic contact dermatitis skin lesions from PPD are mostly seen as erythema multiforme-like eruption, a bullous contact dermatitis or as an exudative erythema. Herein, we report on our finding on a 15 year-old adolescent female who had been unaware of being previously sensitized to PPD from a black henna tattoo, and angioedema-like reaction which occurred after her first exposure to hair dye.

Introduction

In hairdressing, the main contact allergen is PPD, followed by its derivatives paratoluenediamine, para-aminodiphenylamine and o-nitrop-phenylenediamine (1). Although pure henna is known to be harmless, the addition of PPD, which gives a darker brown to black color and is sometimes used to speed up the process of hair dyeing with henna, yields what is called the black henna mixture (2-4). Herein, we will discuss our finding, regarding a 15 year-old woman with an angioedema-like reaction that occurred after her first exposure to hair dye. She came to us, unaware of having been previously sensitized to PPD by a black henna tattoo she had received a year before on her left forearm, which, at the time, had caused an allergic reaction.

Case report

A 15 year-old adolescent female presented herself at Erciyes University Pediatric Emergency Services with severe edema involving the upper and lower eyelids, the forehead, scalp and face (**figure 1**). She consulted with our allergy department and was initially diagnosed with angioedema. After using hair dye for the first time in her life, one hour later pruritus started on her scalp, forehead and face. Two days after the exposure occurred, she also experienced severe edema on the scalp, forehead, upper and lower eyelids and face. We started methylprednisolone, H1 (cetirizine, hydroxyzine), H2 (ranitidine) for 5 days. After the 7 days of medication**,** clinical improvement was observed (**figure 2**). She described an allergic reaction in the application area (pruritic, erythematous, edematous reaction) of a black henna tattoo she had on her left forearm one year earlier. We performed a patch test including the European standard series

(True test®) of allergens on the patient 2 weeks after the end of the medication. At the end of the 48 hours a positive result to PPD with bullous reaction on erythematous test area was observed (**figure 3**). She and her family were told that she had an allergy to PPD and cross-reacting substances. We cautioned her about dyeing her hair with any products containing PPD or other para-dye ingredients, and to avoid getting black henna tattoos. A list of products that may contain PPD or cross-react with it was also given.

Figure 1 *- Severe edema of eyelids and frontal area extending to the scalp*

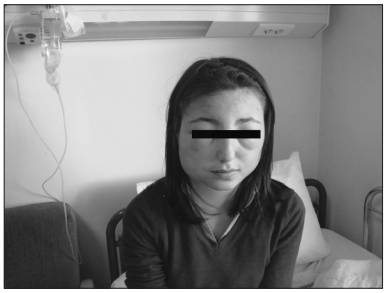


Figure 2 *- Clinical improvement after management*

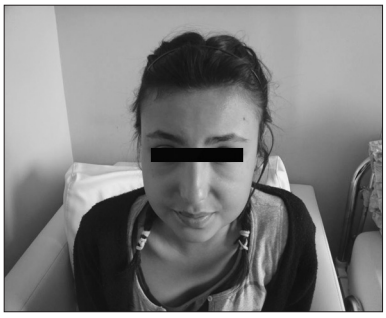
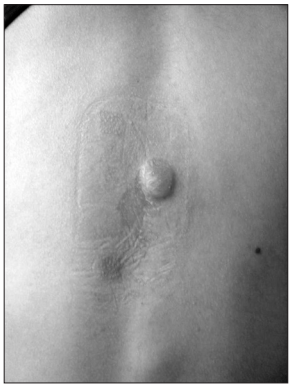


Figure 3 *- Positive patch test result to Paraphenylenediamine*



Discussion

Allergic contact reactions according to hair dyes occur mostly due to the sensitization to PPD. Sensitization to PPD derivatives could cause cross-reactions. As an ingredient, PPD has several applications, including the coloring used in fabric dyes, rubber, lacquers, leather, eye shadow, and shoe polish. It has also been used as an antioxidant in fax machines, photographic products, plastics, printing ink, and liquid for x-ray film, as well as in lithography (1,2). Prevalence of PPD sensitization based on population patch test studies in Europe has been found to be between 0.1% and 1% (5). In temporary henna tattoos, the PPD concentration has been shown to be as high as 15.7%, which is much stronger than the concentrations used in hair dyes (4). In most of the samples, the PPD concentration was higher than is recommended for hair dyes. Acute effects, caused by short-term exposure to high levels of PPD, may include eye irritation and tearing, severe dermatitis, renal failure, asthma, gastritis, vertigo, convulsions, tremors and coma in humans (6). Kligman reported that a single application of 10% of 1.0 mL solution would sensitize about 80% of the population (7). Combined with the extended period of skin exposure without neutralization, higher PPD concentration causes potent skin sensitization to PPD (8). Oxidative hair dyes (permanent hair dyes) contain primary intermediates (such as PPD) and couplers. When mixing intermediates and couplers, the primary intermediates initially react with hydrogen peroxide (neutralizing agent) to form a diimine, and the diimine then reacts with couplers to form dinuclear, trinuclear or polynuclear structures. Unreacted primary intermediates and couplers (small molecules) diffuse into hair, start coupling reactions and then become trapped in hair and increase the risk of skin sensitization (9). Even in low concentrations such as those in hair dyes, subsequent exposure to PPD can then result in a delayed type-IV hypersensitivity reaction, manifesting as an acute contact dermatitis (4). These contact dermatitis symptoms usually begin after the initial application (10). Contact dermatitis associated with PPD in hair dye often extends beyond the scalp to include the forehead, neck, eyelids and face. It usually manifests as pruritic, edematous, erythematous scaly patches and plaques; vesicular lesions sometimes occur as well (4). Differential diagnosis for the hair dye allergic reactions includes contact urticaria syndrome and angioedema that appears immediately (mostly within 5-20 min, exceptionally later) upon contact with the causal agent (11). We excluded these diseases because the reaction occurred 2 days after the hair dye exposure in our patient. In the literature, it is reported that 'contact dermatitis with severe scalp swelling and upper airway (is compromised) due to black henna hair dye' (12). In the literature the reports of hair loss in the scalp due to hair dyes containing PPD is rare, citing only two reported cases (13). In our patient, only a severe edema from scalp to forehead, upper and

lower eyelids, and face was prominent initially and a misdiagnosis of angioedema was made in the pediatric emergency room. Positive allergic reaction to PPD was confirmed with the patch test bullous reaction on the erythematous test area. Our patient was a 15 year-old, had applied hair dye for the first time in her life and the sensitization phase of allergic reaction had been initiated by a black henna tattoo which had been performed one year before. An angioedema-like reaction occurred two days after hair dyeing, supporting a delayed type of hypersensitization. Therefore, patch tests results confirmed this relationship between hair dye and black henna tattoos.

We concluded that the patients with severe edematous reactions after the first application of a hair dye might have previously been sensitized from other PPD-containing materials. Black henna tattoos contain PPD. It may sensitize users. Allergic contact dermatitis ought to be considered in patients presenting with angioedema. Patch testing should be done on patients who react to hair dyes (PPD and its derivatives) to elucidate the cause and to prevent further severe reactions.

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The needle in the haystack: allergic anaphylaxis caused by the local anesthetic articaine

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Local anesthetics (LA) are extensively used drugs with an excellent benefit-risk profile. The vast majority of immediate-type adverse reactions can be attributed to non-immune mediated pharmacological effects of LA or psycho-vegetative reactions. Clinical symptoms of these reactions often closely resemble anaphylaxis, including hypotension, tachycardia and subjective feelings such as general weakness, heat or vertigo. However, true IgE-mediated allergic anaphylaxis due to LA is so exceedingly rare, that the question arises whether it does occur at all. Consequently, neither commercial skin test reagents nor validated IgE-measurements are available (1).

A 28-year-old man requiring dental procedures was referred to our allergy clinic with suspected LA-associated anaphylaxis for further evaluation. In March 2011 the patient underwent a dental anesthesia by local injection of the LA Septanest™, containing articaine, epinephrine, as well as the preservatives sodium meta-bisulfite and methyl-4-hydroxybenzoate. Only a few minutes after injection and before starting dental treatment, he suffered a feeling of heat and subsequently developed generalized wheals. The patient experienced dizziness due to measurable cardiovascular depression and nausea. After emergency treatment including fluid replacement, corticosteroids (betamethasone) and antihistamines (clemastine) the symptoms quickly resolved. In the context of this anaphylaxis episode no other potential elicitors such as drugs, foods or concomitant infectious diseases could be evaluated.

Allergologic workup was performed 3 months later. Initially, IgE-mediated natural latex allergy was excluded by an inconspicuous history, negative latex-specific IgE and negative skin testing. Thereafter, skin prick testing with a series of different undiluted LA including articaine, articaine combined with epinephrine, mepivacaine, procaine, prilocaine and prilocaine combined with epinephrine, revealed unequivocally positive immediate wheal-and-flare responses to both articaine preparations. Intradermal testing with serial dilutions of articaine showed positive immediate reactions even down to the highest dilution. A wheal diameter ≥ 5mm was considered as a positive reaction, according to international guidelines. Positive responses, even in case of diminished erythema by the vasoconstrictor epinephrine, could be clearly distinguished from the wheal caused by the intradermal injection itself (**table 1**). Therefore, an IgE-mediated hypersensitivity to articaine was strongly suggested. A basophil activation test (BAT) with articaine failed to reveal any positive results. Following negative skin testing

Table 1 *- Results of intradermal testing (–, negative test result)*

Active substances	Dose [mg/mL]	Wheal diameter
Articaine	1	14 mm
	0.1	11 mm
	0.01	11 mm
	0.001	9 mm
Articaine (multi-dose preparation)	4	14 mm
(Epinephrine	0.0006)	
(Sodium meta-bisulfite	0.0025)	
(Methyl-4-hydroxybenzoate	0.005)	
Articaine (multi-dose preparation)	0.4	9 mm
	0.04	9 mm
	0.004	8 mm
Mepivacaine	1	7 mm
	0.1	–
	0.01	–
	0.001	–
Procaine	1	–
Prilocaine	2	–
Prilocaine (multi-dose preparation)	1	–
(Epinephrine	0.00091)	
(Sodium meta-bisulfite	0.001)	
(Methyl-4-hydroxybenzoate	0.002)	

results, controlled challenge testing was done with the alternative LA procaine, prilocaine combined with epinephrine, and mepivacaine. These LA were injected subcutaneously into the extensor side of the upper arms in incremental doses, starting with 0.1 mL of the undiluted LA followed by 0.2, 0.5, 1.0 and 2.0 mL. These LA were all well tolerated without any side effects up to the cumulative dosage of 3.8 mL.

Throughout the world, about 6 million patients every day receive LA injections. Adverse reactions, occurring in 0.1 to 1% of applications, are rare and may be attributed to different pathomechanisms (2,3). A delayed and localized oedematous swelling could represent a type IV allergy or an episode of hereditary angioedema, triggered by intraoral manipulations. Toxic effects of LA on the central nervous or the cardiovascular system can occur after a high dosage, large-area mucosal application or after accidental intra- or paravasal injection (2,3). Pharmacological side effects associated with epinephrine, a vasovagal reflex or a psychosomatic panic reaction should be also taken into account (4). The symptoms of these "pseudo-allergic" reactions may closely imitate IgE-mediated anaphylaxis (3). Moreover, preservatives in LA preparations have to be considered as causative agents for anaphylaxis.

Even if immediate-type allergic reactions to LA are extremely rare, the potential of IgE-mediated allergy against this class of drugs still exists, as shown by case reports. Venemalm et al. were able to demonstrate mepivacaine-specific IgE-antibodies (5). Calderon et al. described anaphylaxis after regional anesthesia with levobupivacaine and ropivacaine (6). Immediate-type LA allergy was diagnosed based on the timing of serum histamine and tryptase levels as well as positive skin prick test results (6).

In our patient several facts and results strongly suggested an IgE-mediated allergy against articaine. First, clinical symptoms of anaphylaxis, such as generalized wheals, cardiovascular and gastrointestinal symptoms appearing within a few minutes after injection were convincing. Second, we ascertained positive intradermal test results with articaine in dilutions from 1:10 down to 1:10.000 in view of a huge number of negative test results in 220 control patients tested within the last 6 years in our allergy clinic. The 1:10 dilution of articaine produced false positive immediate reactions in a rather small number of patients (in 10 out of the 220 mentioned). But in these 10 patients, further dilutions proved to be clearly negative. Third, allergy was substance-specific as demonstrated by the tolerated LA procaine, prilocaine and mepivacaine. The structural differ-

ences between articaine, which is a thiophene derivative (with presence of a thiophene ring and an additional ester group), and the amino-acylamides prilocaine and mepivacaine, containing a methylated phenyl ring, reasonably explain this apparent lack of cross-reactivity (7,8). Fourth, by skin and challenge testing a series of LA containing the preservatives sodium meta-bisulfite and methyl-4-hydroxybenzoate, a causative role of these agents could be excluded. BAT was proposed as a complementary method for *in-vitro* diagnosis of drug allergy. But until now, the sensitivity of BAT for confirming drug hypersensitivity is generally low.

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Extraordinary response to omalizumab in a child with severe chronic urticaria

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Key words
Chronic urticaria; omalizumab; ciclosporin; therapy

Summary
A case of immediate and definitive response to a single dose of omalizumab in a child with severe ciclosporin-resistant chronic urticaria is reported.

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An 11-year-old boy was recently seen at the allergy department of the Clinica San Carlo. He suffered from very severe urticaria with angioedema for 3 months (Urticaria Activity Score = 5) (1), and had a history of seasonal allergic rhino-conjunctivitis for several years, induced by both grass and pellitory pollen. The boy suffered from sensineural deafness. At the first visit, the patient was taking Hydroxyzine 25 mg/day + Levocetirizine 5 mg/day + Montelukast 10 mg/day + Prednisone 25 mg/day with very little benefit. The chronic use of prednisone had caused an increase in body weight of 9 Kg during the last 3 months. Exams showed basopenia, an increase in RCP and normal total IgE, normal complement fractions and negative antinuclear antibodies and thyroid autoantibodies. In view of the sensineural deafness and to exclude a Muckle-Wells disease, gene-sequencing analysis was carried out. Analysis of GJB2 and GJB3 genes showed a compound heterozygosity for V153I polymorphic mutation in GJB2 and a -3224G>A mutation in its promoter UTR region. The two mutations were each present in heterozygote state in patient's parents, and it is improbable that, associated in trans, these might cause the bilateral sensineural deafness present in patient. Sequencing of exon3 of gene CIASI/NLRP3 was also performed to exclude cold urticaria or Muckle-Well syndromes due to alterations in this gene and no pathogenic mutations were found.

In view of the very poor response to current treatment and of the severe side effects of oral corticosteroids, after an informed written consent was obtained by the parents, ciclosporin 3 mg/Kg/day was started, maintaining a single dose of levocetirizine 5 mg in the evening. Ciclosporin dosage was increased to 3.8 mg/kg/day after 2 weeks due to the insufficient response, and this dose (250 mg/day) led to an improvement of the clinical situation by 80-90%. A further increase in dosage of ciclosporin to 4.6 mg/kg/day (300 mg/day) led to the complete disappearance of both wheals and angioedema. After one month, some viral respirato-

ry infections were followed by a relapse of urticaria (UAS = 4), which did not respond to levocetirizine at higher than licensed dose. At this point, a marked increase in D-dimer plasma levels (2450 ng/ml; normal 500 ng/ml) and a moderate kidney failure were observed: BUN 70 mg/dl (normal range 19-50 mg/dl).

After an informed written consent was obtained by the parents, omalizumab (Xolair) 300 mg was administered subcutaneously. The dosing was based on the present recommendations for asthma. The day after the administration urticaria completely disappeared, and the boy is still completely urticaria free after 6 months.

The effectiveness of omalizumab (Humanized monoclonal anti IgE antibodies) in different subsets of CU/angioedema unresponsive to antihistamines is supported by several case-reports (2-11), and lately by some multi-center randomized placebo-controlled studies as well (12-14). Two points make our case novel. First, the fact that the patient was a 11-year-old boy, and second that the drug worked very quickly and apparently in a resolutive way in a patient that had not been satisfactorily controlled neither by systemic corticosteroids (plus leukotriene receptor antagonists and both first and second generation antihistamines) nor by ciclosporin. The mechanism by which omalizumab works in patients with chronic idiopathic urticaria is all but established. Previous studies showed that the binding of circulating IgE by the drug eventually leads to a down regulation of the high affinity IgE receptors, within 2 weeks on basophils and after 8 weeks on mast cells (15-17). Other authors showed that omalizumab induces eosinophil apoptosis, a down-regulation of inflammatory cytokines (18) and a reduction in B-cell activation and homing. (19). However these observations cannot explain a clinical response that occurs within few days in most cases.

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Exclusively breastfed infants at risk for false negative double blind placebo controlled milk challenge

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Key words
Cow's milk allergy; infants; false negative; double-blind placebo controlled food challenge; open food challenge

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Summary
The double blind placebo controlled food challenge (DBPCFC) is the gold standard for diagnosing cow's milk allergy (CMA). However, false-negative DBPCFC have been reported. We present 2 cases with a false negative DBPCFC in exclusively breastfed infants suspected of CMA. These cases highlight the occurrence of severe allergic reactions of infants who were exclusively breastfed. Several reported causes of a false negative DBPCFC will be discussed. However, there is currently no clear understanding of the cause of a false negative DBPCFC. This paper highlights that a negative outcome of a DBFCFC must be interpreted with caution, because a severe allergic reaction might occur upon re-introduction of cow's milk. Therefore, an additional open food challenge under medical supervision is recommended in exclusively breastfed infants with a negative DBPCFC.

Abbreviations:
AAF: Amino acid formula
CMA: Cow's milk allergy
CMP: Cow's milk protein
DBPCFC: Double-blind placebo controlled food challenge
OFC: Open food challenge
pHF: Partially hydrolysed formula
w-eHF: Whey-based extensively hydrolysed formula
SPT: Skin prick test

Background

Cow's milk allergy (CMA) is a common food allergy in infants (1,2). The double-blind placebo controlled food challenge (DBPCFC) is the gold standard for diagnosing CMA (3). However, false-negative DBPCFC have been reported with a prevalence varying from 3-13% (4-6). We encountered two cases of exclusively breastfed infants who underwent a DBPCFC with a negative outcome, followed by an allergic reaction upon introduction of cow's milk protein (CMP).

Case A

A full term boy with a normal birth weight and APGAR scores had postnatal complications of persisting pulmonary hypertension of the neonate and perinatal sepsis, for which he was managed with surfactant, breathing support and antibiotics. He was exclusively breastfed from birth on an unrestricted maternal diet until 8 weeks of age, with the exception of one bottle of partially hydrolysed cow's milk based infant formula (pHF) in the first week of life. At 6 weeks he developed severe irritability, persistent crying and eczema. CMA was suspected. Since he was taking part in the EuroPrevall Birth Cohort Study, investigations were carried out according to the protocol (7). He

***Table 1** - Skin prick test results before and after DBPCFC for Case A*

	Wheal size before DBPCFC (mm)	Allergen-histamine ratio before DBPCFC	Wheal size after DBPCFC (mm)	Allergen-histamine ratio after DBPCFC
Histamine	3		5.5	
CMP	0	0	3.5	0.64
Fresh w-eHF	N.D.		0	0
Fresh standard infant formula	N.D.		5.5	1
Fresh semi-skimmed milk	N.D.		6.5	1.2

DBPCFC = double blind placebo controlled food challenge; mm = millimeter; CMP = cow's milk protein; w-eHF = whey-based extensively hydrolysed formula; N.D. = not done

was successfully managed on a maternal CMP elimination diet followed by an amino-acid based formula (AAF), according to protocol (7).

Both skin prick test (SPT) for CMP (ALK-Abelló, Hørsholm, Denmark) and CMP specific IgE measurement (Phadia Diagnostics, Uppsala, Sweden) were negative. At 3 months of age a DBPCFC was performed (7). He developed eczema on the chest at dose 7 and redness/flushed skin around the nose at dose 8 at the placebo-day, and had no symptoms on the active day. The DBPCFC was determined negative. As he had a positive atopic family history, a pHF was introduced (8,9). He immediately developed urticaria, angioedema and wheezing. He was diagnosed with anaphylaxis and managed accordingly. Three weeks later, the SPT was repeated for CMP, fresh whey-based extensively hydrolyzed formula (w-eHF), fresh standard infant formula and fresh semi-skimmed milk, and showed allergen-histamine ratios of 0.64, 0.1 and 1.2 respectively (**table 1**). Specific IgE for cow's milk was 8.17 kU/L. As the parents refused a second DBPCFC, an open food challenge (OFC) was carried out to confirm CMA. After a dose of 3 mg CMP (equivalent of 90.4 µl cow's milk) (7), he developed urticaria around the mouth and swelling of the right side of the lip. A CMP elimination diet was continued including an AAF to maintain nutritional adequacy (9). Also an adrenaline auto injector was prescribed. He was re-challenged annually and after 3 years he became tolerant to cow's milk.

Case B

A full term girl with a normal birth weight and APGAR scores presented with eczema at 3 months. She was exclusively breastfed, had abdominal cramps since birth and she was vomiting on consumption of breast milk. Family history was positive for atopic diseases. On physical examination she had a dry skin and moderate eczema; Scoring Atopic Dermatitis score was 36 out of 103 (objective score 34 out of 83) (10). CMA was suspected. As she was taking part in the EuroPrevall Birth Cohort Study, investigations were carried out according to protocol (7). SPT carried out for CMP (ALK-Abelló, Hørsholm, Denmark) and fresh standard infant formula, w-eHF and semi-skimmed milk were negative. IgE for cow's milk was negative (< 0.35kU/L). Maternal CMP elimination diet was successful. At 4 months AAF was initiated, because of significant reduction in breast milk supply. A DBPCFC was postponed until baby B was willing to drink adequate amounts of AAF, required for testing. The DBPCFC at 6 months of age was negative. Additionally, an OFC was performed with a pHF. Within 1.5 hours after receiving the top dose (251 ml = 4.0 gram CMP) she had erythema and oedema in the face as well as on the arms and legs, while no skin lesions were reported at the start of the OFC. Within 24 hours she also developed diarrhoea. CMA was confirmed, the elimination diet was continued and w-eHF was introduced. Within 2 weeks after introduction of w-eHF she again developed diarrhoea 4-6 times a day, which persisted for 3 months. Stool cultures for Salmonella, Shigella, Yersinia, Campylobacter, and triple faeces test remained negative. She was switched from a w-eHF to a casein-based eHF, however there was no improvement in diarrhoea. At reintroduction of AAF the diarrhoea disappeared. At scheduled follow-up at 18 months, all allergy tests that where carried out, including DBPCFC, were negative and CMP was successfully introduced.

Discussion

Both exclusively breastfed infants had initially a false negative outcome of a DBPCFC and a severe reaction during reintroduction of cow's milk. Our cases are not the first to describe false negative outcomes and several explanations have been discussed (4,5,11). Firstly, the possibility of a masked reaction due to medication was ruled out since both infants were not given any medication, including so called "over the counter medication" (11). Secondly, the dose of challenge food could have been too

small to elicit symptoms (12). In both cases mentioned above the infants reacted to a lower dose compared to the dose used in the DBPCFC. Formula samples were analysed in a laboratory and exchange of active and placebo foods was ruled out. Thirdly, Niggemann and Beyer described a so called short-term specific oral tolerance, which means that the infant develops tolerance for the allergenic food during the increasing doses of the DBPCFC, but loses this tolerance quickly after the DBPCFC (11). Another possibility may be that during the challenge the infants become sensitised, while the actual clinical reaction occurs upon re-introduction of CMP.
We would like to add that exclusive breastfeeding might be a risk factor of having a false negative DBPCFC, especially since all mentioned studies were performed in children of several ages, while we only describe young infants who were exclusively breastfed (4,5,11,12).

Implications

Despite the fact that DBPCFC is being considered as the gold standard for diagnosing CMA, a false negative outcome remains possible. Exclusively breastfed infants are at risk of experiencing a false negative DBPCFC outcome compared to formula-fed infants. This could result in severe allergic reactions occurring when CMP is re-introduced. Therefore, it is recommended that in exclusively breastfed infants an additional OFC with the formula of choice (standard formula or pHF) is performed under medical supervision, rather than introduction of the formula at home (5).
After the occurrence of these severe reactions on re-introduction of CMP we have adapted our protocol accordingly.

Conclusion

We described two cases of exclusively breastfed infants with a severe allergic reaction after a negative DBPCFC. Despite the fact that we are not able to provide a clear explanation for the false negative DBPCFC, an additional OFC with the formula of choice, performed under medical supervision, is necessary in exclusively breastfed infants to avoid severe allergic reactions.

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Competing interests

The EuroPrevall study is funded by the European Commission (FOOD-CT_2005-514000). The Dutch cohort received additional funding from Nutricia, the Netherlands and unrestricted grants from Nutricia Advanced Medical Nutrition, the Netherlands, AstraZeneca, the Netherlands, TEVA, the Netherlands and GlaxoSmithKline, the Netherlands. These companies were not involved in the design of the study or collecting the data.

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A case of anaphylaxis to Pollinex® Quattro MPL-4

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Key words

Anaphylaxis; subcutaneous allergen immunotherapy (SCIT); allergoid; monophosphoryl lipid A

Summary

We described the first case reported in literature of anaphylactic shock after administration of pollen extract vaccine chemically modified (allergoid) adsorbed onto L-Tyrosine depot adjuvanted with monophosphoryl lipid A (Pollinex® Quattro MPL-4).

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Introduction

Subcutaneous allergen immunotherapy (SCIT) is an approved efficacious treatment for respiratory allergy, confirmed by WHO Position Paper (1).

The incidence of fatal reactions, as reported in an American study, in twelve years of surveillance (1990-2001) is 3.4 per year (41 fatal immunotherapy reactions in 12 years). In particular, the fatality rate is estimated at 1 per 2.5 million injections approximately, according to the incidence rates reported by Lockey et al. and Reid et al. (2). An Italian prospective study conducted on 1738 subjects for a total of 60785 injections over a mean immunotherapy duration of 3 years (3) suggests that SCIT is safe, since systemic reactions occurred only in 3.6% of patients and 0.15% of injections, with only one grade 4 reaction per > 2000 courses.

Sublingual immunotherapy (SLIT) is generally considered to have a better safety profile: most reactions are local and transient and usually do not lead to interruption or cessation of treatment. Calderon et al. estimated that the incidence of SLIT-induced anaphylaxis is 1 case per 100 million SLIT administrations or per 526000 treatment years, no reaction observed was fatal (4).

Modified vaccines with pollen allergoid (chemically modified allergen with a lowered recognition by IgE allergen-specific antibodies) have been developed in order to make immunotherapy more effective, reduce side effects and improve compliance.

Allergy Pollinex® Quattro MPL-4 is a pollen extract vaccine (chemically modified by glutaraldehyde) adsorbed onto L-tyrosine with addition of the immunostimulatory adjuvant monophosphoryl lipid A (MPL). 4

MPL is a detoxified, attenuated form of the lipid A component of the lipopolysaccharide of *Salmonella Minnesota,* a Toll-like receptor 4 agonist.

The inclusion of MPL reduces the number of required injections (only 4) for effective SCIT and improves compliance (5).

In vitro studies on peripheral blood mononuclear cells from patients with seasonal allergic rhinitis to grass pollen showed that MPL added to grass pollen extract resulted in the suppression of allergen-induced peripheral Th2 cell responses, in favour a protective Th1 response (6).
Several studies demonstrated its clinical efficacy, safety, tolerability (7,8,9,10), improvement of patient's symptoms and combined medication/symptom scores (8,5).
In addition, this treatment is usually well tolerated. Local reactions, such as redness and swelling in injection site, are the most common reported symptoms. Systemic reactions are rare and mild (5).
For example, Crivellaro et all (11) reported in their study that only 1.37% of patients (510 in total) experienced an adverse systemic reaction (SR): all SRs were delayed (> 30 minutes), at grade 1 or 2; epinephrine was not required for any of the reactions, all resolved spontaneously or after administration of oral antihistamine.
No report of anaphylaxis is described in literature with this vaccine.

Case Description

We report the first case of anaphylaxis after administration of Pollinex® Quattro MPL.
A 50-year-old Caucasian male with a history of asthma and rhinitis since 1992, sensitized to grass, ragweed and birch, usually controlled his symptoms with frequent administration of inhaled salbutamol or disodium cromoglycate and oral antihistamine.
In February 2012 he started SCIT with Pollinex® Quattro MPL for grass pollen (concentrations at the end of the therapeutic cycle are of 21.5 mcg/ml and 13.64 mcg/ml for the major allergens, Phl p 1 and Phl p 5).
His clinical history presented arterial hypertension in therapy with beta-blocker, that was pre-emptively substituted with an angiotensin-II receptor blocker (olmesartan) before the start of the immunotherapy.
The patient, in good health, tolerated the first three injections (300, 800 and 2000 standardized unit SU). However, 10 minutes after the fourth administration (2000 SU), that had been properly executed, he presented palpebral swelling, conjunctivitis, rhinitis, asthma with serrated bronchospasm, hypotension (PA 70/60). The patient was initially treated with oral antihistamine and methylprednisolone IV but since the symptoms were fast-growing, he was administered twice with epinephrine 0.3 cc IM and beta agonist inhaler, and treatment with hydrocortisone was repeated. No biphasic anaphylaxis was observed.

Discussion

This case documents that although an allergoid, Pollinex® Quattro MPL is not risk free from systemic reaction. It is very important to underline that anaphylaxis must be treated immediately with an intramuscular injection of epinephrine, as death can occur within minutes. All anaphylaxis guidelines recommend prompt intramuscular injection of epinephrine, although they differ with regard to the importance of H1-antihistamines, H2-antihistamines, corticosteroids, and bronchodilators other than epinephrine (12).
Information should be collected on the role of potential amplifying factors and co-factors, such as concurrent use of beta-blockers or other medication (ACE inhibitors, FANS), viral infection, fever, emotional stress, disruption of routine, premenstrual status in females, and exercise (12).
Although ARBs are associated with a lower anaphylactic risk than angiotensin-converting enzyme (ACE) inhibitors (13), in our report we cannot exclude that this drug played a facilitating role.

Disclosure

The manuscript has not been published elsewhere and it's not under consideration elsewhere. The authors disclose any financial or personal relationship which could result in a conflict of interest.

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Skin prick test: the only predictive tool of anaphylaxis? A case report

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Key words

Skin prick test; specific immunoglobulin E antibodies; anaphylaxis; cow's milk; atopic dermatitis

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Summary

Currently, in the literature there is a lack of definite predictive values parameters to identify patients with the risk to develop anaphylaxis. The controlled oral food challenge remains the gold standard for food allergy diagnosis. We report a case of a girl allergic to cow's milk with low levels of specific IgE and large skin prick test wheal sizes for cow's milk.
In some cases the high diameter of skin prick test wheal may be more reliable than specific IgE levels in predicting an anaphylactic reaction.

Introduction

The diagnostic workup of suspected food allergy includes the patient's history, skin prick tests (SPTs), and the measurement of food specific immunoglobulin E antibodies (sIgE). Because none of these parameters can accurately predict food allergy, the gold standard for diagnosing is still the controlled oral food challenge (OFC). However, it is an expensive investigation, with risks of possible severe allergic symptoms for patients and one cannot always make the OFC even if appropriate. Moreover, in the literature there are no unique predictive values of food tolerance (1-4) and Mehl et al. (1), in a recent study, showed that the SPTs and sIgEs should not be used interchangeably.

Case report

In this report we describe the case of a 25-year-old girl allergic to cow's milk (CM), with low levels of CM-sIgE but presenting large wheal sizes of CM-SPTs.
At the age of 2 months the child came to our Pediatric Allergology Outpatients clinic for severe atopic dermatitis (AD) with impetigo at typical sites. The family history was negative for allergy and she was exclusively breastfed.
At 10 months she underwent SPTs that showed a sensitization to hen's egg white (wheal diameter 6 mm), cereal mix (4 mm) and CM (8 mm). The sIgE were positive for egg (++++), CM (++++), wheat (++++), barley (++++), peanut (+---), hazelnut (+++-) and negative for inhalant allergens. The child was advised to continue maintaining a normal weaning, excluding from the diet CM, hen's egg, nuts and dairy products; in the case of lack of breast milk, soy milk or extensive CM protein hy-

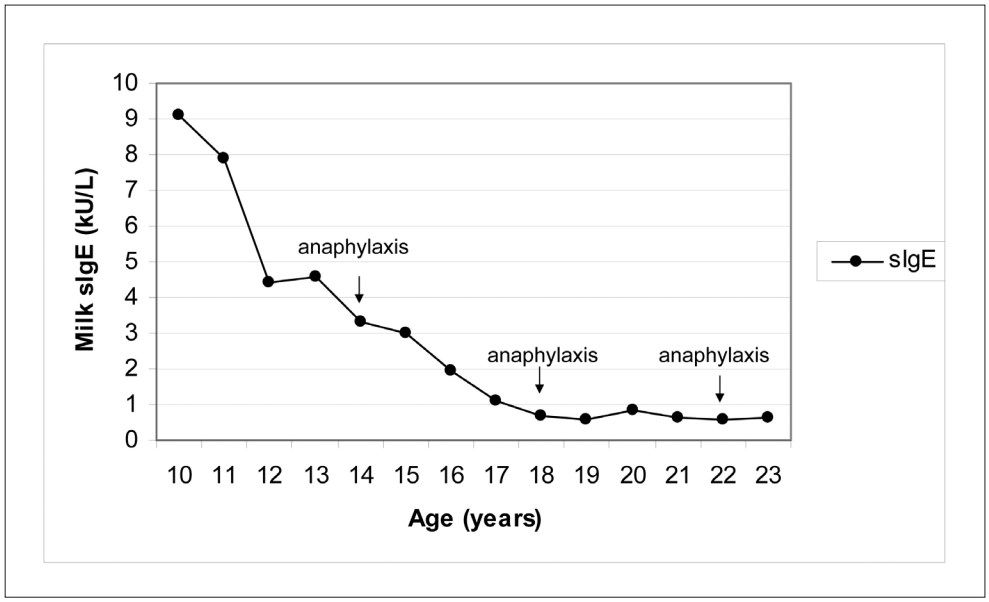

Figure 1a *- Specific IgE levels for cow's milk*

drolysate were permitted. The patient and her family refused the proposed OFC with CM. AD disappeared at the age of 4 years. At the age of 10 years asthma and allergic rhino-conjunctivitis with sensitization to grass pollen (sIgE for Cynodon dactylon 16 kU/L, for Phleum pratense 45 kU/L; total IgE 299 kU/L) appeared.

At the age of 14 years, soon after eating a lemon ice cream containing traces of CM, she presented an immediate reaction characterized by nausea, vomiting, abdominal pain and hypotension. As a consequence, epinephrine (Fastjekt) was prescribed. At that time, the sIgE for CM were weakly positive (3.3 kU/L) while the wheal SPT for CM was large (10 mm) (**figures 1a and b, 2**). It is worthy of note that the patient had never eaten CM. At age of 18 years, immediately after taking a piece of bread containing CM, the girl presented an anaphylactic reaction (vomiting, abdominal pain, syncope). Even in this case epinephrine was administered in an emergency setting. The allergometric tests confirmed the data previously recorded: low values of CM-sIgE (0.67 kU/L) and a large wheal diameter of CM SPT (12 mm) (**figures 1a and b, 2**).

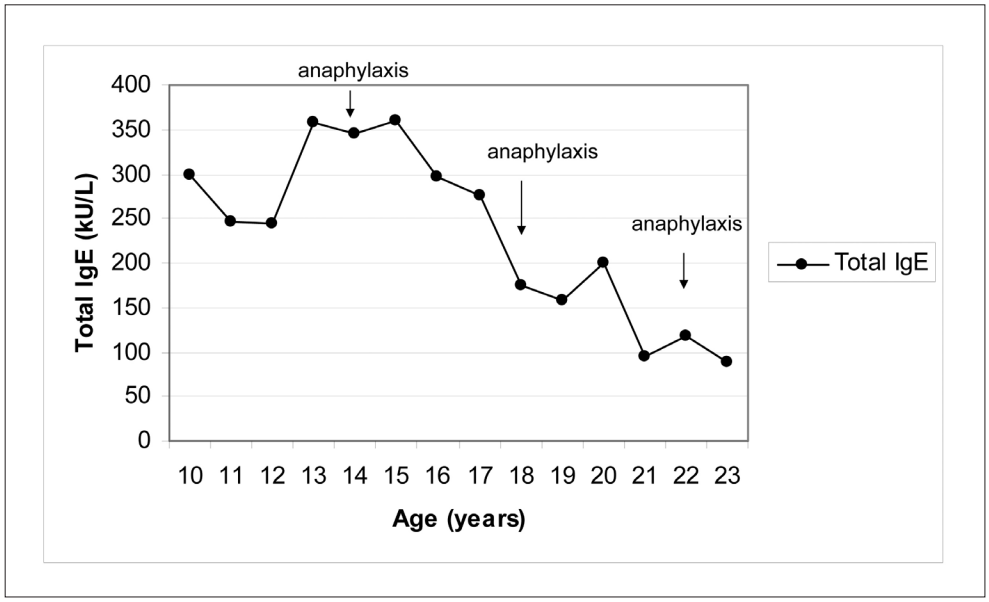

Figure 1b *- Total IgE levels*

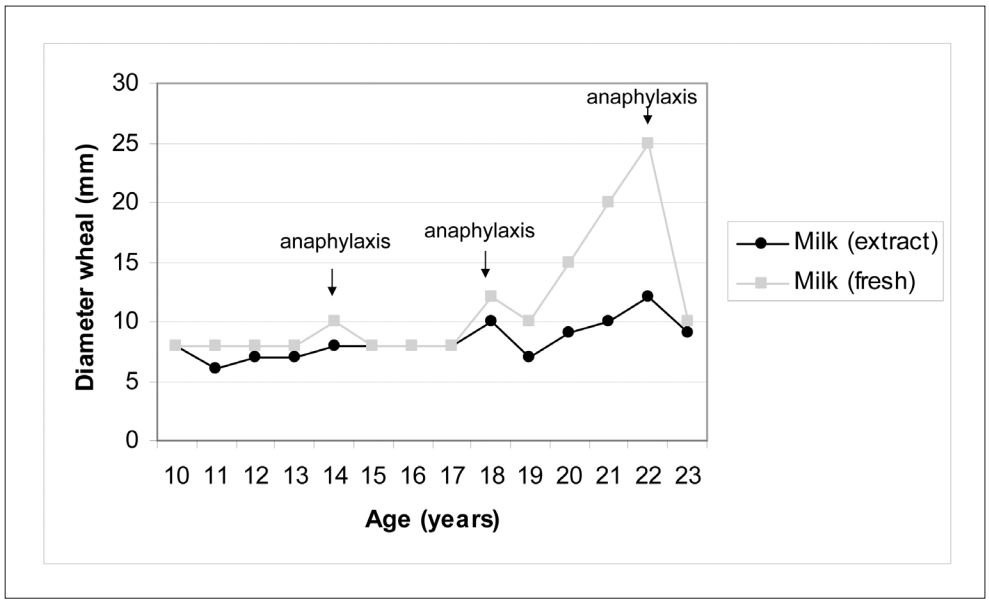

Figure 2 *- Diameter wheal for cow's milk*

At the age of 22, while she was having a meal at a restaurant, despite clear previous recommendations concerning what she could eat, she was served a first course containing CM, of which she was unaware. After a few minutes, a further anaphylactic reaction intervened. On that occasion the patient administered epinephrine by herself with a good clinical response. At that time, the CM-sIgE were 0.57 kU/L and the diameter of the CM-SPT wheal was 25 mm (**figures 1a and 2**).

Over the years the patient has had a good growth height-weight, and even if she and her family were offered on several occasions to perform an OCT with CM, they always refused. Currently she is on a free CM, hen's egg, nuts and dairy products diet, and she is suffering from asthma and allergic rhinitis with a sensitization to grass pollen.

Discussion

The limitation of our study is that in 1987 total serum IgE and sIgEs were measured with the IgE RIA and Phadebas RAST kits, respectively (Pharmacia Diagnostics, Uppsala, Sweden); in 2000, the determination of total serum IgE level was performed by PRIST (Pharmacia, Uppsala, Sweden), and the determination of sIgE was performed by Immuno-CAP™ (Pharmacia, Sweden). So it is not possible a comparison between the levels of specific and total IgE at baseline and follow-up.

In agreement with the data of literature (1,5,6), our case shows that the CM-SPT and the measurement of sIgE to CM cannot be used interchangeably (**figures 1a and b, 2**).

Mehl et al. (1) compared the sIgE levels (kU/L) with the mean wheal diameter size of SPTs for CM in children with CM allergy. It was clearly shown that the correlation between the levels of sIgE and the SPT with the same food allergen is poor, and children with CM allergy (positive OFC) who had the lowest levels of sIgE presented a wheal of up to 15 mm, similarly to our patient who presented a diameter of the wheal of 25 mm for CM, whilst the specific CM IgE were 0.57 kU/L (**figures 1a and 2**).

In a recent study, Cianferoni et al. (7) showed that the mean size for CM-SPT was 8.15 mm in those who failed oral food challenge vs. 4.8 mm in those who passed. In a work (2) on a population of 139 Portuguese children with CM allergy, the higher sIgE level to CM (> 17.5 kU/L) during the follow-up period was associated with a reduced likelihood of acquiring CM tolerance. According to the study of Vazquez-Ortiz et al. (3) CM-sIgE > 50 kU/L resulted as an independent risk factor of reaction persistence. Also in the work of Vanto et al. (4) CM-sIgE level > 2 kU/L are useful prognostic indicators of the development of tolerance to milk in infants with CM allergy. On the contrary, our case suggests that even if a patient shows low CM-sIgE levels, he can have a severe systemic reaction to CM. Therefore, patients with low sIgE levels and high CM-SPT wheal diameter should be followed closely during the follow-up. For both SPTs (in vivo test) and sIgE (in vitro assay), there may be factors that influence the magnitude of the response, such as the number and/or density of IgE epitopes to a particular allergen or the affinity/avidity of sIgE antibodies. However, this influence may act differently for the two test systems. Furthermore, the presence of specific IgG antibodies may unequally affect the result of the two test systems (1). As has already been demonstrated in other studies, SPTs are better than sIgE with regard to specificity and negative predictive values (8-14).

We conclude that in some cases the positivity of SPTs to CM proteins may be more reliable than sIgE levels in predicting an anaphylactic reaction, especially in case of very high diameter of the CM-SPT wheal.

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Desensitization to clopidogrel: a tailor-made protocol

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Keywords
Clopidogrel; desensitization; hypersensitivity reaction

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Summary
Clopidogrel is an antiplatelet drug widely used for treatment and prevention of a variety of cardiovascular diseases. We report a successful desensitization to clopidogrel in a 70-year-old Caucasian man with delayed hypersensitivity (HS) reaction. He developed lip, hand and foot swelling, erythematous papular non-pruritic lesions and arthralgias 2 weeks after starting treatment with clopidogrel 75 mg/d. A 3-hour desensitization protocol was started, achieving a cumulative dose of 154 mg without any reaction, and a daily dose of 75 mg was recommended. On the 4th day, the patient developed skin lesions similar to the previously described. He was treated with topical steroids and oral antihistamines, and the daily dose of clopidogrel was reduced to 20 mg. A new desensitization protocol was established, with a slow dose increment, according to the patient's response. It was only possible to achieve the dose of 75 mg/d after 2 months. Although well tolerated by most patients, HS reactions with clopidogrel may occur and desensitization is rising as a safe alternative in those patients. In delayed reactions with cutaneous lesions, a slower desensitization protocol may be necessary, as in this case.

Case Report

Clopidogrel is a thienopyridine antiplatelet drug widely used for treatment and secondary prevention of a variety of cardiovascular diseases (1). Its combination with acetylsalicylic acid is considered essential to reduce the risk of stent thrombosis in patients undergoing coronary stenting (2). Although clopidogrel is well tolerated in the majority of patients, mucocutaneous reactions have been described: fixed drug eruption (1), oral erosive lichen planus (3), hemorrhagic herpes zoster (4), maculopapular pruritic skin rashes (5) and angioedema (1). The alternative thienopyridine, ticlopidine, is more expensive and associated with serious side effects, such as neutropenia, thrombotic thrombocytopenic purpura, bone marrow aplasia and renal failure, limiting its clinical safety and utility (6). Clopidogrel is, therefore, the preferred thienopyridine in all cases. When hypersensitivity reactions occur, as other treatment options are limited and frequently not tolerated, desensitization is rising as a safe alternative (7). Drug desensitization is defined as the induction of a temporary state of tolerance to a compound responsible for a hypersensitivity reaction, which can only be maintained by continuous administration of the drug (8). If the drug involved is discontinued, tolerance is lost within a period of time that can vary from a few hours to a few days (8). In this procedure, increasing doses of the drug are administered over a short period of time until the total cumulative therapeutic dose is achieved and tolerated (8).

We report a successful desensitization to clopidogrel in a patient with delayed hypersensitivity reaction.

A 70-year-old Caucasian man with allergic rhinitis, hypertension, dyslipidemia and chronic gastritis was submitted to coronary catheterization with placement of a stent and prescribed clopidogrel 75 mg/day. Longstanding medication included ramipril, carvedilol, nitrates, indapamide, acetylsalicylic acid

Table 1 *- Desensitization protocols to clopidogrel*

	Day	Time (min)	Concentration	Dose (mg)	Cumulative Dose (mg)
1st protocol	1	00	**0.5 mg/mL**	0.05	0.05
		20		0.1	0.15
		40		0.5	0.65
		60	**5 mg/mL**	1.0	1.65
		80		2.5	4.15
		100		5	9.15
		120		10	19.15
		140		20	39.15
		160		40	79.15
		180	**75 mg**	75	154.15
	2-8		**75 mg**	75	
	Day	**Time (days)**	**Concentration**	**Dose (mg)**	
2nd protocol	9-15	7	**5 mg/mL**	20	
	16-18	3		30	
	19-41	23		40	
	42-44	3		45	
	45-50	6		50	
	51-53	3		55	
	54-56	3		60	
	57-59	3		65	
	60-63	4		70	
	64		**75**	75	

and simvastatin. Two weeks after starting treatment with clopidogrel, he developed lip, hand and foot swelling, erythematous papular non-pruritic lesions on the neck and abdomen, and arthralgias. He was admitted at the emergency department, treated with oral deflazacort and hydroxyzine, and clopidogrel was stopped. Laboratory evaluation, which included complete blood count, erythrocyte sedimentation rate, immunoglobulins (G, A, M and E), rheumatoid factor and antinuclear antibodies revealed no abnormalities. Cutaneous lesions resolved in five days and arthralgias in three weeks. He was referred to our outpatient clinic for suspected drug allergy. Patch tests with different concentrations of clopidogrel (10%, 30% and 50% pet) and oral challenge (cumulative dose of 75 mg) were negative. One week later, the patient started continuous administration of the drug and 24 hours after a single dose he developed erythematous papular pruritic lesions. Skin biopsy suggested drug related dermatitis: epidermis with multifocal mild spongiosis, exocytosis of lymphocytes and rare vacuolar degeneration of the basal layer, multifocal dermal edema, perivascular inflammatory infiltrate of lymphocytes and rare eosinophils, and macrophages. He was advised to avoid clopidogrel and treatment with ticlopidine was started but it was not well tolerated due to gastrointestinal symptoms. Since dual antiplatelet therapy was required and therapeutic alternatives to clopidogrel are limited, a three-hour desensitization protocol was started, achieving a cumulative dose of 154 mg (**table 1**, 1st protocol) without any reactions. A daily dose of 75 mg was recommended. On the 4th day, the patient developed skin lesions similar to the previously described. The daily dose of 75 mg was continued for 4 more days, with simultaneous treatment with topical mometasone and oral rupatadine. Due to persistence of skin lesions, the dose of clopidogrel was reduced to 20 mg/day, and maintained for

7 days. A new desensitization protocol was established, with a slow dose increment, according to the patient's response (**table 1**, 2nd protocol). Clopidogrel administration was performed at home and the patient was evaluated in our outpatient department every 3 days, or less if the clinical situation warranted earlier evaluation. During the 2nd protocol, when skin lesions reappeared they were treated with topical corticosteroids and oral antihistamines previously used, and clopidogrel dose was increased only when there was a marked improvement of skin lesions. The dose of 75 mg/day was only achieved after two months. Even though clopidogrel was well tolerated, the patient decided to stop its administration one month later. This means that he did not understand the principle of drug desensitization, which was explained several times and it was included in the written informed consent. The case was discussed with the cardiothoracic surgeon and given the patient's non-compliance it was decided not to start a new desensitization procedure.

Although well tolerated by most patients, hypersensitivity reactions with clopidogrel may occur. Since other treatment options are limited and frequently not tolerated, desensitization is an alternative in patients who require prolonged dual antiplatelet therapy. Some protocols for desensitization to clopidogrel have been published, demonstrating its safety and effectiveness (6,7,9,10).

Cutaneous hypersensitivity reactions to drugs may be caused by different pathogenic mechanisms. Despite negative patch tests to clopidogrel, the authors consider that a specific involvement of T cells may be suggested by histological findings on skin biopsy of dermal perivascular inflammatory infiltrate with lymphocytes and scarce eosinophils and macrophages.

According to a recent publication of the European Drug Allergy Interest Group there is no universal or consensus drug desensitization protocol for delayed-type hypersensitivity reactions, and protocols vary in duration, ranging from a few hours to several weeks. Patients with delayed reactions may benefit from longer protocols, with repetitive and slowly increasing doses, since rush protocols frequently have a higher failure rate (11).

The authors present a tailored-made desensitization protocol to clopidogrel that can be used in an outpatient setting. This case also highlights the importance of patient awareness for the need of continuous drug administration so that tolerance can be maintained.

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Efficacy of omalizumab in severe asthma with fungal sensitisation: a case report

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Key words

Severe asthma with fungal sensitisation; omalizumab

Summary

Severe asthma with fungal sensitisation (SAFS) is characterized by poor symptoms control and frequent hospital admissions for exacerbations despite treatment with high dose inhaled steroids, long-acting beta-2 agonists and leukotriene receptor antagonists. Treatment with oral steroids is usually necessary and courses of antifungal therapy may improve asthma symptoms. We report a case refractory to conventional inhaled therapies, continuous oral steroids and antifungal therapy courses, who was effectively treated with omalizumab.

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The link between fungi and severe asthma is complex and not fully understood. Both allergy to fungi (spores, hyphae) and the effects of cell wall constituents (glucan, volatile organic compounds) on bronchi are involved in asthma (1,2).

Distinct pathways of exposure to fungal allergens may be associated with asthma, mainly inhalation of airborne fungal allergens (spores or hyphae) with symptoms due to both persistent indoor exposure (in particular in mouldy and damp houses) or to outdoor acute exposure to very high concentration of these allergens (3,4). Fungal infection outside the respiratory tract, most often dermatophyte infection of the skin or nails may also be associated with immediate hypersensitivity, and in these patients courses of antifungal therapy may improve asthma symptoms (5).

Chronic or intermittent colonization of the airways by the ubiquitous fungus *Aspergillus Fumigatus* in conjunction with immediate hypersensitivity may cause an abnorm allergic response leading to allergic bronchopulmonary aspergillosis (ABPA). However, many other species of fungi, including *Cladosporium, Alternaria, Penicillium, Trichophyton and Candida*, may be involved leading to a less specific disease named allergic bronchopulmonary mycosis (ABPM) (1,2,6). Criteria for diagnosis of ABPA and ABPM include presence of severe asthma, peripheral blood eosinophilia, elevated total serum IgE level (> 1000 IU/ml), migrant chest radiographic infiltrates and central bronchiectasis easily detected by chest CT scan. Additional criteria are usually considered mucus plugs production, *Aspergillus* or other fungi species immediate skin prick test reactivity, and/or elevated specific IgE antibodies.

Many patients with severe asthma do not have the diagnostic criteria of ABPA and ABPM and are not demonstrably colonized

by fungi in the respiratory tract, yet are sensitized to fungi such as *Aspergillus, Cladosporium, Alternaria, Penicillium, Trichophyton, Candida* and other species (1,2). In these patients direct external exposure to fungi ubiquitous in air or limited airways colonization for saprophytic fungi may enhance the specific allergic response, and consequently the pulmonary inflammation in a persistent severe asthma condition.

The severe asthma with fungal sensitization (SAFS) is a particular phenotype of severe asthma, in which a therapeutic effect of antifungal therapy was documented (1,7). In SAFS fungal colonization is not easily demonstrable as it is limited and intermittent. Moreover the airborne fungi implicated are barely capable of growth at 37°C and inept at establishing themselves within the human host, whereas saprophytic fungi are not isolated from respiratory biologic samples if meticolous procedures for processing the materials are not used (7).

The antifungal therapy impact on asthma may be explained by the reduction of allergen exposure due to the direct killing of viable filamentous fungi. However, daily interactions between fungi and humans and their effects on asthma remain to be better understood, as within SAFS patients nearly 40% of non-responders to antifungal therapy with symptoms unchanged or relatively worse were documented (7).

We report the case of a 57 year-old female, former smoker (about 10 cigarettes a day for 20 years), who complained for almost 5 years of poorly productive cough, progressive fatigue and dyspnea after moderate exertion. The patient was initially diagnosed with COPD on the basis of a spirometry flow volume curve which showed moderate obstructive ventilatory defect. She remained severely symptomatic despite inhaled therapy with long-acting anticholinergic (tiotropium 18 ug once daily) and combination of topical steroid with long-acting B2 agonist (fluticasone 500 ug - salmeterol 50 ug twice daily). She was diagnosed also of a hiatal hernia with minimum gastroesophageal reflux, treated with proton pump inhibitors in high doses, with no improvement in respiratory symptoms. Despite the maximal inhalation therapy, the patient frequently had to use inhaled short acting B2 agonist (salbutamol 100-200 ug) as needed, with side effects such as tremors and tachycardia, as well as several cycles of oral steroid (prednisone 25 mg). In the last 12 months she experienced four significant exacerbations and two hospitalizations for acute respiratory failure. The patient was referred to our attention for further investigations and to evaluate a more effective therapeutic strategy. Spirometry (**table 1**) showed a very severe obstructive ventilatory defect with hyperinflation; the bronchodilation test with salbutamol 400 ug was positive, with partial reversibility of the obstruction. The Asthma Control Test (ACT score 12) was indicative of poor control of symptoms. Routine blood tests (erythrocyte sedimentation rate, C-reactive protein, liver and renal function indices, coagulation parameters) were normal. White blood cell count showed a mild peripheral hypereosinophilia (percentage of eosinophils 18% and absolute value 0.8x10^3 ul). Total serum IgE level was 279 KU/L. Skin prick test with the most important allergens of the mediterranean area, along with *Aspergillus sp, Alternaria sp, Cladosporium sp and Penicillium sp* were negative (Lofarma Allergeni, Milano, Italy). Serum specific IgE were positive for *Candida albicans* (4.31 KUA/L), *Aspergillus fumigatus* (2.53 KUA/L), *Cladosporium herbarum* (1.14 KUA/L), *Penicillium notatum* (0.6 KUA/L) and negative for *Saccaromices Cerevisiae* (0.10 KUA/L) (Immunocap Termofisher, Uppsala, Sweden). A microarray-based immunoassay (ISAC 112, Termofisher, Uppsala, Sweden) was positive for Fel d1 (10 ISU-E), Der f 1 (03 ISU-E), Der f 2 (06 ISU-E) and Der p 2 (04 ISU-E). Serum immunoglobulins were normal and there was not any IgG subclass deficiency. Paranasal sinuses CT scan and rhinoscopy were normal. High-resolution chest CT scan showed only bronchial wall thickening and areas of pulmonary hyperinflation. Bronchoalveolar lavages obtained from a previous bronchoscopy and sputum culture, performed several times during phases of productive cough, were negative for bacterial and fungal growth.

These investigations allowed us to rule out diseases such as ABPA or ABPM and the Churg Strauss syndrome. We established instead the diagnosis of severe asthma with fungal sensitization (SAFS) also considering inadequate control and instability of the disease despite the maximal inhalation and systemic therapy. Patient's house indoor walls, which were very damp and probably contaminated by ubiquitous moulds, were repainted. However, there was not any symptoms improvement despite the environmental sanitation. The patient was then started with a course of oral itraconazole (200 mg twice daily) for a scheduled period of 32 weeks. Antifungal therapy was suspended at 24 weeks due to lack of clinical response with symptoms and respiratory functional parameters unchanged. Peripheral blood eosinophils and serum total IgE were also unchanged. Therefore, we decided to start a course of treatment with the anti-IgE recombinant humanized monoclonal antibody omalizumab. According to the level of circulating total IgE (279 KU/L) and body weight (54 kg), we decided to administer a subcutaneous dose of 300 mg (2 pre-filled syringes of 150 mg) every 4 weeks. After 12-14 weeks the patient already reported an improvement in her respiratory symptoms as documented by the significant increase in ACT score (from 12 to 21). The oral steroid was reduced gradually and then suspended. Symptoms control persisted (ACT score 21) and in this time there was not any asthma exacerbation. Spirometry was performed after 16 weeks of treatment and showed a significant improvement of the functional parameters compared to the values obtained before therapy with omalizumab (**table 2**). The patient has never accused any side effects during treatment with omalizumab, has continued to

Table 1 *- Spirometry parameters and reversibility before omalizumab treatment*

	Pred	Pre Meas	Pre % Pred	Post Meas	Post % Pred	Post % Chg
FVC (L)	2.00	1.94	97	2.42	121	25
FEV1 (L)	1.63	0.54	33	0.73	45	35
FEV1/ FVC %	76	28	37	30	40	8
RV (L)	1.89	3.02	160	/	/	/

Reversibility with 400 µg salbutamol

Table 2 *- Spirometry parameters and reversibility after omalizumab treatment*

	Pred	Pre Meas	Pre % Pred	Post Meas	Post % Pred	Post % Chg
FVC (L)	2.00	2.12	106	2,44	122	15
FEV1 (L)	1.63	1.12	69	1.48	91	32
FEV1/ FVC %	76	53	70	61	75	7
RV (L)	1.89	2.15	114	/	/	/

Reversibility with 400 µg salbutamol

show good asthma control without the use of oral steroids and her quality of life has much improved.

This case report provides two important recommendations for clinical practice. Firstly, it shows the persistent unreliability of the diagnostic tools for fungal allergy, and secondly confirms the efficacy of omalizumab in severe asthma refractory to conventional therapies.

We found negative results of fungal skin test and allergen microarray in comparison with several positive results of fungal allergens serological test. This is consistent with previous findings, and suggests the need to perform both cutaneous and serological test in patient with severe asthma (8). The presence of relatively high levels of serum IgE against *Candida sp* seems common in severe asthma with fungal sensitization (7), but their pathological role remains poorly understood. Sensitisation to saprophytic fungi such as *Candida sp* may be undoubtedly elicited by frequent and transient airways colonizations favored by systemic steroidal treatments used in severe asthmatic patients. It is possible that these fungi, having the ability to actively germinate and colonise the host skin or the respiratory tract, may produce toxins and enzymes that have an accessory role in triggering allergy (1).

The clinical course of this case clearly demonstrates the effectiveness of omalizumab treatment in severe allergic asthma despite high level of allergen exposure (9,10). The antifungal treatment therapeutic failure and its lack of any effect on total serum IgE (11), suggest that the severity of symptoms was not consequent to an hidden fungal colonization, but rather to a consistent and prolonged environmental fungal allergens indoor exposure. The insignificant clinical effect of the indoor environment sanitation may be explained by several reasons. Probably the indoor quality of air was still unhealthy. Many fungi are present in great numbers also in the outdoor environment if some climatic conditions occur. Finally there are evidences that some fungal antigens may generate cross-reactive responses and induce a self-perpetuating allergic inflammation (1,12)

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A case of hereditary angioedema who presented with difficulty in urination and globe

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Key words
Hereditary angioedema; C1 inhibitor deficiency; difficulty in urination; globe

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Summary
Hereditary angioedema (HEA) is a disease characterized by decreased levels or function of C1 esterase inhibitor (C1-INH). The symptoms of HEA in pediatric age group generally consist of recurrent episodes of soft tissue swelling. These symptoms can be transient, subtle, and varied in severity. Genitourinary system is rarely affected in this disease. Here, a three-year-old girl who presented with angioedema on her hands, fingers, and face, and had difficulty in urination and globe is reported. The aim of this case is to focus on this rare disease, hereditary angioedema, which presented with difficulty in urination and urinary globe.

Introduction

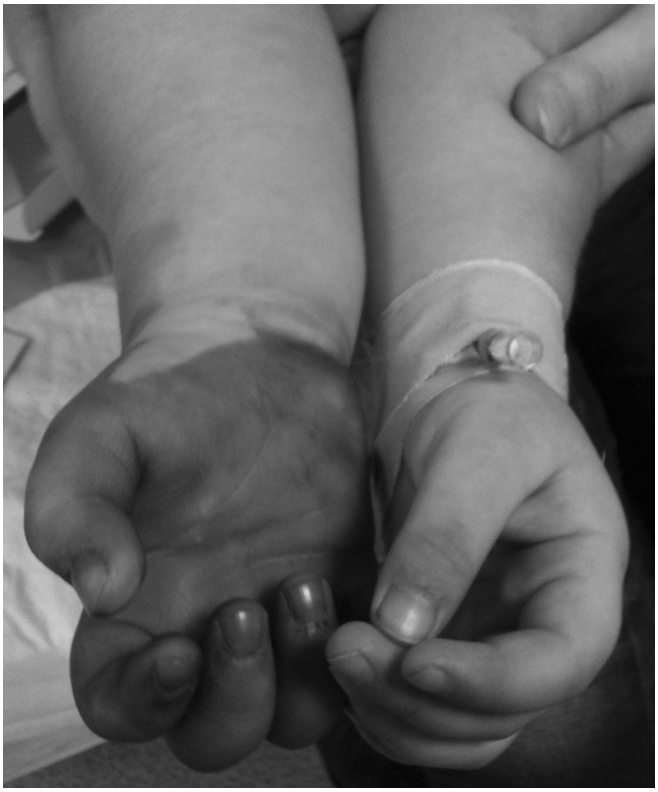
C1-INH deficiency can be genetic or acquired. HAE is a rare, life-threatening condition manifested by acute attacks of facial, laryngeal, peripheral or genital edema. The estimated prevalence of HAE is between 1 in 10,000 and 1 in 150,000 people (1,2). The genetic HEA deficiency is due to mutations in one of the two alleles of the C1-INH gene that result in reduced protein levels in plasma (type I HEA) or in normal protein levels but always in reduced function (type II HEA). A third type has also been reported, occurring exclusively in women who have normal C1-INH levels and function (Type III HEA). It is estimated that 20% to 25% of HAE cases are caused by spontaneous mutations in patients with no family history of the disease (3,5). The development of angioedema in C1-INH-deficient patients involves the inappropriate generation of kinins (particularly bradykinin) that stimulate vascular smooth-muscle relaxation and induce increased permeability. C1-INH is a serine protease inhibitor (serpin), also known as SERPING1, that blocks the activity of some complement components (e.g. C1r, C1s, Mannose binding lectin-associated serine protease; MASP-1 and MASP-2). C1-INH also controls contact-kinins, coagulation, and fibrinolytic cascades (6).
In this paper, we report a case with rarely manifested hereditary angioedema who presented with difficulty in urination and globe.

Case report

One day, a three-year-old girl with HEA was admitted to our emergency pediatric department with swelling of right forearm and urinary globe, and difficulty in urination (**figure 1**). She didn't have dysuria, fever or pelvic pain. Urinary globe, mild external genital swelling and right forearm angioedema were examined in her physical examination. Her urine analyses,

complete blood counts, biochemical tests, C reactive protein were normal. The patient was treated with C1 esterase inhibitor concentrate. Within a few hours, her difficulty in urination decreased, and urinary globe as well as right forearm angioedema resolved. She was being followed in pediatric allergy department. In her past life, the patient has experienced a lot of angioedema on her hands, fingers and face. When she was 1.5 year old she had experienced her first angioedema attack, which had been treated with intravenous corticosteroids and antihistamines without effect in a private hospital. At first, the symptoms usually subsided spontaneously within a few days. On the day of her first pediatric allergy clinic visit (she was 20 months old), on her physical examination blood tests [eosinophil counts (3.1%, 370/mm^3), nephelometric IgE 70 (0-90 iU/mL)] and skin prick test were normal. C1 inhibitor [41.7 and 63 (210-345 mg/dL)] and C4 [2.9 and 2.7 (16-38 mg/dL)] levels were low for two times. There was no similar history in her family. Father's and mother's C4 and C1 inhibitor levels were normal. These laboratory data and clinical features were compatible with a diagnosis of type I HAE.

Figure 1 *- Angioedema on forearm*



Discussion

Hereditary angioedema accounts for approximately 2% of all cases of angioedema. Three types of HAE have been described in the literature: type I HAE (approximately 85% of cases), type II HAE (approximately 15% of cases) and type III HEA (less than 1% of cases) without abnormalities of complement or C1 inhibitor characterized by a coagulation factor XII gene mutation and seen primarily in females. In 50% of patients with HAE, the initial symptoms appear during the first decade of life, in 35% symptoms appear during the second decade, and in the remaining 15% symptoms appear after 20 years of age. Almost 20-25% of patients lack a family history, which can make difficulty in diagnosis (4,5).

The C1NH gene maps onto chromosome 11q12-q13.1 and it is organized into 8 exons and 7 non introns, particularly rich in repetitive *Alu* sequences (7). Although the de novo mutation also made diagnosis difficult because of the lack of family history, this type of mutation seems not so rare. In the literature, more than 300 deficiency-causing mutations have been identified, and approximately 25% of them occur de novo. De novo mutations can belong to all types of deleterious changes (single nucleotide changes, microdeletions-insertions, and gross deletions) and their distribution according to these types is similar to that found for hereditary angioedema in general (8). In our patient's family, there was no similar history for HEA. Anamnesis and laboratory finding suggested that our patient most likely had de novo mutation.

In the literature, episodes affecting the urinary bladder were very rare (3:1000) (9). Hematuria association with HEA was rarely published in the literature (10,11). Our patient was admitted with forearm swelling, difficulty in urination and globe, without hematuria and dysuria, and her symptoms were decreased with the administration of C1 esterase inhibitor concentrate. Her laboratory tests (urine, blood) were normal for the other etiologies of urinary globe and difficulty in urination.

In summary, we have described a case of type I HEA with most likely de novo mutation. The patient had recurrent episodes of angioedema, urinary globe and difficulty in urination, which resembled manifestations of urinary infections and other etiologies of urinary globe. HAE should be taken into consideration for the differential diagnosis of urinary globe etiology.

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NSAIDs are the most frequent medicaments involved in hypersensitivity drug reactions

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Dear Sir

The editorial by Asero et al. in issue n° 1 of Vol. 45 of the European Annals of Allergy and Clinical Immunology (1) points out important data on cutaneous hypersensitivity to multiple non-steroidal anti-inflammatory drugs (NSAIDs) in the sense that a certain number of patients may also respond to selective COX-2 inhibitors. Although the cases of the two patients reported had good tolerance to paracetamol, the authors noted that tolerance to selective COX-2 inhibitors may vary according to previous tolerance to paracetamol. Comparing reactivity to different coxibs, figures lower than 10% could correspond to cases who had good tolerance to paracetamol, and higher figures could correspond to those who previously had skin problems after taking paracetamol, as reported by our group (2). However, a more detailed analysis (from the table) shows that tolerance to paracetamol was not tested in every patient (1).

It is important also to emphasize the relevance of NSAIDs in the induction of hypersensitivity reactions. Asero et al stated in the article that NSAIDs are one of the common causes inducing drug hypersensitivity. For this purpose, a detailed analysis of the manuscript published by Gomes et al. (3) indicates that NSAIDs were the second on the list of implicated drugs in hospital-based populations as well as in outpatients and general populations. However, a recent study published by our group (4) showed that after a detailed evaluation of 4460 patients over a period of 6 years, 966 patients were finally confirmed as positive to NSAIDs, this representing the most frequent group of drugs involved and confirmed as causing allergy. A previous study focusing on the hypersensitivity patterns of responses to the NSAID group suggests that 60% were cross-intolerant (5). We believe that with the data now available, these references should be taken into account when referring to drugs involved in hypersensitive reactions.

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Reply

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Doña and co-workers' comments on our editorial (1) underline two aspects of NSAIDs hypersensitivity. The first one is paracetamol tolerance in NSAIDs-hypersensitive patients, particularly in those reacting to different NSAIDs, including selective COX-2 inhibitors (coxibs). As reported in the editorial, Doña and co-workers found that intolerance to paracetamol could represent a "marker" of reactivity to etoricoxib and to other coxibs (2). This was not the case in our two patients. On the other hand, reviewing the outcome of paracetamol tolerance in coxibs-reactive patients was out of scope of our article, dealing with challenge tests with coxibs in patients with cutaneous hypersensitivity to multiple NSAID. The two cases we presented underlined the need of checking tolerability of alternative NSAIDs (including selective COX2-inhibitors) in a proper setting. The same holds obviously true also for paracetamol, as highlighted by Doña et al. (2).

The second aspect is the prevalence of hypersensitivity reactions to NSAIDs: definitely this class of drugs is one of the most common causes of adverse reactions (3), and in some populations is the most frequent one, as recently reported by Doña et al. (4). Differences in the prevalence rate of drug hypersensitivity may depend on study populations (ethnicities, inpatients or outpatients, adults or children), differences in methods for assessing offending drug, and different methods of data analyses (5). However, we believe that an exact ranking of prevalence doesn't appear very relevant in the evaluation of coxibs as alternative drug in cutaneous hypersensitivity to multiple NSAIDs.

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