edra | Issn 1764-1489 | Volume 51 N. 6/2019 – Novembre 2019

European Annals of Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF AAIITO | ASSOCIAZIONE ALLERGOLOGI IMMUNOLOGI ITALIANI TERRITORIALI E OSPEDALIERI
THE OFFICIAL JOURNAL OF SPAIC | SOCIEDADE PORTUGUESA DE ALERGOLOGIA E IMUNOLOGIA CLINICA

6/2019

Clinical aspects of hymenoptera venom allergy and venom immunotherapy

Assessing clinical and psychological features: who are patients showing a nocebo reaction during the drug challenge test?

Occupational allergy to dog among police dog trainers

Can an otorhinolaryngological visit induce the suspect of allergic rhinitis in children?

Anaphylaxis to baobab fruit: the paradox of "natural healthy food"

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Ph. 0039 (0)2-88184.317
Italy subscription: 60 euro
World subscription: 85 euro

Printing

Rotomail Italia S.p.A., Strada Rivoltana (SP 14), 12/AB 20060 Vignate (MI), Italy

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Via G. Spadolini, 7
20141 Milano - Italy
Tel. 0039 (0)2-88184.1
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www.edizioniedra.it

"European Annals of Allergy and Clinical Immunology" registered at Tribunale di Milano - n. 336 on 22.10.2014

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Clinical aspects of hymenoptera venom allergy and venom immunotherapy

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Key words

venom allergy; immunotherapy; anaphylaxis; adrenaline; diagnosis; treatment

Abbreviations

AAI, adrenaline auto-injectors; HVA, hymenoptera venom allergy; SR, systemic reaction; VIT, specific venom immunotherapy.

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Doi
10.23822/EurAnnACI.1764-1489.113

Summary

Hymenoptera venom allergy (HVA) is the most frequent cause of anaphylaxis in Europe, accounting for most of the severe reactions occurring in adults, and being the second cause of anaphylaxis in children. Prevention of further episodes in patients who developed a systemic reaction (SR) is based on the correct management of the allergic emergency, the referral to an allergist for a correct diagnosis, prescription of adrenaline auto-injectors (AAI) and specific venom immunotherapy (VIT), if recommended.

Diagnosis is based on the classification of the type of reaction, confirmation of an IgE-mediated pathogenesis and the identification of the offending insect. The use of component resolved diagnostics may be helpful in case of poly-sensitization or negative allergy tests with a proven history of previous SRs. When a severe SR occurs, baseline serum tryptase levels should always be assessed.

The prescription of AAI is recommended or suggested for specific untreated patients, patients undergoing VIT and after discontinuation of treatment, according to multiple evidence.

VIT is the most effective treatment available for HVA patients, as confirmed by recent European guidelines. VIT has an early, sustained and persistent protective effect and modifies the natural course of the disease. Moreover, VIT proved to be safe and well tolerated. According to a recent systematic review, no treatment-related fatalities were recorded to date. Compared to AAI, VIT significantly improves the quality of life of HVA patients by reducing the anxiety and limitations in daily activities caused by the fear of stinging insects. The memory of a life-threatening experience is the most likely reason why adherence to VIT is higher compared to immunotherapy with inhalant allergens.

Several risk factors in HVA have been identified that can influence not only the severity of sting reactions in untreated patents, but also the occurrence of side effects, treatment effectiveness and the risk of relapse after discontinuation of VIT. Patient and treatment-related risk factors must be considered while selecting the best candidates for VIT, the type and duration of treatment. In this paper we address the most important issues related to HVA and VIT that may have an impact on daily clinical practice.

Introduction

Hymenoptera venom allergy (HVA) is a potentially life-threatening allergic condition frequently observed in the general population. In Europe, the prevalence of systemic reactions in the adult population is 0.3 - 8.9%, being lower in children and higher in beekeepers (1). According to the European Anaphylaxis Registry, HVA is the major cause of anaphylaxis in adult subjects (48.2%), while it accounts for 20.2% of anaphylactic episodes in pediatric patients (2).

Stinging insects that most frequently cause HVA in developed countries are bees of the *Apidae* family, and wasps of the *Vespidae* family. Among bees, the most commonly observed stinging species that causes HVA is the common bee (*Apis mellifera*), while among wasps, several species of both *Vespinae* (i.e. *Vespula* spp., *Dolichovespula* spp., *Vespa* spp.) and *Polistinae* (i.e. *Polistes dominula*, *Polistes annularis*) subfamilies cause allergic reactions. Venoms produced by red wood ants (*Formica rufa*) and fire ants (*Solenopsis invicta*), usually found in rural areas of North and Central America, and Australia, although sporadic in Europe, are also potent sensitizing agents and cause of allergic reactions upon biting (3). Allergens of bee and vespids venoms are summarized in **table I**.

Given its unpredictable nature, patients with HVA usually have a poor quality of life, even in the case of mild severe reactions (SR) (4).

It is especially daunting to properly diagnose patients with HVA, choose the right treatment and manage the long-term follow-up. Furthermore, there are several risk factors for SR that must be taken into consideration, from the diagnosis to the discontinuation of treatment, that might complicate HVA treatment and management and are often unrecognized.

The purpose of this review is to provide to clinicians relevant and updated information on HVA diagnosis, clinical management and treatment in adult and pediatric populations, with special interest to high-risk HVA patients, and suggestions on how to manage HVA effectively in daily practice.

Table I *- Allergens of bee and vespid venoms according to WHO/IUIS nomenclature.*

Family	Species	WHO/IUIS nomenclature	Biochemical name
Apidae	*Apis mellifera*	Api m 1	phospholipase A2
		Api m 2	hyaluronidase
		Api m 3	acid phosphatase
		Api m 4	mellitin
		Api m 6	dipeptidyl-peptidase iv
		Api m 6	serine protease inhibitor
		Api m 7	CUB serine protease
		Api m 8	carboxylesterase
		Api m 9	serine carboxypeptidase
		Api m 10	icarapin
		Api m 11.0101	major royal jelly protein 8
		Api m 11.0201	major royal jelly protein 9
		Api m 12	vitellogenin
	Bumblees	Bom p 1	phospholipase A2
		Bom t 1	
		Bom p 4	protease
		Bom t 4	
Vespidae	*Polistes dominula*	Pol d 1	phospholipase A1
		Pol d 3	dipeptidyl-peptidase IV
		Pol d 4	serine protease
		Pol d 5	antigen 5
	Vespula vulgaris	Ves v 1	phospholipase a1
		Ves v 2	hyaluronidase
		Ves v 3	dipeptidyl-peptidase IV
	Vespa crabro	Ves v 5	antigen 5
		Ves v 6	vitellogenin
		Ves c 1	phospholipase A1
		Ves c 5	antigen 5

Methods

We performed a PubMed search for most relevant state-of-the-art guidelines, position papers, reviews, expert opinions and articles, with focus on clinical aspects, diagnosis, self-treatment and management of acute reactions, specific venom immunotherapy and long-term management of HVA.

Results and discussion

Clinical aspects and diagnosis of HVA

Collection of clinical history

In HVA, it is of vital importance to collect as many relevant information to formulate a correct diagnosis, but also aimed at recognizing potential risk factors that might increase the risk of severe reactions (5).

Information on the stinging insect, although challenging and sometimes misleading, is helpful to guide the diagnosis and the selection of VIT. A detailed history of the stinging event (i.e. number of stings, previous and subsequent re-stings), with questions on the appearance and behavior of the insect (day / night encounter, information on hives / nests) and the type of sting (i.e. extraction of sting, death of offending insect), when available, should be documented from each subject. Information on occupational or recreational activities linked to a higher likelihood of sting (e.g. farmers, beekeepers, outdoor sports) are also important pieces of information to collect, guiding the treatment strategy and future management (5).

The type of elicited reaction is also a crucial step during the collection of the clinical history from HVA patients: reactions

are divided in large local (LLR) and systemic, according to the extent of involvement. Usually the toxic local reaction induced by venoms is transient, self-limiting and completely resolving in less than 24 - 48 hours; in allergic patients, LLRs are defined as edema exceeding 10 cm in diameter, increasing within 24 - 48 hours after the sting, and lasting longer than 72 hours (5).
LLRs, although worrisome for HVA patients, have a low risk of evolution in SR (2-7%), especially in case of repeated LLRs (6,7), even though a recent paper on a large population shows that the risk of a SR, after a previous LLR, occur more frequently than that reported by previous literature (8). LLRs should not be underestimated if causing reduced quality of life, or when the risk of multiple simultaneous stings is high (i.e. beekeepers, farmers).
Allergic SR may involve one or more organ systems (i.e. cutaneous, respiratory, gastrointestinal, neurologic and cardiovascular systems), while the simultaneous involvement of two or plus organ systems during an acute allergic event is diagnostic for anaphylaxis (9-11).
Cutaneous involvement (e.g. acute generalized urticaria / angioedema) is more frequently observed in both adults and children, accounting for 80% and more than 90% of HVA reactions, accordingly (5,12). Respiratory involvement (e.g. bronchospasm, acute upper airway obstruction due to angioedema) is observed in around half of SRs (5). As for the involvement of the cardiovascular system, hypotension (60% of cases) and loss of consciousness (50%) might occur independently of other associated symptoms, especially in case of systemic indolent mastocytosis, and are more frequently observed in adults than children (13). Gastrointestinal involvement (e.g. vomiting, diarrhea, abdominal pain, nausea), uterine cramps (with possible miscarriage), and neurologic symptoms (e.g. dizziness, convulsions), are also reported (13). Other symptoms like rhabdomyolysis, disseminated intravascular coagulation, intravascular hemolysis, acute hepatic and renal failure might also occur, and are generally due to direct toxic effects of hymenoptera venom (5).
It is important to also investigate the recurrence of symptoms after 4 - 12 hours from the resolution of the first anaphylactic episode, without re-exposure to stings, since biphasic anaphylaxis is reported in 0.4 - 14.7% of cases. Known risk factors for biphasic reactions are history of previous anaphylactic episodes, and delayed treatment with adrenaline (14,15).
Several classifications were proposed to assess the degree of severity of anaphylaxis; the most used in clinical practice are Mueller's and Ring's, both of which however show some important limitations; Mueller's classification tends to underestimate cardiovascular collapse without onset of associated cutaneous symptoms, while Ring's underestimates respiratory involvement (16,17). New proposed severity scores from Brown and EAACI guidelines suggest simpler criteria, namely dividing reactions in mild, moderate or severe, or in grades according to local (grade 1) or systemic involvement (grade 2,3) (18-20). In the latter, however, such proposed grading might be confusing for HVA, given that local reactions are referred to local cutaneous involvement (i.e. LLR), rather than generalized urticaria.
During the collection of clinical history, it is important to assess concomitant conditions that might increase the severity of the HVA reactions (i.e. heart disease, clonal mast cell disorders) (12,21-24), conditions that might influence future treatment strategies (i.e. active systemic autoimmune diseases, severe acquired and/or primary immunodeficiencies, malignancies, pregnancy) (25-27) and use of medications that might hinder HVA treatment response (i.e. beta-blockers, ACE [angiotensin-converting enzyme] inhibitors) (25,28-30).

Diagnosis of HVA

Skin testing

Both skin tests and serologic tests should be performed in patients with a positive history of systemic reactions. In patients with LLRs, diagnostic tests can be optionally performed, especially when bothersome or with high risk of recurrence, possibly to start VIT (5,31,32). They are not recommended for screening the general population, since 10-30% of subjects without any previous history can be found positive (13,19,31,33).
Skin tests are safe to perform even in subjects with history of severe anaphylaxis or with clonal mast cell disorders, if executed by experienced professionals in a hospital setting with access to emergency care (22,34).
The gold standard for HVA diagnosis is skin testing with venom extracts, which should be performed not less than two weeks after the last sting to prevent false negative tests due to the refractory period (5,19,31).
Skin prick test (SPT) at 100 μg/mL concentration can be used as first assessment for HVA. Cut-off for positivity is the appearance of a wheal of ≥ 3 mm diameter compared to the negative control in the pricked area after 15 - 20 minutes (35).
Regardless of SPT results, it is recommended to also perform intradermal testing (IT); briefly, venom extracts, serially diluted to reach end concentrations ranging from 0.001 to 1 μg/mL, are administered at increasing concentrations with intradermal needle injection (5). The test is stopped at the concentration causing the formation of a wheal (threshold concentration) after 15-20 minutes, or when reaching 1 μg/mL concentration, since higher concentrations of venom extracts might exert an irritant effect (36). Multiple venoms can be assessed at once, given that the same concentration is used (13). The outline of the positive wheal reaction should be marked with a drawing pen, transferred to paper using transparent tape and stored in clinical records for both diagnostic and VIT monitoring purposes (37).
The sensitivity of SPT alone is estimated around 64%, while a combination of SPT and IT reaches a 94% sensitivity, hence it

is recommended to perform both tests sequentially, when available (5,19,31).
In case of negative skin tests but presence of a suggestive history of SR, cutaneous tests should be repeated after 1-2 months, along with serologic testing.
As for other in vivo tests, it is recommended to refrain from using the sting challenge with a live insect for diagnostic purposes, since this procedure is at high risk for severe reactions and has low negative predictive value (38).

Serologic testing for IgE antibodies

The detection of specific IgE antibodies is an important step for HVA diagnosis to improve the diagnostic accuracy, therefore current guidelines recommend performing both skin and serologic tests (5,19,31).
IgEs are antibodies produced after the very first sensitizing event and can be detected immediately in the serum after the first allergic reaction, although it is recommended to determine their levels 1-4 weeks after the last sting (13).
Sensitivity of serological tests is different according to the type of venom tested: typically, the detection of specific IgEs against *Vespula* spp. is less sensitive than *Apis mellifera*'s, showing 83 - 97% and 98 - 100% sensitivity, respectively (39-41).
A new in vitro method enriched with recombinant allergen Ves v 5 demonstrated a greater sensitivity compared to traditional methods (42).
When assessing venom-specific IgE, it is important to also dose total IgE levels; such test is especially helpful to correctly interpret low venom-specific IgE levels and suggests concomitant atopy if excessively high (13).
Conversely, component-resolved diagnostics (CRD) allows the identification of molecule-specific IgEs, using recombinant or natural allergenic epitopes, with important consequences for both diagnosis and therapeutic management. Nevertheless, CRD plays also an important role in the diagnostic assessment of negative skin test results with a positive history of systemic reaction (43-46).

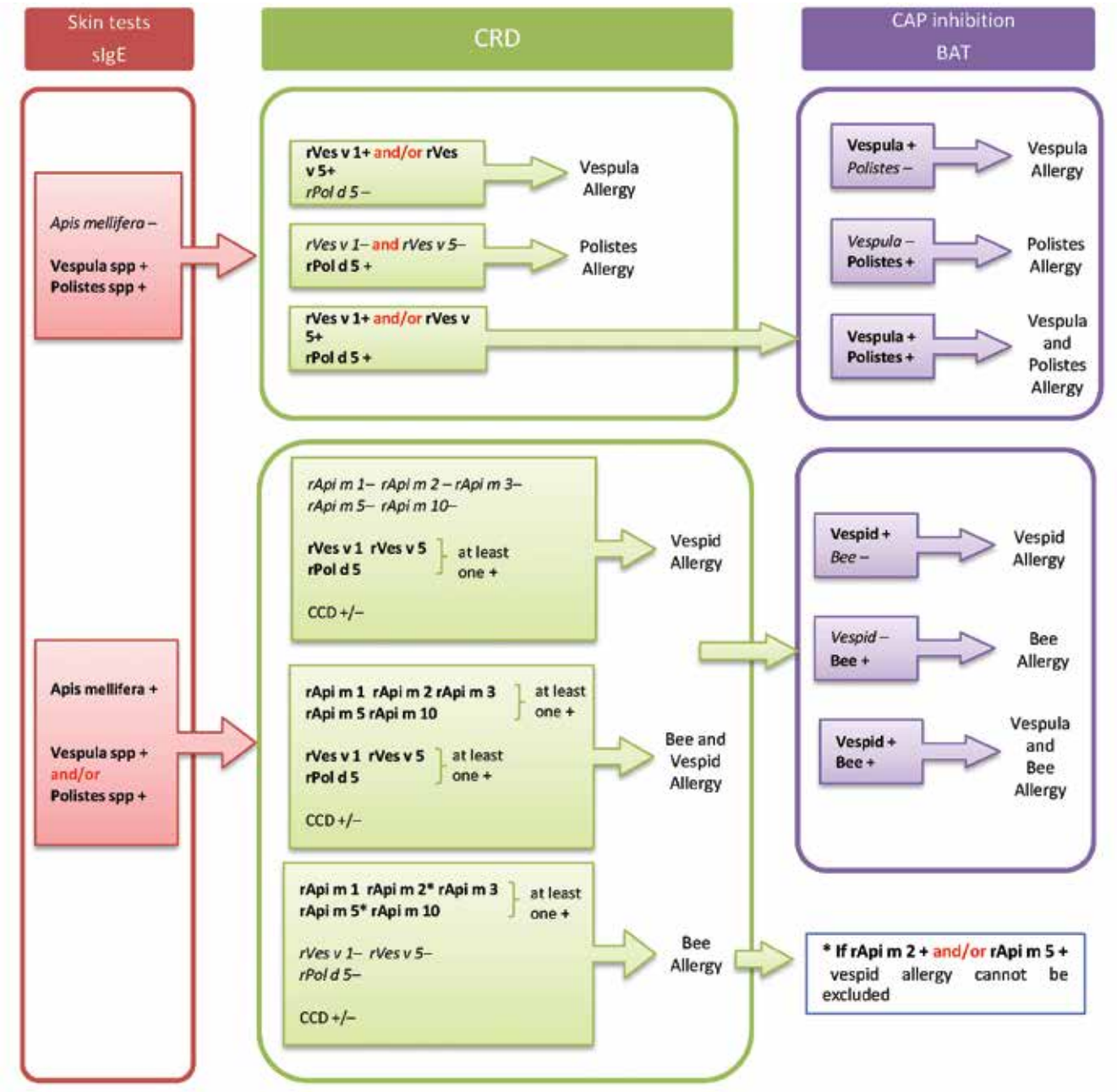
Discriminating cross-reactivity from multiple sensitizations

When the stinging insect cannot be identified, and skin and/or serologic tests show positivity to multiple venoms (i.e. *Vespula* spp. and *Apis mellifera* in 25 - 40% of the cases, *Vespula* spp. and *Polistes* spp. in over 50% of cases), it is important to discriminate between cross-reactivity and multiple sensitizations for an accurate HVA diagnosis and treatment with VIT (47-49).
Cross-reactivity between different venoms can occur due to high homology in the structural composition of allergenic molecules produced by different species (e.g. Api m 5 - Ves v 3, Api m 2 and Ves v 2, and Api m 12 - Ves v 6) (44) or cross-reactive carbohydrates (CCD), like MUXF3 or bromelain, that can be detected in most venoms, with the exception of *Polistes dominula* venom (40,50).
Several recombinant major allergens of different species are commercially available, and the specific sensitization profiles obtained can dramatically increase the specificity of HVA diagnosis (42). For instance, positive detection of 6 of the major allergens of bee venom (Api m 1 to 5 and Api m 10) increases the specificity of bee allergy diagnosis to 94.4%, compared to 84.4% if only two allergens are detected (51). Similarly, patients with concomitant Ves v 1 and Ves v 5 sensitization identifies 92 - 98% of *Vespula* spp. allergic patients (44). Of note, none of the cross-reactive recombinant pairs (rApi m 2 / rVes v 2, rApi m 5 / rVes v 3, and rApi m 12 / rVes v 6) are commercially available (with the exception of Api m 2), thus preventing physicians from identifying a primary sensitizer in cases of sensitization to those allergens (46). Conversely, the discrimination between *Vespula* spp. and *Polistes* spp. sensitization is more challenging, due to high phylogenetic overlap between the two species, for which CRD testing has proven to be less efficient (47-49). In clinical practice, assessing serum levels of Ves v 5 and Pol d 5 is considered helpful to discriminate between sensitizations, given that the levels of one recombinant allergen is at least double than the other (52,53). However, a recent study showed that such proposed ratio was less accurate than CAP-inhibition and poorly agreed with CAP-inhibition results, while a slight diagnostic improvement was obtained using Ves v 5 - Pol d 5 to total IgE ratios (54). Therefore, increasing the number of commercially available *Polistes dominula* recombinant antigens (e.g. rPol d 3) for *Vespula-Polistes* discrimination is an important asset to increase the diagnostic accuracy (55). Other diagnostic tests are also useful to discriminate between cross-reactivity and multiple sensitizations, especially when CRD results are inconclusive. While CAP-inhibition is particularly useful in discriminating *Vespula-Polistes* double sensitization (49,52,54), BAT has several other applications; in fact, it can be used also as confirmation test in case of negative or inconclusive results of conventional diagnostic tests (56,57). However, both CAP-inhibition and BAT are reserved for selected situations, since both are time consuming, expensive and performed by selected laboratories only. **Figure 1** summarizes current diagnostic algorithms to assess multiple sensitizations using CRD.

Baseline serum tryptase

During the diagnostic workup of HVA, basal serum tryptase levels should be assessed in each patient with SR, to properly identify subjects at a higher risk of developing severe reactions to stings, due to unrecognized clonal mast cell disorders. However, high tryptase levels can also be found in other conditions (e.g. hematologic malignancies, parasitic infections, end-stage chronic renal disease, aneurysms of the abdominal aorta) (58,59).
Patients with history of severe reactions upon stinging, especially if hypotensive episodes in the absence of cutaneous involvement, with increased baseline serum levels of tryptase, especially if above 25 µg/ml, are at high risk of clonal mast cell disease or

Figure 1 *- Workflow for HVA diagnosis in Apis-Vespula and Vespula-Polistes double sensitizations.*



mast cell disorders. For this reason, the validated REMA score was created to identify patients with potential mast cell-related conditions; if the score is ≥ 2, further diagnostic tests are warranted (i.e. skin inspection and biopsy, bone marrow analysis, testing for somatic c-kit mutations) (60). Of note, patients with syncope without urticaria and/or angioedema should be investigated for mastocytosis, even in in the presence of normal baseline tryptase level (61).

Practical considerations for diagnosis of HVA for everyday practice are summarized in **table II**.

Table II *- Practical considerations for the diagnosis of HVA.*

Modality	Test type	Considerations
in vivo	skin tests	- gold standard for HVA diagnosis - to avoid false negatives, to be performed at least 2 weeks after stinging, if negative repeat after 1-2 months - generally safe even in patients with mastocytosis, when performed by trained personnel in a safe environment
	prick tests	- need to be integrated with intradermal testing, even if positive
	intradermal tests	- simultaneous testing of the same concentration of more venoms is preferred, with incremental increase only if negative
in vitro	serum sIgE	- validated tests should be preferred when determining serum specific IgE to hymenoptera venoms
	CRD	- use in poly-sensitization or in case of negative tests, with suggestive history of systemic reaction
	CAP-inhibition	- useful to discriminate multiple sensitizations, if CRD results are unclear
	BAT	- highly specific diagnostic technique to be performed in selected laboratories in specific situations - controversial use in patients with mast cell disorders and negative venom sIgE
	baseline serum tryptase	- to be assessed in case of systemic reactions, especially if severe - high baseline levels in repeated measurements suggest mast cell disorders, to be further investigated
both	skin tests Serum sIgE	- no correlation with disease severity and the scores/levels - no predictive value for reactions at re-sting

Treatment and management of HVA

After an appropriate diagnosis of HVA, it is of utmost importance to provide patients both a strategic plan to manage acute reactions upon re-sting and a long-term management plan, to reduce the occurrence of severe reactions, by adopting avoidance measures and prescribing immunotherapy with specific venoms.

Self-treatment and management of acute episodes

In the management of an acute allergic reaction, it is vital that the patient, caregivers and/or parents, have been adequately informed and trained on recognizing the early signs and symptoms of anaphylaxis, on the use of self-medication treatments to be administered without any delay or hesitation, and the precautionary actions to be performed after resorting to self-treatment (10).

Self-medication is the mainstay for the treatment of acute events, since in most cases the re-sting occurs outdoors, distant to emergency departments, and the quick onset of symptoms after stinging requires immediate treatment to avoid severe, and sometimes fatal outcomes. The type of treatment may differ according to the severity of the acute allergic reaction. Onset of cutaneous systemic reactions (i.e. urticaria and/or angioedema, without any evidence of other systemic involvement) requires the administration of double dose oral anti-histamines and 4 tablets of prednisone 16 mg, or equivalent (62).

Treatment of choice for severe reactions in adults is the administration of 0.3 mg of adrenaline by intramuscular injection in the vastus lateralis muscle of the thigh (9,10,62). AAIs should be provided to any patient that experienced anaphylaxis upon stinging, although the availability and type of AAI (i.e. cartridge-based, syringe-based) might differ according to country and local regulations. Patients must be advised to bring AAIs and other rescue medications (i.e. anti-histamines and corticosteroids) along with them at all times, especially in situations at high risk of stinging (i.e. outdoor activities) or in out of reach locations, distant to emergency departments (10,62).

Current position papers and guidelines suggest the following indications for AAI prescription in adults and children, also according to treatment with VIT (9,10, 19,62,63):

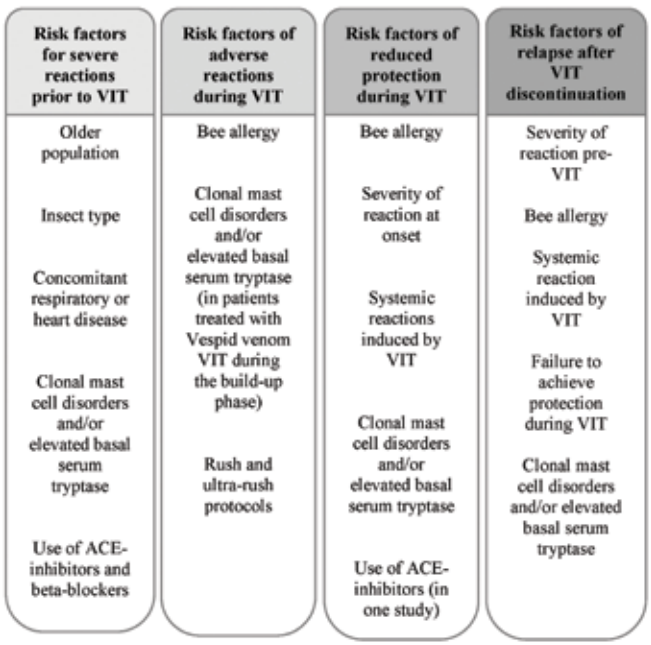
1. untreated patients: if history of systemic reactions is not limited to cutaneous involvement, or with a high risk of re-exposure to stings (i.e. occupational or recreational exposure);
2. patients treated with VIT: if risk factors of reduced protection are present (**figure 2**);

3. patients who discontinued VIT: if risk factors for incomplete protection are present (**figure 2**);
4. patients with clonal mast cell disorders and/or elevated baseline serum tryptase, regardless of VIT.

Prescription of AAI in LLR is usually not recommended (62,64). However, if patients with LLRs are at risk of multiple stings, or in case of a single reported LLR, when the severity of subsequent reactions cannot be predicted, AAIs can be prescribed (6,13).
In terms of efficacy, no major differences between different commercially available AAIs can be observed in adults (62,65,66).
Double AAIs can be prescribed, according to current EAACI position paper and guidelines, in the following situations (10,62):
1. patients living, working or performing outdoor activities in out of reach locations or distant from emergency rooms;
2. history of severe reactions, requiring multiple adrenaline administrations;
3. patients with clonal mast cell disorders and/or elevated levels of baseline serum tryptase;
4. subjects for which the available AAI dose is lower than recommended for body weight.

These indications on AAI prescription are however different to the European Medicines Agency (EMA) provisions and the American Academy of Asthma Allergy and Immunology (AAAAI) practice parameter. Both suggest to prescribe two AAIs to each HVA subject, taking into consideration several factors that might influence the correct administration of adrenaline (i.e. type of AAI, needle length, ability to follow the instructions, force required to activate the AAI, angle and pressure applied to the skin) (67,68). In children, the dose of adrenaline to be administered depends on body weight; the fixed 0.15 mg pediatric dose is reserved for children weighing less than 15 kg, while for children > 15 kg it is possible to use the adult dose, although it might sometimes be over dosed (69,70). Therefore, it is especially important in children weighing between 15 and 30 kg to dose adrenaline according to the severity of symptoms; the adult dose should be prescribed in case of previous severe symptoms, or concomitant bronchial asthma (70,71).
Delays and hesitation in treating anaphylactic episodes with adrenaline by patients have been reported, mostly out of fear of the side effects of adrenaline (72,73); stressing the importance of promptly treating SR is vital, since the known side effects of adrenaline administration (e.g. tachycardia, vasoconstriction, tremors, nervousness) are transient, and outweigh the potential risk of a fatal anaphylactic episode (74).
Prescription of AAIs to patients with heart disease undergoing treatment with beta-blockers is not contraindicated and, although beta-blockers could potentially reduce the efficacy of adrenaline in treating anaphylaxis, this reduced efficacy was not observed in patients with anaphylaxis using beta-blockers in the emergency department (28). However, given the increased risk of cardiac anaphylaxis, it is of utmost importance that such patients are also treated with VIT, to reduce overall severity of symptoms upon stinging and the need for AAIs (13). The use of AAIs is not contraindicated to treat anaphylaxis also in pregnant women (75). After resorting to self-medication, patients should be advised to call for help and immediately transported to the closest emergency department to receive care, document the event and, if available, dose tryptase levels. Patients that experienced an anaphylactic episode should be monitored from 6 up to 24 hours, depending on the severity and features of the anaphylactic episodes and treatment received, or if any comorbidities and risk factors for severity or biphasic anaphylaxis are present (9,10,13,15). Unlike corticosteroid treatment, prompt use of adrenaline to treat the anaphylactic episode seems to prevent the occurrence of biphasic anaphylaxis (14,76).

***Figure 2** - Risk factors for severe reactions in HVA before, during and after discontinuation of VIT.*



Specific Venom Immunotherapy (VIT)

To date, the only disease-modifying treatment for HVA is VIT; VIT is a safe and effective therapy, capable of inducing selective tolerance to specific venoms (protection against vespids reported in 91 - 96% of cases, 77 - 84% for bee allergy (32). Nonetheless, VIT offers long lasting protection upon re-sting even after discontinuation of treatment, and increases dramatically the quality of life of HVA patients (19,32,33,63).

VIT is currently indicated for treating the following adult and pediatric subjects:

a) history of systemic reaction involving other apparatuses besides the skin in both children and adults (32,63);
b) in adults, systemic cutaneous reactions at high risk of re-sting and/or impaired quality of life (32,33,63,77). In children, VIT is not usually recommended when only skin involvement is present, due to low risk of SR after re-sting (10%), unless the subject is at high risk of re-sting, and/or distant from emergency care facilities, and/or impaired quality of life for the patient and/or parents / caregivers (32,33,78);
c) clonal mast cell disorders with history of systemic reaction (79,80).

VIT is not indicated in subjects with history of LLRs, except for recurrent and particularly severe LLRs for which VIT might help reduce the extent of symptoms (32,81,82). VIT is also not indicated for treating toxic manifestations or unusual reactions (32,63). VIT should not be initiated during pregnancy, although it should not be interrupted in pregnant women if ongoing and tolerated (25,32).

When prescribing VIT, it is essential to choose the proper venom for each patient, by performing a correct clinical, in vivo and in vitro diagnosis. When the diagnosis is complicated due to multiple sensitization, if the discrimination of the insect is difficult, it is possible to perform VIT using multiple venoms (32).

Standard target protective dose (i.e maintenance dose) is 100 µg of venom, that can be increased up to 200 µg in specific situations, namely reduced protection after re-sting (i.e. in mastocytosis patients), or in beekeepers at risk for multiple stings with bee venom (13,32,83). To reach the maintenance dose, a build-up phase is required, during which venom extracts are administrated to both adults and children at incremental concentrations at selected intervals (19,32,63,84,85); conventional protocols require up to 15 weeks from the first administration to reach maintenance dose, while cluster, rush and ultra-rush protocols take several non-consecutive days, 3 - 5 consecutive days and 3 - 5 hours, respectively. The starting dose for the build-up phase ranges between 0.001 - 0.01 µg of venom, according to the type of protocol used, although studies reported that 1 - 5 µg of venom can also be used safely, even in rush protocols (13,19,63,86). No differences in terms of efficacy between conventional, rush and ultra-rush protocols are observed in adults and children (13,19,63,84,85). Moreover, ultra-rush protocols offer rapid protection from re-sting as early as the maintenance dose is achieved (87).

Commercially available aqueous extracts from different manufacturers are available for *Vespula* spp., *Apis mellifera* and *Polistes dominula,* while aluminium hydroxide adsorbed (depot) formulations are available only for *Vespula* and *Apis mellifera* (88).

The VIT protocol should be flexible, to accommodate both patients' and clinicians' necessities; for instance, switching from aqueous to depot formulations of the same manufacturer can be easily done, without any reduced safety or efficacy for the patient (89). In case of shortage of venom extracts, the switch to another manufacturer can be performed safely, according to a recently proposed switch protocol, using the same maintenance dose in subjects that previously tolerated a long-term VIT, while in case of documented SR during VIT, a safe option is to restart VIT from the build-up phase (90,91).

Once maintenance dose is reached, recommended administration interval is 4 weeks for the first year of VIT, and slowly increased up to 6-8 weeks (or 12 weeks, according to some authors) in the subsequent years, to maintain the achieved tolerance with no loss of efficacy over time (32,92). In case of bee allergy or mastocytosis, lengthening of dosing intervals should be performed with caution (13).

According to recent guidelines, the recommended duration of VIT is 3 - 5 years in both adults and children (32,93). It is estimated that, after the third year of VIT, 83 - 100% of patients are protected from further SR upon stinging, and such protection usually lasts for 1 - 3 years after discontinuation; however, long lasting results are more likely to be obtained after at least 5 years of treatment (32,94,95). In selected cases (i.e. very severe pre-treatment anaphylactic reactions, clonal mast cell disorders with history of SR) VIT should be continued lifelong (96).

The protection induced by VIT is also responsible for the increased perceived quality of life in treated patients, even compared to AAI prescription alone (77,97).

However, therapeutic failure in VIT might still occur, and is more frequently observed in adults rather than children (13,32,63).

Reasons for reduced protection are briefly summarized in **figure 2**. Among them, a possible reason for reduced protection is the variable amount of major specific allergenic components in venom extracts used for bee venom immunotherapy. It was demonstrated that the major allergenic molecule Api m 10 is underrepresented in several commercial extracts used for VIT, thus suggesting a reduced VIT efficacy in patients with a prevalent Api m 10 sensitization profile (98,99).

Furthermore, there may be a difference in the protective effect of *Polistes* spp. venoms according to species: venom extracts of European *Polistes dominula* show incomplete cross-reactivity with the American *Polistes*, therefore European *Polistes* extracts should be used for treating European HVA patients (100,101).

Adverse reactions during VIT are observed in around 2.8 - 5.8% patients treated for Vespid allergy and 14.2 - 28.9% of bee-allergic subjects, such reactions especially occurring during the build-up phase (1.9%) (32,63,102).

Adverse events are more frequently observed using non-purified extracts compared to purified, among which aqueous formu-

lations tend to cause more local reactions compared to depot (88,103,104).
Risk factors for SR during VIT are listed in **figure 2**.
The choice of rush and ultra-rush build-up protocols might pose some increased risks of adverse reactions according to some authors, while others report both to be even safer than conventional build-up phases (32,105-108). To minimize the risk of serious events, rush and ultra-rush protocols should be performed only by experienced centers, with access to emergency care, while conventional therapy can be safely used in an outpatient setting.
The appearance of a large local reaction at the administration site is not correlated with an increased risk of subsequent adverse events and therefore no dose adjustments are required. Conversely, the appearance of a SR requires to step down and temporarily to continue VIT with the last tolerated dose (32). Pre-treatment with anti-histamines was shown to reduce local and mild systemic adverse reactions, increasing VIT tolerability without compromising its efficacy, and is currently recommended by EAACI guidelines (32). However, expert panels suggest it as optional, due to the risk of masking warning signs of SR, especially when using rush and ultra-rush protocols (13). Omalizumab might also be used as premedication strategy in subjects experiencing SR during VIT, although its use is still off-label (32).
Treatment with VIT can be safely discontinued when both skin and serologic test are negative, although complete negative results are rarely observed (63). To date no validated tests to predict the risk of recurrence of allergic symptoms upon discontinuation are available (109,110). The decision to interrupt VIT should account for several factors, including age, quality of life, severity of allergic symptoms and presence of risk factors. Inadvertent field sting challenges offer important information on the effectiveness of VIT in preventing SR; however, they do not occur in every VIT treated patient, due to avoidance strategies, therefore the current gold standard is the sting challenge with live insects to be performed in specialized centers. The sting challenge, although useful, is a procedure that poses both ethical and management problems in some countries and is therefore difficult to perform (13).
Practical considerations for VIT in clinical practice are listed in **table III**.

Long-term management

In clinical practice it is useful, once a proper diagnosis and treatment plan is made, to re-assess HVA patients at proper intervals, to collect updated information on subsequent stings (if any), type of elicited reaction, cutaneous threshold concentrations, newly occurring sensitizations, use of AAIs and rescue medications, and compliance to treatment. It is also important to renew the prescription of adrenaline, when applicable, checking that AAI devices have not expired or stored not properly, and

Table III *- Practical considerations for venom immunotherapy.*

VIT recommended	- adults and children with HVA and systemic sting reactions, not limited to skin symptoms - adults with systemic reactions limited to skin symptoms, if high risk factors or impaired quality of life - patients with clonal mast cell disorders
VIT NOT recommended	- subjects sensitized to insect venom with no clinical symptoms upon stinging - unusual / toxic reactions, not immediate type systemic reactions - patients with active, systemic autoimmune disorders - patients with severe immunodeficiency - pregnancy (initiation of VIT)
special populations	- patients with cardiovascular disease may undergo VIT, but disease should be stabilized before initiation - high-risk HVA subjects with malignancy may undergo VIT, only if stable or in remission - patients with organ-specific autoimmune diseases should undergo VIT, only if stable or in remission - children below 5 years of age should undergo VIT, only if positive history of severe sting reactions, and if cooperative - ongoing VIT can be continued during pregnancy, if tolerated - beta blocker and ACE inhibitor therapy may be continued during VIT, but the patient should be informed about possible risks
maintenance dose	- the standard maintenance dose to be administered is 100 μg of venom. If patients still react to field stings or sting challenge, a dose increase to 200 μg of venom can be recommended
adverse reactions	- purified venom preparations have a lower frequency of local and systemic adverse events than non-purified aqueous preparations
dosing interval	VIT injections should be administered every 4 weeks in the first year of treatment, every 6 weeks in the second year, and in case of a 5-year treatment, every 8 weeks from year 3-5. In the case of lifelong therapy, 12-week intervals may be still safe and effective
duration of VIT	VIT should be performed for at least 3 years. In patients with severe initial sting reactions, at least a 5-year treatment is recommended - lifelong VIT may be recommended in highly exposed patients with bee venom allergy, patients with very severe initial sting reactions, patients with systemic side-effects during VIT, and patients with mast cell disease
risk factors	- patient-related as well as treatment-related risk factors must be taken into account, and patients with one or more risk factor should be treated and monitored with special care

also retrain patients, caregivers and/or parents on treatment and management of acute events.

Current guidelines do not specify long-term management strategies, therefore in this review we summarized the recommendations suggested by a panel of HVA experts (13). Patients not treated with VIT, who were prescribed AAIs for SR, or subjects at high risk for multiple stings or showing risk factors for relapse after VIT interruption, should be reassessed if re-stung and information on clinical history should be collected at the renewal of each AAI prescription. Subjects that were not re-stung, not treated with VIT, who were prescribed AAIs for SR, should undergo a complete re-evaluation once every two years. Conversely, subjects treated with VIT should be reassessed in case of SR after re-sting, or in scheduled clinical re-evaluations after 3 and 5 years of treatment (13). According to recent data, compliance to VIT is usually higher compared to other allergen immunotherapies; however, it should be reassessed regularly, especially if performed in different centers (111).

Conclusions

The appropriate diagnosis, treatment and management of HVA is important to modify the natural course of the disease, and increase dramatically the quality of life of affected patients. Recognizing specific risk factors for severity and treatment failure, and knowing the strengths and weaknesses of diagnostics and currently available treatments should make dealing with HVA a less daunting task.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution statement

MMB, CT, MM, SA, AC and LA reviewed literature, MBB and CT wrote the article, MMB, CT, MM, SA, AC, LA revised and approved the article.

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Assessing clinical and psychological features: who are patients showing a nocebo reaction during the drug challenge test?

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Key words

adverse drug reaction; nocebo effects; challenge test; psychological assessment; risk factors

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Doi
10.23822/EurAnnACI.1764-1489.116

Summary

The nocebo reaction, namely the undesirable effect of an inert substance (placebo), is a phenomenon rarely investigated in literature. A better knowledge of this reaction may help clinicians in the management of these patients in clinical practice. Patients with drug adverse reactions (ADR) undergoing the drug challenge test are an ideal model for studying the nocebo effect, and the study aims to investigate their clinical and psychological features. One hundred and twenty patients (M_{age} = 46.59, SD = 15.5; 82% female), of which 90 non responders and 30 with nocebo reactions (25%) were recruited, and completed a battery of psychological measures: State-Trait Anxiety Inventory X1-X2, Beck Depression Inventory II, Symptoms Checklist-90-R, Difficulties in Emotion Regulation Scale, Toronto Alexithymia Scale. Clinical features (individual characteristics and ADR clinical history) were collected by clinicians. The results show that older age (p = 0.002), low level of education (p = 0.039) and a depressive tendency (p = 0.030) appear to be potential risk factors for nocebo effects. Although none of the features related to the previous clinical history appear to represent a risk factor for the nocebo reactions (p > 0.05), significant correlations between some of the clinical and psychological characteristics considered (p values from 0.005 to 0.042) help to better delineate the profile of these reactive patients. A specific training of the sanitary team about psychological aspects is recommendable.

Introduction

The term "nocebo" was originally used to differentiate the undesirable effects following the administration of an inert substance that the patient believes to be an active drug (during both pharmacological treatment and challenge test experimental studies). It is defined as the negative equivalent of the known placebo phenomenon. The nocebo reaction usually occurs in a subjective way with nonspecific symptoms (gastrointestinal symptoms, dizziness, headache, itching, paresthetic and thermal sensations), but sometimes also with objective signs (cutaneous, respiratory, gastric, cardiac rhythm or blood pressure changes). Like the placebo effect, the nocebo effect can have an important impact on the clinical practice and the outcome of clinical trials (1,2,3,4,5).

Patients with adverse drug reactions (ADR) are an ideal model for studying the nocebo effect, because their previous experience can generate a negative expectation conditioning their acceptance and results of subsequent therapies. In many ADR cases, the allergy diagnostic workup includes the systemic challenge (oral or parenteral), to confirm the responsibility of a drug in the reaction and to identify alternative drugs that can be safely used (6). The experience of allergists is that some patients may show negative reactions to the administration of an inert substance (placebo) which usually precedes the active drug. The practice using placebo has the purpose to better evaluate the test results, evidencing a possible adverse reaction - the nocebo effect - that is reported by literature in percentages ranging from 3% to 27% (7,8,9). Lombardi and colleagues (8) stressed that the

quality of medical-patient communication, and the empathetic approach by medical and nursing staff could be associated with a lower frequency of nocebo reactions.

Therefore, it is important to improve the clinical practice checking whether there are variables regarding the type of reactions experienced by patients, or particular psychological features enabling specialists to identify such subjects before submitting them to challenge tests (10). This may require specific training of specialists, but could be useful to increase the reliability of the allergy diagnostic workup.

The present study aims to investigate the clinical (individual characteristics and ADR clinical history) and psychological features (anxiety, depression, psychological symptoms, emotion dysregulation, and alexithymia) of patients with nocebo effect to oral challenge test compared with patients without reactions. At the exploratory level, the association between clinical and psychological features in nocebo patients are also investigated, to better delineate the profile of the reactive patients.

Materials and methods

Among all the patients with a clinical history of ADR, 120 were recruited consecutively from the Allergy Unit of the San Martino IST University Hospital (Genoa, Italy) in the first months of 2016, because they needed to be submitted to challenge test. The allergist proposed and asked the patient consent to take part in the study. This study was conducted following the ethical standards established in the Declaration of Helsinki of 1946. Based on the clinical features of the ADR, the allergist decided the diagnostic workup in agreement with the patient, including the oral challenge test for one or more drugs. In the first day, after clinical evaluation of the patient including heart rate, blood pressure, and respiratory function, four doses of a placebo (water or talc) were administered at 30' intervals in a single-blind way. The patient was observed for one hour after the last dose and re-evaluated by the sanitary team before leaving the hospital. The day after, before the active drug administration, patients were required to report any symptoms at home. At the beginning of the test, a battery of psychological questionnaires was proposed by the psychologists to the patient, with the task of completing them by the first day.

Two groups of patients, negative (n = 90) and positive (n = 30) for nocebo effects occurring after administration of the "placebo" were selected and compared in this study. Socio-demographic features, associated pathologies and clinical characteristics of the ADR were analyzed (i.e. number of reactions, number and class of drugs involved, timing and severity of symptoms, emergency services access, history compatible with drug hypersensitivity diagnosis). The battery of psychological self-report administered (validated in Italian context) are summarized in **table I**.

Table I *- Battery of psychological questionnaire for psychological assessment.*

Measure	Questionnaire (authors)	Number of items	Subscales
anxiety	state-trait anxiety inventory X1-X2 (CBA, 12)	20 20	state anxiety (STAI-X1) trait anxiety (STAI-X2)
depression	Beck depression inventory II (BDI, 13)	21	total score
psychological symptoms	symptoms checklist-90-R (SCL-90-R, 14)	90	somatization (SOM) obsessive-compulsive (O-C) interpersonal sensitivity (I-S) depression (DEP) anxiety (ANX) anger hostility (HOS) phobic anxiety (PHOB) paranoid ideation (PAR) psychoticism (PSY) global severity index (GSI)
emotional regulation-dysregulation	difficulties in emotion regulation scale (DERS, 15)	36	non-acceptance of emotional response (Non-acceptance) difficulties engaging in goal-directed behavior (Goal) impulse control difficulties (Impulse) lack of emotional awareness (Awareness) limited access to emotion regulation strategies (Strategies) lack of emotional clarity (Clarity) total score (DERS Total)
alexithymia	Toronto alexithymia scale (TAS-20, 16)	20	difficulty in identifying feelings (DIF) difficulty in describing feelings (DDF) external oriented thinking (POE) total score (TAS-20 Total)

Clinical data collected by the clinicians in a data sheet were analyzed in aggregated form using the Statistical Package for Social Science (Version 23.0; IBM Corp., Armonk, NY, USA). Chi square test and independent-sample t test were used to compare the clinical and psychological features of two groups. The multivariate analysis was used to control the effect of socio-demographic data differing two groups. Point biserial correlations were used to compare the association between nominal (clinical data) and quantitative variables (psychological measures). The level of significance for all analyses was $p < 0.05$.

Results

One hundred and twenty patients (M_{age} = 46.59, SD = 15.5; 82% female) evaluated for an ADR clinical history completed the allergy and psychological tests. A nocebo effect was observed in thirty patients (25%), of which 18 complained symptoms during or within one hour after the last dose of placebo administration and 12 reported reactions later, at home. Most of the symptoms were subjective, with a few cases of objective reactions: 27% skin symptoms (itching, burning sensation, paresthesia), 33% neurological symptoms (agitation, tremors, dizziness, headache), 20% gastrointestinal symptoms (nausea, abdominal pain, diarrhea), 10% respiratory symptoms (dyspnea, laryngeal obstruction sensation), 10% cardiovascular symptoms (hypo or hypertension, tachycardia). Some patients complained more than one symptom, in four cases both in hospital than later, at home. In almost all cases the reactions were mild and patients immediately evaluated by the responsible allergist. Only one patient refused to continue the test, the day after, with active drug.

Comparing patients with nocebo reactions (n = 30) with non responders (n = 90) respect to some socio-demographic data and associated pathologies (**table II**), significant differences on age (nocebo group: M_{age} = 54.20, SD = 12.77; non responders group: M_{age} = 44.06, SD = 15.56; $t_{(118)} = 3.22$, $p = 0.002$) and educational level (nocebo group: 33% had the junior high school license; non responders group: 16% had the junior high school license; $X^2_{(2)} = 6.47$, $p = 0.039$) were found. The two groups differed only marginally for gender (nocebo group: 93% female; non responders group: 78% female; $X^2_{(1)} = 3.64$, $p = 0.057$). No significant difference resulted for associated pathologies, including atopy.

Table III shows the clinical features (i.e. number of drug reactions, number of implicated drugs, timing of reactions, symptoms, severity of reactions, emergency services access, and compatible diagnosis of drug hypersensitivity) comparing patients with nocebo reactions with non responders. No significant difference was found ($p > 0.05$).

In **table IV** are shown the results of psychological assessment (i.e. anxiety, depression, psychological symptoms, emotional regulation-dysregulation, and alexithymia), comparing patients with nocebo reactions with non responders. The only significant difference (even if minimal) between the two groups was found on SCL-90-R Depression ($t_{(118)} = 2.19$, $p = 0.030$), showing higher level of depression symptoms in nocebo patients (M = 2.00, SD = 0.88) than in non responders (M = 1.69, SD = 0.58).

Table II *- Socio-demographic data and associated pathologies of patients.*

		Patients with nocebo reactions (n = 30)	Non responders (n = 90)	Statistics
Gender (%)	female	93	78	$X^2_{(1)} = 3.64$, $p = 0.057^2$
	male	7	22	
Mean age (SD)		54.20 (12.77)	44.06 (15.56)	$t_{(118)} = 3.22$, $p = 0.002^1$
Educational level (%)	junior high school license	33	16	$X^2_{(2)} = 6.47$, $p = 0.039^1$
	high school diploma	40	65	
	degree	27	19	
Association with other pathology (%)	yes	40	42	$X^2_{(1)} = 0.046$, $p = 0.831$
	no	60	58	
Associations with atopy (%)	yes	30	30	$X^2_{(1)} = 0.00$, $p = 1.000$
	no	70	70	

$p < 0.05^1$; $p < 0.10^2$

Table III *- Clinical features of ADR patients: the comparison between patients with nocebo effects and non responders.*

		Patients with nocebo reactions (n = 30)	Non responders (n = 90)	Statistics
Number of drug reactions (%)	1	20	25.6	$X^2_{(1)} = 0.38$, p = 0.538
	> 1	80	74.4	
Number of implicated drugs (%)	1	43.3	55.6	$X^2_{(1)} = 1.35$, p = 0.246
	> 1	56.7	44.4	
Timing of reactions (%)	immediate	63.3	46.7	$X^2_{(2)} = 5.30$, p = 0.071
	not immediate	13.3	35.6	
	both	23.3	17.8	
Symptoms (%)	mono-symptomatic	80	62.9	$X^2_{(1)} = 2.97$, p = 0.085
	multi-symptomatic	20	37.1	
Severity of reactions (%)	mild	37.9	29.9	$X^2_{(2)} = 0.65$, p = 0.722
	moderate	48.3	54	
	severe	13.8	16.1	
Emergency services access (%)	yes	50	51.7	$X^2_{(1)} = 0.02$, p = 0.873
	no	50	48.3	
Compatible drug hypersensitivity diagnosis (%)	probable	86.2	85.4	$X^2_{(1)} = 0.01$, p = 0.914
	not probable	13.8	14.6	

Table IV *- Values and differences in psychological data resulting from the specific questionnaires.*

		Patients with nocebo reactions M (SD)	Non responders M (SD)	Statistics t (118)
CBA	State Anxiety	46.21 (33.27)	40.83 (28.58)	0.86
	Trait Anxiety	37.52 (28.36)	37.55 (26.60)	-0.01
Beck-II	Depression	11.60 (10.30)	7.96 (7.83)	2.03
SCL-90-R	SOM	1.96 (0.67)	1.67 (0.55)	2.34
	O-C	1.83 (0.67)	1.70 (0.53)	1.05
	I-S	1.68 (0.69)	1.54 (0.54)	1.14
	DEP	2.00 (0.88)	1.69 (0.58)	2.19[1]
	ANX	1.78 (0.73)	1.57 (0.53)	1.68
	HOS	1.67 (0.65)	1.48 (0.50)	1.72
	PHOB	1.32 (0.68)	1.25 (0.42)	0.68
	PAR	1.88 (0.71)	1.68 (0.58)	1.56

Table IV *(continued)*

		Patients with nocebo reactions M (SD)	Non responders M (SD)	Statistics t (118)
	PSY	1.40 (0.54)	1.29 (0.33)	1.31
	GSI	1.77 (0.60)	1.58 (0.42)	1.91
DERS	Non-Acceptance	12.27 (5.13)	12.49 (4.99)	-0.21
	Goals	13.10 (3.27)	13.29 (3.60)	-0.25
	Impulse	11.43 (2.86)	11.61 (3.14)	-0.27
	Awareness	23.43 (4.41)	22.67 (3.55)	0.96
	Strategy	16.87 (5.22)	15.81 (4.01)	1.15
	Clarity	12.80 (1.19)	13.20 (1.36)	-0.14
	Total	89.90 (15.29)	89.07 (13.03)	0.29
TAS-20	DIF	13.00 (5.46)	13.29 (5.23)	-0.26
	DEF	13.00 (4.50)	12.42 (3.25)	0.76
	POE	27.07 (3.61)	26.82 (3.73)	0.31
	Total	106.60 (14.43)	104.32 (14.98)	0.73

[1]$p < 0.05$

To control age and educational level differences on psychological subscales scores between patients with nocebo group and non responders, multivariate analysis was applied. Findings showed no significant effect for educational level and for group $F < 1$, but significant effect for age, $F (1, 119) = 1.88$, $p = 0.018$, $eta^2 = 0.32$. Significant interaction between educational level and group was found, $F (1, 119) = 0.51$, $p = 0.025$, $eta^2 = 0.28$. At the exploratory level, the association between clinical and psychological features on nocebo group was analyzed. Significant correlations are shown in **table V**. Emergency services access (0 = access, 1 = non access) are negatively correlated with trait anxiety of CBA ($r_b = -0.437$, $p = 0.016$), and with TAS total ($r_b = -0.447$, $p = 0.013$). The time of previous drug reactions (0 = immediate; 1= non immediate reactions) is positively correlated with various subscales of DERS (Non-acceptance, Impulse, Strategy, DERS Total) and SCL-90-R subscales (SOM, O-C, DEP, HOS, GSI) with p values from 0.042 to 0.005.

Discussion

This is one of the few studies focused on patients with nocebo reactions to the placebo administration during the pharmacological challenge. The result as frequency of patients with nocebo reactions (25%) is in line with other studies (7,9), and higher than that of 3% reported and attributed by Lombardi (8) to the absence of cases of severe reactions in his sample of patients.
As in other studies, the nocebo symptoms were subjective and of mild severity in almost all cases, such as not able to hinder the continuation of the test after clinical examination, but perceived as troublesome by patients. Notably, more than one-third of responders experienced reactions after several hours at home, despite a history of previous immediate drug reactions. The remainder complained about immediate symptoms or within one hour from the last dose of placebo administered in the hospital. This is not comparable with other studies, but deserves attention because it could be a key factor for the responders and have relevant practical implications, as the need to instruct the patient about

Table V *- Point biserial correlations between clinical and psychological data in nocebo patients.*

		The time of reactions	Healthcare service
CBA	Trait Anxiety	-	-0.437[1]
SCL-90-R	SOM	0.452[1]	-
	O-C	0.566[2]	-
	DEP	0.506[1]	-
	HOS	0.550[2]	-
	GSI	0.487[1]	-
DERS	Non-acceptance	0.467[1]	-
	Impulse	0.507[1]	-
	Strategy	0.428[1]	-
	Total	0.470[1]	-
TAS-20	Total	-	-0.447[1]

[1]$p < 0.05$; [2]$p < 0.01$

their possible appearance and their management. In any case, interpretation and discussion of the reactions with the patient may represent a problem for the clinicians and nurses involved.

The analysis of the socio-demographic data shows a prevalence of the female gender in line with the literature (7) in the nocebo group compared to non responders. Age and level of education are variables not previously reported as influential, while in our study higher age and lower level of education characterize the responders.

Considering that patient's expectation and previous experiences of untoward reactions to drugs are the main factors influencing the nocebo effect (2,3), some of the clinical features of the previous drug reactions can be assumed as risk-factors. In our study, although 80% of responders reported more than one ADR, predominantly immediate (over 60%), with compatible symptoms of hypersensitivity to drugs, and of moderate-severe degree (in 62% of cases), no significant difference has been demonstrated between the two groups.

According to the aim of this work to outline the profile of responders, various psychological variables were also analyzed (i.e. anxiety, depression, psychological symptoms, emotional regulation-dysregulation, and alexithymia). The only factors associated in the literature to the nocebo phenomenon are somatization tendency, anxiety and depression (8,11), the latter suggested as a general feature of the ADR population (10). In our study, the only datum that seems to delineate the psychological profile of responders (controlling the effects of age and educational level) is the presence of depressive symptoms that confirm the data of the literature, while anxiety and somatization tendency, are not confirmed. These findings could reinforce the key-role that other factors, as negative expectation and pavlovian conditioning process with the consequent involvement of neurobiological mediators, play in nocebo reactions, as evidenced by studies of Benedetti (17) and Colloca (3). A more detailed analysis of the individual variables, however, shows that higher levels of trait anxiety and alexithymia appears to be associated with more frequent access to emergency services. In other words, this datum suggests that anxiety and alexithymia are individual features making the nocebo patients more vulnerable to call for help. Besides, psychological symptoms and emotional dysregulation appear to be associated with the late-onset drug reactions. This could be interpreted with the greater vulnerability of nocebo patients to psychological discomfort, and with the greater difficulty of these patients to acceptance, evaluation and dealing with the drug reactions.

Conclusions

Despite the limitations of the study, such as the low sample size and the use of self-report measures, the findings seem to be clinically relevant. Female sex, older age and low level of education combined with a depressive tendency appear to be potential risk factors for nocebo effects appearing during oral challenge test in one among four patients. However, none of the features related to the previous clinical history of ADR appear to be associated with the possibility of nocebo reactions. Although various psychological features do not seem to outline a typical profile of responders, some of these patients show psychological symptoms and emotional problems significantly associated with the time of previous drug reactions and with the use of emergency services. In view of this, the training of the sanitary team dedicated to pharmacological challenges must include the psychological aspects (18). The verbal communication between health caregivers and patient, the patient education with respect to possible reactions, the understanding of what the patient needs to know about adverse effects, and the general clinical context are key factors for a proper assessment of this diagnosis, burdened with time and human resources high costs (5,11).

Aknowledgements

We thanks the doctors S. Ravazza, A. Rametti, and D. Scopece for their help with data collection.

Conflict of interest

The authors declare that they have no conflict of interest.

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Occupational allergy to dog among police dog trainers

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Key words
animal allergy; atopy; skin prick test; allergic rhinitis; sensitization

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Doi
10.23822/EurAnnACI.1764-1489.102

Summary
This study was aimed to reveal the prevalence of dog allergy and other common allergy and allergic symptoms in police dog trainers. Fifty-six police dog trainers and 150 workers as control group were included in this study. Medical records of dog trainers including respiratory, skin, eye symptoms and physical examinations and skin prick test results are compared with the medical records of control group. Positive SPT to dog was present in 21.4% of dog trainers, whereas the frequency of sensitization to dog in the control group was 1.3% ($p < 0.001$). Dog allergy development risk is found 20 times greater in dog trainers than control group. In multiple logistic regression analysis, it was found that atopy was associated with dog allergy likelihood. Sensitization to dog allergens is an important occupational problem for dog trainers.

Introduction

Dog allergy is a worldwide problem that affects 5 - 10% of the adult population and is a common cause of asthma and allergic rhinitis (1-3). Animal allergy as an occupational hazard was reported especially in animal laboratory workers. There are few studies on occupational dog allergy. The respiratory and cutaneous allergic symptoms in occupations that are exposed to animal proteins have been reported particularly in veterinarians, veterinary technicians, animal laboratory workers and pet shop workers (4-8). The main sources of mammalian allergens are hair, dander, saliva and serum (9-10).

Allergy to mammals is usually caused by recurrent contact with mammalian allergens. It was determined that 70% of laboratory workers have developed allergies to animals in 2 - 4 years after exposure. In case of prolongation of exposure, one third of sensitized individuals could develop occupational asthma (11). In this situation atopy is a significant risk factor. Atopy is defined as an increased propensity to mount an IgE antibody response to low-dose environmental aeroallergens. Atopy is generally established by detection of IgE antibodies to common environmental allergens, such as pollen and house dust mite.

In the literature, dog allergies have been reported among pet shop workers, veterinarians, workers in animal hospital, in animal shelters, and animal caretakers (12-16). There is no occupational allergy described in the literature in the profession group of police dog trainer.

In this article, we aimed to reveal the prevalence of allergic diseases in police dog trainers. We also evaluated allergic symptoms, skin prick test results, dermatological, respiratory system findings of police dog trainers. In addition, we investigated factors that were associated with the presence of allergy among these participants. As a result of this study, we aimed to find out whether there is a need for preventive programs against allergic

and respiratory diseases among this occupational group in Turkey, that is a country with a low pet-keeping rate.

Materials and methods

Study design and participants

This study was conducted in Ankara Occupational Diseases Hospital. In this hospital, different occupational groups are routinely examined at certain times. Fifty-six police dog trainers and 150 workers as control group were included in this study. Non-animal workers were selected as a control group from 5 different occupations (indoor workers). Medical records of dog trainers including respiratory, skin, eye symptoms and physical examinations, and skin prick test results were compared with the medical records of control group. The study was carried out in accordance in the Declaration of Helsinki. Institutional ethic committee approved the study and written informed consent was obtained from all participants. There were no subjects that had a dog as a pet at any time. Exclusion criteria of the study were taking antihistamine drugs in 15 days prior to hospital visit, severe common cold, dermatographism, and pregnancy.

Clinical history and examination

From each participant, we obtained demographic details, smoking history, family history of atopy (at least one parent or sibling), detailed information about animal contact, occupational and non-occupational symptoms, pets at home and animal contact during previous jobs or education, and medical and occupational history. Rhinorrhea, sneezing and nasal congestion were considered as allergic rhinitis; cough, wheezing and shortness of breath were considered as pulmonary symptoms; itchy rash and urticaria were considered as skin symptoms; and eye itching and redness were considered as conjunctivitis. Symptoms were considered as work-related if they started after exposure to dogs at work in dog trainers' group.

Skin prick testing

Skin prick tests (SPT) were performed using a common panel including feather mix, cat epithelia, dog epithelia, cow epithelia, goat epithelia, poultry, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria*, *Aspergillus fumigatus*, tree and weed mix pollens, ash (*Fraxinus excelsior*), walnut, willow tree (*Salix caprea*), poplar (*Populus alba*), beech (*Fagus silvatica*), pine tree, latex, wheat, cockroach allergen extracts, a positive control (histamine, 10 mg/mL), and a negative control (Allergopharma, Stockholm, Sweden). Allergens were applied on the volar side of the forearm using lancets. Skin prick test results were read after 15 minutes, and were considered positive if the largest wheal diameter was at least 3 mm and surrounded by erythema. Additionally, results of the negative control test were considered negative when the wheal diameter was less than 1 mm in the absence of erythema.

Statistical analyses

Data were analyzed using the SPSS version 21.0 software program (Statistical Package for Social Sciences v.21, IBM, Chicago, IL). Pearson chi square test and Fisher's exact test were, where appropriate, used to investigate the association between categorical variables. The Student t test was used to compare continuous numerical variables between groups. To analyze risk of group, odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated for each allergen in SPT. To predict skin prick test positivity to dog allergen, binary logistic regression was used for multivariate analysis of all potential predictors associated with sensitization to dog. All variables were forced to enter the equation in regression models.

Results

General data

This study included 206 subjects, including 56 in the dog trainer group and 150 in the control group. There was no difference in age between groups (p 0.835). There was no difference in the proportion of female proportion between groups (p 0.295). Characteristics of the study population are shown in **table I**.

Control group characteristics

Of the control group (n = 150), 10 (6.6%) were female and 140 (93.3%) were male. The mean age of control group was 33.18 years (standard deviation SD ± 14.83; min - max 18 - 75 years). The current smoking rate was 21.3%. Subjects in control group worked at 5 different facilities (indoor workers), and their workplaces were free of exposure to animals. No worker worked in outdoor work.

Of the control group (n = 150), 44 (29.3%) subjects reported having rhinitis, 19 (12.6%) reported skin symptoms, 15 (10%) reported conjunctivitis, 6 (4%) reported ever having asthma.

Of the control group (n = 150), 31 (20.6%) subjects were sensitized to at least 1 common allergen in skin prick test. A summary of the skin prick test results of the subjects is given on the **table II**.

Dog trainer group characteristics

Fifty-six dog trainer were examined. Of these 56 subjects, 1 (1.7%) was female and 55 (98.2%) were male. The mean age of dog trainer group was 33.6 years (SD ± 6.37, min - max 25 - 52 years). The current smoking rate was 10.7%. The mean

Table I *- Characteristics of the study population.*

	Dog trainer group (n = 56)	Control group (n = 150)	p
characteristics of the population			
age (y), mean ± SD (min - max)	33.6 ± 6.37 (25 - 52)	33.18 ± 14.83 (18 - 75)	0.835[1]
sex (female/male)	1/55	10/140	0.295[2]
data from clinical history			
smoking, yes (%)	6 (10.7%)	32 (%21.3)	0.080[2]
rx smoker	12 (21.4%)	44 (29.3%)	0.257[2]
family history of atopy, n (%)	17 (30.3%)	31 (20.61%)	0.143[2]
time of dog work, year ± SD (min - max)	6.02 ± 5.82 (0.5 - 20)	-	
pet seeing (any kind of pets at home)			
bird in the home	4 (7.1%)	9 (6.0%)	0.764[2]
cat in the home	2 (3.5%)	12 (8.0%)	0.261[2]
allergic symptoms			
rhinitis	39 (69.6%)	44 (29.3%)	**< 0.001**[2]
rhinoconjunctivitis	7 (12.5%)	15 (10%)	0.605[2]
allergic skin symptoms	13 (23.2%)	19 (12.6%)	0.063[2]
asthma	1 (1.7%)	6 (4%)	0.435[2]
work related symptoms	19 (33.9%)	0 (0%)	**< 0.001**[2]

[1]Student t test, [2]Pearson chi square test.

Table II *- The comparison of dog trainer group and control group in terms of the results of SPT.*

Skin prick test	Dog trainer group (n = 56)	Control group (n = 150)	p value[1]	OR[1]	95% CI[1]
dog	12 (21.4%)	2 (1.3%)	**< 0.001**	20.18	4.35 - 93.60
feather	1 (1.7%)	0 (0%)	0.272	0.982	0.94 - 1.01
cat	10 (17.8%)	9 (6%)	**0.009**	3.406	1.30 - 8.89
cow	1 (1.7%)	0 (0%)	0.272	0.982	0.94 - 1.01
poultry	2 (3.5%)	3 (2%)	0.615	1.815	0.29 - 11.15
goat	3 (5.3%)	1 (0.6%)	0.062	8.434	0.85 - 82.85
Der p	7 (12.5%)	6 (4%)	**0.047**	3.429	1.09 - 10.69
Der f	5 (8.9%)	6 (4%)	0.174	2.353	0.68 - 8.04
Alternaria	6 (10.7%)	5 (3.3%)	0.073	3.480	1.01 - 11.90
Asp fum	4 (7.1%)	1 (0.6%)	**0.020**	11.462	1.25 - 104.89
tree pollen	2 (3.5%)	1 (0.6%)	0.180	5.519	0.49 - 62.09
weed	13 (23.2%)	14 (9.3%)	**0.018**	2.937	1.28 - 6.72
ash	6 (10.7%)	8 (5.3%)	0.213	2.13	0.70 - 6.44
walnut	5 (8.9%)	3 (2%)	**0.036**	4.80	1.10 - 20.81
willow	4 (7.1%)	2 (1.3%)	**0.048**	5.69	1.01 - 31.99
poplar	1 (1.7%)	1 (0.6%)	0.471	2.709	0.16 - 44.06
beech	2 (3.5%)	1 (0.6%)	0.180	5.519	0.49 - 62.09
pine	5 (8.9%)	0 (0%)	**0.001**	0.911	0.83 - 0.98
latex	2 (3.5%)	0 (0%)	0.073	0.964	0.91 - 1.01
wheat	2 (3.5%)	2 (1.3%)	0.298	2.741	0.37 - 19.94
cockroach	4 (7.1%)	2 (1.3%)	**0.048**	5.692	1.01 - 31.99

Der p, *Dermatophagoides pteronyssinus*; Der f, *Dermatophagoides farinae*; Asp fum, *Aspergillus fumigatus*; OR, odds ratio; CI, confidence interval. [1]Odds ratio chi square test.

working duration was 6.02 years (SD; ± 5.82, min - max 0.5 - 20 years).

Allergic symptoms were mainly reported by dog trainers. Of the dog trainers (n = 56), 35 (62.5%) reported ever having rhinitis, 13 (23.2%) reported skin symptoms, 7 (12.5) reported ever having conjunctivitis, 1 (1.7%) reported ever having asthma. 6 (10.7%) dog trainers reported work related symptoms. The distribution of symptoms according to the presence or absence of dog allergy is given in **table III**.

Of the dog trainers (n = 56), 37 (66%) were sensitized to at least 1 common allergen in skin prick test. Of the sensitized subjects (37 cases), 1 (1.7%) was sensitized only to dog allergen. Twelve subjects were sensitized to dog allergen. There was cat-feeding history in two participants' report. One of these participants had a positive SPT for cat. But no participant reported ever seeing dog in his or her homes. A summary of the skin prick test results of the subjects is given on **table II**.

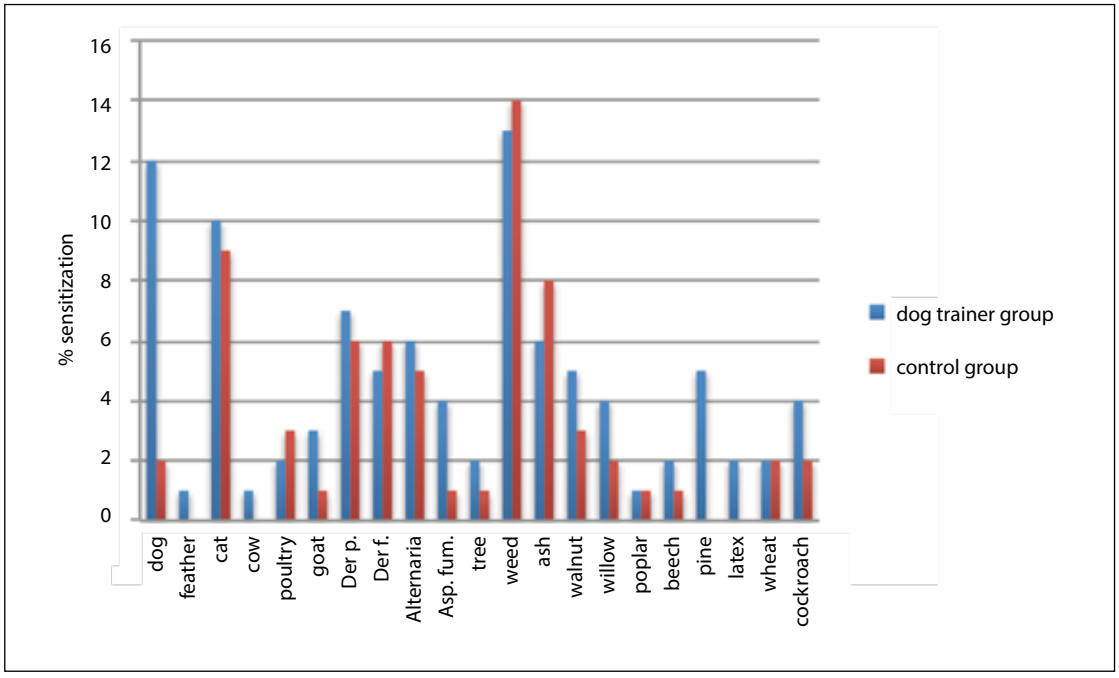
Table II and **figure 1** are showing the prevalence of positive skin prick test to common allergens in the dog trainer group and the control group. A positive SPT to dog was observed in 21.4% of dog trainers, whereas the frequency of sensitization to dog in the control group was 1.3% ($p < 0.001$, odds ratio OR 20.18, 95% CI 4.35 - 93.60). Dog allergy development risk is found 20 times greater for dog trainers than control group.

Table III is showing comparison of characteristics of the dog trainer with and without dog allergy. Contrary to expectation, there was no statistically significant difference between the subjects with and without family history of atopic disorders in terms of sensitization to dog. Only rhinitis symptom was statistically more significant in the subjects with sensitization to dog, while the other allergic symptoms were not. Reporting work-related allergic symptoms was related to positive skin prick test results to dog allergens by 83.3%. Two dog trainers with positive dog allergen SPT reported no clinical symptoms after exposure to dogs. There was no statistically significant difference between individuals with and without dog allergy in terms of accompanying allergy other than aspergillus fumigatus allergy.

Multiple logistic regression

A logistic regression was performed to ascertain the effects of age, smoking status, pet keeping, working duration, family history of atopic disorders and skin prick test positivity (against allergens other than the dog allergen) on the likelihood that dog trainers have dog allergy. The logistic regression model was statistically significant, $p = 0.039$. The model explained 37.0% (Nagelkerke R^2) of the variance in the dog allergy, and correctly classified 85.7% of cases. Skin prick test positivity (against aller-

Figure 1 *- The rate of sensitization against 21 common allergens in dog trainer group and control group.*



Der p, *Dermatophagoides pteronyssinus*; Der f, *Dermatophagoides farina*; Asp fum, *Aspergillus fumigatus*.

Table III *- Comparison of the dog trainer with and without dog allergy in dog trainer group.*

	Dog trainer group (56)		
	dog allergy + (n = 12)	**dog allergy - (n = 44)**	**p values**
age, years (± SD)	32.08 ± 4.87	34.02 ± 6.71	0.355[1]
sex (male)	12 / 12	43 / 44	0.786[2]
smoking			
current smokers, n (%)	2 (16.6%)	4 (9.0%)	0.599[2]
ex smokers, n (%)	3 (25.0%)	9 (20.4%)	0.734[3]
pet seeing			
bird in the home	1 (8.3%)	3 (6.8%)	0.630[2]
cat in the home	1 (8.3%)	1 (2.2%)	0.386[2]
skin prick test positivity (another allergy from dog allergy)	11 (91.6%)	25 (56.8%)	**0.026**[3]
family history of atopic disorders	4 (33.3%)	13 (29.5%)	0.529[3]
working years, (mean ± SD)	3.9 ± 4.94	6.6 ± 5.96	0.159[1]
symptoms			
rhinitis	11 (91.6%)	28 (63.6%)	**0.061**[3]
rhinoconjunctivitis	0 (0%)	7 (15.9%)	0.140[3]
allergic skin symptoms	2 (16.6%)	11 (25.0%)	0.544[3]
asthma	1 (8.3%)	0 (0%)	0.214[2]
work related symptoms	10 (83.3%)	9 (20.4%)	**< 0.001**[3]
SPT positivity, n (%)			
feather	0 (0%)	1 (2.2%)	0.786[2]
cat	4 (33.3%)	6 (13.6%)	0.114[3]
cow	1 (8.3%)	0 (0%)	0.214[2]
poultry	0 (0%)	2 (4.5%)	0.614[2]
goat	1 (8.3%)	2 (4.5%)	0.522[2]
Der p	3 (25%)	4 (9.0%)	0.326[2]
Der f	3 (25%)	2 (4.5%)	0.060[2]
Alternaria	2 (16.6%)	4 (9.0%)	0.599[2]
Asp fum	4 (33.3%)	0 (0%)	**0.001**[2]
tree pollen	0 (0%)	2 (4.5%)	0.614[2]
weed	3 (25%)	10 (22.7)	0.869[3]
ash	3 (25%)	3 (6.8%)	0.105[2]
walnut	2 (16.6%)	3 (6.8%)	0.289[2]
willow	1 (8.3%)	3 (6.8%)	0.630[2]
poplar	0 (0%)	1 (2.2%)	0.786[2]
beech	0 (0%)	2 (4.5%)	0.614[2]
pine	2 (16.6%)	3 (6.8%)	0.289[2]
latex	0 (0%)	2 (4.5%)	0.614[2]
wheat	1 (8.3%)	1 (2.2%)	0.386[2]
cockroach	0 (0%)	4 (9.0%)	0.567[2]

[1]Student t test; [2]Fisher's exact test; [3]Pearson chi square.

gens other than the dog allergen) was associated with dog allergy likelihood; age, smoking, cat keeping, bird keeping, working duration and family history of atopic disorders were not associated with dog allergy likelihood. The subjects with positive skin prick test against allergens other than the dog allergen were 27.81 times more likely to exhibit dog allergy than the subjects with negative skin prick test (95% CI 1.630 - 474.847, p 0.022). Having pets other than the dog was not associated with positive skin prick test to dog (**table IV**).

Discussion

This study aimed to reveal the prevalence rate of allergic diseases among police dog trainers by using skin prick test. It has been estimated that sensitization to dog confirmed by skin prick test can cause rhinitis, eczema and asthma (17). Skin prick testing (SPT) is informative and safe for detecting IgE-mediated allergen sensitization. No subject kept dogs at home in the past and current. For this reason, the potential confounder of keeping dog at home was excluded. Dogs were living always in the stations and trainers were not allowed to take dogs to their homes, and trainers were spending time with dogs only in the workplace. Thus, this study reflects the real effect of workplace exposure on the development of dog sensitivity. This is the first study investigating work-related symptoms and allergic sensitivity in dog trainers.

Table IV *- Multivariate analysis (logistic regression) of factors for development of sensitization to dogs.*

Risk factor	OR	95% CI	p value
age	0.91	0.734 - 1.149	0.458
smoking	0.50	0.020 - 12.415	0.674
working duration	1.17	0.918 - 1.503	0.201
pet seeing			
bird in the home	14.417	0.367 - 565.830	0.154
cat in the home	0.624	0.013 - 30.060	0.812
family history of atopic disorders	0.35	0.062 - 2.002	0.239
skin prick test positivity (another allergy from the dog allergy)	27.81	1.630 - 474.847	**0.022**

OR, odds ratio; CI, confidence interval.

In this study it was found that sensitization to dog allergens was higher among dog trainers (21.4%) than control group (1.3%). Krakowiak et al. found allergies to animals (dog, cat, rat, mouse, rabbit, guinea pig and hamster) in 26% of zoo workers (18). In many studies, it has been determined that animal workers have an increased risk of animal allergy (11,15,19,20). Current study recommend that police dog trainers should also be accepted as animal workers in terms of allergy because they spend nearly all of their work time with dogs. Airborne dog allergens can be deposited in the workplace (21). Additionally, dog saliva is an allergen source for dog allergy. There is variability between the IgE-binding protein profiles of saliva from different dogs (22). It has been found there are at least 12 protein bands in dog saliva that can be recognized by IgE of dog-allergic patients. Also, it has been determined that there is a great variation in the IgE-binding profile, when investigating saliva from different dog breeds. On account of this, contact with many dogs and different breed dogs can increase the likelihood of allergy.

Other than dog allergies, weed was the allergen with the highest prevalence of sensitization among the dog trainers. Frequency of sensitization to weed differed significantly between dog trainers and controls (23.2% versus 9.3%). Also, sensitization to cat, *Dermatophagoides pteronyssinus*, *Aspergillus fumigatus*, walnut, willow, pine, and cockroach were significantly more frequent in dog trainers than controls (**table II**). Allergenic cross-reactivity between dog and cat was explored (23). An increased risk of sensitization to dogs 20.1-fold, to *Dermatophagoides pteronyssinus* 3.4-fold, and to *Aspergillus fumigatus* 11.4-fold was found in dog trainers group. There are also endotoxin or other microbial agent exposures from dogs. It has been found that mites feed on animal scales, so sensitization to mite allergens may be due to occupational factors (21). Also, dog trainers had a 4.8-fold increased risk of sensitization to walnut, a 5.6-fold increased risk of sensitization to willow. Dog trainers may come in contact with these allergens at work. The important question at this point is whether the results of dog exposure specifically influence only the risks of dog allergy, or the risks of allergy to multiple allergens. This study has been conducted in a country with a low pet-keeping rate. In this country, it has been found that dog allergen exposure due to passive transport is a less important problem than in countries with high pet-keeping ratios (16). Therefore, it was thought that results reflect the real effect of workplace exposure.

It was observed that the prevalence of rhinitis in dog trainers was higher than in the control group. Respiratory, skin and eye symptoms were found similar between study and control groups, although it was found that sensitization to dog allergen in 21.4% work-related symptoms was declared in 33.9% of dog trainers. Nineteen animal workers with allergy symptoms had negative animal allergen SPT. Symptom and atopy rates were

quite high, while sensitivity to animal allergens was less than expected. Negative skin tests in symptomatic individuals may be due to non-IgE mediated mechanisms. Dog trainers reported frequent work-related symptoms in this study. Dog trainers have close contact with dogs; also, dogs contain high levels of allergens such as mite and fungal allergens. Because of this, work-related symptoms may have occurred more frequently. So, dog trainers are exposed to a variety of allergens, which constitute a risk factor for allergic sensitization and symptoms. The presence of work-related symptoms could be explained by exposure to other allergens or non-specific irritants in the workplace. Two dog trainers with sensitization to dog (by using skin prick test) reported no clinical symptoms after exposure to dogs. Similarly, in a laboratory workers study, it has been found that sensitization rates were 12.7% and 16.3% in those exposed to mice and rats, respectively, and work-related complaints occurred in 33.7% and 37.8% of employees (24).

The multivariate logistic regression analysis revealed a significant role of skin prick test positivity (against allergens other than the dog allergen) associated with dog allergy likelihood. Age, smoking, working duration, pet seeing and family history of atopic disorders were found not an independent risk factor for the development of sensitization to dogs. Although there weren't pre-employment SPT of workers, it has been asserted that skin prick test positivity is associated with atopy. Of the sensitized subjects (37 cases), 1 (1.7%) was sensitized only to dog allergen. In a study about occupational allergy, it was found that other factors associated with atopy, such as having a positive skin test response for house dust mite or pollen and a number of positive allergy test results, likewise showed positive associations with occupational sensitization to laboratory animals (25).

Risk factors for developing allergic sensitization to dogs have not been fully elucidated. The main risk factor for the development of laboratory animal allergy was identified to be atopy (15,26). Atopic subjects were found to be up to 12 times more likely to have laboratory animal allergy. In the multivariate logistic regression analysis, having a positive skin prick test created an increase in the odds by a factor of 27.8 (95% CI 1.6 - 474.8). In other words, in our study, subjects with positive SPT have 27.8 times higher risk of dog allergy.

The key question is how can we predict the risk of developing dog allergy after exposure. Although atopy appears to be the main risk factor for occupational allergy, establishing atopy is generally considered inadequate for pre-employment selection, because atopy is common in industrialized countries (27). The algorithm defined by Liccardi and colleagues can be used to detect the susceptible subjects to dog allergy before working with dogs (28,29). In that algorithm, it was suggested that subjects should be evaluated by SPT, specific IgEs and further molecular diagnosis. That molecular diagnosis is done by evaluation of specific IgEs using micro-array technique for lipocalins and albumins, and gives opportunity to evaluate the possibility of cross-reactions between allergens of different animals. Atopic individuals should be identified pre-employment, and screening and counseling should be applied periodically. Prevention programs as legal requirements should base on medical check-ups. These check-ups should include questionnaires and medical examination. Also, education, engineering controls, administrative controls should be made. Work practices should be planned to minimize allergen exposure. Regular washing of the pet, use of denaturants for reservoirs, HEPA air filtration, and regular vacuuming may reduce risk of sensitization by lowering allergen loads.

Selecting hypoallergenic dog breeds as police dogs can be the solution of this occupational health problem but previous studies have been reported that there is no dog breed that can be considered as hypoallergenic (30,31).

Further studies will be needed, to clarify whether working with different breed dogs increases the risk of allergies. Longitudinal studies are needed for determining all of risk factors. This study is the first study to investigate the presence of sensitization to dogs and common allergens in police dog trainers.

Conclusions

Current study indicates that allergic disease is a serious occupational health concern for police dog trainers. Dog trainers are exposed to a variety of different breed dogs that may constitute a risk factor for allergic sensitization and symptoms.

Conflict of interest

The authors declare that they have no conflict of interest.

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Can an otorhinolaryngological visit induce the suspect of allergic rhinitis in children?

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Key words

otorhinolaryngological visit; allergic rhinitis; familial atopy; endoscopy; children

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Doi
10.23822/EurAnnACI.1764-1489.105

Summary

Allergic rhinitis (AR) is very frequent in childhood. AR is commonly associated with some co-morbidities and typical clinical features. This study aimed to test the hypothesis whether an otorhinolaryngological (ORL) visit could induce the suspect of AR.

Globally, 1,002 children (550 males, mean age 5.77 years) were consecutively visited at an ORL clinic. Clinical visit, nasal endoscopy, and skin prick test were performed in all patients. In particular, history investigated atopic familiarity, birth, feeding type, passive smoking, co-morbidities, including asthma, respiratory infections, otitis media, respiratory sleep disorder. Endoscopy assessed the tonsil and adenoid volume, turbinate contacts, mucosal color, and nasal discharge. Univariate and multivariate analysis were performed.

The study showed that 547 (54.6%) children had AR. Some parameters were predicting factor for suspecting AR: middle turbinate contact (OR = 9.27), familial atopy (OR = 6.24), pale nasal mucosa (OR = 4.95), large adenoid volume (OR = 3.02 for score 4), and asthma co-morbidity (OR = 2.95).

In conclusion this real-life study showed that during an ORL visit it is possible to suspect AR in children with turbinate hypertrophy, familial atopy, nasal pale mucosa, adenoid enlargement, and asthma comorbidity.

Introduction

Allergic rhinitis (AR) is the most common immune-mediated disorder in childhood as it may affect up to 40% of children (1). AR is frequently associated with relevant comorbidities, including other allergies, rhinosinusitis, recurrent respiratory infections, otitis, adenoid hypertrophy (AH) and tonsillar hypertrophy (TH), as recently reported by several recent studies (2-6). Moreover, the possible correlation between AR and AH-TH has been investigated by some studies which reported a positive association between the two disorders (7-11). Familiar atopy is also common in AR children.

Actually, the otorhinolaryngology (ORL) specialist visits children with nasal symptoms daily. The desire of every doctor is to diagnose a disease already at the time of the visit thanks to personal background, experience, and practice, and possibly with the instruments present in the clinic. Therefore, predictive diagnostic information could be very fruitful in clinical practice. In this regard, to observe a pale mucosa in the nasal cavity has been traditionally considered a sign suggesting allergic rhinitis by most ORL specialists for a long time until today (12,13). However, it has been evidenced that turbinate hypertrophy is a sign with higher predictive reliability to suspect allergic rhinitis during an ORL visit, both in children and adults (14,15). Consequently, nasal obstruction may be a trustworthy symptom able to suggest the presence of allergy. Consistently, it has been reported that also bronchial airflow limitation, documented by a simple spirometry, may be able to suspect allergy (16,17). Furthermore, it has to be highlighted that to define a diagnostic marker there is the need to fulfill a series of pragmatic requirements as recently pointed out (18).

On the basis of this background, we tested the hypothesis that the ORL visit could suggest the suspect of allergic rhinitis. Therefore, this real-life study aimed to evaluate whether some clinical data and endoscopic findings may be predictive factors of allergic rhinitis in children during an ORL visit.

Materials and methods

Patients. 1002 children (550 males, 452 females, mean age 5.77 + 1.84 years), complaining upper airway symptoms, were consecutively referring to the ORL Unit of the Casa di cura Villa Montallegro (Genoa, Italy) during the period 2015-2017. They were consecutively enrolled into the study. Inclusion criteria were: i) age between 3 and 10 years; ii) to have complaints of upper airways (i.e. nasal obstruction, rhinorrhea, otalgia, sore throat, cough, snoring). Exclusion criteria were: i) a craniofacial syndrome, ii) recent facial trauma, and iii) current treatment able to interfere with the findings. The study was approved by the local Review Board and an informed written consent was obtained by the parents.

Study design. All children were evaluated by clinical visit, nasal endoscopy, and skin prick test.

Clinical visit. included detailed medical history, concerning premature birth, feeding type (breastfeeding or artificial), familiar atopy, passive smoking, documented diagnosis of: asthma, recurrent respiratory infections, recurrent acute otitis media, otitis media with effusion, and respiratory sleep disorders.

Endoscopy. It was performed with a pediatric rigid endoscope diameter 2.7 mm with 30° angle of vision (Karl Storz cod 7207 ba) with a 300 W cold light source (Storz Xenon Nova, cod. 20134001) and a light cable of 1.8 mm length. Endoscopy was video recorded by a micro-camera connected to a digital recorder set (Karl Storz Tele Pack, cod. 20043002-020). A flexible endoscope (3 mm diameter) was used in restless children and in those with narrow nasal fossa due to anatomical abnormalities. The child lied supine with his-her head bent by about 45°. Some cotton wool soaked with anesthetic solution (ossibuprocaine 1%) was placed into the nose for 5 minutes. The complete description of the procedure was previously described in detail (11,14,19). In particular, pale nasal mucosa was defined by a lighter color than close mucosal tissues (depending on the edema of the turbinate); it was defined as present or absent (20). Nasal discharge is defined by a draining into the nasal cavity that may have different appearance: clear and watery typically in allergic subjects, and purulent in infective disorders; it was defined as present or absent (15).

Tonsils volume assessment. Tonsils volume was classified according to validated criteria (21) as follows: grade 1, tonsils in the tonsillar fossa barely seen behind the anterior pillar; grade 2, tonsils visible behind the anterior pillar; grade 3, tonsils extended three quarters of the way to med-line; grade 4, tonsils completely obstructing the airway (also known as kissing tonsils).

Adenoids volume assessment. The patients were evaluated by nasal endoscopy for adenoid hypertrophy. The adenoids were graded in according to Parikh's classification, that was created based on the anatomical relationships between the adenoid tissue and the following structures: vomer, soft palate, and torus tubarius (22). The grading is based on the relationship of the adenoids to adjacent structures when the patient is at rest (i.e. when the soft palate is not elevated). Specifically, grade 1 adenoids are non-obstructive and do not contact any of the previously mentioned anatomic subsites; subsequently, grade 2,3 and 4 adenoids contact the torus tubarius, vomer, and soft plate (at rest), respectively.

Turbinate hypertrophy. The contact of turbinate was considered as surrogate marker for turbinate hypertrophy, as previously described and validated (14,15).

Skin Prick Test. Allergy was assessed by the presence of sensitization to the most common classes of aeroallergens by performing a skin-prick test. It was performed as stated by the European Academy of Allergy and Clinical Immunology (23). The allergen panel consisted of the following: house-dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cats, dogs, grasses mix, *Compositae* mix, *P. judaica*, birch, hazel trees, olive trees, cypress, *Alternaria tenuis, Cladosporium,* and *Aspergilli* mix. The concentration of allergen extracts was 100 immune reactivity/mL (Stallergenes-Greer Italia, Milan, Italy). A histamine solution in distilled water (10 mg/mL) was used as positive control and the glycerol-buffer diluent of the allergen preparations was used as negative control. Each patient was skin tested on the volar surface of the forearm using 1 mm prick lancets. The skin reaction was recorded after 15 minutes, by evaluating the skin response in comparison with the wheal given by the positive and the negative control. A wheal diameter of at least 3 mm was considered as a positive reaction.

The AR diagnosis was made if nasal symptom history was consistent with sensitization, such as the demonstration of symptom occurrence after exposure to the sensitizing allergen.

Statistical analysis. Continuous variables were given as means with standard deviations (SD) and categorical variables as number of subjects and percentage values. The univariate logistic regression models were performed to screen the effect of the clinical and demographic variables on the AR diagnosis.

The odd ratios associated with AR were calculated with their 95% confidence interval for each factor from the logistic model. The likelihood ratio (LR) test was used as a test of statistical significance and the estimated p-values were adjusted for multiple comparisons by the Bonferroni correction method.

Those covariates with a p-value < 0.05 were then selected for the multivariate analysis, where the AR was the dependent variable. Possible multicollinearity was assayed using intraclass correlation coefficient (ICC) and those variables with an ICC more than 0.5 were considered associated. Multivariate analysis was

performed using again the logistic regression model, and the model selection was done by the Akaike and information criterion. The sensitivity and specificity of the model were evaluated using confusion matrix (a tabular representation of actual *versus* predicted values). Moreover, multiplicative interaction terms were used to test whether the feeding type was different according to the risk factors. For those results suggestive of an interaction with the feeding type factor (p-value < 0.05), a stratified analysis was then performed based on that variable using penalized logistic model. Differences, with a p-value less than 0.05, were selected as significant and data were acquired and analyzed in R v3.5.1 software environment.

Results

A total of 1002 (550 males) children was enrolled in this study. The demographic and clinical characteristics of the study participants are summarized in **table I**. About the primary outcome, 547 (54.6%) children had AR. The mean age was 5.77 years (SD = 1.84); 77 (7.7%) children were born prematurely; the majority of children (76.4% n = 765) received breastfeeding, while 236 (23.6%) received artificial feeding. Passive smoking was present in 73 (7.3%) cases, 726 (72.7%) children had familial atopy. About comorbidity, asthma was documented in 129 (12.9%) children, recurrent respiratory infections in 633 (63.5%), recurrent acute otitis media in 187 (18.7%), otitis media with effusion in 213 (21.3%), and a respiratory sleep disorder was present in 739 (73.9%) children. About endoscopic findings, only 233 (23.3%) children had a tonsil volume of grade 1; 370 (37%) children had adenoid volume of grade 1, 661 (66.3%) had the inferior turbinate contact and 528 (52.8%) had middle turbinate contact, 319 (31.8%) children showed a pale mucosa, and 515 (51.4%) had nasal discharge.

Descriptive statistics of demographic and clinical factors according to AR diagnosis are reported in **table II**. The percentages of males and females in AR groups were quite similar (range: 44.22% to 55.78%), whereas there was a significant difference about the age: allergic children were older than non-allergic children (p < 0.0001). There were significant differences between the subgroups regarding: feeding, passive smoking, familial atopy, asthma comorbidity, respiratory sleep disorders, tonsil and adenoid volume, turbinate contact, pale mucosa, and nasal discharge. The univariate logistic regression analysis (**table II**), using the complete set of data, demonstrated a significant association among feeding, passive smoking, familial atopy, asthma, respiratory sleep disorder, tonsil volume, adenoid volume, inferior and middle turbinate contact, pale mucosa and AR (p-values < 0.05). Multicollinearity presence was observed between inferior and middle turbinate contact: ICC (95% CI) = 0.51 (0.46 - 0.55). Due to this result, the inferior turbinate contact was not included in the multivariate analysis.

The multivariate analysis (**table III**) confirmed a statistically significant effect of feeding, familial atopy, asthma, adenoid volume, middle turbinate contact, and pale mucosa on AR (p-values < 0.0001). In particular, an increased probability of having AR was shown for the asthma co-morbidity (OR 95% CI) = 2.95 (1.37 - 6.65), the middle turbinate contact (OR 95% CI) = 9.27 (6.05 - 14.43), and the pale mucosa (OR 95% CI) = 4.95 (3.05 - 8.26). As regard multiplicative interaction term, the effect of feeding on AR was significantly different according to the familial atopy presence / absence (p-value for the interaction term = 0.0302). The sensitivity and specificity of the model were 88.08% and 90.07%, respectively.

The subsequent stratification analysis (**table IV**) showed that the breastfeeding was associated with increased risk of having AR, only in children with familial atopy (OR 95% CI) = 2.98 (1.75 - 5.10).

Discussion

Upper airways symptoms are very common in pediatric population. In particular, allergic rhinitis is frequent in children affecting up to 40% of the general population. Allergic rhinitis may be frequently associated with several co-morbidities, including respiratory infections and asthma, and familiar atopy (24).

The present study was based on a real-life setting, such as the studied cohort was constituted of children complaining upper airways symptoms and visited at an ENT office, undergoing nasal endoscopy.

The main outcome was the ability to identify some clinical parameters that could induce the suspect of AR during an ORL visit. In particular, five parameters could predict AR: middle turbinate contact (OR = 9.27), familial atopy (OR = 6.24), pale mucosa (OR = 4.95), adenoid hypertrophy (OR = 3.02 for the volume 4), and asthma comorbidity (OR = 2.95).

The current study demonstrated that middle turbinate contact was the main predictor factor for AR; the turbinate contact depends essentially on the hypertrophy of the turbinate. This outcome confirmed previous studies that reported consistently a significant association between this sign and AR diagnosis (14,15,20). So, this endoscopic finding may be reasonably considered as a surrogate marker for turbinate hypertrophy (18).

The familial atopy represents another relevant predictive factor for having AR; this finding was expected and was consistent with the literature evidence as recently reported in an International Consensus on AR (25). The genetic background of allergy is well known as allergy is widespread in allergic families (26). In this regard, breastfeeding is strongly recommended, as necessary for the healthy growth of infants (27) particularly in children with high risk for atopy. However, a real protective role in preventing allergic disorders is not clear. Indeed, there are conflicting results about the prevention of allergy as provided by

Table I *- Demographic and clinical characteristics of study participants (n = 1002). The results are expressed as mean with standard deviation or as number of subjects with percentage.*

Characteristic	Overall
allergic rhinitis	
no	455 (45.4%)
yes	547 (54.6%)
age (years)	5.77 (1.84)
gender	
female	452 (45%)
male	550 (55%)
premature birth	
no	924 (92.31%)
yes	77 (7.69%)
feeding	
artificial	236 (23.58%)
breastfeeding	765 (76.42%)
passive smoking	
no	929 (92.71%)
yes	73 (7.29%)
familiar atopy	
no	273 (27.33%)
yes	726 (72.67%)
asthma	
no	872 (87.11%)
yes	129 (12.89%)
recurrent respiratory infections	
no	364 (36.51%)
yes	633 (63.49%)
recurrent acute otitis media	
no	792 (79.04%)
yes	187 (18.66%)
ongoing	23 (2.3%)
otitis media with effusion	
no	695 (69.36%)
yes	213 (21.26%)
ongoing	94 (9.38%)
respiratory sleep disorder	
no	262 (26.17%)
snoring	553 (55.24%)
sleep apnoea	186 (18.58%)
tonsil volume	
1	233 (23.3%)
2	310 (31%)
3	294 (29.4%)
4	163 (16.3%)
adenoid volume	
1	370 (36.96%)
2	218 (21.78%)
3	215 (21.48%)
4	198 (19.78%)
inferior turbinate contact	
no	336 (33.7%)
yes	661 (66.3%)
middle turbinate contact	
no	472 (47.2%)
yes	528 (52.8%)
pale mucosa	
no	683 (68.16%)
yes	319 (31.84%)
nasal discharge	
no	487 (48.6%)
yes	515 (51.4%)

Table II *- Contingency tables and output of the univariate analysis.*

Characteristic	Descriptive statistic		Univariate analysis	
	allergic rhinitis		OR (95% CI)	p-value
	no 455 (45.4%)	yes 547 (54.6%)		
age	5.46 (1.85)	6.05 (1.8)	1.2 (1.12 - 1.29)	< 0.0001
gender				0.9999
female	211 (47.11%)	239 (52.89%)	1	
male	241 (44.22%)	304 (55.78%)	1.12 (0.87 - 1.44)	
premature birth				0.5325
no	408 (44.4%)	511 (55.6%)	1	
yes	44 (57.14%)	33 (42.86%)	0.6 (0.37 - 0.96)	
feeding[1]				< 0.0001
artificial	143 (60.59%)	93 (39.41%)	1	
breastfeeding	309 (40.66%)	451 (59.34%)	2.24 (1.67 - 3.03)	
passive smoking[1]				< 0.0001
no	396 (42.86%)	528 (57.14%)	1	
yes	57 (78.08%)	16 (21.92%)	0.21 (0.12 - 0.36)	
familiar atopy[1]				< 0.0001
no	237 (86.81%)	36 (13.19%)	1	
yes	215 (29.82%)	506 (70.18%)	15.49 (10.67 - 23.09)	
asthma[1]				< 0.0001
no	429 (49.48%)	438 (50.52%)	1	
yes	23 (17.83%)	106 (82.17%)	4.51 (2.87 - 7.39)	
recurrent respiratory infections				0.3993
no	147 (40.61%)	215 (59.39%)	1	
yes	304 (48.03%)	329 (51.97%)	0.74 (0.57 - 0.96)	
recurrent acute otitis media				0.2076
no	341 (43.06%)	451 (56.94%)	1	
yes	99 (54.4%)	83 (45.6%)	0.63 (0.46 - 0.88)	
ongoing	13 (56.52%)	10 (43.48%)	0.58 (0.25 - 1.34)	
otitis media with effusion				0.4498
no	304 (43.74%)	391 (56.26%)	1	
yes	94 (45.19%)	114 (54.81%)	0.94 (0.69 - 1.29)	
ongoing	55 (58.51%)	39 (41.49%)	0.55 (0.35 - 0.85)	
respiratory sleep disorder[1]				< 0.0001
no	99 (38.52%)	158 (61.48%)	1	
snoring	226 (40.87%)	327 (59.13%)	0.91 (0.67 - 1.23)	
sleep apnoea	127 (68.28%)	59 (31.72%)	0.29 (0.19 - 0.43)	

Table II *(continued)*

Characteristic	Descriptive statistic		Univariate analysis	
	allergic rhinitis		OR (95% CI)	p-value
	no 455 (45.4%)	yes 547 (54.6%)		
tonsil volume[1]				< 0.0001
1	39 (16.74%)	194 (83.26%)	1	
2	127 (40.97%)	183 (59.03%)	0.17 (0.12 - 0.23)	
3	176 (59.86%)	118 (40.14%)	1.52 (1.14 - 2.04)	
4	109 (68.99%)	49 (31.01%)	0.98 (0.77 - 1.25)	
adenoid volume[1]				< 0.0001
1	57 (15.41%)	313 (84.59%)	1	
2	80 (36.7%)	138 (63.3%)	0.1 (0.07 - 0.13)	
3	164 (78.1%)	46 (21.9%)	1.88 (1.39 - 2.55)	
4	151 (76.26%)	47 (23.74%)	1.78 (1.32 - 2.42)	
inferior turbinate contact[1]				< 0.0001
no	305 (90.77%)	31 (9.23%)	1	
yes	144 (21.95%)	512 (78.05%)	34.98 (23.48 - 53.77)	
middle turbinate contact[1]				< 0.0001
no	368 (77.97%)	104 (22.03%)	1	
yes	85 (16.25%)	438 (83.75%)	18.23 (13.33 - 25.21)	
pale mucosa[1]				< 0.0001
no	384 (56.22%)	299 (43.78%)	1	
yes	69 (21.97%)	245 (78.03%)	4.56 (3.37 - 6.23)	
nasal discharge[1]				< 0.0001
no	328 (68.05%)	154 (31.95%)	1	
yes	125 (24.27%)	390 (75.73%)	6.65 (5.05 - 8.8)	

Characteristic, variable taken into account in the analysis; OR (95% CI), odd ratios with 95% confidence interval; p-value, Likelihood Ratio p-value. [1]Variables entering in the multivariate analysis (see the text for abbreviations and further details).

different meta-analysis and reviews (28-32). Consistently, the current study showed that breastfeeding was not significantly associated with AR even though breastfeeding combined with atopic familiarity may predict AR. This finding should be considered cautiously as the predictivity is closely dependent on the genetic predisposition.

Nasal pale mucosa also significantly predicted AR diagnosis. Pale mucosa depends on tissue edema consequent to allergic inflammation. Notably, we found conflicting results in a previous study that reported no predictive role of this endoscopic sign (14). The possible explanation could be related to the smaller sample analyzed in the previous study. Similarly, we reported previously an inverse relationship between adenoid hypertrophy and AR (11). Probably, the limited sample size could account for the negative result. However, the present study showed the AR predictivity of adenoid hypertrophy, namely with an impressive size-dependent progression. Moreover, the current outcome is consistent with a previous study that showed a positive association between adenoid hypertrophy and AR (33).

Asthma comorbidity was another predictive factor for AR. Association with asthma is well known in patients with allergic rhinitis (34) and underlines the close relationship between upper and lower airways, successfully defined by the term "allergic march", such as the progression from the nose to the bronchi of the allergic reaction (35).

The current study identified a series of clinical parameters with increased odds for having AR. Therefore, it demonstrated that it is conceivably possible to characterize some predictive factors for AR diagnosis during an ENT visit. However, AR diagnosis should be based on other criteria, including documented sensitization, such as IgE production, and proved consistency between exposure to sensitizing allergen and immediate symptom

Table III *- Multivariate analysis, the predictor effects on AR. Results are expressed as odds ratio (OR) with 95% confidence interval (95% CI); p-value, likelihood ratio p-value.*

Characteristic	Multivariate analysis	
	OR (95% CI)	**p-value**
(intercept)	0.02 (0.01 - 0.06)	
feeding		< 0.0001
artificial	1	
breastfeeding	0.75 (0.26 - 2.26)	
familiar atopy		< 0.0001
no	1	
yes	6.24 (2.29 - 18.3)	
asthma		< 0.0001
no	1	
yes	2.95 (1.37 - 6.65)	
adenoid volume		< 0.0001
1	1	
2	0.15 (0.1 - 0.23)	
3	2.88 (1.87 - 4.48)	
4	3.02 (1.94 - 4.78)	
middle turbinate contact[1]		< 0.0001
no	1	
yes	9.27 (6.05 - 14.43)	
pale mucosa		< 0.0001
no	1	
yes	4.95 (3.05 - 8.26)	
familiar atopy[1] feeding		0.0302
familiar atopy (no) artificial	1	
familiar atopy (yes) breastfeeding	3.91 (1.14 - 12.95)	

Table IV *- Stratification analysis for familiar atopy presence / absence on the risk of AR. Results are expressed as odds ratio (OR) with 95% confidence interval (95% CI), keeping constant asthma, adenoid volume, middle turbinate contact and pale mucosa.*

	Familiar Atopy					
	no			**yes**		
Characteristic	**descriptive statistics**		**OR (95% CI)**	**descriptive statistics**		**OR (95% CI)**
(intercept)			0.03 (0.01 - 0.10)			0.15 (0.09 - 0.25)
Feeding						
Artificial	42 (80.77%)	10 (19.23%)	1	97 (54.8%)	80 (45.2%)	1
Breastfeeding	189 (88.32%)	25 (11.68%)	0.99 (0.32 - 3.17)	115 (21.42%)	422 (78.58%)	2.98 (1.75 - 5.10)

occurrence. This study once more confirms that obtaining an adequate history and a thorough clinical examination are most important for suspecting AR.

The main limitations of the present study are: i) the cross-sectional design; ii) the selected population; iii) the lack of standardized score for some endoscopic signs, and iv) the absence of immunological investigation, able to clarify the pathogenic mechanisms. Therefore, further studies should be performed to address these issues.

However, the strength of this study is the large number of children, the careful work-up, and the real-life setting, so the outcomes may mirror what could occur in daily practice.

Conclusions

This real-life study showed that during an ORL visit it is possible to suspect AR in children with turbinate hypertrophy, familial atopy, nasal pale mucosa, adenoid enlargement, and asthma comorbidity.

Conflict of interest

The authors declare that they have no conflict of interest.

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Anaphylaxis to baobab fruit: the paradox of "natural healthy food"

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To the Editor

Baobab (*Adansonia digitata*) is a woody plant, characteristic of Africa and North-West Australia, whose fruits and leaves have been largely used by the local African populations as food and in traditional medicine (1-3). The cosmetic and wellness industries are currently promoting the baobab-based products for their claimed moisturizing, non-irritating, and highly penetrating properties, particularly useful in the skin care. Similarly, the food industries, to address the growing consumers' interest in natural / healthy products in the developed countries (e.g. vegan lifestyle), have started a huge trading to import baobab fruit's extracts for their products (4,5). In addition, recent studies have focused on health-promoting properties of the baobab fruit (6) and its possible effect on weight maintenance (7). Consequently, there has been a remarkable increase in the sales of products derived from the baobab fruit. In 2005, the industry of natural products was valued at $ 65 billion / year, with an impressive annual increase of 15-20% (4,5).

We describe a case of anaphylaxis, few minutes after the ingestion of a snack, in a 31-year-old Caucasian woman who presented at the emergency room for oral pruritus, generalised urticaria, facial angioedema, throat tightness, abdominal pain, and diarrhea. The vital signs were normal, and she was treated with systemic corticosteroids, antihistamines, and saline solution. The clinical history revealed that the woman, willing to start a lifestyle based on vegan foods, tasted for the first time a vegan snack (Lifebar Plus™) at lunch, without any other foods, physical exercise, alcohol, or medications taken before or after. The ingredients reported on the product's label were dates, almonds, dried cherries, raw cashew nuts, baobab fruit pulp, dried cranberry powder, maca powder, and crystal pink himalayan salt, with possible traces of other nuts and sesame. The patient, after the episode, ate all the above-mentioned foods without reactions, with the exception of baobab, maca, and sesame, which were not ingested again. No previous food allergy episodes, comorbidities or concomitant medications were reported, except for a mild rhinitis. We started the allergologic diagnostic work-up two months after the episode. Skin prick testing was performed with food (egg, milk, flour, fish, shrimp, almond, walnut, peanut, hazelnut, peach, tomato, apple, celery, soy, sesame, profilin) and airborne (alternaria, birch, cat, cladosporium, cypress, *Dermatophagoides farinae* and *pteronyssinus*, dog, hazel, mugwort, olive, parietaria, penicillium, ragweed, timothy grass) commercial extracts (ALK-Abelló). The whole snack and its ingredients, namely maca and baobab fruit powder, were tested

by prick-to-prick using raw products. Two healthy subjects were also tested, as negative controls. Singleplex ImmunoCAP (Thermo Fisher Scientific, Uppsala) was used for specific IgE measure of food (almond, cashew nut, date, sesame, omega-5 gliadin) and airborne molecular allergens (Alt a1, Cup a1, Der p1, Der P2, Der p23, Der P10, Par j2, Phl P1, Phl P2, Phl P4, Phl P5, Phl P6, Phl P7, Phl P11, Phl P12, Cyn d1). ELISA and IgE-immunoblot tests were conducted as previously described (8). ELISA inhibition experiments were performed using as inhibitors grass and cypress pollen extracts, or alternaria and house dust mite extracts or peel peach extract. All the extracts were used at two different concentrations (30 and 3 µg/ml of extract).

Food skin testing resulted positive only to the whole snack and the baobab fruit (5 mm), being negative to all the other foods. Skin tests to airborne allergens revealed several sensitizations, consistent with the mild rhinitis (i.e. dust mite, timothy grass, cypress, alternaria, parietaria, dog, and cat). Specific IgE resulted negative for all the tested foods, and the airborne pattern was consistent with the skin results. The direct ELISA test confirmed the IgE reactivity of the patient to the baobab fruit's extract (1307 ODx1000), compared to the negative control (354 ODx1000). IgEs to cross reactive carbohydrate determinants (CCD) were negative. SDS-PAGE profile of the baobab fruit's extract showed the presence of different protein components (**figure 1**, lane 3); the subsequent IgE-immunoblot analysis evidenced two IgE-binding regions at about 40 and 60 kDa in the patient's serum (**figure 1**, lane 1). The ELISA inhibition experiments did not show any significant inhibition for all the inhibitors used, taking into account that no increase of inhibition level was observed between 3 and 30 µg/ml inhibitor concentrations. On the contrary, when using the baobab fruit's extract as inhibitor, an inhibition of 92% was observed (**figure 2**). Due to the severity of her reaction, the patient refused a food challenge with the baobab fruit. She was discharged with the indication of strict avoidance of all kind of baobab-based products (e.g. foods and cosmetics), and provided with self-injectable adrenaline.

To our knowledge, this is the first case of food allergy to baobab fruit, probably driven by two baobab-specific allergens (40 and 60 kDa, respectively) that we identified as responsible for a genuine sensitization, leading to anaphylaxis. The study raises some considerations. First of all, prick-to-prick test endorses its diagnostic reliability to identify unknown allergens. Furthermore, the way of sensitization and the paradox of "natural healthy" foods are noteworthy. The IgE-mediated reaction occurred, apparently, without a previous ingestion of baobab. Since the ELISA inhibition results did not reveal cross-reactions, an over-

Figure 1 *- SDS-PAGE/IgE-immunoblot: lane 1, patient's serum; lane 2, healthy control serum; lane 3, protein profile of the baobab fruit's extract.*

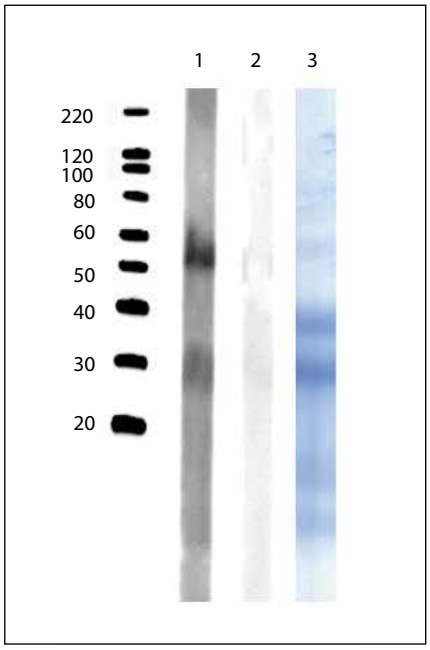
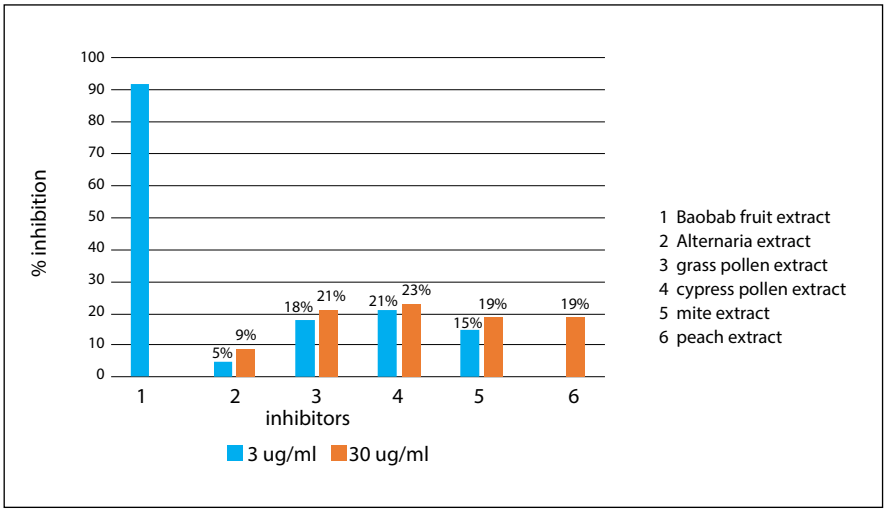


Figure 2 *- ELISA inhibition test.*



looked previous sensitization to baobab-derived products (i.e. foods or cosmetics (9)), cannot be excluded. The first paradox is related to the myth that natural products cannot be harmful. Another paradox concerns the baobab fruit, that albeit widely used in traditional medicine in the native regions, becomes a dangerous allergen in western countries. Consistently with the model of the peanut allergy (10), the first exposure in adult age and the different processing methods of the baobab fruit in the developed countries, compared to the native countries, could promote its allergenic properties.

In conclusion, the baobab fruit may trigger severe food allergy reactions. Taking into account the increasing market of natural products, similar cases should be expected in the near future.

Conflict of interest

The authors declare that they have no conflict of interest.

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