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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).

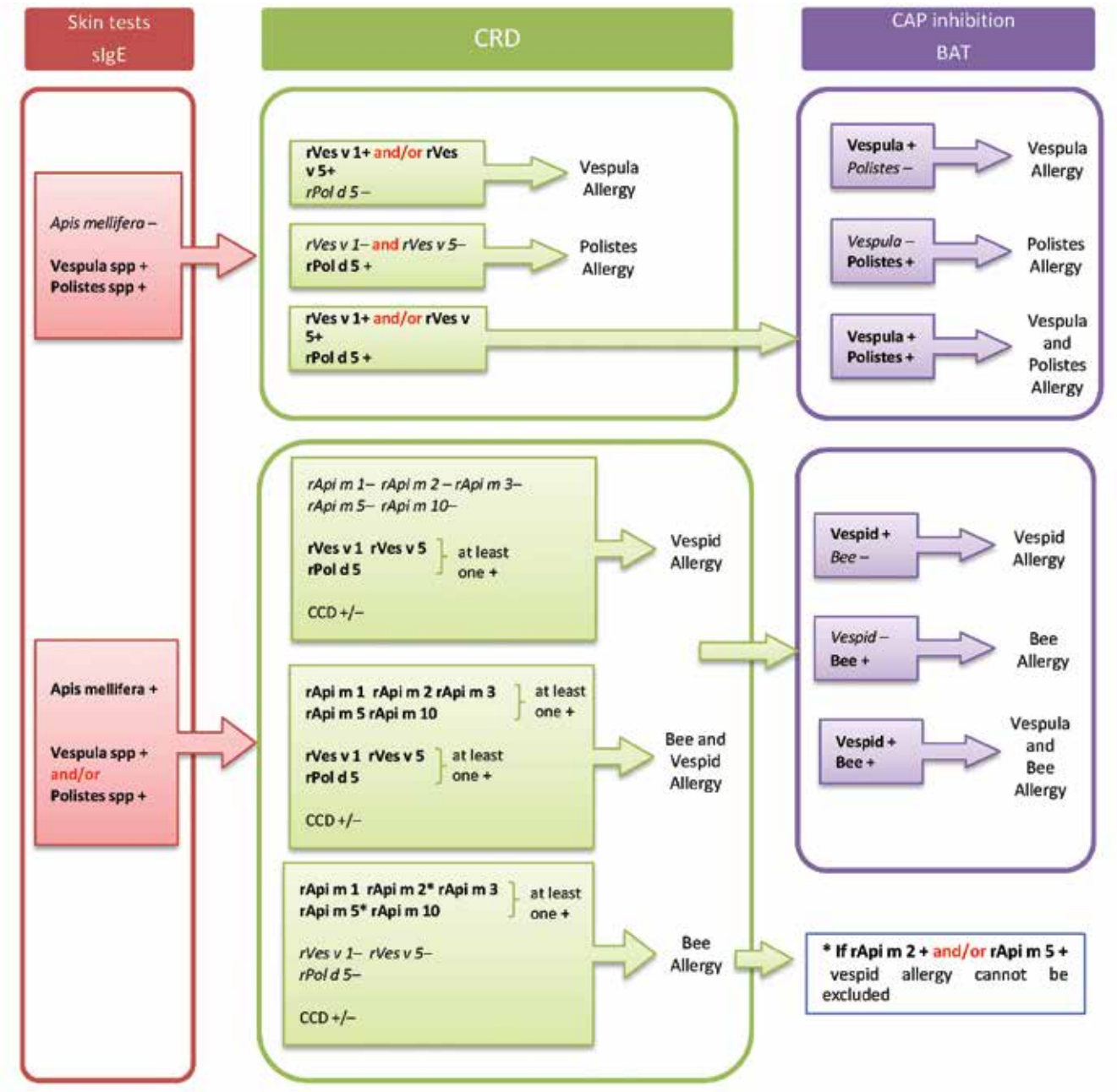
Discriminating cross-reactivity from multiple sensitizations

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):

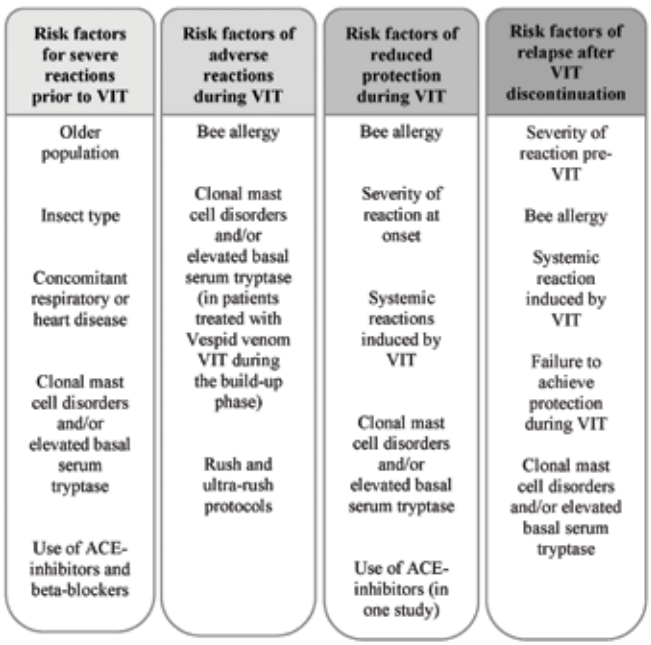
1. untreated patients: if history of systemic reactions is not limited to cutaneous involvement, or with a high risk of re-exposure to stings (i.e. occupational or recreational exposure);
2. patients treated with VIT: if risk factors of reduced protection are present (**figure 2**);

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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