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Review article

Diagnosis of Hymenoptera venom allergy

The purpose of diagnostic procedure is to classify a sting reaction by history, identify the underlying pathogenetic mechanism, and identify the offending insect. Diagnosis of Hymenoptera venom allergy thus forms the basis for the treatment. In the central and northern Europe vespid (mainly *Vespula* spp.) and honeybee stings are the most prevalent, whereas in the Mediterranean area stings from *Polistes* and *Vespula* are more frequent than honeybee stings; bumblebee stings are rare throughout Europe and more of an occupational hazard. Several major allergens, usually glycoproteins with a molecular weight of 10–50 kDa, have been identified in venoms of bees, vespids. and ants. The sequences and structures of the majority of venom allergens have been determined and several have been expressed in recombinant form. A particular problem in the field of cross-reactivity are specific immunoglobulin E (IgE) antibodies directed against carbohydrate epitopes, which may induce multiple positive test results (skin test, *in vitro* tests) of still unknown clinical significance. Venom hypersensitivity may be mediated by immunologic mechanisms (IgE-mediated or non-IgE-mediated venom allergy) but also by nonimmunologic mechanisms. Reactions to Hymenoptera stings are classified into normal local reactions, large local reactions, systemic toxic reactions, systemic anaphylactic reactions, and unusual reactions. For most venom-allergic patients an anaphylactic reaction after a sting is very traumatic event, resulting in an altered health-related quality of life. Risk factors influencing the outcome of an anaphylactic reaction include the time interval between stings, the number of stings, the severity of the preceding reaction, age, cardiovascular diseases and drug intake, insect type, elevated serum tryptase, and mastocytosis. Diagnostic tests should be carried out in all patients with a history of a systemic sting reaction to detect sensitization. They are not recommended in subjects with a history of large local reaction or no history of a systemic reaction. Testing comprises skin tests with Hymenoptera venoms and analysis of the serum for Hymenoptera venom-specific IgE. Stepwise skin testing with incremental venom concentrations is recommended. If diagnostic tests are negative they should be repeated several weeks later. Serum tryptase should be analyzed in patients with a history of a severe sting reaction.

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The first EAACI position paper on immunotherapy with Hymenoptera venoms was published in 1987 (1). Six years later a revised version appeared (2). As then many papers on the diagnosis and treatment of Hymenoptera venom allergy have been published, making a review of the last EAACI position paper necessary.

This paper focuses on Hymenoptera venom allergy, as allergic reactions caused by stings of insects other than Hymenoptera are rare and standardized extracts for the diagnosis and treatment of allergic reactions to non-Hymenoptera insects are not available (3).

Taxonomy

Most authors follow the Chinery classification (4), although over the last few years a few minor changes have been introduced. Aculeatae are a suborder of Hymenoptera (Fig. 1).

The family Apidae consists of the honeybees (*Apis mellifera*) who are brown in color and moderately hairy and the bumblebees (Genus *Bombus*) which are bigger than honeybees, much more hairy and characterized by distinct yellow or white bands on their abdomen. Vespidae are divided into the Vespinae and Polistinae subfamilies, with differences at the junction of the thorax and abdomen. Vespinae have a truncated junction while Polistinae are more oval in shape. Vespidae are almost hairless and have black and yellow striped abdomens.

Vespula, *Dolichovespula* and *Vespa* make up the three genera of the Vespinae. *Vespula* (called wasps in Europe, yellow jackets in the USA) are the most important species in Europe. The *Vespula* spp. (*V. germanica* and

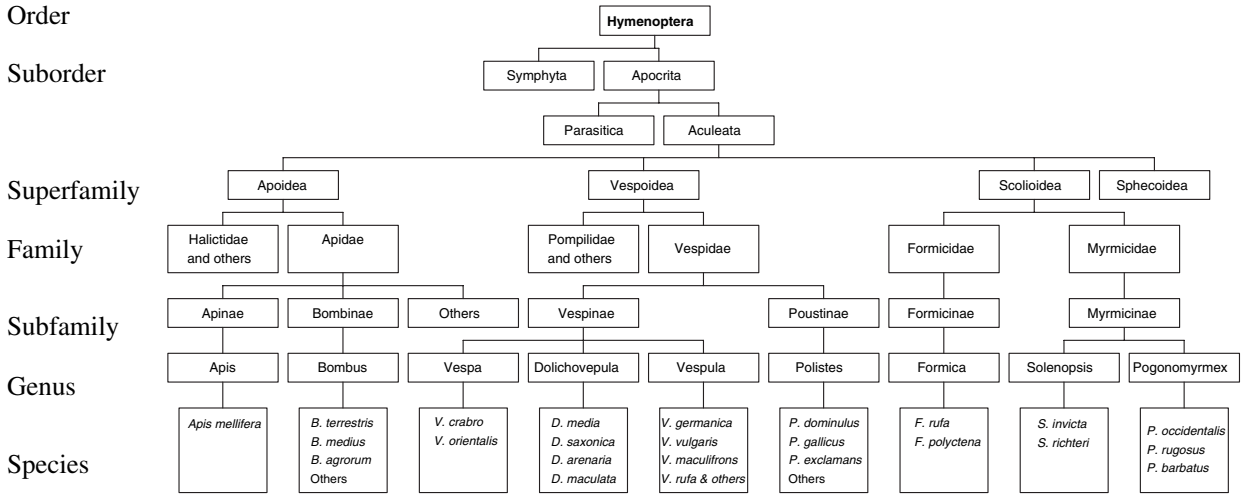

Figure 1. Taxonomy of Hymenoptera.

V. vulgaris) are easily distinguished from insects of the genus *Vespa* (hornets) by their smaller size, but much harder from those of the genus *Dolichovespula* by the shorter distance between their eyes and upper jaws (short-headed wasps).

Dolichovespula media, *D. saxonica* and *D. sylvestris* are the commoner species of the genus *Dolichovespula* in Europe.

In the genus *Vespa*, *Vespa crabro* (European hornet) is the most prevalent in Europe. Among Polistinae (called wasps in Europe and the USA), in Europe *Polistes gallicus*, *P. nimpha* and above all *P. dominulus* are widespread especially in the Mediterranean areas (5).

The Formicidae family (ants) has the following characteristics: antennae geniculate or folded, one or two nodes at the thin waist and generally lack wings.

With respect to allergic sting reactions mainly social Aculeatae – Vespidae (vespids), Apidae (bees), and Formicidae (ants) – are of importance. The entomology and the behavior of insects are described extensively elsewhere (6–8).

Venom allergens

Knowledge of the composition of venoms and structure of allergens is a prerequisite for the accurate diagnosis and treatment of insect venom allergy (Table 1).

The sequences and structures of the majority of major venom allergens have been cloned and sequenced; several major allergens have been expressed in recombinant form (http://www.allergen.org). Most of them are glycoproteins of 10–50 kDa containing 100–400 amino acid residues (9). However, some venom components are smaller, e.g. honeybee venom melittin (Api m 4), a 2.9 kDa peptide, and the recently described Api m 6 with a molecular weight of 7.9 kDa (10).

Venom dose per sting

The amount of venom which is released during a sting varies from species to species and even within the same species: bee stings release an average of 50 μg (11) up to 140 μg (12) of venom protein per sting; however, venom sacs may contain up to more than 300 μg of venom (13). Bumblebee stings release 10–31 μg of venom (11). In contrast Vespinae, which are capable of repeated stings, generally inject less venom per sting: *Vespula* stings release 1.7–3.1 μg of venom, *Dolichovespula* stings 2.4–5.0 μg and *Polistes* stings from 4.2 to 17 μg of protein (11). The amount of venom injected by a single European hornet sting is not known. The dry weight of venom per sac was found to be 260 μg (14).

Composition of venoms

The most important allergen in honeybee venom is phospholipase A_2, which is a glycoprotein with 134 amino acid residues (Table 1). The enzyme acts as a cytotoxin and an indirect cytolysin (15). Phospholipase A_2 comprises 12–15% of the dry weight of bee venom (16). Hyaluronidase, another major allergen of honeybee venom, shares a 50% sequence identity with vespid venom hyaluronidase (17). Acid phosphatase is an enzyme of 49 kDa, which has been partially cloned and sequenced (18). Like protease, an enzyme of around 39 kDa, it is probably a major allergen. Bee venom contains 1–2% of Api m 6, which consists of 71 amino acids (10). Melittin is a major bee venom component (50% of dry weight), it consists of 26 amino acids residues (18) but only 28% of patients have specific immunoglobulin E (IgE) antibodies against this peptide (19).

Bumblebee venom contains phospholipase A_2 (Bom p 1), protease (Bom p 4), hyaluronidase, acid phosphatase, and

Table 1. Allergens of Hymenoptera venoms

Venom	Allergen	Common name	Molecular weight (kDa)	Major/minor
Apis mellifera	Api m 1	Phospholipase A_2	16	Major
	Api m 2	Hyaluronidase	43	Major
	Api m 3	Acid phosphatase	49	Major?
	Api m 4	Melittine	2.9	Minor
	Api m 6		7.9	Minor
		Protease	39	Major?
Bombus pennsylvanicus	Bom p 1	Phospholipase A_2		Major
	Bom p 4	Serine protease		Major?
Vespula vulgaris (accordingly in *V. germanica, maculifrons,* etc.)	Ves v 1	Phospholipase A_1	35	Major
	Ves v 2	Hyaluronidase	45	Major
	Ves v 5	Antigen 5	25	Major
Dolichovespula maculata (accordingly in *D. arenaria, D. media,* etc.)	Dol m 1	Phospholipase A_1	35	Major
	Dol m 2	Hyaluronidase	45	Major
	Dol m 5	Antigen 5	25	Major
Polistes annularis (accordingly in *P. dominulus, gallicus, fuscatus,* etc.)	Pol a 1	Phospholipase A_1		Major
	Pol a 2	Hyaluronidase		Major
	Pol a 5	Antigen 5	25	Major
Vespa crabro	Vesp c 1	Phospholipase A_1		Major?
	Vesp c 5	Antigen 5		Major?
Solenopsis invicta	Sol i 1	Phospholipase A_1	37	Major?
	Sol i 2		13.2	
	Sol i 3	Antigen 5	24	Major?
	Sol i 4		13.4	

several other proteins not found in honeybee venom (20) (Table 1).

The major allergens in vespid venoms are phospholipase A_1 (Ves v 1), hyaluronidase (Ves v 2), and antigen 5 (Ves v 5) (11, 21). Phospholipase A_1 comprises 6–14% of the total dry weight of vespid venom (22). Antigen 5, is a major allergen in all vespid venoms (23).

Solenopsis venom contains four known allergens, phospholipase A_1 (Sol i 1), Sol i 2, antigen 5 (Sol i 3), and Sol i 4. Sol i 1 has a partial sequence identity with PLA_1 from vespids (24). Sol i 3 have about a 50% sequence identity with antigen 5 from vespid venoms.

Cross-reactivity

Double or even multiple positive tests can be caused by true double sensitization or by cross-reactive IgE antibodies which recognize similar epitopes of different venoms, especially carbohydrate-containing epitopes of venoms and common allergens (25). The distinction between cross-reactivity and 'true' double-sensitization is important for the choice of venom(s) for immunotherapy (VIT).

Cross-reactivity within the Apidae family. Available data suggest that venoms and major allergens of different honeybees worldwide are very similar, and that the structure of the major allergen phospholipase A_2 is highly identical (26, 27). By comparison, the variability of allergens within bumblebee venoms is higher (20). Bumblebee PLA_2 is only 53% identical to honeybee PLA_2. However, immunologic cross-reactivity does exist between honeybee and bumblebee venoms and concurrent sensitization can be found in many patients (28, 29).

Cross-reactivity within vespid venoms. Cross-reactivity among vespids is strong, due to similarities of venom composition and structure of single allergens (30, 31). The allergens from different *Vespula* species show identities up to 95% (30, 31). Correspondingly, different *Vespula* venoms strongly cross-react (22, 32). There is also substantial cross-reactivity between *Vespula*, *Vespa*, and *Dolichovespula* venoms (32–37). Cross-reactivity of the Vespinae (*Vespula*, *Dolichovespula*, and *Vespa*) with paper wasps (*Polistes*) is generally lower than cross-reactivity within the Vespinae (21, 33, 35, 36, 38). The cross-reactivity among European species of *Polistes* (*P. dominulus*, *P. gallicus*) is very strong, whereas that between European and American species weaker (5, 39).

Cross-reactivity between venoms of Apidae and Vespidae. The enzyme hyaluronidase shows an approximately 50% sequence identity between honeybee and vespid venoms (31), and has been identified as the major cross-reactive component (40–44).

Clinical presentation of sting reactions and quality of life

Venom hypersensitivity, as defined in the recently revised nomenclature for allergy, may be mediated by immunologic mechanisms (IgE-mediated or non-IgE-mediated venom allergy) but also by nonimmunologic mechanisms

(45). Reactions to Hymenoptera stings are classified into normal local reactions, large local reactions, systemic anaphylactic reactions, systemic toxic reactions (6, 46, 47), and unusual reactions (6, 48–50). The most frequent clinical patterns are large local and systemic anaphylactic reactions.

Large local reaction is defined as a swelling exceeding a diameter of 10 cm which lasts longer than 24 h; blisters may rarely be present. The underlying mechanisms of large local reactions are unknown. In some patients the clinical course, skin and *in vitro* tests indicate an IgE-mediated mechanism (51–55), in others a cell-mediated allergic pathogenesis (6) or a combination of both have been suggested.

Systemic anaphylactic reactions are most often IgE-mediated. Rarely, they may be due to short-term sensitizing IgG antibodies or complement activation by IgG–venom complexes. In patients with mastocytosis, the possible role of toxic mediator release from mast cells has been discussed (56–58). However, venom sensitization is demonstrable in the majority of patients with mastocytosis and previous anaphylactic sting reactions (59).

The skin, the gastrointestinal, respiratory, and cardiovascular systems can be involved. Various classifications of the degree of the severity of systemic reactions have been proposed. The most frequently used are those by Mueller (60) and by Ring and Messmer (61) (Table 2).

Severe reactions or a status after resuscitation may leave patients with a permanent disorder: hypoxic brain damage with permanent neurologic deficits, and myocardial infarction (6). Fatal reactions after insect stings may occur. Autopsies after fatal sting reactions revealed significant cardiopulmonary comorbidity in 50% (62) or even the majority of the unlucky victims (63).

For most patients as well as for their families, an anaphylactic reaction after a Hymenoptera sting is very traumatic event. It has been demonstrated that patients with anaphylactic responses following yellow jacket stings experienced impairment in their quality of life especially because of the emotional distress associated with having to be constantly on the alert while leading their everyday 'normal' lives (64).

Epidemiology

The prevalence of large local reactions ranges from 2.4% (65), 4.6% (52), 18.6% (51), up to 26.4% (54). In children the prevalence yielded by one study is 19% (66) and in beekeepers as high as 38% (67, 68).

Epidemiologic studies report a prevalence of self-reported systemic anaphylactic sting reactions between 0.3% and 7.5% (52, 54, 65, 66, 69–73) (Table 3). The prevalence of systemic reactions among beekeepers is high and falls between 14% and 43% (68, 74). In children prevalence rates are lower: questionnaires in several thousand girl and boy scouts in the USA (75, 77) and children in Europe (66) resulted in a prevalence of only 0.15–0.3%.

The incidence of insect sting mortality is low, ranging from 0.03 to 0.48 fatalities per 1 000 000 inhabitants per year (6, 62, 71, 78–80). However, the true number may be underestimated (81). Around 40 (63) to 85% (62) of the subjects with fatal reactions after Hymenoptera stings had no documented history of previous anaphylactic reactions.

Risk factors of Hymenoptera venom allergy

A distinction has to be drawn between risk factors, which are associated with a higher risk of stings and those increasing the risk to develop a severe sting reaction. Zone, climate, temperature, insect behavior, certain occupations or activities will influence the risk of receiving a sting. Beehives or vespid nests located in the near vicinity of dwellings, work places and also outdoor sport, have to be taken into account as risk factors.

Risk factors influencing the outcome of an anaphylactic reaction

When patients who received placebo or whole body extract in controlled studies on venom immunotherapy (82–84) were exposed, 57–75% of the patients with a history of systemic anaphylactic sting reaction develop systemic symptoms once again when re-stung. Several factors are associated with the occurrence and the severity of a systemic anaphylactic resting reaction.

Table 2. (a) Classification of systemic reactions to insect stings by Mueller (60), (b) classification of systemic reactions modified according to Ring and Messmer (61)

(a)	
Grade I	Generalized urticaria, itching, malaise, and anxiety
Grade II	Any of the above plus two or more of the following: angioedema, chest constriction, nausea, vomiting, diarrhea, abdominal pain, dizziness
Grade III	Any of the above plus two or more of the following: dyspnea, wheezing, stridor, dysarthria, hoarseness, weakness, confusion, feeling of impending disaster
Grade IV	Any of the above plus two or more of the following: fall in blood pressure, collapse, loss of consciousness, incontinence, cyanosis
(b)	
Grade I	Generalized skin symptoms (e.g. flush, generalized urticaria, angioedema)
Grade II	Mild to moderate pulmonary, cardiovascular, and/or gastrointestinal symptoms
Grade III	Anaphylactic shock, loss of consciousness
Grade IV	Cardiac arrest, apnea

Table 3. Prevalence of systemic anaphylactic sting reactions (recent epidemiologic studies in Europe)

Author	Country	Study population	*n*	Methods	Systemic reactions (%)
Charpin et al. (69)	France	General population	8271	Qu	0.6–3.3
Björnsson et al. (72)	Sweden	General population	1815	Qu, skin test, IgE	1.5
Strupler et al. (73)	Switzerland	General population	8322	Qu, IgE	3.5
Schäfer and Przybilla (71)	Germany	Rural area population	277	Qu, skin test, IgE	3.3
Kalyoncu et al. (70)	Turkey	Cellulose paper factory and family members	786	Qu, skin test, IgE	7.5
Grigoreas et al. (52)	Greece	Hellenic air force	480	Qu, skin test, IgE	3.1
Novembre et al. (66)	Italy	School children	1175	Qu, skin test	0.34
Incorvaia et al. (65)	Italy	Conscripts	701	Qu	2.7
Fernandez et al. (54)	Spain	Rural area population	1600	Qu, skin test, IgE	2.3

Qu, questionnaire; IgE, *in vitro* tests for venom-specific immunoglobulin E; skin test, skin prick test and/or intradermal test.

Time interval between stings, number of stings. A short interval between stings increases the risk of a systemic reaction to the later one (85). With increasing interval between stings the risk generally declines steadily, but remains in the range of 20–30% even after 10 years (86).

On the contrary, being stung very frequently appears to induce tolerance: 45% of beekeepers who were stung < 25 times a year had a history of systemic sting reactions,- when compared to none of those with more than 200 stings per year (67, 75).

Venom sensitization. Irrespective of the previous history, beekeepers with an increased preseason concentration of bee venom-specific IgE (> 1.0 kU/l) had a 12-fold increased risk of systemic reactions (87).

In adult subjects without a history of a previous systemic anaphylactic sting reaction and a positive skin test the risk of a later anaphylactic sting reaction was 17% *vs* 0% in skin test-negative individuals (86).

Severity of the preceding reaction. After a large local sting reaction, between 5% and 15% (6, 53) will develop a systemic reaction when next stung. In those with mild systemic reactions the risk of subsequent systemic reactions was found to be about 18% in children (88, 89) and 14–20% in adults with mild (90) to 79% in adults with severe reactions (91).

Age. In children about 60% of systemic sting reactions are mild (92), whereas in adults respiratory or cardiovascular symptoms occur in about 70% (93). Elderly patients more often develop particularly severe sting reactions (93–95), and the fatality rate is higher than in children and young adults (6). Children also have a better prognosis than adults with respect to the risk of systemic reactions to re-stings. Both sting challenges (96, 97) and studies of the natural course (88, 89, 91) of insect venom allergy show lower risks in children than adults.

Cardiovascular diseases, β-blockers. Studies on larger groups of patients identified cardiovascular diseases (62, 94), or treatment with β-blocking drugs (94) to be associated with particularly severe sting reactions. β-blockers do not however, seem to increase the overall risk of a systemic reaction.

Insect. Bee venom-allergic patients are at a greater risk of a systemic reaction on next sting than those with vespid venom allergy (82, 98–100). A recent study comparing the relative risk for life-threatening sting reactions in a Mediterranean area showed that this risk was about three times higher for hornet (*Vespa crabro*) stings than for honeybee or wasp stings (101).

Elevated serum tryptase, mastocytosis. Several case reports suggest that particularly severe, even fatal sting reactions may occur in patients with mastocytosis (56–59, 102, 103).

In Hymenoptera venom-allergic patients even without diagnosed mastocytosis, elevated baseline serum tryptase levels were found to be associated with very severe anaphylactic reactions to stings (104, 105).

Diagnosis

History

Information should be collected on: number and date of sting reactions, sort and severity of symptoms, interval between sting and the onset of symptoms, emergency treatment, sting site, retained or removed stinger, environment and activities before sting, risk factors of a particular severe reaction, risk factors for repeated re-stings, tolerated stings after the first systemic reactions, and other allergies.

Skin tests

It is recommended to perform skin tests at least 2 weeks after the reaction to a sting to avoid the possibility of false-negative results during the refractory period (6). Because the duration of refractoriness may be longer, they should, if negative in the presence of a definitive history of a systemic sting reaction, be repeated after 1–2 months.

Skin tests are performed by skin prick or intradermal testing. General procedural recommendations are outlined elsewhere (106). Stepwise incremental venom skin tests are recommended. If the patient has a conclusive reaction at a set concentration the test can be stopped. For skin prick test venom concentrations of 0.01–100 μg/ml are usually used. Intradermally a 0.02 ml venom concentration ranging from 0.001 to 1 μg/ml is injected into the volar surface of the forearm.

Even at 100 μg the sensitivity of skin prick tests is definitely lower than that of the intradermal test (107). In patients with a negative prick test it is therefore recommended to confirm this in the intradermal test. According to a number of studies, the sensitivity of intradermal testing may be estimated at about 90% or higher for a 1 μg/ml concentration (108–112). The specificity of skin tests with Hymenoptera venoms is difficult to define, because exposed patients who never developed a systemic reaction may have been sensitized following their last sting.

In vitro tests

Allergen-specific IgE. In vitro radioallergosorbent test (RAST) and a variety of methods derived from this test can be applied, the newer usually being more sensitive (113).

In the first few days after a sting the IgE specific to the injected venom may be low or may not even be demonstrable. Venom-specific IgE usually increases within days or weeks after a sting. Following this initial phase specific IgE declines slowly with a large individual variation (114, 115). In patients with no detectable specific IgE to the presumptive relevant venom, the tests should be repeated after a few weeks (114). A rapid change of venom-specific IgE antibodies shortly after a sting may provide an additional indication of the relevant venom (116–118).

Venom immunotherapy induces an initial rise of venom-specific IgE antibodies followed by a decline after a few months, with a large individual variation (115, 119). There is no clear correlation between the concentration of venom-specific IgE and the reactivity status of the individual patient.

Sensitivity of venom-specific IgE serum tests in patients with a history of systemic sting reactions is somewhat lower than that of intradermal skin tests, especially after the first year following a reaction (6). With regard to specificity, similar problems are found as with skin tests.

Double positivity of diagnostic tests to both bee and vespid venoms is not infrequently observed and may be due to actual double sensitization or to cross-reactivity between epitopes of the hyaluronidases of the two venoms (31). A particular problem in the field of cross-reactivity are specific IgE antibodies directed against carbohydrate epitopes, which may induce multiple positive test results of unknown clinical significance (25). The RAST inhibition test is helpful in distinguishing between cross-reactivity and double sensitization. This may be a relevant issue, when venom immunotherapy is being considered (36). The test for detection of venom-specific IgE is modified with the inclusion of an initial inhibition phase during which the patient's serum is incubated with venom extract from both species separately (44) or with carbohydrate epitope-containing aeroallergens (25).

Allergen-specific IgG. The level of specific IgG primarily reflects exposure. Venom-specific IgG increases after a sting and does not correlate with the presence or absence of an allergic sting reaction (6). Specific IgG initially decreases more rapidly than specific IgE (115).

In beekeepers bee venom-specific IgG correlates to the number of annual stings and to the number of years spent in bee-keeping (120). Venom immunotherapy is accompanied by an increase in allergen-specific IgG (115, 118), although neither concentration (or a change in concentration) of these antibodies nor the IgE/IgG ratio correlate closely to the clinical response to immunotherapy (121). Routine assessment of venom-specific IgG in the diagnosis of Hymenoptera venom allergy before or after treatment is not recommended.

Baseline serum tryptase. Concentration should be determined in all patients with a history of severe reactions (104, 105).

Other in vitro tests. When venom skin tests and the measurement of venom-specific IgE antibodies in serum by RAST or an equivalent method yield negative results in patients with a history of a systemic anaphylactic sting reaction, additional *in vitro* tests may be used to demonstrate immunologic sensitization (like immunoblotting, the basophil histamine release test, basophil activation test and leukotrine release test) (122–126).

Because of high costs the majority of these tests can only be performed in specialized laboratories. As these tests are not standardized, their results cannot be directly compared between centers. Moreover, data on sensitivity and specificity of this test, especially in relation to re-exposure, are still scarce.

Interpretation of skin test and *in vitro* test results

In subjects with a history of a previous anaphylactic sting reaction, sensitization is confirmed by the demonstration of venom sensitization by a skin test reaction to venom or the detection of venom-specific IgE-antibodies. To date it has not been possible to find a predictive marker that indicates more than sensitization. In particular, future systemic reactivity of untreated or treated patients cannot be predicted from skin test results or from any *in vitro* test: 25–84% of subjects with skin test reactions to venom

do not react to a subsequent sting from the culprit insect; on the contrary, 0–22% of subjects with negative skin tests will develop a systemic reaction (86, 98, 99, 127–129).

'*Negative*' *test results*. A small group of patients reporting systemic reactions to insect stings had no detectable venom-specific IgE in their serum and were 'negative' at skin testing (127). This could be due to insufficient sensitivity of tests, or to a long interval from the sting-induced reaction to testing with spontaneous decline in venom-specific IgE (130). The failure to detect venom-specific IgE provides no guarantee that the clinical reactivity has waned. A recent study (127) reported subsequent systemic reactions to sting challenge in 11 of 51 patients with positive histories but negative intracutaneous tests. Notably, when using a very sensitive RAST technique, nine of the 11 subjects had a positive RAST result with an analytic sensitivity of 1 ng/ml, indicating that a very low level of venom-specific IgE, not detected by the current technique of skin testing, is enough to elicit systemic reactions (127).

Sting challenge tests

As already evident from the fact that some patients tolerate VIT very well, but still have systemic reactions to a sting from the same insect, challenge tests with subcutaneously or intracutaneously administered venom are not reliable (82, 131). Therefore, if challenge tests are to be performed in Hymenoptera venom-allergic patients these should be performed using live insects; the practical aspects are described extensively elsewhere (132).

Sting challenge tests have been used in untreated patients with (96, 98, 100, 127, 128, 133) or without (86, 128) a history of anaphylactic sting reactions, mostly in order to identify those who need immunotherapy. The prognostic reliability of a tolerated sting challenge with respect to the outcome of a later field sting was found to be 85 (134) to 95% (135) in selected patients. If repeated sting challenges were performed several weeks (96) or a mean of 12 months (100) after a first tolerated sting, 6.5% of pediatric (96) and 21% of adult (100) patients had a systemic reaction only on exposure to the second sting. As a tolerated sting challenge does not fully predict the outcome of future stings in an individual patient and as untreated patients may develop very severe reactions to a sting challenge (99), testing of this sort is generally not recommended for diagnostic purposes in untreated patients (132, 136, 137).

Sting challenges are recommended in patients on maintenance VIT to identify those who are not yet protected. The effectiveness of VIT should be assessed by a sting challenge particularly in those patients who are at increased risk of re-stings due to high exposure or due to their proneness to very severe anaphylaxis. This could be of important practical use, as full protection may be achieved by an increase of the venom maintenance dose (138).

There are only few data on patients with repeated sting challenges during VIT. These indicate that the results of a tolerated sting challenge in patients on VIT are reliable as long as the treatment continues (92, 132).

Sting challenges have also been performed 1 year or more after stopping VIT in order to monitor the duration of the protection afforded by the treatment (132, 139–141). Sting challenges for these purposes should be restricted to scientific studies. This procedure is not recommended as a routine diagnostic method, as there is a risk that these stings might boost already decreased sensitization or even re-sensitizes the patient (132).

Future strategies

Potentially there is still much can be undergone to improve the diagnosis of Hymenoptera venom allergy. Thanks to modern molecular biology technology, a considerable number of major venom allergens both from the honeybee and various vespids are available today in recombinant form (Table 4) (18, 142). Recombinant venom allergens will certainly improve the diagnosis of venom allergy in the near future. There is a very close correlation with regard to their IgE-binding capacity (143) (comparison with the respective natural purified preparations). Some disparities have, however, been disclosed by RAST-inhibition and Western blot studies, which revealed that all natural preparations were contaminated with trace amounts of other venom allergens. Recombinant allergens will therefore be superior to highly purified natural preparations when it comes to the determination of the true clinical relevance of an individual allergen. Recombinant technology has also been very helpful in clarifying cross-reactivities between venom allergens from different species, genera or even families of Hymenoptera. Finally, the use of recombinant cocktails for diagnosis is promising (144).

Table 4. Recombinant Hymenoptera venom allergens (142)

Species	Allergen	Common name	Molecular weight (kDa)
Apis mellifera	Api m 1	Phospholipase A_2	16–20
	Api m 2	Hyaluronidase	43
	Api m 3	Melittin	2.9
	Api m 4	Acid phosphatase	49
Vespula vulgaris	Ves v 1	Phospholipase A_1	35
	Ves v 2	Hyaluronidase	45
	Ves v 5	Antigen 5	25
Dolichovespula maculata	Dol m 1	Phospholipase A_1	35
	Dol m 2	Hyaluronidase	45
	Dol m 5	Antigen 5	25
Polistes annularis	Pol a 5	Antigen 5	25

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