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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

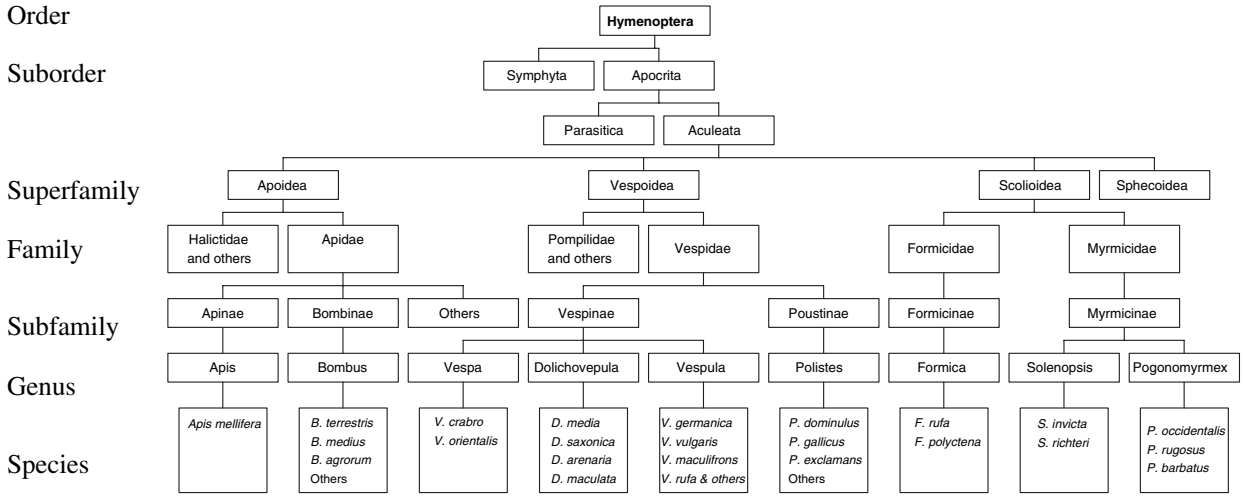


*Figure 1.* Taxonomy of Hymenoptera.

*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 |

## References


1. Bousquet J, Müller UR, Dreborg S, Jarish R, Malling HJ, Mosbech H et al. Immunotherapy with Hymenoptera venoms. Allergy 1987;**42**:401–413.
2. Müller U, Mosbech H. Position paper: Immunotherapy with Hymenoptera venoms. EAACI Allergy 1993;**48**:36–46.
3. Hoffman DR. Allergic reaction to biting insects. In: Levine MI, Lockey RF, eds. Monograph on Insect Allergy, 4th edn. Pittsburgh: Dave Lambert Associates, 2003: 161–173.
4. Chinery M. A field guide to the insects of Britain and Northern Europe. London, UK: William Collins Sons & Co. Ltd, 1984.
5. Sánchez F, Blanca M, Fernández J, Miranda A, Terrados A, Torres MJ et al. Comparative study between European and American species of *Polistes* using sera from European sensitised subjects. Clin Exp Allergy 1995;**25**:281–287.
6. Müller UR. Insect sting allergy. Stuttgart, Germany: Gustav Fischer, 1990.
7. de Groot H, de Graaf-in't Veld C, Gerth van Wijk R. Allergy to bumblebee venom: I. Occupational anaphylaxis to bumblebee venom: diagnosis and treatment. Allergy 2001;**1995**:581–584.
8. Seebach JD, Bucher Ch, Anliker M, Schmid-Grendelmeier P, Wüthrich B. Ameisengift: ein seltene Ursache für allergische Reaktionen in der Schweiz (Ant venoms: a rare cause of allergic reactions in Switzerland). Schweiz Med Wochenschr 2000;**130**(Nr47):1805–1813.
9. King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. Int Arch Allergy Immunol 2000;**123**:99–106.
10. Kettner A, Hughes GJ, Frutiger S, Astori M, Roggero M, Spertini F et al. Api m 6: a new bee venom allergen. J Allergy Clin Immunol 2001;**107**:914–920.
11. Hoffman DR, Jacobson RS. Allergens in Hymenoptera venom: XII. How much protein is in a sting? Ann Allergy 1984;**52**:276–278.
12. Schumacher MJ, Tveten MS, Egen NB. Rate and quality of delivery of venom from honeybee stings. J Allergy Clin Immunol 1994;**93**:831–835.
13. Schumacher MJ, Schmidt JO, Egen NB, Dillon KA. Biochemical variability of venoms from individual European and Africanized honeybees (*Apis mellifera*). J Allergy Clin Immunol 1992;**90**:59–65.
14. Edery H, Ishay J, Gitter S, Joshua H. Venoms of Vespidae. In: Bettini S, editor. Arthropod venoms. Berlin, New York, USA: Sprinter Verlag, 1978:691–77.
15. Owen MD, Pfaff LA, Reisman RE, Wypych J. Phospholipase A2 in venom extracts from honey bees (*Apis mellifera* L.) of different ages. Toxicon 1990;**28**:813–820.
16. Habermann E. Bee and wasp venoms. Science 1972;**177**:314–322.
17. Markovic-Housley Z, Miglierini G, Soldatova L, Rizkallah PJ, Müller U, Schirmer T. Crystal structure of hyaluronidase, a major allergen of bee venom. Structure Fold Des 2000;**15**:1025–1035.
18. Müller U. Recombinant venom allergens. Allergy 2002;**57**:570–576.
19. Paull BR, Yunginger JW, Gleich GJ. Melittin: an allergen of honeybee venom. J Allergy Clin Immunol 1977; **59**: 334–338.
20. Hoffman DR, Jacobson RS. Allergens in Hymenoptera venom: XXVII. Bumblebee venom allergy and allergens. J Allergy Clin Immunol 1996;**97**:812–821.
21. King TP, Kochoumian L, Joslyn A . Wasp venom proteins: phospholipase A1 and B. Arch Biochem Biophys 1984;**230**:1–12.
22. King TP, Alagon AC, Kuan J, Sobotka AF, Lichtenstein LM. Immunochemical studies of yellow jacket venom proteins. Mol Immunol 1983;**20**:297–308.
23. Henriksen A, King TP, Mirza O, Monsalve RI, Meno K, Ipsen H et al. Major venom allergen of yellow jackets, Ves v 5: structural characterization of a pathogenesis-related protein superfamily. Proteins 2001;**45**:438–448.
24. Hoffman D. Hymenoptera venom: XXIV. The amino acid sequences of imported fire ant venoms allergens Sol i 2, Sol i 3 and Sol i 4. J Allergy Clin Immunol 1993;**91**:71–78.
25. Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wöhrl S et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. J Allergy Clin Immunol 2001;**108**:1045–1052.
26. Nelson DR, Collins AM, Hellmich RL, Jones RT, Helm RM, Squillace DL et al. Biochemical and immunochemical comparison of Africanized and European honeybee venoms. J Allergy Clin Immunol 1990;**85**:80–85.
27. Schumacher MJ, Schmidt JO, Egen NB, Lowry JE. Quantity, analysis, and lethality of European and Africanized honey bee venoms. Am J Trop Med Hyg 1990;**43**:79–86.
28. Stapel SO, Waanders-Lijster Raadt de J, van Toorenenbergen AW, de Groot H. Allergy to bumblebee venom: II. IgE cross-reactivity between bumblebee and honeybee venom. Allergy 1998;**53**:769–777.
29. Bucher C, Korner P, Wüthrich B. Allergy to bumblebee venom. Curr Opin Allergy Clin Immunol 2001;**1**:361–365.
30. Hoffman DR. Allergens in Hymenoptera venom: XXV. The amino acid sequences of antigen 5 molecules and the structural basis of antigenic cross-reactivity. J Allergy Clin Immunol 1993;**92**:707–716.
31. King TP, Lu G, Gonzales M, Qian N, Soldatova L. Yellow jacket venom allergens, hyaluronidase and phospholipase. Sequence similarity and antigenic cross-reactivity with hornet and wasp homologs and possible implications for clinical allergy. J Allergy Clin Immunol 1996;**98**:588–600.
32. Wicher K, Reisman RE, Wypych J, Elliott W, Steger R, Mathews RS et al. Comparison of the immunogenicity of venoms of various species of yellow jackets (genus *Vespula*). J Allergy Clin Immunol 1980;**66**:244–249.
33. Hoffman DR. Allergens in Hymenoptera venoms: XVI. Studies of the structures and crossreactivities of vespid venom phospholipases. J Allergy Clin Immunol 1986;**78**:337–343.
34. Bischof M, Müller U, Volkart HD. How important are *Dolichovespula* (DV) in insect sting allergy? J Allergy Clin Immunol 1986;**77**:142 [abstract].
35. Blanca M, Garcia F, Miranda A, Carmona MJ, Garcia J, Fernandez J et al. Determination of IgE antibodies to *Polistes dominulus*, *Vespula germanica* and *Vespa crabro* in sera of patients allergic to vespids. Allergy 1991;**46**:109–114.
36. Hamilton RG, Wiesenauer JA, Golden DBK, Valentine MD, Adkinson FA. Selection of Hymenoptera venoms for immunotherapy on the basis of patient's IgE antibody crossreactivity. J Allergy Clin Immunol 1993;**92**:651–659.
37. Panzani R, Blanca M, Sanchez F, Juarez C. Sensitivity to European wasps in a group of allergic patients in Marseille: preliminary results. J Investig Allergol Clin Immunol 1994;**4**:42–46.

38. Hoffman DR. Allergens in Hymenoptera venoms: XV. The immunologic basis of vespid venom cross-reactivity. J Allergy Clin Immunol 1985;**75**:611–613.
39. Pantera B, Hoffman DR, Carresi L, Cappugi G, Turillazzi S, Manao G et al. Characterization of the major allergens purified from the venom of the paper wasp *Polistes gallicus*. Biochim Biophys Acta 2003;**1623**:72–81.
40. Wypych J, Abenounis C, Reisman R. Analysis of differing patterns of cross-reactivity of honey bee and yellow jacket venom-specific IgE: use of purified venom fractions. Int Arch Allergy Appl Immunol 1989;**89**:60–66.
41. Reisman RE, Müller UR, Wypych JI, Lazell MI. Studies of coexisting honeybee and vespid venom sensitivity. J Allergy Clin Immunol 1984;**73**:246–252.
42. Reisman RE, Wypych JI, Lazell MI. Further studies in patients with both honeybee- and yellow-jacket venom-specific IgE. Int Arch Allergy Appl Immunol 1987;**82**:190–194.
43. Schlenvoigt G, Müller M, Jäger L, Wenz W. In-vitro-Untersuchungen zum Auftreten von Doppelsensibilisierungen und ihre Charakterisierung bei Insektengiftallergikern. Allergologie 1996;**19**:461–464.
44. Straumann F, Bucher C, Wüthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Value of FEIA inhibition for the identification of the cross-reacting IgE antibodies in double-sensitized patients to honeybee and wasp venom. Int Arch Allergy Immunol 2000;**123**:268–274.
45. Johansson SGO, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001;**56**:813–824.
46. Vachvanichsanong P, Dissaneewate P, Mitarnun W. Non-fatal acute renal failure due to wasp stings in children. Pediatr Nephrol 1997;**11**:734–736.
47. Daher Ede F, da Silva GB Jr, Bezerra GP, Pontes LB, Martins AM, Guimaraes JA. Acute renal failure after massive honeybee stings. Rev Inst Med Trop Sao Paulo 2003;**45**:45–50.
48. De Bandt M, Atassi-Dumont M, Kahn MF, Herman D. Serum sickness after wasp venom immunotherapy: clinical and biological study. J Rheumatol 1997;**24**:1195–1197.
49. Creange A, Saint-Val C, Guillevin L, Degos JD, Gherardi R. Peripheral neuropathies after arthropod stings not due to Lyme disease: a report of five and review of the literature. Neurology 1993;**43**:1483–1488.
50. Boz C, Velioglu S, Ozmenoglu M. Acute disseminated encephalomyelitis after bee sting. Neurol Sci 2003;**23**:313–315.
51. Golden DBK, Marsh DG, Kagey-Sobotka A, Freidhoff L, Szklo M, Valentine MD et al. Epidemiology of insect venom sensitivity. JAMA 1989;**262**:240–244.
52. Grigoreas Ch, Galatas ID, Kiamouris Ch, Papaioannou D. Insect venom allergy in Greek adults. Allergy 1997;**52**:51–57.
53. Mauriello PM, Barde SH, Georgitis JW, Reisman RE. Natural history of large local reactions from stinging insects. J Allergy Clin Immunol 1984;**74**:494–498.
54. Fernandez J, Blanca M, Soriano J, Sanchez J, Juarez C. Epidemiological study of the prevalence of allergic reactions to Hymenoptera in a rural population in the Mediterranean area. Clin Exp Allergy 1999;**29**:1069–1074.
55. Charpin D, Vervolet D, Haddi E, Segalen C, Tafforeau M, Birnbaum J et al. Prevalence of allergy to Hymenoptera stings. Allergy Proc 1990;**11**:29–32.
56. Müller UR, Horat W, Wütrich B, Conroy M, Reisman RE. Anaphylaxis after Hymenoptera stings in three patients with urticaria pigmentosa. J Allergy Clin Immunol 1983;**72**:685–689.
57. Kors JW, van Doormaal JJ, de Monchy JG. Anaphylactoid shock following Hymenoptera sting as a presenting symptom of systemic mastocytosis. J Intern Med 1993;**233**:255–258.
58. Bucher C, Simic P, Furrer J, Wütrich B. Mastocytosis: an important differential diagnosis in anaphylactoid reactions to Hymenoptera sting. A case report and overview of clinical aspects, diagnosis and current therapy of mastocytosis. Schweiz Rundsch Med Prax 2000;**89**:411–418.
59. Fricker M, Helbling A, Schwartz L, Müller U. Hymenoptera sting anaphylaxis and urticaria pigmentosa: clinical findings and results of venom immunotherapy in ten patients. J Allergy Clin Immunol 1997;**100**:11–15.
60. Mueller HL. Diagnosis and treatment of insect sensitivity. J Asthma Res 1966;**3**:331–333.
61. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet 1977;**1**(8009):466–469.
62. Mosbech H. Death caused by wasp and bee stings in Denmark 1960–1980. Allergy 1983;**38**:195–200.
63. Sasvari T, Müller U. Fatalities from insect stings in Switzerland 1978 to 1987. Schweiz Med Wochenschr 1994;**124**:1887–1894.
64. Oude Elberink JNG, de Monchy JGR, Brouwer JLP, Golden DBK, Guyatt GH, Dubois AEJ. Development and validation of the vespid allergy quality of life questionnaire (VQLQ). J Allergy Clin Immunol 2002;**109**:162–170.
65. Incorvaia C, Mauro M, Pastorello EA. Hymenoptera stings in conscripts. Allergy 1997;**52**:680–681.
66. Novembre E, Cianferoni A, Bernardini RA, Ingargiola A, Lombardi E, Vierucci A. Epidemiology of insect venom sensitivity in children and its correlation to clinical and atopic features. Clin Exp Allergy 1998;**28**:834–838.
67. de la Torre-Morin F, Garcia-Robaina JC, Vazquez-Moncholi C, Fierro J, Bonnet-Moreno C. Epidemiology of allergic reactions in beekeepers: a lower prevalence in subjects with more than 5 years exposure. Allergol Immunopathol (Madr) 1995;**23**:127–132.
68. Annila IT, Karjalainen ES, Annila PA et al. Bee and wasp sting reactions in current beekeepers. Ann Allergy Asthma Immunol 1996;**77**:423–427.
69. Charpin D, Birnbaum J, Lanteaume A, Vervolet D. Prevalence of allergy to Hymenoptera sting in different samples of the general population. J Allergy Clin Immunol 1992;**90**:331–334.
70. Kalyoncu AF, Demir AU, Ozcan U, Ozkuyumcu C, Sahin AA, Baris YI. Bee and wasp venom allergy in Turkey. Ann Allergy Asthma Immunol 1997;**78**:408–412.
71. Schäfer T, Przybilla B. IgE antibodies to Hymenoptera venoms in the serum are common in the general population and are related to indications of atopy. Allergy 1996;**51**:372–377.
72. Bjornsson E, Janson C, Plaschke P, Norrman E, Sjoberg O. Venom allergy in adult Swedes: a population study. Allergy 1995;**50**:800–805.
73. Strupler W, Wüthrich B, Schindler CH, SAPALDIA-Team. Prävalenz der Hymenopterengiftallergien in der Schweiz: eine epidemiologische und serologische Studie der SAPALDIA-Stichprobe. Allergo J 1997;**6**(Suppl. 1):S7–S11.
74. Bousquet J, Menardo JL, Aznar R, Robinet-Levy M, Michel FB. Clinical and immunological survey in beekeepers in relation to their sensitization. J Allergy Clin Immunol 1984;**73**:332–340.

75. Abrishami MH, Boyd GK, Settipane GA. Prevalence of bee sting allergy in 2010 girl scouts. Acta Allergol 1971;**26**:117–120.
76. Settipane GA, Boyd GK. Prevalence of bee sting allergy in 4992 boy scouts. Acta Allergol 1970;**25**:286–291.
77. Settipane GA, Newstead GJ, Boyd GK. Frequency of Hymenoptera allergy in an atopic and normal population. J Allergy Clin Immunol 1972;**50**:146–150.
78. Charpin D, Birnbaum J, Vervloet D. Epidemiology of Hymenoptera allergy. Clin Exp Allergy 1994;**24**:1010–1015.
79. Barnard JH. Studies of 400 Hymenoptera sting deaths in the United States. J Allergy Clin Immunol 1973;**52**:259–264.
80. Antonicelli A, Bilò MB, Bonifazi F. Epidemiology of Hymenoptera allergy. Curr Opin Allergy Clin Immunol 2002;**2**:1–6.
81. Schwartz HJ, Squillace DL, Sher TH, Teigland JD, Yunginger JW. Studies in stinging insect hypersensitivity: postmortem demonstration of antivenom IgE antibody in possible sting-related sudden death. Am J Clin Pathol 1986;**85**:607–610.
82. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. N Engl J Med 1978;**299**:157–161.
83. Müller U, Thurnheer U, Patrizzi R, Spiess J, Hoigne R. Immunotherapy in bee sting hypersensitivity. Bee venom versus wholebody extract. Allergy 1979;**34**:369–378.
84. Brown S, Wiese M, Blackman K, Heddle R. Ant venom immunotherapy: a double blind, placebo-controlled cross-over trial. Lancet 2003;**361**:1001–1006.
85. Pucci S, Antonicelli L, Bilò MB, Garritani MS, Bonifazi F. The short interval between two stings as a risk factor for developing Hymenoptera venom allergy. Allergy 1994;**49**:894–896.
86. Golden DBK, Marsh DG, Freidhoff LR, Kwiterovich KA, Addison B, Kagey-Sobotka A, Lichtenstein LM. Natural history of Hymenoptera venom sensitivity in adults. J Allergy Clin Immunol 1997;**100**:760–766.
87. Annila IT, Annila PA, Morsky P. Risk assessment in determining systemic reactivity to honeybee stings in beekeepers. Ann Allergy Asthma Immunol 1997;**78**:473–477.
88. Schuberth KC, Lichtenstein LM, Kagey-Sobotka A, Szklo M, Kwiterovich KA, Valentine MD. Epidemiologic study of insect allergy in children: II. Effect of accidental stings in allergic children. J Pediatr 1983;**102**:361–365.
89. Valentine MD, Schuberth KC, Kagey-Sobotka A, Graft DF, Kwiterovich KA, Szklo M, Lichtenstein LM. The value of immunotherapy with venom in children with allergy to insect stings. N Engl J Med 1990;**323**:1601.
90. Engel T, Heinig JH, Weeke ER. Prognosis of patients reacting with urticaria to insect sting. Results of an in-hospital sting challenge. Allergy 1988;**43**:289–293.
91. Reisman RE. Natural history of insect sting allergy: relationship of severity of symptoms of initial sting anaphylaxis to re-sting reactions. J Allergy Clin Immunol 1992;**90**:335–339.
92. Chipps BE, Valentine MD, Kagey-Sobotka A, Schuberth KC, Lichtenstein LM. Diagnosis and treatment of anaphylactic reactions to Hymenoptera stings in children. J Pediatr 1980;**97**:177–184.
93. Lockey RF, Turkeltaub PC, Baird-Warren IA, Olive CA, Olive ES, Peppe BC et al. The Hymenoptera venom study I, 1979–1982: demographics and history-sting data. J Allergy Clin Immunol 1988;**82**:370–381.
94. Lantner R, Reisman RE. Clinical and immunologic features and subsequent course of patients with severe insect-sting anaphylaxis. J Allergy Clin Immunol 1989;**84**:900–906.
95. Przybilla B, Ring J, Grieshammer B. Association of features of atopy and diagnostic parameters in Hymenoptera venom allergy. Allergy 1991;**46**:570–576.
96. Hauk P, Friedl K, Kaufmehl K, Urbanek R, Forster J. Subsequent insect stings in children with hypersensitivity to Hymenoptera. J Pediatr 1995;**126**:185–190.
97. Schuetze GE, Forster J, Hauk PJ, Friedl K, Kuehr J. Bee-venom allergy in children: long-term predictive value of standardized challenge tests. Pediatr Allergy Immunol 2002;**13**:18–23.
98. Blaauw PJ, Smithuis LO. The evaluation of the common diagnostic methods of hypersensitivity for bee and yellow jacket venom by means of an in-hospital insect sting. J Allergy Clin Immunol 1985;**75**:556–562.
99. van der Linden PW, Hack CE, Struyvenberg A, van der Zwan JK. Insect-sting challenge in 324 subjects with a previous anaphylactic reaction: current criteria for insect-venom hypersensitivity do not predict the occurrence and the severity of anaphylaxis. J Allergy Clin Immunol 1994;**94**:151–159.
100. Franken HH, Dubois AE, Minkema HJ, van der Heide S, de Monchy JG. Lack of reproducibility of a single negative sting challenge response in the assessment of anaphylactic risk in patients with suspected yellow jacket hypersensitivity. J Allergy Clin Immunol 1994;**93**:431–436.
101. Antonicelli L, Bilò MB, Napoli G, Farabollini B, Bonifazi F. European hornet (*Vespa crabro*) sting: a new risk factor for life-threatening reaction in Hymenoptera allergic patients? Allerg Immunol (Paris) 2003;**35**:199–203.
102. Biedermann T, Ruëff F, Sander CA, Przybilla B. Mastocytosis associated with severe wasp sting anaphylaxis detected by elevated serum mast cell tryptase levels. Br J Dermatol 1999;**14**:1110–1112.
103. Oude Elberink JN, de Monchy JG, Kors JW, van Doormaal JJ, Dubois AE. Fatal anaphylaxis after a yellow jacket sting, despite venom immunotherapy, in two patients with mastocytosis. J Allergy Clin Immunol 1997;**99**:153–154.
104. Ludolph-Hauser D, Ruëff F, Fries C, Schopf P, Przybilla B. Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to Hymenoptera stings. Lancet 2001;**357**:361–362.
105. Haeberli G, Bronnimann M, Hunziker T, Müller U. Elevated basal serum tryptase and Hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy 2003;**33**:1216–1220.
106. Position paper: Allergen standardisation and skin tests. The European Academy of Allergology and Clinical Immunology. Allergy 1993;**48**(Suppl. 14):48–82.
107. Bjorkander J, Belin L. Diagnostic skin testing in Hymenoptera sensitivity. In: Oehling A, editor. Advances in allergology and applied immunology. New York, USA: Pergamon Press, 1980:733.
108. Hunt KJ, Valentine MD, Sobotka AK, Lichtenstein LM. Diagnosis of allergy to stinging insects by skin testing with Hymenoptera venoms. Ann Intern Med 1976;**86**:56–59.
109. Patrizzi R, Müller U, Yman L, Hoigné R. Comparison of skin tests and RAST for the diagnosis of bee sting allergy. Allergy 1979;**34**:249–256.
110. Meriney D, Nall TH, Wallace D, Rosenzweig D, Goel Z. Comparison of venom and wholebody RAST and intradermal testing in vespid-sensitive patients. Int Arch Allergy Appl Immunol 1980;**62**:442–452.

111. Wüthrich B, Wick H, Crass B, Wyss S. Zur Diagnostick der Hymenopterenstich-Allergie: ein Vergleich zwischen Anamnese, Hauttesten und IgE-Bestimmungen (RAST) mit Giftextrakten. Praxis 1981;**70**:934–943.
112. Georgitis JW, Reisman RE. Venom skin tests in insect-allergic and insect-nonallergic populations. J Allergy Clin Immunol 1985;**76**:803–807.
113. Jeep S, Kircholf E, O'Connor A, Kunkel G. Comparison of Phadebas RAST with the Pharmacia CAP system for insect venom. Allergy 1992;**47**:212–217.
114. Goldberg A, Confino-Cohen R. Timing of venom skin tests and IgE determinations after insect sting anaphylaxis. J Allergy Clin Immunol 1997;**100**:182–184.
115. Mosbech H, Christensen J, Dirksen A, Söborg M. Insect allergy. Predictive value of diagnostic tests: a three-year follow-up study. Clin Allergy 1986;**16**:433–440.
116. Heinig JH, Engel T, Weeke ER. Allergy to venom from bee or wasp: the relation between clinical and immunological reactions to insect stings. Clin Allergy 1988;**18**:71–78.
117. Rieger-Ziegler V, Rieger E, Kranke B, Aberer W. Hymenoptera venom allergy: time course of specific IgE concentrations during the first weeks after a sting. Int Arch Allergy Immunol 1999;**120**:166–168.
118. Ruëff F, Werfel S, Przybilla B. Change of the serum concentration of Hymenoptera venomspecific IgE antibodies after a systemic sting reaction – a possible diagnostic tool? Allergy 2003;**58**(Suppl. 74):99 [abstract].
119. Kemeny DM, Lessof MH, Patel S, Youlten LJ, Williams A, Lambourn E. IgG and IgE antibodies after immunotherapy with bee and wasp venom. Int Arch Allergy Appl Immunol 1989;**88**:247–249.
120. Annila I, Hurme M, Miettinen A, Kuusisto P, Nieminen MM. Lymphocyte subpopulations, cytokine release and specific immunoglobulin G in reactive and nonreactive beekeepers. J Investig Allergol Clin Immunol 1998;**8**:109–114.
121. Müller U, Helbling A, Bischof M. Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honey bee venom. Allergy 1989;**44**:412–418.
122. Zollner TM, Spengler K, Podda M, Ergezinger K, Kaufmann R, Boehncke WH. The Western blot is a highly sensitive and efficient technique in diagnosing allergy to wasp venom. Clin Exp Allergy 2001;**31**:1754–1761.
123. Przybilla B, Ring J, Wielgosch J. Der Basophilen-Histamin-Freisetzungstest als diagnostische Methode bei Hymenopterengift-Allergie. Hautarzt 1988;**39**:662–670.
124. Radermecker MF, Leclerq MD, Mariz SD, Louis RE. Basophil histamine releasability in patients with Hymenoptera venom allergy. Int Arch Allergy Immunol 1993;**101**:283–287.
125. Maly FE, Marti-Wyss S, Blumer S, Cuhat-Stark I, Wüthrich B. Mononuclear blood cell sulfidoleukotriene generation in the presence of interleukin-3 and whole blood histamine release in honey bee and yellow jacket venom allergy. J Investig Allergol Clin Immunol 1997;**7**:217–224.
126. Sainte-Laudy J, Sabbah A, Drouet M, Lauret MG, Loiry M. Diagnosis of venom allergy by flow cytometry. Correlation with clinical history, skin tests, specific IgE, histamine and leukotriene C4 release. Clin Exp Allergy 2000;**30**:1166–1171.
127. Golden DBK, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein M. Insect sting allergy with negative venom skin test responses. J Allergy Clin Immunol 2001;**107**:897–901.
128. Parker JL, Santrach PJ, Dahlberg MJE, Yunginger JW. Evaluation of Hymenoptera-sting sensitivity with deliberate sting challenges: inadequacy of present diagnostic methods. J Allergy Clin Immunol 1982;**96**:200–207.
129. Mosbech H. Insect allergy. A comparative study including case histories and immunological parameters. Allergy 1984;**39**:543–549.
130. Kontou-Fili K. Patients with negative skin tests. Curr Opin Allergy Clin Immunol 2002;**2**:353–357.
131. Schultze-Werninghaus C, Wahn U, Niggemann B. Evaluation of the risk of anaphylactic reactions by wasp venom-extract challenges in children. Pediatr Allergy Immunol 1999;**10**:133–137.
132. Ruëff F, Przybilla B, Müller U, Mosbech H. The sting challenge test in Hymenoptera venom allergy. Allergy 1996;**51**:216–225.
133. Halmerbauer G, Hauk P, Forster J, Urbanek R, Kaufmehl K, Koller DY. In vivo histamine release during the first minutes after deliberate sting challenges correlates with the severity of allergic symptoms. Pediatr Allergy Immunol 1999;**10**:53–57.
134. Blaauw PJ, Smithuis LOMJ. Report of the meeting of the Dutch Society for Allergology. Ned Tijdschr Geneeskd 1993;**137**:1903.
135. Van Halteren HK, van der Linden P-WG, Burgers SA, Bartelink AKM. Hymenoptera sting challenge of 348 patients: relation to subsequent field stings. J Allergy Clin Immunol 1996;**97**:1058–1063.
136. Dubois A. Investigational and clinical use of the sting challenge. Curr Opin Allergy Clin Immunol 2003;**3**:283–285.
137. Bilò MB, Brianzoni MF, Garritani MS, Antonicelli L, Farabollini B, Bonifazi F. The sting challenge test in Hymenoptera venom allergy: pros and cons. Allerg Immunol (Paris) 2003;**35**:377–381.
138. Ruëff F, Wenderoth A, Przybilla B. Patients still reacting to a sting challenge while receiving Hymenoptera venom immunotherapy are protected by increased venom doses. J Allergy Clin Immunol 2001;**108**:1027–1032.
139. Lerch E, Müller U. Long-term protection after stopping venom immunotherapy: results of re-stings in 200 patients. J Allergy Clin Immunol 1998;**101**:606–612.
140. Müller U, Berchtold E, Helbling A. Honeybee venom allergy: results of a sting challenge 1 year after stopping successful venom immunotherapy in 86 patients. J Allergy Clin Immunol 1991;**87**:702–709.
141. Van Halteren HK, van der Linden P-WG, Burgers JA, Bartelink AKM. Discontinuation of yellow jacket venom immunotherapy: follow-up of 75 patients by means of deliberate sting challenge. J Allergy Clin Immunol 1997;**100**:767–770.
142. Müller U. Recent developments and future strategies for immunotherapy of insect venom allergy. Curr Opin Allergy Clin Immunol 2003;**3**:299–303.
143. Müller U, Fricker M, Wymann D, Blaser K, Crameri R. Increased specificity of diagnostic tests with recombinant major bee venom allergen phospholipase A2. Clin Exp Allergy 1997;**27**:915–920.
144. Müller U, Soldatova L, Weber M. Bee venom allergy: comparison of IgE-binding capacity of purified natural and recombinant-synthetic venom allergens. J Allergy Clin Immunol 1998;**101**:33.