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EAACI POSITION PAPER

DIAGNOSIS OF HYMENOPTERA VENOM ALLERGY

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SUMMARY

The purpose of diagnostic procedure is to classify a sting reaction by history, identify the underlying pathogenetic mechanism, and identify the offending insect. Several major allergens, usually glycoproteins with a molecular weight of 10-50 kDa, have been identified in venoms of bees, vespids and ants. Diagnostic tests comprise skin tests with hymenoptera venoms and analysis of the serum for venom-specific IgE; they should be done in all patients with a history of a systemic sting reaction. If negative they should be repeated several weeks later. Serum tryptase should be analysed in patients with a history of a severe sting reaction.

Keywords: allergy, diagnosis, hypersensitivity, hymenoptera venom

1. INTRODUCTION

The first EAACI position paper on immunotherapy with Hymenoptera venoms was published in 1987 (1). Six years later a revised version appeared (2). Since 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 review considers also the evidence of individual statements and recommendations according to new guidelines (3).
This Position Paper was split up into two parts: **Diagnosis** and **Prevention and Treatment**. It focuses on Hymenoptera venom allergy, as allergic reactions caused by stings of insects other than Hymenoptera are rare and standardised extracts for the diagnosis and treatment of allergic reactions to non-Hymenoptera insects are not available.

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.

2. TAXONOMY

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

The family ***Apidae*** consists of the honeybees *(Apis mellifera)* who are brown in colour and moderately hairy **(Fig. 1a)** and the bumble-bees *(*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 USA) are the most important species in Europe. The *Vespula spp* (*V. germanica* and *V. vulgaris* **(Fig. 1b)** 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 **(Fig. 1d).**

Polistinae (called wasps in Europe and USA): In Europe *Polistes gallicus, P. nimpha* and above all *P. dominulus* **(Fig. 1c)** 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.

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Table 1

Taxonomy of hymenoptera

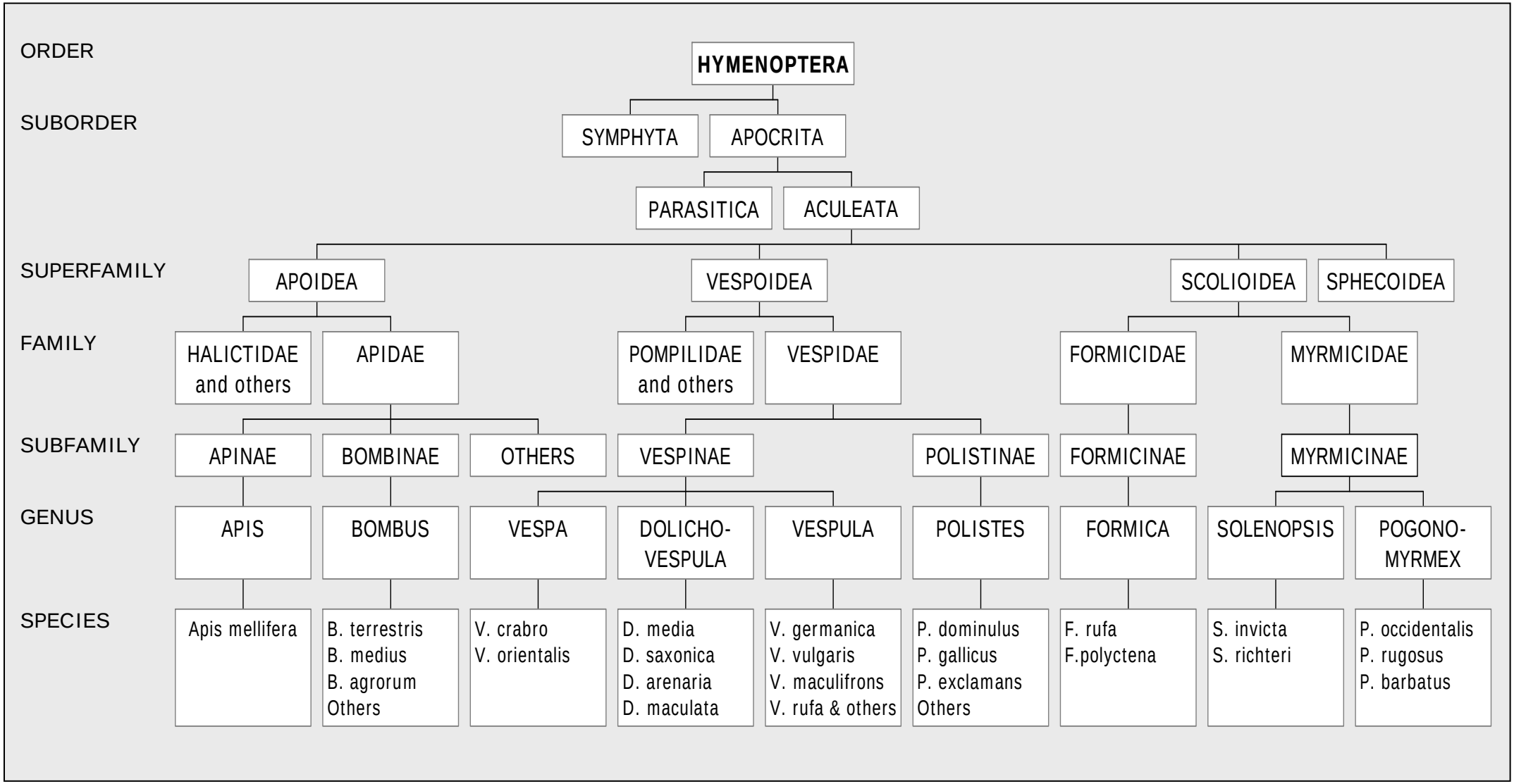
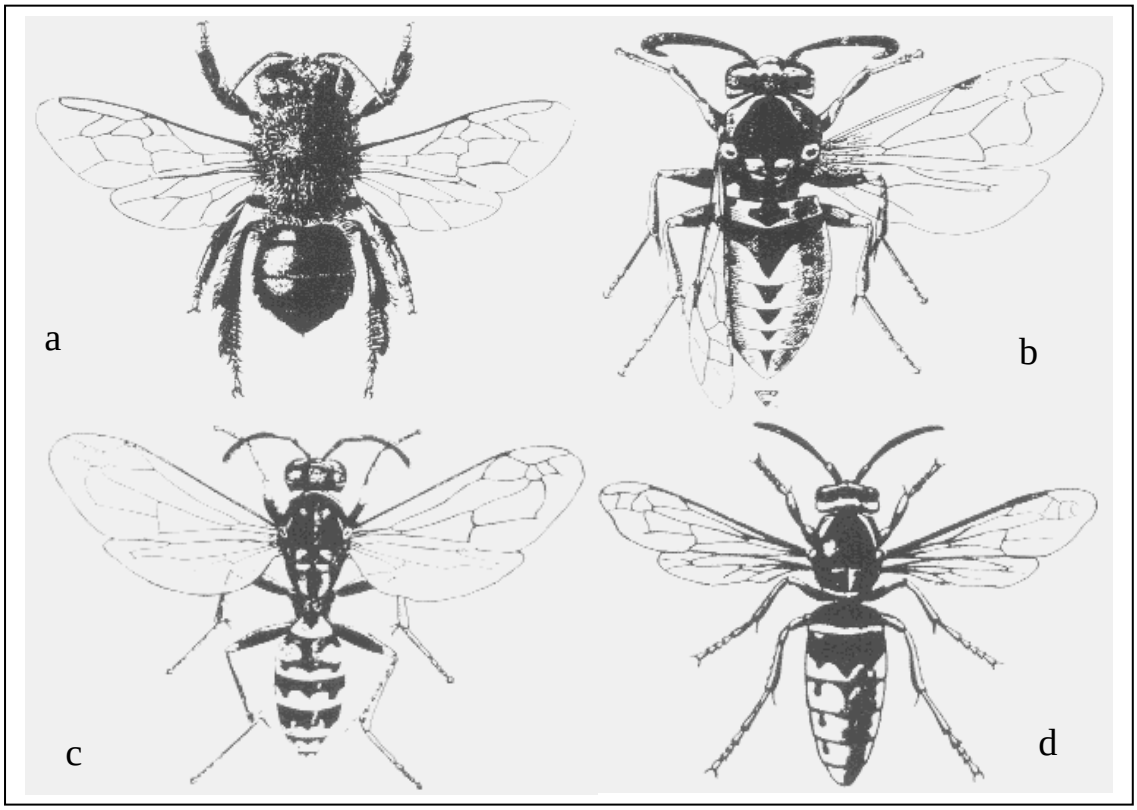


Figure 1

Drawings of the main European hymenoptera: a) Apis mellifera b) Vespula spp. c) Polistes dominulus and d) Vespa crabro. With permission of Carlos Perez Santos.



3. ENTOMOLOGY, BEHAVIOUR OF INSECTS AND RISK OF STINGS

With respect to allergic sting reactions mainly social Aculeatae - *Vespidae* (vespids), *Apidae* (bees), and *Formicidae* (ants) - are of importance. Social insects have developed a division of labour, in which sterile females form a class of workers, and take care of nest-building, brood care, and defence of the nest or hive. Female workers have a stinger, which is formed from the ovipositor and is not usable for the laying of eggs.
The stinging apparatus in female *Aculeatae* consists of a supporting stiletto and two lancets covering a poison canal. The venom is produced in the acid gland and stored in the venom sac, from which it is squeezed. Since the sting lancet has barbs, especially in bees, it works its way deeper into the elastic skin of mammals, but not into the chitinous surface of other insects. Bees can therefore remove their stinger from other insects, but normally their stinger remains in human skin.
Knowledge of insect behaviour and biology may help to identify the stinging insect and to avoid further stings. Insects which forage on human foods pose the greatest threat to man. Humans are not only stung by these insects in the vicinity of the nest site but also in everyday routine activities. Human food is mainly attractive for *Vespula spp*. However, honeybees and other insects are also attracted by sweets and fruits.

Apidae: Honey bees and bumble-bees tend only to sting in defence or when a critical distance to the nest is overpassed (6). Honeybees are bred by man all over the world for

pollination, honey and wax production. Bumble-bees are increasingly used in green houses for their efficiency as pollinators of certain plants (7).
The whole beehive colony usually survives through winter and even on warm winter days honeybees may fly and sting. In contrast to vespids, large colonies of honey bees exist already in spring and therefore bee stings are common already at that time of the year.

Vespidae: Since only the queens survive the winter, larger populations are only observed in summer, which is when most vespid stings occur. *Vespula* preferably build their nests underground, in attics or shelters. *V. germanica* and *V. vulgaris* are more aggressive. Stings may often occur without any obvious reason. *Dolichovespula* build their nests often hanging freely on tree branches or under the roofs of houses. Stings are rare and occur almost exclusively in the vicinity of the nests.
Vespa crabro (European hornet) build their nests in hollow tree-trunks or in birds' nesting-boxes (8). Hornet stings are rare and occur almost exclusively in the vicinity of the nests.
European species of *Polistes* are well adapted to the climate of the Mediterranean basin. However, small colonies of *Polistes* may be observed all over Europe except for the British islands. Their paper nests hang from tree branches or roofs, contain only one womb and few individuals.

Ants*:* The species *Pogonomyrmex* (harvesting ant) and *Solenopsis* (*S. invicta,* red imported fire ant) are the most important species responsible for allergic sting reactions in Northern and Central America (9) and *Myrmecia (M. pilosula,* the jack jumper ant) in Australia (10). In Europe, allergic reactions to ants seem to be extremely rare and only a few cases have been reported, mostly to *Formica rufa*~~,~~ (11).

In 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; bumble-bee stings are rare throughout Europe and more of an occupational hazard.
The stinger of honeybees usually remains in human skin. Bumble-bees and vespids normally remove their stinger from the human skin.

4. VENOM ALLERGENS (TABLE 2)

Knowledge of the composition of venoms and structure of allergens is a prerequisite for the accurate diagnosis and treatment of insect venom allergy.
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 (www.allergen.org). Most of them are glycoproteins of 10-50 kDa containing 100 – 400 amino acid residues (12). However, some venom components are smaller, e.g. honeybee venom melittin (Api m 4), a 2.9 kDa peptide, and the recently described Api m6 with a molecular weight of 7.9 kDa (13).

Table 2

Allergens of hymenoptera venoms

Venom	Allergen	Common name	MW (KD)	major / minor
Apis mellifera	Api m 1 Api m 2 Api m 3 Api m 4 Api m 6	phospholipase A2 hyaluronidase acid phospatase melittine protease	16 43 49 2.9 7.9 39	major major major? minor minor major?
Bombus pennsylvanicus	Bom p 1 Bom p 4	phospholipase A2 serine protease		major major?
Vespula vulgaris Accordingly in V.germanica, maculifrons=etc.	Ves v 1 Ves v 2 Ves v 5	phospholipase A1 hyaluronidase antigen 5	35 45 25	major major major
Dolichovespula maculate accordingly in D. arenaria, D.media etc	Dol m 1 Dol m 2 Dol m 5	phospholipase A1 hyaluronidase antigen 5	35 45 25	major major major
Polistes annularis Accordingly in P.dominulus, gallicus, fuscatus etc	Pol a 1 Pol a 2 Pol a 5	phospholipase A1 hyaluronidase antigen 5	 25	major major major
Vespa crabro	Vesp c 1 Vesp c 5	phospholipase A1 antigen 5		major? major?
Solenopsis invicta	Sol i 1 Sol i 2 Sol i 3 Sol i 4	phospholipase A1 antigen 5	37 13.2 24 13.4	major? major?

4.1 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 (14) up to 140 μg (15) of venom protein per sting; however, venom sacs may contain up to more than 300 μg of venom (16). Bumble-bee stings release 10 to 31 μg of venom (14). In contrast *Vespinae*, which are capable of repeated stings, generally inject less venom per sting: *Vespula* stings release 1.7 to 3.1 μg of venom, *Dolichovespula* stings 2.4 to 5.0 micrograms and *Polistes* stings from 4.2 to 17 micrograms of protein (14). 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 (17).

4.2 Composition of venoms (Table 2)

4.2.1 *Apidae* venoms

Honeybee venom contains five allergens of known sequence: phospholipase A_2 (Api m1), hyaluronidase (Api m2), mellitin (Api m 4), Api m 6 and acid phosphatase (Api m 3). The most important allergen in honeybee venom is phospholipase A_2, which is a glycoprotein

with 134 amino acid residues. The enzyme acts as a cytotoxin and an indirect cytolysin (18). Phospholipase A_2 comprises 12-15 % of the dry weight of bee venom. Hyaluronidase is another major allergen of honeybee venom. It shares a 50% sequence identity with vespid venom hyaluronidase (19). Acid phosphatase is an enzyme of 49 kD which has been partially cloned and sequenced (20). 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 (13). Melittin is a major bee venom component (50 % of dry weight) but only 28 % of patients have specific IgE antibodies against this peptide (21). Melittin consists of 26 amino acids residues (22).

Bumble-bee venom contains phospholipase A_2 (Bom p 1), protease (Bom p 4), hyaluronidase, acid phosphatase and several other proteins not found in honeybee venom (23).

4.2.2 *Vespinae* venoms

The major allergens in vespid venoms are phospholipase A_1 (Ves v 1), hyaluronidase (Ves v 2) and antigen 5 (Ves v 5) (14, 24).
Phospholipase A_1 comprises 6-14 % of the total dry weight of vespid venom (25). Antigen 5 is a major allergen in all vespid venoms (26).

4.2.3 Ant venoms

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 (27). Sol i 3 has about a 50 % sequence identity with antigen 5 from vespid venoms.

4.3 Cross-reactivity

Double or even multiple positive tests can be caused by true double sensitization or by cross-reactive IgE-antibodies which recognise similar epitopes of different venoms, especially carbohydrate-containing epitopes of venoms and common allergens (28). The distinction between cross-reactivity and “true” double-sensitisation is important for the choice of venom(s) for immunotherapy.

4.3.1. Cross-reactivity within the Apidae family

Available data suggests that venoms and major allergens of different honeybees world-wide are very similar, and that the structure of the major allergen phospholipase A_2 is highly identical (29, 30). By comparison, the variability of allergens within bumble-bee venoms is higher (23). Bumble-bee PLA_2 is only 53 % identical to honeybee PLA_2. However, immunological cross-reactivity does exist between honeybee and bumble-bee venoms and concurrent sensitisation can be found in many patients (31, 32).

4.3.2. Cross-reactivity within vespid venoms

Cross-reactivity among vespids is strong, due to similarities of venom composition and structure of single allergens (33,34). The allergens from different *Vespula* species show identities up to 95 % (33,34). Correspondingly, different *Vespula* venoms strongly cross-react (25,35). There is also substantial cross-reactivity between *Vespula*, *Vespa*, and *Dolichovespula* venoms (35-40). Cross-reactivity of the *Vespinae* (*Vespula*, *Dolichovespula*, and *Vespa*) with paper wasps (*Polistes*) is generally lower than cross-reactivity within the *Vespinae* (24,36,38,39,41). The cross-reactivity among European species of Polistes (*P. dominulus, P. gallicus*) is very strong, whereas that between European and American species weaker (5,42).

4.3.3. Cross-reactivity between venoms of Apidae and Vespidae

The enzyme hyaluronidase shows an approximately 50 % sequence identity between honeybee and vespid venoms (34), and has been identified as the major cross-reactive component (43-47).

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 major venom allergens have been determined and several have been expressed in recombinant form.
A particular problem in the field of cross-reactivity are specific IgE antibodies directed against carbohydrate epitopes, which may induce multiple positive test results (skin test, in vitro tests) of still unknown clinical significance.

5. CLINICAL PRESENTATION AND PATHOGENESIS OF STING REACTIONS

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 non-immunologic mechanisms (48). Reactions to Hymenoptera stings are classified into normal local reactions, large local reactions, systemic toxic reactions, systemic anaphylactic reactions, and unusual reactions.

5.1 Normal local sting reactions

The injection of Hymenoptera venom by a sting into the skin of non-allergic subjects elicits a local reaction with pain, erythema and slight swelling around the sting site. Normally, local symptoms subside within 24 hours and only a small sting reaction may remain visible for a few days.

5.2 Large local reactions

As yet, there is no universally accepted definition of a large local reaction (LLR) (49-53). To easily distinguish between normal and large local reaction, the latter is defined in this position paper as a swelling exceeding a diameter of 10 cm which lasts longer than 24 hours; blisters may rarely be present. LLRs may be very disturbing and cause a great deal of discomfort, especially when they last several days or weeks and involve a whole limb or affect the eyes or the lips. LLRs may also be accompanied by unspecific systemic inflammatory symptoms, e.g., a feeling of sickness, shivering, fever, or headaches. In the extremities, LLRs are sometimes accompanied by swollen lymph glands and even lymphangitis.
The underlying mechanisms of large local reactions are unknown. According to some studies LLRs are associated with a positive skin test in 70 - 90% (50,54,55) or demonstration of venom-specific IgE antibodies in 26 – 50 % (54,56-58). These findings suggest an underlying IgE-mediated mechanism in some patients. In others the clinical course, skin and in vitro tests suggest a cell- mediated allergic pathogenesis (6) or a combination of both.

5.3 Systemic anaphylactic reactions

Systemic anaphylactic reactions are most often IgE-mediated. Rarely, non-IgE-mediated anaphylactic reactions are observed, which may be due to short term sensitising 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

(59-61). However, venom sensitisation is demonstrable in the majority of patients with mastocytosis and previous anaphylactic sting reactions (62).
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 (63) and by Ring (64), which are given in **Tables 3 and 4**.
Most often symptoms appear within a few minutes to one hour after the sting (6), but rarely they can occur hours or even days later (65). Normally, the patient recovers from anaphylactic reactions within a few hours. Rarely a biphasic course is observed with an early onset, an apparent recovery and a subsequent relapse after 4-24 hours. Fatal reactions after insect stings may occur. Autopsies after fatal sting reactions revealed significant cardiopulmonary co-morbidity in 50 % (66) or even the majority of the unlucky victims (67).
Severe reactions or a status after resuscitation may leave patients with a permanent disorder: hypoxic brain damage with permanent neurological deficits, and myocardial infarction (6). In pregnant women impaired blood circulation may impair the foetus and lead to premature birth (68) or to malformations of the central nervous system (69).

Table 3

Classification of systemic reactions to insect stings by H. L. Mueller (63)

Grade I	Generalized urticaria, itching, malaise and anxiety
Grade II	Any of the above plus two of 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.

Table 4

Classification of systemic reactions modified according to J. Ring and Messmer (64)

Grade I	Generalised skin symptoms (e.g. flush, generalised urticaria, angioedema)
Grade II	Mild to moderate pulmonary, cardiovascular, and/or gastrointestinal symptoms
Grade III	Anaphylactic shock, loss of consciousness
Grade IV	Cardiac arrest, apnoea

5.4 Effect of a systemic anaphylactic reaction on the quality of life

For most patients as well as for their families, an anaphylactic reaction after a Hymenoptera sting is very traumatic event. Patients therefore will do their utmost to prevent a re-sting. This can result in a dramatically altered lifestyle which can and often has adverse effects on emotional, social and, in some cases, professional functioning. Recently a disease specific questionnaire, the Vespid Allergy Quality of Life Questionnaire, was designed and validated

for assessing Health Related Quality of Life in patients with anaphylactic responses following yellow jacket stings. The survey showed that patients 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 (70).

5.5 Systemic toxic reactions

Some components of Hymenoptera venom (such as phospholipase and hyaluronidase) have a toxic effect (71-73).
In principle, toxic reactions are influenced by the venom composition and are dose-dependent, occurring only after multiple, usually 50 to several hundred stings.
The appearance of symptoms ranges from hours to days (6,73). Symptoms may comprise rhabdomyolysis, myocardial damage, hepatic dysfunction (74), intravascular haemolysis (75,76), acute renal failure (72,77,78) and coagulation disorders with bleeding and disseminated intravascular coagulation. The number of stings, which may induce a fatal reaction varies between 200 and 1000 (79). In younger children less than 50 stings may prove lethal (80). However, several case reports document that adults have survived 500 (81), or even more than 2000 (74) bee stings after adequate treatment and a child more than 800 (82).

5.6 Unusual reactions

Unusual reactions are rare and systematic studies are not available. In some cases toxic or non-IgE-mediated immunologic mechanisms may be responsible. The causal relation to the sting for many of these unusual reactions is doubtful, especially if they are based on only one or a few case reports. Serum sickness like syndromes with fever, arthralgias, urticaria/angioedema, lymphadenopathy and neurological symptoms (65,83,84) as well as hypersensitivity vasculitis (6) have been reported in relation with hymenoptera stings.
Peripheral neuropathy (85), Guillain-Barré-like polyradiculomyelitis (86), extrapyramidal syndromes (87) and acute disseminated encephalomyelitis (88) have also been reported. Some of these reactions were fatal. Occasionally glomerulonephritis (86,89), acute allergic interstitial nephritis (90), haemolytic anemia and thrombocytopenia have also been observed after single stings (6).

6. EPIDEMIOLOGY

6.1 Large local reactions

The prevalence of large local reactions ranges from 2.4 % (91), 4.6 % (55), 18.6% (54), up to 26.4 % (57). In children the prevalence yielded by one study is 19 % (92) and in beekeepers as high as 38 % (93, 94).

6.2 Systemic anaphylactic reactions

Epidemiological studies report a prevalence of self-reported systemic anaphylactic sting reactions between 0.3 % and 7.5 % (55, 57, 91,92, 95-100) (**Table 5**). The prevalence of systemic reactions among beekeepers is high and falls between 14 % and 43 % (94, 101). In children prevalence rates are lower: questionnaires in several thousand girl and boy scouts in USA (102-104) and children in Europe (92) resulted in a prevalence of only 0.15-0.3 %.

Table 5

Prevalence of systemic anaphylactic sting reactions. Recent epidemiological studies in Europe.

Author	Country	Study population	n	Methods*	Systemic reactions (%)
Charpin, 1992 (95)	France	General population	8271	Qu	0.6-3.3
Björnsson, 1995 (99)	Sweden	General population	1815	Qu, skin test, IgE	1.5
Strupler, 1995 (100)	Switzerland	General population	8322	Qu,IgE	3.5
Schäfer, 1996 (98)	Germany	Rural area population	277	Qu, skin test, IgE	3.3
Kalyoncu, 1996 (97)	Turkey	Cellulose paper factory and family members	786	Qu, skin test, IgE	7.5
Grigoreas, 1997 (55)	Greece	Hellenic air force	480	Qu, skin test, IgE	3.1
Novembre, 1998 (92)	Italy	School children	1175	Qu, skin test	0.34
Incorvaia, 1997 (91)	Italy	Conscripts	701	Qu	2.7
Fernandez, (57)	Spain	Rural area population	1600	Qu, skin test, IgE	2.3

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

6.3 Mortality

The incidence of insect sting mortality is low, ranging from 0.03 to 0.48 fatalities per 1.000.000 inhabitants per year (6, 66, 96, 97, 105, 106)**.** However, the true number may be underestimated: in one study (107) the authors reported the presence of venom-specific IgE in 23 % of post-mortem serum samples taken from subjects, who had died outdoors suddenly and inexplicably between the end of May and the beginning of November. Around 40 % (67) to 85 % (66) of the subjects with fatal reactions after Hymenoptera stings had no documented history of previous anaphylactic reactions.

7. RISK FACTORS OF HYMENOPTERA VENOM ALLERGY

A distinction has to be drawn between risk factors that are associated with a higher risk of stings and those increasing the risk to develop a severe sting reaction. Zone, climate, temperature, insect behaviour and personal exposure will influence the risk of receiving a sting. Certain occupations or activities are associated with an increased risk of Hymenoptera stings, e.g. gardeners, farmers, beekeepers (and their family members), greenhouse workers, food handlers, bakers. 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.

7.1 Risk factors influencing the outcome of an anaphylactic reaction

When patients who received placebo or wholebody extract in controlled studies on venom immunotherapy (108-110) were exposed, 57 to 75% of the patients with a history of systemic anaphylactic sting reaction develop further systemic symptoms when re-stung. Several factors are associated with the occurrence and the severity of a systemic anaphylactic resting reaction:

7.1.1 Time interval between stings, number of stings

A short interval between stings increases the risk of a systemic reaction to the later one (111). With increasing interval between stings the risk declines steadily, but remains in the range of 20 to 30 % even after ten years (112).
On the other hand, being stung very frequently appears to induce tolerance: 45 percent of beekeepers who were stung less than 25 times a year had a history of systemic sting reactions, as compared to none of those with more than 200 stings per year (93, 101).

7.1.2 Venom sensitisation

Irrespective of the previous history, beekeepers with an increased pre-seasonal concentration of bee venom-specific IgE (> 1.0 kU/L) had a 12-fold increased risk of systemic reactions (113). 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 % versus zero in skin-test-negative individuals (112).

7.1.3 Severity of the preceding reaction

After a large local sting reaction, between 5% and 15 % (6, 50, 56) 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 (114, 115) and 14 % to 20% in adults with mild (116) to 79 % in adults with severe reactions (117).

7.1.4 Age

In children about 60 % of systemic sting reactions are mild (118), whereas in adults respiratory or cardiovascular symptoms occur in about 70 % (119). Elderly patients more often develop particularly severe sting reactions (119-121), 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 (122, 123) and studies of the natural course (114, 115, 117) of insect venom allergy show lower risks in children than adults.

7.1.5 Cardiovascular diseases, beta-blockers

Studies on larger groups of patients identified cardiovascular diseases (66, 120), or treatment with beta-blocking drugs (120) to be associated with particularly severe sting reactions. Beta-blockers do not however seem to increase the over-all risk of a systemic reaction.

7.1.6 Insect

Bee venom-allergic patients are at a greater risk of a systemic reaction on next sting than those with vespid venom allergy (108, 124-126). 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 (127).

7.1.7 Elevated serum tryptase, mastocytosis

Several case reports suggest that particularly severe, even fatal sting reactions may occur in patients with mastocytosis (59-62, 128, 129).
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 (130, 131).

8. DIAGNOSIS

8.1 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.

8.2 Skin tests

It is recommended to perform skin tests at least two 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 to 2 months.
Skin tests are performed by skin prick or intradermal testing. General procedural recommendations are outlined elsewhere (132). 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 tests venom concentrations of 0.01 up to 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 (133). 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 (134-138).
The specificity of skin tests with Hymenoptera venoms is difficult to define, since exposed patients who never developed a systemic reaction may have been sensitized following their last sting (134-138).

8.3 In vitro tests

8.3.1 Allergen specific IgE

In vitro Radio Allergo Sorbent Test (RAST) and a variety of methods derived from this test can be applied, the newer usually being more sensitive (139).
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 (140, 141). In patients with no detectable specific IgE to the presumptive relevant venom, the tests should be repeated after a few weeks (140).
A rapid change of venom-specific IgE-antibodies shortly after a sting may provide an additional indication of the relevant venom (142-144).

Venom immunotherapy induces an initial rise of venom-specific IgE antibodies followed by a decline after a few months, with a large individual variation (141, 145). 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 crossreactivity between epitopes of the hyaluronidases of the two venoms (34). 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 (28). The RAST-Inhibition test is helpful in distinguishing between crossreactivity and double sensitization. This may be a relevant issue, when venom immunotherapy is being considered (39). 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 (47) or with carbohydrate epitope containing aeroallergens (28). In case of true double sensitization there is only inhibition by the homologous allergen.

8.3.2 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 (141).
In beekeepers bee venom specific IgG correlates to the number of annual stings and to the number of years spent bee-keeping (146). Venom immunotherapy is accompanied by an increase in allergen specific IgG (141,145), although neither concentration (or a change in concentration) of these antibodies nor the IgE/IgG ratio correlate closely to the clinical response to immunotherapy (147).
Routine assessment of venom-specific IgG in the diagnosis of Hymenoptera venom allergy before or after treatment is not recommended.

8.3.3 Baseline serum tryptase

Concentration should be determined in all patients with a history of severe reactions.

8.3.4 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 sensitisation (148-152).
Specific IgE and IgG/IgG4-antibodies to individual venom allergens can be identified by **immunoblotting** (148). In the **basophil histamine release test** peripheral blood leukocytes are incubated with venom allergens, which react with cell-bound IgE antibodies and thus stimulate cells, mainly basophils, to release histamine (149-150). In the **leukotriene release test** (cellular antigen stimulation test, CAST) blood leukocytes pre-stimulated with IL-3 are exposed to venom allergens and the release of sulfidoleukotriens is determined by ELISA (151).
The **basophil activation test** is a novel method based on the flow cytometric demonstration of an altered membrane phenotype of basophils activated by allergen

exposure. The currently most commonly used marker to demonstrate basophil activation is CD63 (152).
Because of high costs the majority of these tests can only be performed in specialised laboratories. As these tests are not standardised, their results cannot be directly compared between centres. Moreover data on sensitivity and specificity of this test, especially in relation to re-exposure, are still scarce.

8.4 Interpretation of skin test and in-vitro test results

In subjects with a history of a previous anaphylactic sting reaction, sensitisation is confirmed by the demonstration of venom sensitisation 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 which indicates more than sensitisation. In particular, future systemic reactivity of untreated or treated patients cannot be predicted from skin test results or from any *in-vitro* test: 25 to 84 % of subjects with skin test reactions to venom do not react to a subsequent sting from the culprit insect; on the other hand, up to 22 % of subjects with a history of systemic reactions and negative skin tests will develop a systemic reaction on exposure (112, 124, 125, 153-155).

8.4.1 "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 (153). 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 (156). The failure to detect venom-specific IgE provides no guarantee that the clinical reactivity has waned. A recent study evaluated a group of 51 patients with positive histories but negative intracutaneous tests with concentrations of up to 1 μg/ml and reported subsequent systemic reactions to sting challenge in 11 subjects (22 %). Notably, when using a very sensitive RAST technique, 9 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 (153).

8.5 Sting challenge tests

Challenge tests aim to reveal the clinical reactivity of an individual upon exposure to a defined allergen. In Hymenoptera venom allergy a challenge test which could be administered stepwise with incremental venom doses is considered desirable. However, 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 sub- or intracutaneously administered venom are not reliable (108, 157). Therefore if SCT are to be performed in hymenoptera-venom-allergic patients these should be performed using live insects. The practical aspects of performing sting challenges are described extensively elsewhere (158).

8.5.1 Individuals not treated by VIT

Sting challenge tests have been used in untreated patients with (122-124, 126, 153, 154, 159) or without (122, 154) 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 % (160) to 95 % (161) in selected patients. If repeated sting challenges were performed several weeks (122) or a mean of 12 months (126) after a first tolerated sting, 6.5 % of paediatric (122) and 21 % of adult (126) 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 (125), testing of this sort is not recommended for diagnostic purposes in untreated patients (158, 162, 163).

8.5.2 Individuals on VIT

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 (164).
However, only a “positive” reaction gives evidence of clinical reactivity, whereas a “negative” reaction cannot definitely prove clinical tolerance. 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 (118, 165).

8.5.3 After VIT

Sting challenges have also been performed one year or more after stopping VIT in order to monitor the duration of the protection afforded by the treatment (166-170). 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 sensitisation or even re-sensitise the patient (158).

- Diagnostic tests should be done in all patients with a history of a systemic sting reaction to detect sensitisation.
- Diagnostic tests 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 skin prick tests are negative subsequently intradermal tests should be done.
- If diagnostic tests are negative they should be repeated several weeks later.
- If both skin tests and specific IgE stay negative additional in-vitro tests should be carried out.
- Serum tryptase should be analysed in patients with a history of a severe sting reaction.

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