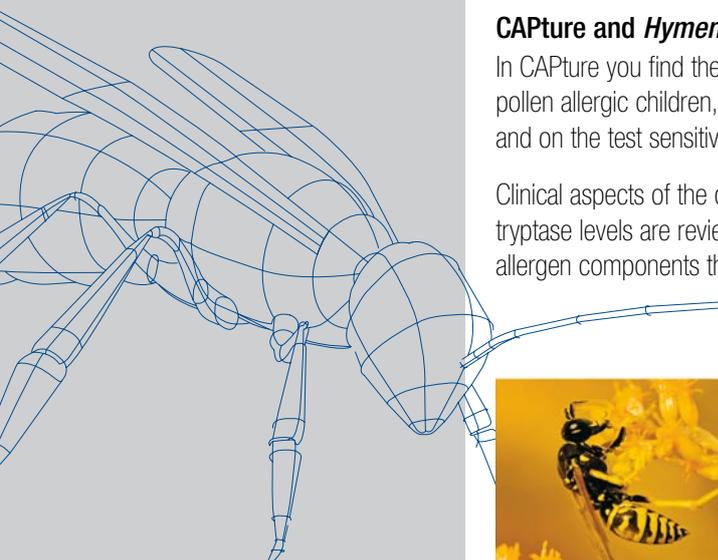


Scientific news,
opinions and reports

Journal

ImmunoDiagnostics



CAPture and *Hymenoptera* allergy diagnosis

In CAPture you find the synopsis of three interesting articles; on the sensitization pattern of grass pollen allergic children, on how ImmunoCAP® ISAC results support the concept of the allergy march, and on the test sensitivity and specificity of wasp and bee allergen components.

Clinical aspects of the diagnosis of *Hymenoptera* allergy, risk factors such as age, patient history and tryptase levels are reviewed in the second part of this issue. You will also find a description of the allergen components that help to make the diagnosis much more exact.



3 CAPture

4 Allergy to stinging insects

With a focus on Allergy



For almost 40 years, Thermo Scientific ImmunoDiagnostics, previously known as Phadia, have maintained global leadership in allergy testing and become one of the world's leading autoimmune disease test

providers. Through clinical excellence, laboratory efficiency and our dedication, we strive to deliver the highest quality and clinical value in our diagnostic tests, as well as providing clinical expertise and scientific information.

You are now holding ImmunoDiagnostics Journal in your hand, our recently introduced customer journal and channel for scientific and clinical information. In this journal you will find articles relevant to aspects of Allergy and Autoimmunity and their diagnosis.

The first two issues were on autoimmunity topics; calprotectin as a useful tool in the diagnosis of inflammatory bowel diseases and the 8th international Congress of Autoimmunity, respectively.

This is the first issue with a focus on Allergy consisting of two parts; CAPture - which gives you brief summaries of a few recent publications - and a review on *Hymenoptera* allergy and its diagnosis. This article covers clinical aspects of the diagnosis of *Hymenoptera* allergy, important factors – such as tryptase levels - to take into account and how to interpret test results. You will also find a description of the main allergen components in bee and wasp venoms.

We hope that the ImmunoDiagnostics Journal will provide you with an easy way to catch up with or learn more on topics of interest to you.



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Editor

Malin Berthold

Contributor(s)

Jan Hed, Anita Kober

CAPture – A selection of recent allergy papers

SYNOPSIS

- Sera from children (n=82, age 1-18 years) and adults (n=48, age 19-70 years) with positive tests to timothy and clinical respiratory symptoms of pollen allergy were recruited.
- Serum IgE to allergen components Phl p 1, Phl p 5, Phl p 7, Phl p 12 of timothy pollen were analyzed with ImmunoCAP® technology. (Phadia Laboratory System, Thermo Fisher Scientific, Uppsala, Sweden)
- The frequency of sensitization to timothy components reported were the following: Phl p 1 - 92%; Phl p 5 - 69.5%; Phl p 12 - 16.6% and Phl p 7 14.8%.
- Almost all (96%) of the patients' sera had specific IgE to the major timothy allergens.
- A small proportion (5%) of children with positive test to timothy extract was negative to all four tested components.

Citation: Sekerkova A et al. Detection of Phl p 1, Phl p 5, Phl p 7 and Phl p 12 specific IgE antibodies in the sera of children and adult patients allergic to Phleum pollen. *Allergol Int.* 2012;61:339-46.

Some grass-pollen allergic children are only sensitized to minor but not to major allergens in contrast to in adults

Introduction of component-resolved diagnosis in grass pollen allergy, based on individual allergen components of the extract, has revealed very heterogeneous sensitization profiles. The clinical value of such analyzes may be important for designing the allergen composition in immunotherapy but also in monitoring the treatment effects. The purpose of this study was to analyze the sensitization profile of grass pollen allergic adults and children with focus on the major allergen components (Phl p 1 and Phl p 5) and cross-reacting minor allergen components (Phl p 7 and Phl p 12) of timothy grass.

Adults as compared to children, had a 20% higher prevalence of sensitization to Phl p 5 (79.1% vs. 59.8%, $p=0.023$) but sensitization to Phl p 1 was similar (90.2% vs. 93.8%). Most patients (71.4%) were sensitized only to major allergens and 24.4% to both major and minor allergens. All adults were sensitized to major allergens whereas a small group of children (3.6%) were sensitized only to minor allergens. Furthermore, the frequency of sensitization to minor allergens at levels ≥ 17.5 kU_A/l was higher in children compared to adults.

The authors conclude that the knowledge that sensitization only to minor cross-reacting allergens is seen in some grass allergic children is of importance for immunotherapy, and that this finding also may be linked to the increase in plant related food allergy that is seen world-wide.

SYNOPSIS

- Serum samples (n=901) from patients (1 month to 65 years) with atopic allergy were selected.
- Serum IgE to 103 different allergen components were measured by ImmunoCAP® ISAC microarray test.
- The percentage of components to which any patient was sensitized increased from 30% before 3 years of age to more than 92% in adolescents and adults (>18 years of age).
- Average ISAC score, defined as the total score of all 103 allergen scores divided by 103, decreased in patients above 17 years in parallel to total IgE
- The sensitizing profile was significantly different in age groups above and below 6 years, respectively, mainly due to the appearance of IgE to inhalant allergens after 3 years of age.
- Before 3 years of age the most prevalent sensitization to allergen component groups were Mites (39.6%) > Egg (29.2%) > Cat (22.9%) > Milk (16.7%).

Citation: Melioli G et al. The IgE repertoire in children and adolescents resolved at component level: a cross-sectional study. *J Pediatr Allergy Immunol.* 2012;23:433-40.

Sensitization profiles shown by the ISAC microarray changed according to age supporting the clinical features of the allergy march

In recent years it has been possible to differentiate sensitization to various allergen components, some of which are species-specific and others are cross-reactive. In the present study IgE sensitization to 103 different allergen components were analyzed by the ImmunoCAP ISAC microarray test and the results were related to the patients' age. The authors emphasize the importance of being cautious when interpreting the difference between the age groups since this is a cross-sectional study.

The mean IgE value of all the tested allergen components in individual patients showed a significant correlation to total serum IgE ($r = 0.92$, $p=0.003$). Sensitization to mite allergen components were the most common sensitization in all age groups including children below 3 years of age.

Sensitization to storage proteins was highest in age groups below 10 years (23-27%) and decreased to 4.3% in the age group above 18 years. This contrasted to the sensitization to LTP which reach a level of roughly 20% at 10 years of age and then remained rather stable at that level into adulthood.

The authors' conclusion is that the pattern of allergen recognition as tested with ISAC microarray is modified according to age supporting the clinical features of the allergy march.

SYNOPSIS

- Patients (n=121) with documented *Hymenoptera* allergy were recruited in a retrospective study.
- Serum IgE to extract and allergen components from bee (Phospholipase A₂) and wasp (Phospholipase A₁, Antigen 5) were measured by ImmunoCAP with a cut off at >0.35 kU_A/l.
- Serum IgE to Cross-reactive Carbohydrate Determinants (CCD) was measured by ImmunoCAP with the same cut off.
- No patient with single positivity to extract gave a positive test to species-specific allergen from the other species.
- Serum IgE to CCDs were significantly ($p<0.0001$) higher in double positive patients compared to single positive (54% vs. 2.2%).

Citation: Müller U et al. IgE to recombinant allergens Api m 1, Ves v 1, and Ves v 5 distinguish double sensitization from cross reaction in venom allergy. *Allergy.* 2012;67:1069-73.

ImmunoCAP test sensitivity and specificity were 100% for wasp components while the sensitivity for bee tests would improve if including additional components

A clinical focus of today in bee and wasp venom allergy is to find out if positive tests to both are due to a true double sensitization, cross-reactivity between the two, or depend on clinically irrelevant sensitization to carbohydrates (CCDs). The introduction of species-specific recombinant *Hymenoptera* major allergens in allergy testing has made it possible to address this issue prior to selection of immunotherapy.

The purpose of the present study was to evaluate these species-specific tests recently introduced on the ImmunoCAP technology platform. Only 47.4% of *Hymenoptera* allergic patients with double-positivity to extract tests had positive test results to species-specific allergen components from both species. All single positive test results were consistent with the suspected culprit insect and the patients showed a low frequency (2.2%) of IgE to CCDs. All patients positive only to wasp extracts showed positive results to the selected wasp-specific allergen components (Ves v 1, Ves v 5). The selected bee-specific venom (Api m 1) was positive in 78.3% of patients single-positive to bee extract. Roughly 50% of patients double-positive to venom extract were sensitized to CCDs either to either species or just one.

The authors concluded that the specificity was optimal (100%) for the selected wasp and bee components and so was the sensitivity for wasp components. The sensitivity for bee components was high (78.3%) and might be improved by including further bee components.

Allergy to stinging insects (*Hymenoptera*)

Clinical background

A systemic reaction to *Hymenoptera* stings is a serious condition that is relatively common – a life time prevalence of 1.2-3.5% has been estimated. Allergic reactions to *Hymenoptera* sting can lead to instant death due to anaphylactic shock and the rate of deaths related to sting reactions ranges between 0.09 to 0.45 deaths per year and one million inhabitants. The prevalence varies between countries depending on the aggressiveness of the *Hymenoptera* species in the region. In Europe most deaths are caused by wasps, while in the United States the African killer bee is the main species causing fatal reactions.

In contrast to many other IgE mediated allergic reactions, also individuals without an atopic background may be sensitized by venom stings and get IgE-mediated reactions upon later stings.

Hymenoptera sting reactions are often classified in three categories:

- mild local (immediate reactions),
- large local reaction (often late reactions)
- systemic reactions (immediate reactions)

The earliest and mildest expressions of a systemic reaction often are skin symptoms which then expand to the respiratory tract and cardiovascular system, thereby leading into life threatening conditions requiring immediate treatment.

While systemic reactions primarily are due to IgE mediated mechanisms, the large local reactions also may include other toxic mechanisms.

Both the EAACI and AAAAI position papers state that all patients with systemic reaction to insect stings and some

with large local reaction should be tested for baseline serum tryptase as a marker for mast cell load and risk for severe systemic reaction at a resting, and that specific IgE to *Hymenoptera* should be investigated.

Major clinical questions

From a clinical perspective there are important questions that need to be answered for a complete and correct diagnosis of the *Hymenoptera* allergy. A correct diagnosis will guide in selecting optimal SIT treatment and in addition it is important to monitor the effectiveness of the treatment.

Patients with history of clinical reactions to stinging insects

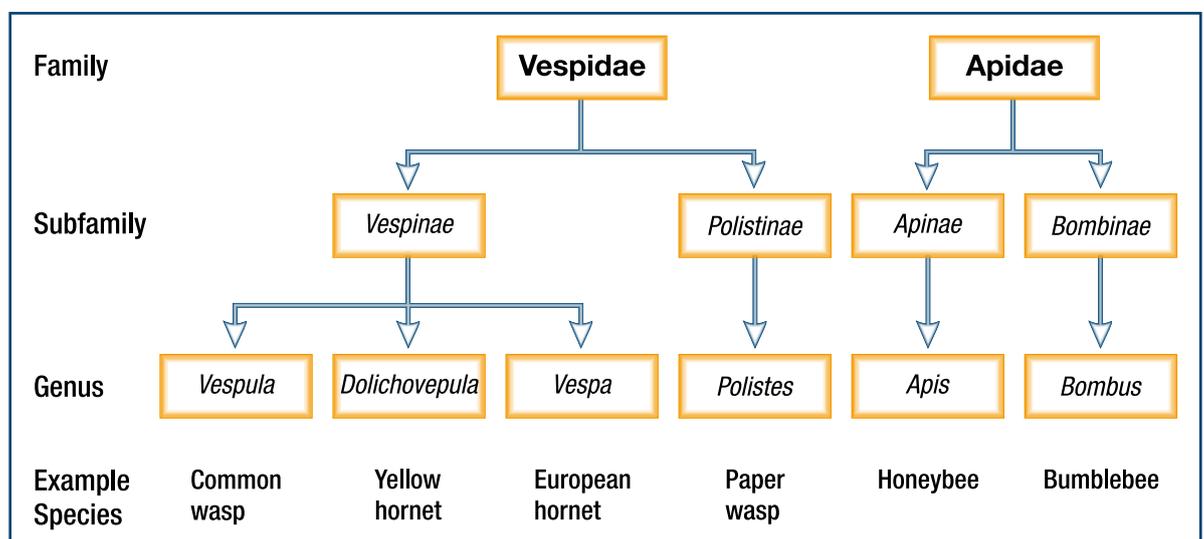
- Does the clinical picture fit with an IgE-mediated systemic reaction?
- How big is the risk for a severe reaction next time when stung by a *Hymenoptera* insect?
- Which *Hymenoptera* species (family/genus level) is the culprit stinging insect?
- Which *Hymenoptera* species extract should be used in immunotherapy?

Patients with no history of venom allergy but with positive test results

- Is it a true sensitization to *Hymenoptera* venoms or a cross reactivity to CCDs of plant or invertebrate allergen components which thus is of less clinical relevance?

Monitoring the effect of immunotherapy

- Is the treatment effective and is tolerance induced?
- Is there a risk for serious side effects?



Important factors for risk assessment

Baseline serum tryptase

The baseline serum tryptase level is a key factor in evaluating the risk for severe systemic reactions. The association between severity of systemic reactions to *Hymenoptera* sting and increased basal serum tryptase levels as a marker of increased mast cell load has been well documented. A non-linear significant correlation between the serum tryptase level and severe systemic reaction was recently shown in a large multicenter study (Ruëff 2010).

The importance of measuring baseline serum tryptase in all patients with a clinical history of severe sting reaction is emphasized in the latest EAACI and AAAAI position papers on *Hymenoptera* immunotherapy. It is stated that baseline serum tryptase shall be measured before the start of venom immunotherapy as a risk marker for future side effects of the treatment.

The WHO has established that a baseline serum level above 20 µg/L is one minor criterion for the diagnosis of systemic mastocytosis.

The serum tryptase test commercially available measures all forms of tryptase that are constitutively released from mast cells and therefore baseline tryptase is a marker of mast cell load in the tissues.

The assay also measures mature tryptase which is de novo released upon mast cell activation (anaphylaxis). Tryptase is released in parallel with histamine in anaphylactic reactions giving a transient increase of tryptase levels that can be measured during a much longer time span (hours) compared with histamine which has a very short half-life in serum (minutes).

A transient increase of tryptase is therefore a confirmation of anaphylactic reaction

Allergen-specific IgE

The presence of IgE antibodies to true CCD-free venom allergens is a risk factor for severe reactions to *Hymenoptera* stings. The availability of both complete extracts and CCD-free specific main venom allergens produced by recombinant technique now give the possibility of making a risk assessment without the confounding effect of CCD specific IgE.

The reaction severity to a *Hymenoptera* sting does not seem to correlate with the level of venom-specific IgE antibodies. It is not uncommon that severe reactions occur also in patients with very low venom specific IgE levels, therefore the sensitivity of the assay used is of high importance.

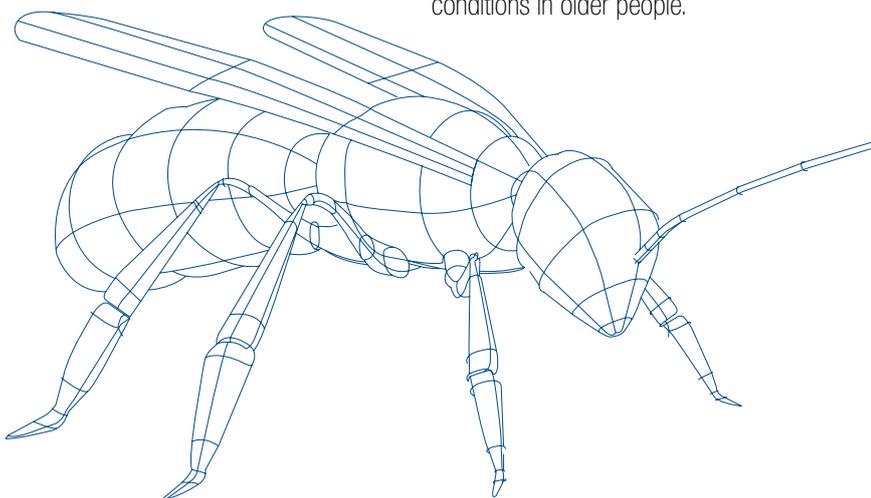
There are a few recent publications where the ratio between *Hymenoptera* allergen-specific IgE and total serum IgE have been used to predict the clinical improvement of allergen-specific immunotherapy however the relevance of this needs to be proven.

Previous severe reactions

Having had a previous systemic reaction suggests an increased risk for severe reaction also at the next *Hymenoptera* sting, whereas there is a lower similar risk after having reacted with large local reaction the first time. The risk to develop a systemic reaction at the next *Hymenoptera* sting has been estimated to be 5-15% in large local reaction, about 20% in mild systemic reaction and up to 80% in severe systemic reaction. However the risk decreases with the interval between stings to about a level of 20-30% after 10 years.

Age

Clinical experience shows that the risk for severe reaction to *Hymenoptera* sting is higher in adults than in children and adolescents. This is suggested to depend on an increased mast cell load (measured as increased basal serum tryptase), lower total serum IgE levels (high ratio spec-IgE/tot-IgE) but also on other contributing clinical conditions in older people.



Central issues for a correct diagnosis

Cross-reaction vs. double-sensitization

The clinical question of cross-reactivity vs. double sensitization is of importance in the selection of venom allergens for immunotherapy. There is a high degree of cross-reactivity between allergen components from closely related *Hymenoptera* species e.g. honey bee and bumblebee, but as always, there may also be specific differences affecting the results of diagnostic tests, which has been demonstrated for *Polistes* species from America and Europe. Therefore it is important to know which insects are most common in the geographic area and thus most likely to be causing the reactions of the patients. A large portion - roughly above 50% - of the cross-reactivity shown in patient's sera is due to IgE binding to CCD epitopes and not peptide epitopes.

Cross-reactive Carbohydrate Determinants (CCD) in *Hymenoptera* venoms

Glycoproteins in plants and invertebrate animals carry N-linked glycans, which do not exist in mammals. Since these carbohydrate determinants are foreign epitopes to humans they are highly immunogenic and will give rise to antibodies such as IgE. The widespread presence of N-linked glycans in plants and invertebrates explains the high degree of cross-reactivity to non-related allergens that has been reported for carbohydrate-specific IgE antibodies. These carbohydrate determinants have been termed Cross-reactive Carbohydrate Determinants (CCDs) and the IgE antibodies are termed anti-CCD IgE.

Approximately 20% of grass pollen allergic patients have IgE antibodies to CCDs and since many allergens in *Hymenoptera* venoms are glycoproteins the anti-CCD IgE antibodies induced by pollen may also bind to *Hymenoptera* allergens in tests. Most *Hymenoptera* allergens contain a single N-linked glycan, however hyaluronidase contains two glycans per molecule which can thus on their own cross-link cell-bound anti-CCD IgE thereby triggering the cellular response. Allergens with only one N-linked glycan need, in addition to anti-CCD, also sensitization to a peptide-epitope to induce cross-linking of IgE on the mast cells.

Timing of testing after a sting reaction

It is today recommended that a patient is tested for IgE antibodies as soon as possible after having reacted to a *Hymenoptera* sting. The clinical advantage of an early testing is that most patients could be considered for immunotherapy immediately. Previously, the common diagnostic routine was to delay the testing of venom specific serum IgE at least two weeks after the *Hymenoptera* sting reaction. More recent studies show, however, that the risk of false-negative results is much overestimated and that most patients show positive test results in direct connection with the sting reaction or at the first visit shortly after.

If there is a strong suspicion that the reaction is due to a sting, but the test results from a sample taken close in time to the reaction are negative, the recommendation is to repeat the testing a couple of weeks later since IgE antibody levels usually increase after a sting.

Interpretation of test results

To correctly diagnose a *Hymenoptera* allergy can be difficult, both as patients not always know which insect they got stung by, and due to that the diagnostic test do not always give unambiguous results. Below is a short guide of how to interpret test results, and what is recommended to do next in the diagnostic work-up for more exact diagnosis.

Positive extract based test to Bee or Wasp but *not both*

- Indicates a true *Hymenoptera* venom sensitization. A positive test due to CCD or to the cross-reacting *Hymenoptera* allergen components such hyaluronidase should give positive results for both/all extract tests.
- Suggested action: continue with component resolved testing to refine the diagnosis.

Positive results with *both* Bee and Wasp extract tests

- This sensitization profile does not tell whether results are due to true co-sensitization to both honeybee and wasp or to cross-reactivity. Cross-reactivity may be caused by antibodies induced by CCDs on hyaluronidases or by CCDS on other CCD-carrying allergens in e.g. grass pollen. In the latter case this sensitization is likely to be of less clinical importance.
- To resolve these questions:
 - Measure specific IgE to recombinant honeybee Phospholipase A₂ (rApi m 1) and Antigen 5 in addition to Phospholipase A₁ from *Vespula* (Ves v 5/Ves v 1) and/or *Polistes* (Pol d 5).
 - Measure specific IgE to CCDs to verify/exclude cross-reactivity based on carbohydrate epitopes (CCDs).

Positive test to CCD but negative to the *Hymenoptera*-specific tests above together with an uncertain clinical history helps to exclude the diagnose *Hymenoptera*-allergy.

Negative tests to Bee and Wasp extracts

- Recent studies have shown that although negative in skin prick tests, wasp allergic patients may have detectable levels of serum IgE levels to wasp venom in the low range (0.1-0.35 kU_A/l). Thus it is important to use tests of high sensitivity:
 - If an extract based test to wasp is negative, component testing with rVes v 5 and rVes v 1 is recommended since in some cases the pure component tests are more sensitive.
- Negative test results may in rare cases be due to very low/undetectable levels of IgE at the time of sting reaction. If the test sample was taken in close relation to a severe sting reaction a retest after 2 weeks is recommended. If still negative after 2 weeks consider - depending on the clinical expression - retesting after 3-6 months.
- If a strong clinical suspicion exists for *Hymenoptera* allergy to bumblebee (high exposure), test for IgE sensitization to bumblebee allergens.

When and how to measure serum tryptase

Baseline and peak serum tryptase levels give different information and are tested for at different time points.

- For risk assessment it is of value to establish whether the patient has an elevated baseline serum tryptase level, which indicates a high mast cell load and thus increased risk for severe reactions. When considering treatment of *Hymenoptera* allergy using specific immunotherapy, baseline tryptase levels should be measured.
- If the clinical systemic reaction is very typical and related to *Hymenoptera* sting but IgE tests are negative, measure serum tryptase after four weeks as a marker of mast cell load/possible involvement of a mast cell related disease.
- In conjunction with a severe reaction measure the early peak-value of serum tryptase as close to the reaction as possible but at least within three hours from symptom start, and in addition after 24-48 hours to establish if the levels have returned to normal baseline.
 - Initially elevated, and then decreased tryptase levels within the time range described above, verifies that a mast cell dependent systemic reaction has occurred.
 - A persistent high baseline value after 72 hours indicates a high mast cell load.

Sensitizing allergens in venoms

The recent development of IgE tests against species-specific allergen components in *Hymenoptera* venom has offered diagnostic tools that much better differentiate between sensitization to bees and wasps, and helps in discriminating between IgE antibodies directed against peptide epitopes and CCD epitopes.

There are some important differences in the allergen composition between *Hymenoptera* families such as bees and wasps. Knowing these differences is of importance for selecting optimal *Hymenoptera* venom immunotherapy and also in the diagnostic routine since the sensitivity of the tests is high but does not reach 100%.

<p>Wasps – Vespidae</p> <p>Wasps Hornets Paper wasps</p>	<p>Bees – Apidae</p> <p>Honeybees Bumblebees</p>
<p>Wasp Venoms</p> <p>Antigen 5 Phospholipase A₁ Hyaluronidase *</p>	
<p>Bee Venoms</p> <p>Phospholipase A₂* Hyaluronidase * Mellitin</p>	
<p>*Native allergens carry CCDs, recombinant are free of CCDs</p>	

Wasp-venom allergens:**Antigen 5** *Ves v 5; Pol d 5; Dol m 5; Vesp c 5*

Antigen 5 is the major Vespidae venom allergen. It is specific for the Vespidae family and it is free of CCD moieties.

IgE antibodies to *Ves v 5* from the common wasp (Yellow jacket) has a sensitivity of 80-90%, thus it is itself not sufficient to unambiguously identify wasp as the culprit stinging insect. It has been shown that by adding wasp venom Phospholipase A₁ (*Ves v 1*) the sensitivity increases roughly 10% almost up to 100% (97.5%). This indicates that there exist still some more minor allergen components or species specific epitopes that might be of clinical importance.

There is certain but not complete cross-reactivity between Antigen 5 from the four different genera (*Vespa*, *Vespula*, *Dolichovepula*, *Polistes*) within the Vespidae family. Antigen 5 from *Vespa*, *Vespula* and *Dolichovepula* are more closely related to each other than to Antigen 5 from the genus *Polistes*. In geographic areas where sting reactions to both *Polistes* and *Vespula* species are common like the Mediterranean region, the diagnostic efficiency is improved if Antigen 5 and Phospholipase A₁ from both genera are included in testing to increase test sensitivity. Also the relative levels of IgE antibodies to the different components give information on the likely culprit insect.

Phospholipase A₁ *Ves v 1; Pol a 1; Vesp c 1; Dol m 1*

IgE to Phospholipase A₁ in wasp venoms does not cross-react with Phospholipase A₂ in bee venoms and furthermore do not carry carbohydrates such as CCD. The cross-reactivity between phospholipase A₁ from different wasps generally follows their degree of phylogenetic relationship and is therefore strongest between species from *Vespula*, *Vespa* and *Dolichovepula* genera. Serum IgE to Phospholipase A₁ will have additional value in excluding or verifying wasp as the culprit stinging insect if the IgE test to Antigen 5 is negative.

Hyaluronidase *Ves v 2; Dol m 2; Pol a 2*

Hyaluronidase has been regarded as a major allergen in both bee and wasp venoms and is thus an important allergen with true cross-reactivity between bee and wasp venoms. However, a large portion - roughly above 50% - of the cross-reactivity shown in patient's sera is due to IgE binding to CCD epitopes and not peptide epitopes. Hyaluronidase carries more than one N-linked glycan, and thus cross-linking of anti-CCDs IgE antibodies on basophils and mast cells which initiate a cellular response, can take place without a concomitant sensitization to peptide epitopes on the allergen.

Recent studies have shown that the peptide part of the wasp *Ves v 2* has very low allergen activity and that the main IgE binding instead is attributed to CCD epitopes. This is however not the case for honeybee hyaluronidase (*Api m 2*), where the CCD free peptide molecule has been shown to be an important allergen (see below).

Bee-venom allergens:**Phospholipase A₂** *Api m 1; Bom t 1/p 1*

Phospholipase A₂ is a major and important allergen component of the Apidae family, and it has very high allergenicity. While native phospholipase A₂ carries a single CCD moiety, recombinant phospholipase A₂ used in diagnostic routine lacks CCD and is a specific marker for Apidae venom sensitization.

Detection of sensitization to the recombinant honey bee *Api m 1* (r*Api m 1*) has a high sensitivity for proving that the culprit insect belongs to the Apidae family (honeybee/ bumblebee). The sensitivity of r*Api m 1* has been reported to range between 60-85 % in different patient populations. The sensitization patterns to bee venom seem to be more complex than for wasp venoms, thus more bee venom components are needed in addition to *Api m 1* to get a complete component resolved diagnostic picture.

Cross-reactivity between phospholipase A₂ from honeybee (*Apis sp*) and bumblebee (*Bombus sp*) takes place but species-specific epitopes do also exist. This has been shown to become a clinical problem in some patients with an occupational sensitization to bumblebees (flowering/ vegetable industry) where sensitization to epitopes distinct from those in honey bee venom may occur. Those patients might have a low or no reactivity to honeybee phospholipase A₂ in tests and should be treated with extracts from bumblebees in the immunotherapy.

It should be noted that Phospholipase A₂ from honeybee and Phospholipase A₁ from wasp are different proteins with different functions, and that cross-reactivity between their peptide epitopes does not take place.

Hyaluronidase *Api m 2; Bom p 2*

Hyaluronidase is a major allergen in both bee and wasp venoms and regarded as the most important allergen for true cross-reactivity between bee and wasp venoms. However, a large portion of the cross-reactivity seen in patients' sera is due to IgE binding to CCD epitopes. Hyaluronidase has at least two N-linked glycans with the possibility to crosslink anti-CCD on effector cells such as basophils and mast cells without a concomitant sensitization to peptide epitopes.

However, also the pure CCD-free honeybee hyaluronidase peptide molecule - rApi m 2- has been shown to be an important allergen as opposed to the case with rVes v 2 (see above).

Melittin *Api m 4*

Melittin is a small protein (2.9 kDa), abundant in the Apidae family (30-50% dry weight of the venom) but it is regarded as a minor allergen. Due to its high concentration in the venom some *Hymenoptera* allergic patients are sensitized to this component, although mono-sensitization to melittin is rare.

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